A Theoretical Model for the Prediction of Sequence-Dependent Nucleosome Thermodynamic Stability

Claudio Anselmi,* Gianfranco Bocchinfuso,* Pasquale De Santis,* Maria Savino,† and Anita Scipioni*
*Dipartimento di Chimica and †Dipartimento di Genetica e Biologia Molecolare, Istituto Pasteur, Fondazione Cenci Bolognetti, Università degli Studi di Roma "La Sapienza," I-00185 Rome, Italy

ABSTRACT A theoretical model for predicting nucleosome thermodynamic stability in terms of DNA sequence is advanced. The model is based on a statistical mechanical approach, which allows the calculation of the canonical ensemble free energy involved in the competitive nucleosome reconstitution. It is based on the hypothesis that nucleosome stability mainly depends on the bending and twisting elastic energy to transform the DNA intrinsic superstructure into the nucleosomal structure. The ensemble average free energy is calculated starting from the intrinsic curvature, obtained by integrating the dinucleotide step deviations from the canonical B-DNA and expressed in terms of a Fourier series, in the framework of first-order elasticity. The sequence-dependent DNA flexibility is evaluated from the differential double helix thermodynamic stability. A large number of free-energy experimental data, obtained in different laboratories by competitive nucleosome reconstitution assays, are successfully compared to the theoretical results. They support the hypothesis that the stacking energies are the major factor in DNA rigidity and could be a measure of DNA stiffness. A dual role of DNA intrinsic curvature and flexibility emerges in the determination of nucleosome stability. The difference between the experimental and theoretical (elastic) nucleosome-reconstitution free energy for the whole pool of investigated DNAs suggests a significant role for the curvature-dependent DNA hydration and counterion interactions, which appear to destabilize nucleosomes in highly curved DNAs. This model represents an attempt to clarify the main features of the nucleosome thermodynamic stability in terms of physical-chemical parameters and suggests that in molecular systems with a large degree of complexity, the average molecular properties dominate over the local features, as in a statistical ensemble.

INTRODUCTION

The nucleosome, the elemental unit of chromatin, is the association complex of DNA with the histone octamer. The first model was proposed by Kornberg (1977). A few years later, Klug and co-workers, according to the Kornberg proposal, determined its structure at low resolution by electron microscopy image reconstitution (Klug et al., 1980), and x-ray diffraction techniques (Richmond et al., 1984). The nucleosome is characterized by a flat solenoid-like structure in which a DNA tract of 145 bp is wrapped around the proteic core of the histone octamer with a pseudo-dyad symmetry. Recently, a 2.8-Å resolution electron density map was obtained that confirms the main features of the previously proposed structure and contains relevant details of both DNA and the protein core (Luger et al., 1997).

Despite the deeper knowledge of the structure, the question about the preferential positioning and stability of the nucleosome along the DNA chain still seems to be open.

Different authors investigated the phase and the translational positioning of nucleosomes on different DNA sequences (McGhee and Felsenfeld, 1980; Drew and Travers, 1985; Satchwell et al., 1986; Travers and Klug, 1987; van Holde, 1988; Widom, 1989; Blank and Becker, 1996; Flaus et al., 1996). The experimental evidence indicates that nucleosome reconstitution can be obtained with practically any DNA sequence. However, several papers pointed out the existence of preferential positioning enhanced by some base sequences, as well as the occurrence of nucleosome-free tracts in chromatin.

Competitive reconstitution experiments allow the determination of the differential thermodynamic nucleosome affinity along a DNA sequence, providing a sound basis for discovering the sequence effects on the nucleosome stability (Shrader and Crothers, 1989, 1990; Godde and Wolffe, 1996; Godde et al., 1996; Wang et al., 1996; Wang and Griffith, 1996; Widlund et al., 1997; Cacchione et al., 1997; Lowary and Widom, 1998; Rossetti et al., 1998; Dal Cornò et al., 1998; Cao et al., 1998).

The possibility of specific interactions between amino acid residues and certain base pairs and/or the propensity of DNA tracts to wrap over the protein core, depending on their flexibility and intrinsic curvature, have been advanced to explain nucleosome stability.

In this context, several authors have made attempts to find the sequence features that enhance or, on the contrary, reduce the stability of nucleosomes. They have shown that intrinsic curvature, flexibility, and some consensus sequences play relevant roles.

Nucleosome stability was first investigated by means of the competitive nucleosome reconstitution technique, by using synthetic nucleosome sequences (Shrader and Crothers, 1989). Later Wolffe and co-workers (Godde et al., 1996; Godde and Wolffe, 1996) provided evidence that a

Received for publication 28 October 1999 and in final form 17 April 2000.
Address reprint requests to Dr. Pasquale De Santis, Università La Sapienza, Dipartimento di Chimica, P. le A. Moro 5, Box 34, Rome 62, 00185 Rome, Italy. Tel.: 39-06-49913228; Fax: 39-06-4453827; E-mail: p.desantis@caspur.it.

0006-3495/00/08/601/13 $2.00

practically straight DNA, characterized by CGG triplet repeats, shows high affinity in the reconstitution experiments. DNAs characterized by runs of phased three or four adenine residues, extensive CA repeats, and TATA tetranucleotides, which form very stable nucleosomes despite their low curvature, were recently isolated in the mouse genome (Widlund et al., 1997). SELEX experiments, carried out with a large pool of random DNA fragments, allowed the isolation of individuals having the highest affinity with histone octamer so far obtained. The results have also revealed new, statistically significant sequence rules (Lowary and Widom, 1998).

On the other side, a large number of DNA fragments characterized by low affinity for histone octamer were selected through anti-SELEX experiments (Cao et al., 1998). Recently, telomeric sequences were identified as the least stable nucleosomes known so far (Rossetti et al., 1998).

Despite the available data, there is no doubt that our understanding of the physical-chemical origin of the nucleosome stability is still unsettled or at least fragmentary. This is made more difficult by the experimental evidence that the free-energy differences, involved in the affinity of the histone octamer to different DNAs, appear to be restricted to within a few kcal per mole of the nucleosome. This suggests that DNA-histone recognition could be driven by a few chemical determinants, which some authors have localized close to the dyad axis (McGhee and Felsenfeld, 1980; Drew and Travers, 1985; Satchwell et al., 1986; Travers and Klug, 1987; van Holde, 1988; Widom, 1989; Blank and Becker, 1996; Flaus et al., 1996), and/or by simple elastic-energy differences depending on different DNA intrinsic curvature and flexibility.

However, the first hypothesis does not appear to be supported by the crystallographic evidence, as the histone octamer dyad axis unexpectedly does not coincide with the virtual dyad symmetry of the palindromic DNA used in the nucleosome reconstitution (Luger et al., 1997). It appears to lie on a virtual dyad axis of the phosphodiester chain, suggesting a minor role of the base pair-histone interactions in the nucleosome formation.

Furthermore, it is puzzling that Shrader and Crothers (1989, 1990) found that some intrinsically curved DNAs, which were supposed to form highly stable nucleosomes, surprisingly showed lower affinity for the histone octamer than relatively straight DNAs with similar sequences.

To evaluate the thermodynamic equilibrium of the competitive nucleosome reconstitution, a model based on the nearest-neighbor approximation was adopted. It calculates the curvature function and flexibility in terms of the sequence, starting from dinucleotide step thermodynamic and structural parameters and adopts first-order elasticity to calculate the pertinent canonical partition functions involved in the DNA-histone octamer equilibrium association (Anselmi et al., 1999). In fact, nearest-neighbor models seem to be rather accurate in predicting the macroscopic behavior of DNA molecules, as demonstrated by the results obtained in our laboratory (De Santis et al., 1996; Anselmi et al., 1998) or by several other authors (Gotoh and Tagashira, 1981; Bolshoy et al., 1991; Gorin et al., 1995; Sugimoto et al., 1996; Olson et al., 1998; SantaLucia, 1998) and recently underlined by Crothers (1998).

In the present paper, we develop this model, justifying some assumptions adopted about DNA flexibility and hydration role. Also the calculation of the elastic canonical partition functions is presented and extensively discussed.

The comparison with the experimental data shows that DNA intrinsic sequence dependent curvature and flexibility play a dual role in determining the nucleosome stability. Besides the apparent decrease in the elastic energy with increasing curvature and flexibility, a destabilizing role emerges, which is probably due to the change in curvature-dependent interactions with water molecules and counterions, in the thermodynamic equilibrium of the nucleosome formation. In the case of flexibility, we have found that the entropy decrease, consequent to nucleosome formation, appears to overwhelm the obvious stabilizing energy effect.

Despite the complexity of the system, which involves a large number of differential interactions between DNA and the histone core, the proposed model appears to be surprisingly capable of predicting the free-energy difference involved in the competitive nucleosome reconstitution experiments performed in different laboratories on a large pool of synthetic and natural DNAs (Shrader and Crothers, 1989, 1990; Godde and Wolffe, 1996; Godde et al., 1996; Widlund et al., 1997; Cacchione et al., 1997; Lowary and Widom, 1998; Rossetti et al., 1998; Dal Cornò et al., 1998; Cao et al., 1998).

METHODS

The model

The nucleosome reconstitution can be treated as resulting from parallel reactions, as pictorially illustrated in Fig. 1. If $\Delta G(k)$ represents the nucleosome reconstitution free energy difference of the kth DNA tract with $L = 144$ bp along a sequence with N bp, the free energy per mole of nucleosome, ΔG, pertinent to the whole DNA is

$$\beta\Delta G = -\ln \sum_{k=L/2}^{N-L/2} \exp[-\beta\Delta G(k)] \qquad (1)$$

where β is $1/RT$.

The relation with the pertinent canonical partition functions allows us to write the nucleosome reconstitution free-energy difference as

$$\beta\Delta G(k) = -\ln \frac{Q_n(k)}{Q_n^*} + \ln \frac{Q_f(k)}{Q_f^*} \qquad (2)$$

where $Q_n(k)$ is the configurational canonical partition function of the kth nucleosomal DNA tract along the sequence; $Q_f(k)$ is that relative to the corresponding uncomplexed free DNA tract. These two functions are calculated under the same physicochemical conditions of competitive

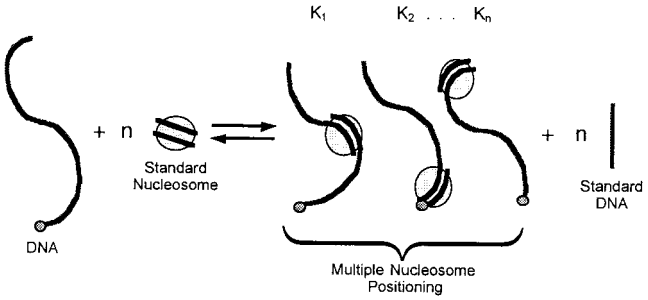

FIGURE 1 Schematic drawing of competitive nucleosome reconstitution. A specific DNA can be long enough to form more than one nucleosome. Each nucleosome positioning is characterized by an equilibrium constant ($K_1, K_2, \ldots, K_n$). An ideal straight random-sequence DNA of 144 bp with uniform average flexibility is adopted as a standard. Adopting a different standard corresponds to adding a constant term to the free energy difference; consequently, it does not affect relative nucleosome stability and positioning.

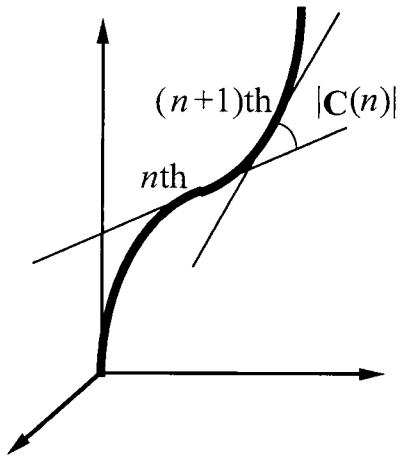

FIGURE 2 Representation of the DNA curvature as the angular deviation between the local helical axes of the turn centered on the *n*th and (n + 1)th basepairs.

nucleosome reconstitution (Shrader and Crothers, 1989). Q_n^* and Q_f^* are those pertinent to an ideal intrinsically straight DNA with a random-sequence 144 bp we assume as a standard. The partition functions of the remaining DNA tracts not involved in the *k*th nucleosome are considered in a first approximation equivalent to those of the free DNA and cancel in the ratio.

In the first approximation we will only consider the elastic energy contribution to the free energy difference between the nucleosomal and the free DNA. These contributions are sequence dependent through the intrinsic curvature, twisting, and flexibility of the DNA tract. The intrinsic curvature and twisting functions can be obtained in terms of the sequence by integrating the dinucleotide step roll, tilt, and twist with rather good confidence, as will be illustrated in the next paragraph. The isotropic bending and twisting flexibility will be obtained as sequence-dependent elastic force constants by modulating the generally accepted standard bending and torsional force constants, with a sequence-dependent factor related to the differential thermodynamic stability of the DNA tract.

Evaluation of the DNA intrinsic curvature and elastic force constants

The curvature of a space line is defined as the derivative, d**t**/d*l*, of the tangent versor, **t**, along the line *l*. Its modulus is the inverse of the curvature radius, and its direction is that of the main normal to the curve (Landau and Lifshitz, 1970).

In the case of DNA, the line corresponds to the helical axis and the curvature is a vectorial function of the sequence. The curvature represents the angular deviation between the local helical axes of the *n*th and (n + 1)th base pairs (Fig. 2). Under similar external conditions, the intrinsic curvature function represents the differential behavior of different DNA tracts and corresponds to the most stable superstructure. The physical origin of curvature is still a matter of debate; it is, however, a result of the chemical and consequently stereochemical inhomogeneity of the sequence, which gives rise to different macroscopic manifestations. These manifestations change with the thermodynamic conditions, such as pH, the ionic force, the kind of counterions, and obviously the temperature as a result of perturbations on the intrinsic curvature depending on the sequence-dependent bendability. Therefore, it is generally found useful to characterize a DNA superstructure with the so-called intrinsic curvature function.

It can be calculated from the local dinucleotide step orientational parameters: roll (ρ), tilt (τ), and twist (Ω) (Fig. 3). In the present work, we adopt the set of angles (Table 1) proposed in our previous papers. They were initially evaluated in the framework of the nearest-neighbor approximation by energy calculations (De Santis et al., 1986) and later refined to improve the correlation between calculated and experimental gel electrophoresis mobility of a very large pool of synthetic as well as natural DNAs (De Santis et al., 1990, 1992; Boffelli et al., 1992). Other authors proposed different base pair orientational parameters on an empirical basis (electrophoresis mobility or x-ray double helix oligonucleotide structural data). Despite their differences, the curvatures predicted for many synthetic or natural DNA tracts appear quite similar, as recently reviewed by Crothers (1998).

Because orientational angles show little variance, it is convenient to adopt a representation of the curvature as a vector in the complex plane, corresponding to the first-order Taylor expansion of the pertinent rotation matrix product (De Santis et al., 1990):

$$\mathbf{C}^{o}(n) = \frac{1}{\nu_n} \sum_{\text{nth turn}} \mathbf{d}_j \exp\left(2\pi i \frac{s}{\nu_n}\right) \tag{3}$$

where ν_n is the local periodicity of the DNA turn evaluated from the twist Ω_j and $\mathbf{d}_j = \rho_j - i\tau_j$.

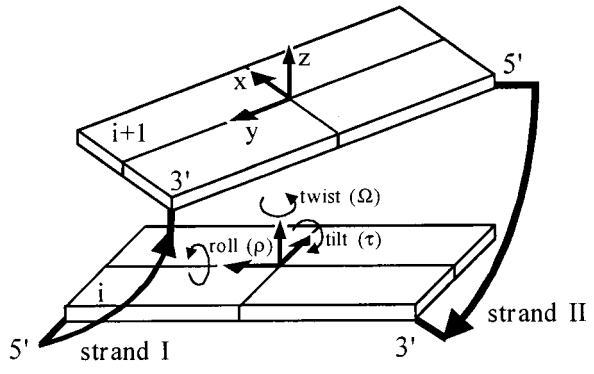

FIGURE 3 Orientational parameters of the basepair average plane in a dinucleotide step.

TABLE 1 Roll (ρ), tilt (τ), and twist (Ω) parameters

d, Ω	A	T	G	C
T	(8.0, 0.0), 34.5	(−5.4, −0.5), 36.0	(6.8, 0.4), 34.1	(2.0, −1.7), 34.6
A	(−5.4, 0.5), 36.0	(−7.3, 0.0), 35.3	(1.0, 1.6), 34.4	(−2.5, 2.7), 33.7
C	(6.8, −0.4), 34.1	(1.0, −1.6), 34.4	(4.6, 0.0), 33.5	(1.3, −0.6), 33.1
G	(2.0, 1.7), 34.6	(−2.5, −2.7), 33.7	(1.3, 0.6), 33.1	(−3.7, 0.0), 33.3

$d = (\rho, -\tau)$ and Ω in degrees.

According to the classical formulation by Landau and Lifshitz (1970), the bending distortion energy ΔE_b, of a DNA tract with N bp is defined as

$$\Delta E_b = \frac{b}{2}\sum_{1}^{N}|\mathbf{C}(n) - \mathbf{C}^o(n)|^2 \tag{4}$$

where b is equal to the product of the Young modulus with the inertia moment of an isotropic rodlike DNA chain and represents the apparent bending force constant; $\mathbf{C}(n)$ and $\mathbf{C}^o(n)$ are the induced (by an external force field) and intrinsic curvatures, respectively.

At the thermal equilibrium, the average energy, corresponding to the nucleotide step curvature fluctuation, $\langle|\mathbf{C}(n) - \mathbf{C}^o(n)|^2\rangle = \langle C^2\rangle$, is equal to RT. Therefore, the apparent harmonic constant is

$$b = \frac{2RT}{\langle C^2\rangle} = \frac{RTP}{l} \tag{5}$$

In fact, $2/\langle C^2\rangle$ coincides with the normalized persistence length, P/l (l = 3.4 Å is the helix rise per base pair in the B-DNA). Consequently, the force constant b in RT units represents the normalized persistence length.

Analogously, the torsional energy

$$\Delta E_t = \frac{t}{2}\sum_{1}^{N}(\Omega(n) - \Omega^0(n))^2 \tag{6}$$

is defined in terms of an apparent harmonic torsional force constant, t, and the squared differences between the intrinsic and the final nucleosomal twist angles of the dinucleotide step. The torsional elastic constant t is related to the torsional rigidity C as

$$t = C\mathcal{N}/l \tag{7}$$

where $\mathcal{N}$ is Avogadro's number.

An evaluation of the sequence-dependent DNA flexibility

Introducing DNA sequence-dependent flexibility implies knowledge of the statistical parameters necessary to evaluate the average flexibility of each DNA tract that would require the determination of the persistence length and the torsional rigidity of a large number of sequences. Several authors addressed this issue by using different experimental data, such as gel electrophoretic retardation (Chastain et al., 1995), circularization kinetics of DNA tracts with different sequences and length (Bacolla et al., 1997), or sequence-dependent deformability in double helix oligonucleotide or protein-oligonucleotide complexes deduced from x-ray crystal structures (Berman, 1997). The information obtained about the sequence-dependent DNA flexibility in terms of dinucleotide step parameters is at present fragmentary and in some cases contradictory. Olson and co-workers obtained two sets of dinucleotide flexibility parameters by surveying x-ray crystal structures of double-helix oligonucleotides (Gorin et al., 1995) and DNA-protein complexes (Olson et al., 1998). They considered the crystal packing (or the interactions with proteins) as external perturbing force fields, so that the dispersion of the roll, tilt, and twist parameters would represent an empirical measure of sequence-dependent flexibility. Actually, these sets of dinucleotide step flexibility appear to be rather uncorrelated and affected by the systematic presence of the AT-rich tracts in the central positions of the examined oligonucleotides, while the GC residues are generally located at the end of the sequences.

In the present uncertainty of data, we tried to evaluate the sequence-dependent DNA flexibility from the double helix thermodynamic stability as proposed by different authors in terms of dinucleotide step parameters (Gotoh and Tagashira, 1981; SantaLucia, 1998, and references therein).

Assuming an elastic rodlike DNA model, we consider the helix-coil transition of a DNA tract as a factor that could modulate the average elastic force constant and quantify the sequence-dependent flexibility.

In fact, because of the catastrophic character of the helix-coil transition, until the temperature is a few degrees lower than that corresponding to the melting point, the double helix still represents the thermodynamic state of DNAs (in fact, spectroscopic and electrochemical properties are only slightly modified with respect to those at room temperature).

Supposing an elastic behavior in such a "premelting" temperature range, the thermal energy can be related to the isotropic bending variance (Hagerman, 1988):

$$\tfrac{1}{2}b\langle C^2\rangle = RT \tag{8}$$

where b is the apparent elastic force constant and $\langle C^2\rangle$ is the average dinucleotide-step bending fluctuation. Therefore, for a standard DNA (namely, a straight chain with random sequence),

$$\tfrac{1}{2}b^*\langle C^2\rangle^* = RT^* \tag{9}$$

We assume that the helix-coil transition starts at the temperature where the energy fluctuations reach a critical value of the base pair libration, corresponding to unstacking the base pairs; this involves the equivalence of the curvature fluctuations at the melting point, $\langle C^2\rangle_m = \langle C^2\rangle^*_m$, and therefore

$$b = b^*\left\langle\frac{T}{T^*}\right\rangle_m \tag{10}$$

The small differences between such "premelting" and melting temperatures do not affect the ratio significantly. Thus the stiffness of a DNA tract can be represented by the ratio of the dinucleotide melting temperatures (in thermodynamic scale), $\langle T/T^*\rangle$, averaged over the tract considered. Similarly, the torsional stiffness of a DNA tract is

$$t = t^*\left\langle\frac{T}{T^*}\right\rangle \tag{11}$$

Gotoh and Tagashira (1981) determined by fitting to observed profiles of a set of restriction fragment DNAs and synthetic polynucleotides the B-DNA conformational stability of the different dinucleotide steps. It is noteworthy that such an evaluation of the sequence-dependent thermody-

namic stability of the double helix below the melting point explains the surprisingly good linear correlation ($R = 0.97$) between the dinucleotide melting points and the quantum mechanics theoretical stacking energies (Ornstein et al., 1978) as reviewed by Gotoh and Tagashira (1981). In fact, such a correlation could be obtained if the melting entropy and the other conformational energy contributions were practically constant for all of the dinucleotide steps. This is rather implausible at the melting point, but as just proposed, it is fully plausible at the "premelting" temperature. Furthermore, a correlated set of flexibility parameters ($R = 0.72$) is found by using the thermodynamic helix-coil parameters, ΔH and ΔS, (at 37°C) of SantaLucia (1998), who averaged data obtained by different authors.

We adopted both sets of flexibility parameters to evaluate the flexibility in the nucleosome stability calculation.

Evaluation of the elastic canonical partition functions

Assuming first-order elasticity, we evaluated the elastic contributions to the partition functions, related to the sum of the bending, $\Delta E_b(k)$, and twisting, $\Delta E_t(k)$, energies necessary to distort the kth DNA tract in the nucleosomal form.

The elastic bending energy contribution can be expressed as

$$\Delta E_b(k) = \sum_{k-L/2}^{k+L/2} \frac{b^*}{2} \left\langle \frac{T}{T^*} \right\rangle |\mathbf{C}_n(s) - \mathbf{C}_f^o(s)|^2 \qquad (12)$$

where b^* is the apparent isotropic bending force constant related to the persistence length. $\mathbf{C}_n(s) - \mathbf{C}_f^o(s)$ is the difference in the complex plane between the curvature of the nucleosome and the free DNA (which corresponds to its intrinsic curvature) relative to the sth bp in the kth tract. As discussed before, the average ratio $\langle T/T^* \rangle$ modulates the force constants, producing a sequence-dependent stiffness for each kth DNA tract.

The bending energy, $\Delta E_b(k)$, can be conveniently expressed, on the basis of the Parseval equality (Spiegel, 1974), in terms of the differences between the Fourier transform amplitudes $\mathbf{A}_n(\upsilon)$ and $\mathbf{A}_f^o(\upsilon)$ with periodicity υ:

$$\sum_{k-L/2}^{k+L/2} |\mathbf{C}_n(s) - \mathbf{C}_f^o(s)|^2 = \frac{1}{L} \sum_{\upsilon} |\mathbf{A}_n(\upsilon) - \mathbf{A}_f^o(\upsilon)|^2 \qquad (13)$$

with

$$\mathbf{A}_n(\upsilon) = \sum_{k-L/2}^{k+L/2} \mathbf{C}_n(s) \exp\left(-2\pi i \upsilon \frac{s}{L}\right)$$
$$\mathbf{A}_f^o(\upsilon) = \sum_{k-L/2}^{k+L/2} \mathbf{C}_f^o(s) \exp\left(-2\pi i \upsilon \frac{s}{L}\right) \qquad (14)$$

because the latter summation in Eq. 13 can be conveniently approximated to a single term by using the same approximation successfully adopted in our recent papers (De Santis et al., 1996; Anselmi et al., 1998):

$$\sum_{k-L/2}^{k+L/2} {}_s|\mathbf{C}_n(s) - \mathbf{C}_f^o(s)|^2 = \frac{1}{L} |\mathbf{A}_n(\mu) - \mathbf{A}_f^o(\mu)|^2 \qquad (15)$$

This ensures the minimization of the distortion bending energy required to transform the free DNA superstructure in the nucleosomal form. In fact, as found in the crystal structure (Luger et al., 1997), the pitch angle, α, of the nucleosomal DNA superhelix is equal to 0.104 rad, corresponding to the twisting number around the helical axis of $-1.75 \cdot \sin \alpha$ (Fuller, 1971; Crick, 1976). It corresponds to the frequency $\mu = -0.18$ in the Fourier representation of the curvature function. The modulus of this Fourier term must be 10.9 rad, corresponding to the integral curvature of one and three-quarter turns of superhelix. Therefore, if we assume all of the amplitude differences to be equal to zero, except that characterized by the periodicity μ, which is needed to constrain a given DNA tract to assume a nucleosome-like superhelix, the maxima of the intrinsic curvature features are preserved and the elastic energy is minimized, as can easily be seen from the Parseval equality (Eq. 13). This is compatible with the hypothesis that the deviations from the ideal uniform superhelix observed in the x-ray structure (Luger et al., 1997) could be due in part to the intrinsic curvature of that DNA sequence.

The elastic twisting contribution can be expressed as well:

$$\Delta E_t(k) = \sum_{k-L/2}^{k+L/2} \frac{t^*}{2} \left\langle \frac{T}{T^*} \right\rangle (\Omega_n(s) - \Omega_f^o(s))^2 \qquad (16)$$

where t^* is the twisting force constant related to the torsional stiffness; $\Omega_n(s) - \Omega_f^o(s)$ is the dinucleotide twisting angle difference between the nucleosome and the free DNA at the sequence number s. We have assumed for $\Omega_n(s)$ a constant value corresponding to a DNA periodicity of 10.15 bp per turn according to the experimental evidence (Drew and Travers, 1985; Shrader and Crothers, 1990; Luger et al., 1997), and $\Omega_f^o(s)$ is the pertinent intrinsic twisting angle as reported in Table 1 (De Santis et al., 1996; Anselmi et al., 1998, 1999).

As a result, the only conditions necessary for a nucleosome-like curvature is to constrain the Fourier term with periodicity $\mu = -0.18$ to the value 10.9 rad and the nucleosomal twist $\Omega_n(s)$ to the average DNA helical periodicity of 10.15.

The elastic partition function of a nucleosomal DNA can be evaluated by integrating in the complex plane. The distortion energy needed to transform the ground state of free DNA in the nucleosomal form depends on the vectorial difference between $\mathbf{A}_n(\mu)$ and $\mathbf{A}_f^o(\mu)$. Only the modulus of the first amplitude is defined by the geometrical constraints. As a consequence, the pertinent partition function involves the integration of all of the possible phase differences (Fig. 4 *A*):

$$Q_n(k) = \exp[-\beta \Delta E_t(k)] \int \exp\left(\frac{\beta b^*}{2L} \left\langle \frac{T}{T^*} \right\rangle \left| \mathbf{A}_n(\mu) - \mathbf{A}_f^o(\mu) \right|^2 \right) d\phi \qquad (17)$$

where ϕ is the phase angle between $\mathbf{A}_n(\mu)$ and $\mathbf{A}_f^o(\mu)$ and $\Delta E_t(k)$ is the twisting energy. Indicating $|\mathbf{A}_n(\mu)| = A_n = 10.9$ rad, $|\mathbf{A}_f^o(\mu)| = A_f^o$ for the sake of clarity, Eq. 17 reduces to

$$Q_n(k) = \exp[-\beta \Delta E_t(k)] \exp\left[-\frac{\beta b^*}{2L} \left\langle \frac{T}{T^*} \right\rangle (A_n^2 + A_f^{o^2}) \right] \int \exp\left(\frac{\beta b^*}{L} \left\langle \frac{T}{T^*} \right\rangle A_n A_f^o \cos \phi \right) d\phi \qquad (18)$$

The last integral is equal to 2π times $J_0(iZ)$, the zero-order Bessel function of the imaginary argument $Z = \beta b^* \langle T/T^* \rangle A_n A_f^o / L$. Therefore,

$$Q_n(k) = 2\pi \exp[-\beta \Delta E^o(k)] \exp(-Z) J_0(iZ) \qquad (19)$$

where $\Delta E^o(k)$ contains both the ground state bending and twisting energy contributions. The integration factor is omitted because it will eventually

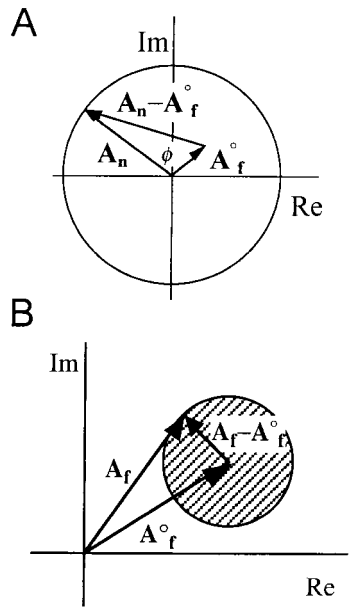

FIGURE 4 Complex plane representation of the curvature function of the nucleosomal and free DNA Fourier amplitudes adopted to evaluate the partition functions. (*A*) The geometrical constraints define only the modulus of $\mathbf{A}_n(\mu)$, so that its tip can span over a circumference in the complex plane. In contrast, $\mathbf{A}^o_f(\mu)$ is an intrinsic feature of the free DNA and is defined in modulus and phase. (*B*) The free-DNA ground-state amplitudes, $\mathbf{A}^o_f(\upsilon)$, and their isotropic changes representing the elastic fluctuations.

disappear in the ratio of the partition functions. Accordingly, for the standard nucleosome where $A^o_f = 0$,

$$Q^*_n = 2\pi \exp[-\beta\Delta E^{o*}(k)] \tag{20}$$

and consequently,

$$\frac{Q_n(k)}{Q^*_n} = \frac{\exp[-\beta\Delta E^o(k)]\exp(-Z)J_0(iZ)}{\exp[-\beta\Delta E^{o*}]} \tag{21}$$

It should be stressed that only the ground states are represented in the partition function ratio of the nucleosomal DNA because the association with the histones practically quenches the elastic fluctuations. Alternatively, these reduced fluctuations can be considered equivalent in all of the DNA tracts, and their contribution cancels in the ratio.

In a first approximation, the canonical partition function ratio between the free *k*th DNA tract and the standard DNA, $Q_f(k)/Q^*_f$, is equal to the product of the bending and twisting fluctuation ratios, if the independence of the bending and twisting modes is assumed. In fact, free DNA fluctuations around the ground-state superstructures, which correspond to the intrinsic curvature (De Santis et al., 1995), contribute significantly to the statistical ensemble properties, as first pointed out by Olson (Olson et al., 1993) and more recently by Schellman and Harvey (1995). $Q_f(k)/Q^*_f$ can easily be evaluated in the complex plane. The elastic fluctuations of DNA involve isotropic changes in all of the curvature Fourier amplitudes, as pictorially represented in Fig. 4 *B*. Consequently, each bending Fourier mode contributes with a term

$$\int \exp\left(-\frac{\beta b^*}{2L}\left\langle\frac{T}{T^*}\right\rangle\left|\mathbf{A}_f(\upsilon)-\mathbf{A}^o_f(\upsilon)\right|^2\right)d(\mathbf{A}_f(\upsilon)-\mathbf{A}^o_f(\upsilon)) \tag{22}$$

A similar conclusion can be derived relative to the twisting, with the difference that the integration is made on real functions. Therefore,

$$\frac{Q_f(k)}{Q^*_f} = \frac{\prod_{-L}^{L}{}_{\upsilon}\int \exp\left(-\frac{\beta b^*}{2L}\left\langle\frac{T}{T^*}\right\rangle\left|\mathbf{A}_f(\upsilon)-\mathbf{A}^o_f(\upsilon)\right|^2\right)d(\mathbf{A}_f(\upsilon)-\mathbf{A}^o_f(\upsilon))}{\prod_{-L}^{L}{}_{\upsilon}\int \exp\left(-\frac{\beta b^*}{2L}\left|\mathbf{A}^*_f(\upsilon)\right|^2\right)d\mathbf{A}^*_f(\upsilon)}$$

$$\cdot\frac{\prod_{1}^{L}{}_{s}\int \exp\left(-\frac{\beta t^*}{2}\left\langle\frac{T}{T^*}\right\rangle(\Omega(s)-\Omega^o_f(s))^2\right)d(\Omega(s)-\Omega^o_f(s))}{\prod_{1}^{L}{}_{s}\int \exp\left(-\frac{\beta t^*}{2}(\Omega^*(s)-\Omega^*_f)^2\right)d(\Omega^*(s)-\Omega^*_f)} \tag{23}$$

where $\mathbf{A}_f(\upsilon)$, $\mathbf{A}^*_f(\upsilon)$, and $\mathbf{A}^o_f(\upsilon)$ are the amplitudes of the bending Fourier modes of periodicity υ, for the free DNA, the standard DNA, and the free-DNA ground state, respectively. The term $\mathbf{A}^o_f(\upsilon)$, pertinent to the ground state of the standard (straight) DNA, is obviously zero. $\Omega(s)$ and $\Omega^o_f(s)$ are the twisting values at sequence number *s* for the free DNA and the free-DNA ground state, respectively. Ω^*_f is the twisting value of the standard DNA and is constant, referring to a uniform chain.

The integrals corresponding to the bending modes reduce to $(2\pi L\langle T^*/T\rangle/\beta b)$; similarly, those pertinent to twisting fluctuations become equal to $(2\pi L\langle T^*/T\rangle/\beta t^*)^{1/2}$ in the absence of coupling. This assumption is plausible for the bending modes but becomes a first-order approximation in the case of twisting fluctuations because the phosphodiester chains introduce a certain degree of coupling.

Consequently, the ratio $Q_f(k)/Q^*_f$ eventually reduces to $\langle T/T^*\rangle^{-(3/2)L}$. It should be noted that $\frac{3}{2}L$ corresponds to the degrees of freedom of orientational parameters of the basepair average planes. A certain degree of coupling between bending and twisting fluctuations would lower the exponent from $\frac{3}{2}L$ to L at most, with small changes in the theoretical results.

Obtaining an analytical expression for $Q_n(k)/Q^*_n$ and $Q_f(k)/Q^*_f$ allows the calculation of the elastic free-energy difference in the *k*th nucleosome competitive reconstitution and consequently, from Eq. 1, the elastic contribution to the thermodynamic affinity, ΔG_{el}, of a DNA as a whole:

$$\beta\Delta G_{el}(k) = \beta\Delta E^o(k) - \tfrac{3}{2}L\ln\left\langle\frac{T}{T^*}\right\rangle + Z - \ln J_0(iZ) \tag{24}$$

where $\Delta E^o(k)$ is the minimum elastic energy required to distort the *k*th tract in the nucleosomal form; $\langle T/T^*\rangle$ is the average dinucleotide empirical melting temperature of the *k*th nucleosomal DNA tract relative to the standard one; *Z* is equal to $(\beta b^*/L)\langle T/T^*\rangle A_n A^o_f$. It is worth noting that $A_n A^o_f$ represents the modulus of the correlation between the superstructure of the nucleosomal DNA and that of the free form, according to the convolution theorem (Spiegel, 1974).

RESULTS AND DISCUSSION

Using the roll, tilt, and twist angles reported in Table 1 to calculate the intrinsic curvature function for a given DNA, a persistence length equal to 450 Å, a torsional rigidity of 2.1×10^{19} erg · cm, and the flexibility parameters, we have

evaluated the elastic free energy difference, $\Delta G_{el}(k)$, of the competitive nucleosome reconstitution for a large pool of DNAs different for sequence and length that were investigated in several laboratories (Shrader and Crothers, 1989, 1990; Godde and Wolffe, 1996; Godde et al., 1996; Widlund et al., 1997; Cacchione et al., 1997; Lowary and Widom, 1998; Rossetti et al., 1998; Dal Cornò et al., 1998; Cao et al., 1998).

The same parameters were used in our previous works to predict the gel electrophoresis retardation of a large pool of synthetic and natural DNAs (De Santis et al., 1990; Boffelli et al., 1992) and later adopted in the theoretical evaluation of the circularization propensity of DNAs, and were in excellent agreement with experimental data (De Santis et al., 1996; Anselmi et al., 1998).

The flexibility parameters were evaluated from the dinucleotide thermodynamic data proposed by different authors as reported above.

Nucleosome reconstitution free energies were calculated by using both the Gotoh and Tagashira parameters and the normalized ratios, $\Delta H/\Delta S$, proposed by SantaLucia (1998), yielding similar results.

The good linear correlation between the melting temperature ratios and the stacking energies evaluated by quantum chemical calculations (Ornstein et al., 1978) suggests the interpretation that the basepair unstacking happens immediately before the melting. This supports the hypothesis that the stacking energies are the major factor in DNA rigidity, as pointed out by Hagerman (1988). We have shown that, as a consequence, the ratios of the melting temperatures could also be a measure of DNA stiffness.

Actually, there is still an open debate about the relative flexibility of some specific repeated sequences, mainly CGG and CTG, based on different experimental data (Chastain et al., 1995; Bacolla et al., 1997). However, with the lack of a complete set of experimental values for the different dinucleotide steps, the relative conformational stability of the double helix seems to be a good measure of sequence-dependent DNA stiffness. In any event, the flexibility seems to play a significant role only in a few cases, as will be pointed out below.

The comparison between the theoretical elastic contributions and the experimental data shows satisfactory agreement for a number of DNAs but major deviations for others. However, this disagreement appears to regularly increase with the free DNA intrinsic curvature, showing that a pure elastic model is not sufficient in the case of highly curved DNAs.

Fig. 5 illustrates the difference between the experimental and theoretical (elastic) nucleosome reconstitution free energy for the whole pool of investigated DNAs, as a function of the pertinent intrinsic effective curvature, represented by the average Fourier amplitude $\langle A_f^o \rangle$. The strikingly significant correlation ($R = 0.98$) with the squared values of $\langle A_f^o \rangle$ suggests the existence of a curvature-dependent contribution to the free energy, which appears to destabilize the nucleosome.

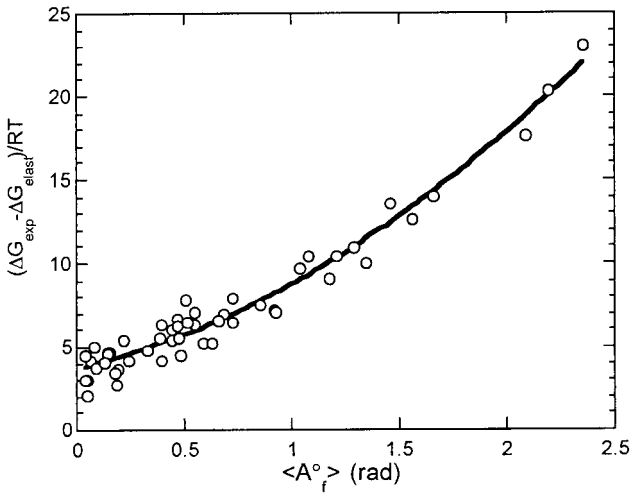

FIGURE 5 Deviations between the experimental and theoretical (elastic) nucleosome reconstitution free energy for the whole pool of the investigated DNAs (Shrader and Crothers, 1989, 1990; Godde and Wolffe, 1996; Godde et al., 1996; Widlund et al., 1997; Cacchione et al., 1997; Lowary and Widom, 1998; Rossetti et al., 1998; Dal Cornò et al., 1998; Cao et al., 1998) as a function of the intrinsic effective curvature represented by the average Fourier amplitude $\langle A_f^o \rangle$.

Therefore, the intrinsic curvature seems to play two opposite roles in nucleosome formation: one stabilizes the nucleosome by reducing the elastic energy required to distort DNA tracts in the nucleosomal structure; the other, clearly related to the curvature of the DNA free form, reduces the affinity with histone octamer.

This is reasonably related to the minor-groove contraction in intrinsically curved DNAs, which stabilizes the curvature-dependent water spine and counterion interactions and adds a further energy cost to the nucleosome formation. In fact, differential hydration effects and counterion interactions in relation to the groove amplitude were recently underlined in crystal structure and NMR investigations of DNA oligomers by different authors (Fack and Sarantoglou, 1991; Liepinsh et al., 1992; Berman, 1994; Shui et al., 1998a, b; McFail-Isom et al., 1999; Hud et al., 1999). In addition, it is noteworthy that the polyamine spermine shows higher affinity for the curved multimeric DNA $d((CA_4T_4G)_n)_2$ than for $d((CT_4A_4G)_n)_2$, which has the opposite sequence polarity but much lower curvature (Bordin et al., 1992).

To take into account these effects, we introduced a semiempirical additive contribution to represent the differential water dipole energy in the electrostatic field of phosphates (see Appendix). Fig. 6 reports the good agreement between experimental and theoretical nucleosome reconstitution free-energy data. The values obtained by adopting the Gotoh and Tagashira dinucleotide melting temperatures

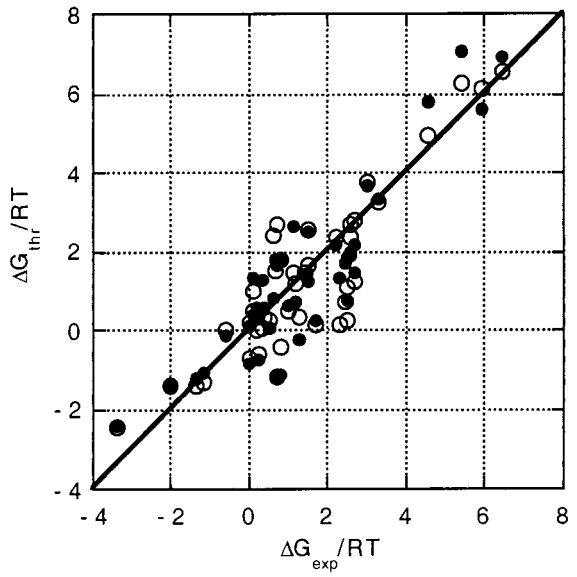

FIGURE 6 Comparison between the experimental and theoretical nucleosome reconstitution free energies. Theoretical results were obtained by adopting the dinucleotide melting temperatures by Gotoh and Tagashira (1981) (●, $R = 0.90$) and those obtained with the thermodynamic parameters of SantaLucia (1998) (○, $R = 0.89$). The data refer to the sequences listed in Fig. 7.

(Gotoh and Tagashira, 1981) ($R = 0.90$) and those obtained with the thermodynamic data, $\Delta H/\Delta S$, of SantaLucia (1998) ($R = 0.89$) are superimposed and do not show significant differences.

Consequently, we adopted the Gotoh and Tagashira parameters, even though they are derived from a limited set of experimental data, because they refer to polynucleotide chains and appear to be more suitable for representing the flexibility of nucleosomal DNA. In contrast, the other proposals were obtained from a larger set of data but refer to oligonucleotides, in which terminal effects could be important.

Fig. 7 illustrates the comparison between experimental and theoretical free energies of the DNA fragments sorted according to increasing curvature. The trend is rather complex because the DNA sequence lengths and flexibility are different, indicating that aside from the curvature, differential intrinsic twist, flexibility, and length are important factors for nucleosome stability.

To clarify the role of the curvature, Fig. 8 reports the comparison between experimental (Shrader and Crothers, 1989, 1990) and theoretical results for a homogeneous pool of DNA fragments of the same length ($N = 162$ bp), sorted according to increasing curvature. Fig. 9 reports the same comparison with the average effective curvature $\langle A_f^o \rangle$. It clearly shows a minimum for an average curvature of ~1.5 rad/144 bp corresponding to the Shrader and Crothers TG (and GT) pentamer (Shrader and Crothers, 1989, 1990), which confirms the dual role of curvature in the nucleosome stability.

The flexibility also seems to have a dual role: decreasing the distortion energy for nucleosome formation and increasing the entropy difference between the flexible free form and the final rigid nucleosomal structure. The two contributions are opposites, as can be seen in Fig. 10, but the entropy term generally appears to be more important. Therefore, DNA flexibility seems to destabilize nucleosomes, contrary to the immediate perception.

It is interesting to note that if we calculate $\Delta G(k)$ along the sequence so that the dyad axis follows the large groove as in the crystal structure (Luger et al., 1997), we obtain the nucleosome phasing in agreement with experimental data. As an example, Fig. 11 shows the satisfactory comparison between the theoretical free-energy profile, $\Delta G(k)$, and the hydroxyl-radical footprint from Shrader and Crothers (1990), averaged over a repeat period of 10 bp, versus the dyad positions of the TG pentamer. The maxima of the experimental profile, which correspond to the cleavage of the external minor groove of the nucleosomal DNA, coincide with the free-energy minima, which represent the preferential positioning of the dyad axis pointing in that direction.

CONCLUSIONS

We can advance the conclusion that the intrinsic curvature is the main factor that controls nucleosome stability and consequently nucleosome positioning. It produces two opposite effects: it decreases the distortion energy of the free DNA tract necessary to assume the nucleosomal shape and increases the free-energy cost corresponding to releasing a part of the water spine and counterions consequent to the nucleosome formation.

Such a destabilizing role due to the curvature was not detected in our previous works on sequence-dependent circularization propensity, where, by adopting a similar elastic model and statistical mechanic approach, we were able to reproduce very satisfactorily the experimental thermodynamic equilibrium constants (De Santis et al., 1996).

However, in the case of nucleosome, it is reasonable that the presence of the histone core changes the topography of the inner regions of the nucleosomal DNA with respect to the circular DNA, because a part of the water molecules and counterions should be replaced by the histone core as found by Woda et al., who analyzed 11 protein-DNA crystal structures. These data suggest that hydration sites generally mark the binding sites at protein-DNA interfaces (Woda et al., 1998).

The sequence-dependent flexibility is well represented by the ratio of the dinucleotide melting temperatures averaged over the DNA tracts, which is justified by the very good linear correlation between the quantum mechanics theoretical stacking energies and the empiric dinucleotide step melting points. In fact, we suppose that melting happens when the amplitude of the basepair libration reaches a critical value, corresponding to the basepair unstacking en-

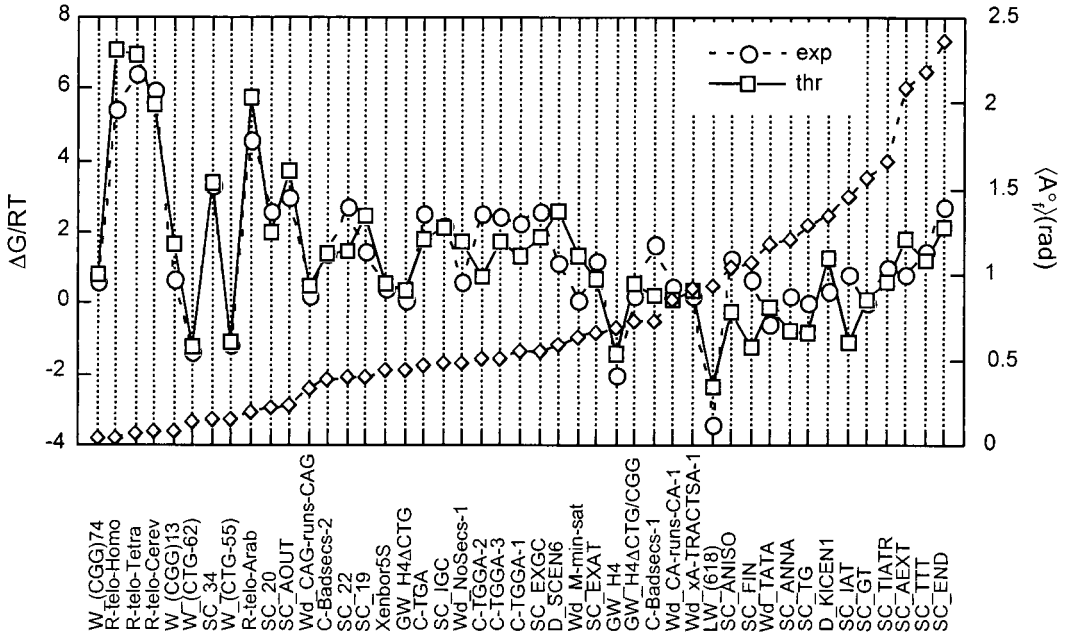

FIGURE 7 Differences between the experimental (○) and theoretical (□) nucleosome reconstitution free energies of synthetic and natural DNA fragments (Shrader and Crothers, 1989, 1990; Godde and Wolffe, 1996; Godde et al., 1996; Widlund et al., 1997; Lowary and Widom, 1998; Rossetti et al., 1998; Dal Cornò et al., 1998; Cao et al., 1998), sorted according to increasing curvature, represented by the average Fourier amplitude $\langle A_f^o \rangle$ (◇). Connecting lines are guides for the eye and have no physical meaning. The experimental data are related to the TG pentamer as a standard (Shrader and Crothers, 1989, 1990). DNA tracts are identified by the prefixes, which refer to the following papers: SC (Shrader and Crothers, 1989, 1990); GW (Godde and Wolffe, 1996); W (Godde et al., 1996); Wd (Widlund et al., 1997); LW (Lowary and Widom, 1998); R (Rossetti et al., 1998); D (Dal Cornò et al., 1998); C (Cao et al., 1998).

ergy. As the libration energy is proportional to the temperature, in the equipartition approximation, the melting points should be related to the DNA elastic force constants, which is representative of its stiffness; the same relationship is observed for temperatures below the melting point. In fact, the force field that controls the basepair libration is considered valid in the whole range of temperatures where the

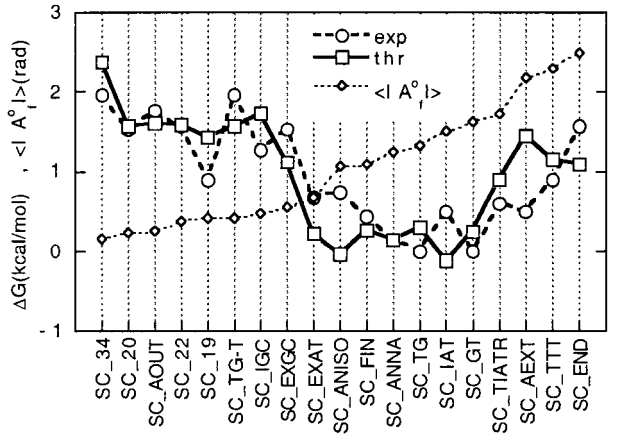

FIGURE 8 Comparison between experimental (○) and theoretical (□) results for a homogeneous pool of DNA fragments of the same length (N = 162 bp), sorted according to increasing effective intrinsic curvature $\langle A_f^o \rangle$ (◇). Connecting lines are guides for the eye and have no physical meaning. The prefix SC refers to the DNA tracts studied by Shrader and Crothers (1989, 1990).

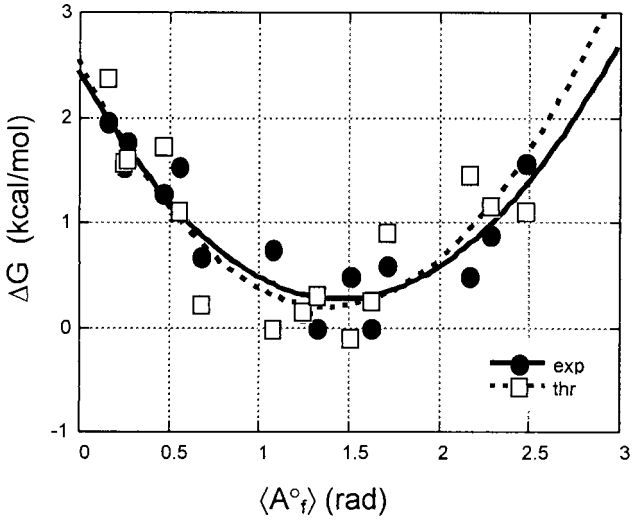

FIGURE 9 Experimental (●) and theoretical (□) nucleosome reconstitution free energy versus the effective curvature $\langle A_f^o \rangle$ for a homogeneous pool of DNA fragments of the same length (N = 162 bp), investigated by Shrader and Crothers (1989, 1990).

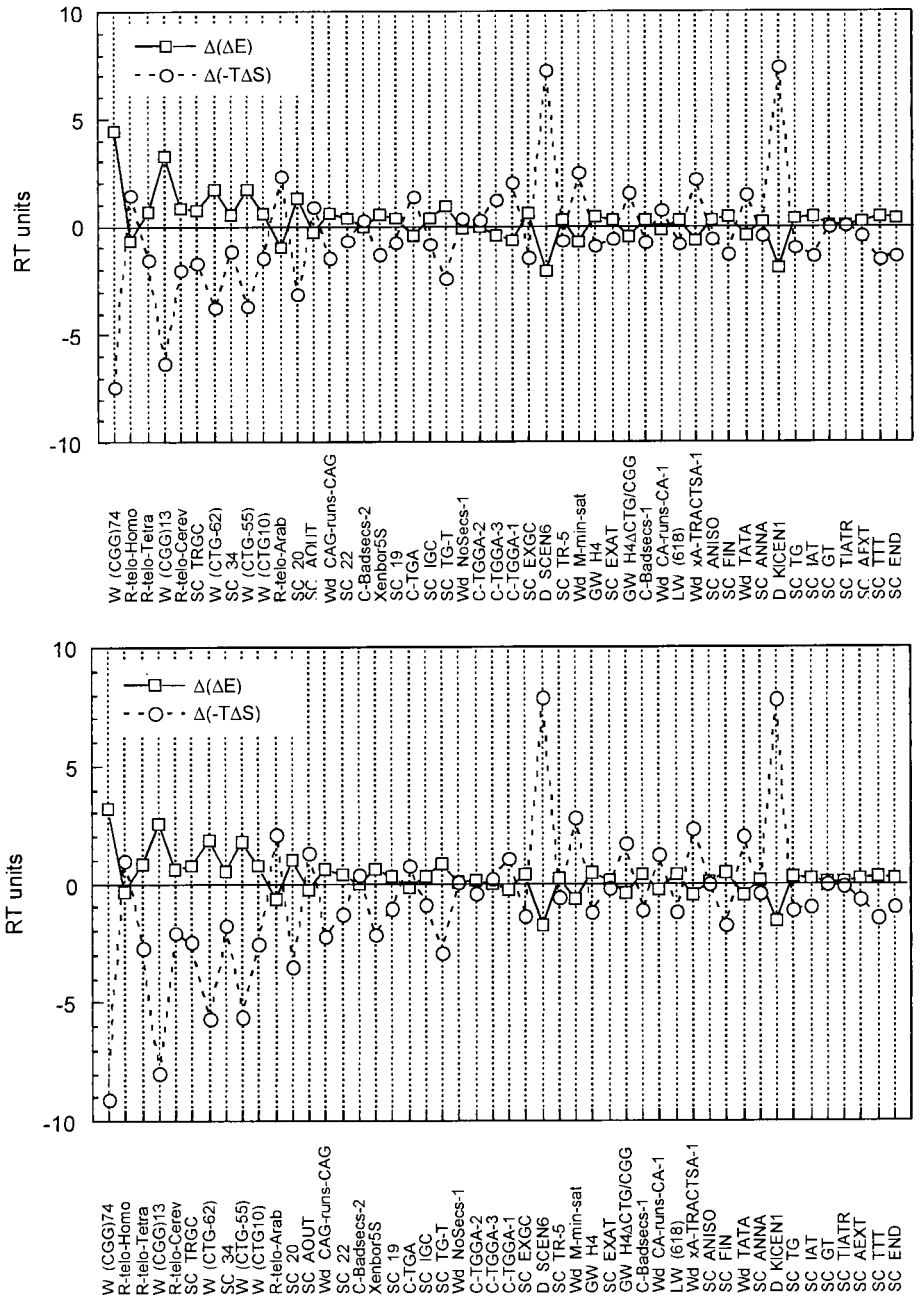

FIGURE 10 Role of the sequence-dependent DNA flexibility: the energy and entropy changes, resulting from the introduction of the flexibility parameters in the model, are reported for the investigated DNAs, sorted according to increasing curvature as in Fig. 8. Theoretical results were obtained by adopting the dinucleotide melting temperatures from Gotoh and Tagashira (1981) (*top*) and those obtained from the thermodynamic parameters of SantaLucia (1998) (*bottom*).

spectroscopic manifestations of the B-DNA structure are conserved. This supports the hypothesis that the stacking energies are the major factor in DNA rigidity.

Flexibility also seems to have a dual role: decreasing the distortion energy for nucleosome formation and increasing the entropy difference between the flexible free form and the final rigid nucleosomal structure. The two contributions are opposites, but the entropy term generally appears to be more important.

Obviously, DNA length always enhances the histone affinity in competitive nucleosome reconstitution by increasing the number of virtual nucleosome positionings. The intrinsic twist also significantly contributes to the distortion energy, particularly in the case of GC-rich sequences, which are characterized by intrinsic values significantly smaller than that of the nucleosomal form.

The other factors concerning hypothetical chemical recognition arising from specific interactions between base pairs and amino acidic residues appear to play a minor role, which, however, requires further investigation.

Nevertheless, it is interesting that recurrence of AA (TT) stretches in phase with the double helix periodicity produces

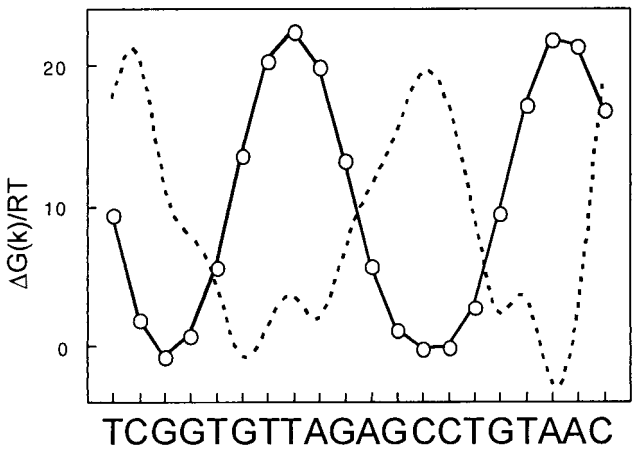

FIGURE 11 Theoretical free-energy profile $\Delta G(k)$ (*empty circles with solid line*) and experimental hydroxyl-radical cleavage pattern (Shrader and Crothers, 1990) (*dashed line*) of TG pentamer versus the dyad positions of the possible nucleosomes. The free-energy minima represent the most stable dyad positioning localized into the large groove along the sequence. Consequently, the corresponding small groove of the sequence faces away from the histone core, resulting in greater sensitivity to hydroxyl-radical cleavage (a maximum in the cleavage profile).

curvature in the free DNA, so that the AA (TT) side inside the small groove faces toward the bending direction as well as toward the histone core in the nucleosome (Drew and Travers, 1985). The presence of AT base pairs adds further stability to the water spine, as first shown by x-ray crystallographic studies (Drew and Dickerson, 1981) and further validated by thermodynamic and NMR data from aqueous solutions (Fack and Sarantoglou, 1991; Liepinsh et al., 1992; Berman, 1994; Shui et al., 1998a, b; Woda et al., 1998; McFail-Isom et al., 1999; Hud et al., 1999). A significant part of such water molecules is displaced by the histone core from the minor groove, increasing the free-energy cost of the nucleosome formation. Therefore AA stretches appear to control the equilibrium by stabilizing and limiting the nucleosome stability. These considerations raise the interesting possibility that hydration effects may significantly contribute to selective nucleic acid recognition processes, in addition to elastic and conformational properties of DNA (Sprous et al., 1995).

The sequence-dependent nucleosome stability is still a widely debated problem concerning the basic elemental structure of chromatin and has an important role in basal gene regulation, DNA compaction, and chromosome segregation. This model represents the first attempt to clarify the main features of such thermodynamic stability in terms of physical-chemical parameters, which, despite its large complexity, works very satisfactorily. Finally, this suggests that in molecular systems with such a large degree of complexity, the average molecular properties dominate over the local features, as in a statistical ensemble.

APPENDIX: AN EVALUATION OF HYDRATION ROLE

The dependence on the groove amplitude of the electrostatic interactions between the water point dipole and the neighbor phosphate charges of a generic and standard DNA is a complex problem. Nevertheless, the energy differences can be approximately written as a function of the DNA groove amplitude:

$$\varepsilon_e - \varepsilon_e^* = \Delta\varepsilon_e \propto -\left(\frac{1}{g^2} + \frac{2}{g^2 + d^2}\right) + \left(\frac{1}{g^{*2}} + \frac{2}{g^{*2} + d^2}\right) \tag{A1}$$

where d is the distance of nearest-neighbor phosphate groups along the strand and g and g^* are the minor groove half-amplitudes, evaluated at the phosphate positions, for a generic and a standard DNA, respectively. Simple geometrical considerations show that the contraction of the minor groove amplitude is proportional to the curvature in the range of curvatures of interest, and a simple proportion is obtained between g and the average curvature, $\langle A\rangle$. Therefore, the local electrostatic charge dipole energy, $\Delta\varepsilon_e$, can be expressed as a function of $\langle A\rangle$.

$\Delta\varepsilon_e$ multiplied by the distribution function gives the differential statistical interaction ΔE_e of water molecules and counterions in terms of the average curvature $\langle A\rangle$:

$$\Delta E_e = \alpha\langle A\rangle\exp(\gamma\langle A\rangle) \tag{A2}$$

α and γ are two constants containing the phosphate and counterion charges, the water electric dipole, the apparent dielectric constant, the temperature, and the relative numbers of DNA interacting water molecules and counterions. ΔE_e represents the additional energy cost when some of the water molecules close to the DNA groove are released into the nucleosome formation, as found in different DNA-protein complexes (Woda et al., 1998).

We determined the value of the constants ($\alpha = 2.9$ and $\gamma = 0.40$) by best fitting the differences between experimental and theoretical elastic free energies as a function of $\langle A\rangle$. However, in the range of DNA curvature explored, it can be fitted with a simple quadratic function of the effective curvature $\langle A\rangle$ with a correlation coefficient of 0.98, $\Delta E_e = \delta\langle A\rangle^2$ with $\delta = 3.2$. This means that the virtual force field acting on water and counterions changes proportionally to the curvature in the range of interest.

It is noteworthy that the parabolic dependence is also compatible with the energy dependence on the inverse third power of the groove amplitude, which represents dipole-dipole interactions.

This research was supported by the Progetto 60% Facoltà of the University la Sapienza and by the Cofinanziamento 40% of the Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

REFERENCES

Anselmi, C., G. Bocchinfuso, P. De Santis, M. Fuà, A. Scipioni, and M. Savino. 1998. Statistical thermodynamic approach for evaluating the writhe transformations in circular DNAs. *J. Phys. Chem. B.* 102: 5074–5714.

Anselmi, C., G. Bocchinfuso, P. De Santis, M. Savino, and A. Scipioni. 1999. Dual role of DNA intrinsic curvature and flexibility in determining nucleosome stability. *J. Mol. Biol.* 286:1293–1301.

Bacolla, A., R. Gellibolian, M. Shimizu, S. Amirhaeri, S. Kang, K. Ohshima, J. E. Larson, S. C. Harvey, B. D. Stollar, and R. D. Wells. 1997. Flexible DNA: genetically unstable CTG · CAG and CGG · CCG from human hereditary neuromuscular disease genes. *J. Biol. Chem.* 272: 16783–16792.

Berman, H. M. 1994. Hydration of DNA: take 2. *Curr. Opin. Struct. Biol.* 4:345–350.

Berman, H. M. 1997. Crystal studies of B-DNA: the answers and the questions. *Biopolymers.* 44:23–44.

Blank, T. A., and P. B. Becker. 1996. The effect of nucleosome phasing sequences and DNA topology on nucleosome spacing. *J. Mol. Biol.* 260:1–8.

Boffelli, D., P. De Santis, A. Palleschi, and A. Scipioni. 1992. Theoretical prediction of sequence dependent DNA superstructures and their implications in recognition mechanisms with proteins. *Int. J. Quantum Chem.* 42:1409–1426.

Bolshoy, A., P. McNamara, R. E. Harrington, and E. N. Trifonov. 1991. Curved DNA without A-A: experimental estimation of all 16 DNA wedge angles. *Proc. Natl. Acad. Sci. USA.* 88:2312–2316.

Bordin, F., S. Cacchione, M. Savino, and A. Tufillaro. 1992. Different interactions of spermine with a "curved" and a "normal" DNA duplex: $(CA4T4G)_n$ and $(CT4A4G)_n$. Gel electrophoresis and circular dichroism studies. *Biochem. Int.* 27:891–901.

Cacchione, S., M. A. Cerone, and M. Savino. 1997. In vitro low propensity to form nucleosomes of four telomeric sequences. *FEBS Lett.* 400: 37–41.

Cao, H., H. R. Widlund, T. Simonsson, and M. Kubista. 1998. TGGA-repeats impair nucleosome formation. *J. Mol. Biol.* 281:253–260.

Chastain, P. D., E. E. Eichler, S. Kang, D. L. Nelson, S. D. Levene, and R. R. Sinden. 1995. Anomalous rapid electrophoretic mobility of DNA containing triplet repeats associated with human disease genes. *Biochemistry.* 34:16125–16131.

Crick, F. H. 1976. Linking numbers and nucleosomes. *Proc. Natl. Acad. Sci. USA.* 73:2639–2643.

Crothers, D. M. 1998. DNA curvature and deformation in protein-DNA complexes: a step in the right direction. *Proc. Natl. Acad. Sci. USA.* 95:15163–15165.

Dal Cornò, M., P. De Santis, B. Sampaolese, and M. Savino. 1998. Superstructure features and nucleosomal organization of the two centromeres of *Kluyveromices lactis* chromosomes 1 and *Sacchoromices cerevisiae* chromosome 6. *FEBS Lett.* 431:66–70.

De Santis, P., M. Fuà, A. Palleschi, and M. Savino. 1995. Influence of dynamic fluctuations on DNA curvature. *Biophys. Chem.* 55:261–271.

De Santis, P., M. Fuà, M. Savino, C. Anselmi, and G. Bocchinfuso. 1996. Sequence dependent circularization of DNAs: a physical model to predict the DNA sequence dependent propensity to circularization and its changes in the presence of protein-induced bending. *J. Phys. Chem.* 100:9968–9976.

De Santis, P., A. Palleschi, S. Morosetti, and M. Savino. 1986. Structures and superstructures in periodical polynucleotides. *In* Structure and Dynamics of Nucleic Acids, Proteins and Membranes. Pergamon Press, Elmsford, NY. 31–49.

De Santis, P., A. Palleschi, M. Savino, and A. Scipioni. 1988. A theoretical model of DNA curvature. *Biophys. Chem.* 32:305–317.

De Santis, P., A. Palleschi, M. Savino, and A. Scipioni. 1990. Validity of the nearest-neighbor approximation in the evaluation of the electrophoretic manifestations of DNA curvature. *Biochemistry.* 29: 9269–9273.

De Santis, P., A. Palleschi, M. Savino, and A. Scipioni. 1992. Theoretical prediction of the gel electrophoretic retardation changes due to point mutations in a tract of SV40 DNA. *Biophys. Chem.* 42:147–152.

Di Gabriele, A. D., M. R. Sanderson, and T. A. Steitz. 1989. Crystal lattice packing is important in determining the bend of a DNA dodecamer containing an adenine tract. *Proc. Natl. Acad. Sci. USA.* 86:1816–1820.

Drew, H. R., and R. E. Dickerson. 1981. Structure of a B-DNA dodecamer. III. Geometry of hydration. *J. Mol. Biol.* 151:535–556.

Drew, H. R., and A. A. Travers. 1985. DNA bending and its relation to nucleosome positioning. *J. Mol. Biol.* 186:773–790.

Fack, F., and V. Sarantoglou. 1991. Curved DNA fragments display retarded elution upon anion exchange HPLC. *Nucleic Acids Res.* 19: 4181–4188.

Flaus, A., K. Luger, S. Tan, and T. J. Richmond. 1996. Mapping nucleosome position at single base-pair resolution by using site-directed hydroxyl radicals. *Proc. Natl. Acad. Sci. USA.* 93:1370–1375.

Fuller, F. B. 1971. The writhing number of a space curve. *Proc. Natl. Acad. Sci. USA.* 68:814–819.

Godde, J. S., S. U. Kass, M. C. Hirst, and A. P. Wolffe. 1996. Nucleosome assembly on methylated CGG triplet repeats in the fragile X mental retardation gene 1 promoter. *J. Biol. Chem.* 271:24325–24328.

Godde, J. S., and A. P. Wolffe. 1996. Nucleosome assembly on CTG triplet repeats. *J. Biol. Chem.* 271:15222–15229.

Gorin, A. A., V. B. Zhurkin, and W. K. Olson. 1995. B-DNA twisting correlates with base-pair morphology. *J. Mol. Biol.* 247:34–48.

Gotoh, O., and Y. Tagashira. 1981. Stabilities of nearest-neighbor doublets in double-helical DNA determined by fitting calculated melting profiles to observed profiles. *Biopolymers.* 20:1033–1042.

Hagerman, P. J. 1988. Flexibility of DNA. *Annu. Rev. Biophys. Biophys. Chem.* 17:265–286.

Hud, N. V., V. Sklenar, and J. Feigon. 1999. Localization of ammonium ions in the minor groove of DNA duplexes in solution and the origin of DNA A-tract bending. *J. Mol. Biol.* 286:651–660.

Klug, A., D. Rhodes, J. Smith, T. J. Finch, and J. O. Thomas. 1980. A low resolution structure for the histone core of the nucleosome. *Nature.* 287:509–516.

Kornberg, R. D. 1977. Structure of chromatin. *Annu. Rev. Biochem.* 46: 931–954.

Landau, L. D., and E. M. Lifshitz. 1970. Theory of Elasticity. Pergamon Press, Oxford and New York.

Liepinsh, E., G. Otting, and K. Wuthrich. 1992. NMR observation of individual molecules of hydration water bound to DNA duplexes: direct evidence for a spine of hydration water present in aqueous solution. *Nucleic Acids Res.* 20:6549–6553.

Lowary, P. T., and J. Widom. 1998. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol.* 276:19–42.

Luger, K., A. W. Mäder, R. K. Richmond, D. F. Sargent, and T. J. Richmond. 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature.* 389:251–260.

McFail-Isom, L., C. C. Sines, and L. D. William. 1999. DNA structure: cations in charge? *Curr. Opin. Struct. Biol.* 9:298–304.

McGhee, J. D., and G. Felsenfeld. 1980. Nucleosome structure. *Annu. Rev. Biochem.* 49:1115–1156.

Olson, W. K., A. A. Gorin, X.-J. Lu, L. M. Hock, and V. B. Zhurkin. 1998. DNA sequence-dependent deformability deduced from protein-DNA crystal complexes. *Proc. Natl. Acad. Sci. USA.* 95:11163–11168.

Olson, W. K., N. L. Marky, R. L. Jernigan, and V. B. Zhurkin. 1993. Influence of fluctuations on DNA curvature. A comparison of flexible and static wedge models of intrinsically bent DNA. *J. Mol. Biol.* 232: 530–554.

Ornstein, R. L., R. Rein, D. L. Breen, and R. D. Macelroy. 1978. An optimized potential function for the calculation of nucleic acid interaction energies. I. Base stacking. *Biopolymers.* 17:2341–2360.

Richmond, T. J., J. T. Finch, B. Rushton, D. Rhodes, and A. Klug. 1984. Structure of the nucleosome core particle at 7 Å resolution. *Nature.* 311:532–537.

Rossetti, L., S. Cacchione, M. Fuà, and M. Savino. 1998. Nucleosome assembly on telomeric sequences. *Biochemistry.* 37:6727–6737.

SantaLucia, J., Jr. 1998. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc. Natl. Acad. Sci. USA.* 95:1460–1465.

Satchwell, S., H. R. Drew, and A. A. Travers. 1986. Sequence periodicities in chicken nucleosome core DNA. *J. Mol. Biol.* 191:659–675.

Schellman, J. A., and S. C. Harvey. 1995. Static contribution to the persistence length of DNA and dynamic contributions to DNA curvature. *Biophys. Chem.* 55:95–114.

Shrader, T. E., and D. M. Crothers. 1989. Artificial nucleosome positioning sequences. *Proc. Natl. Acad. Sci. USA.* 86:7418–7422.

Shrader, T. E., and D. M. Crothers. 1990. Effects of DNA sequences and histone-histone interactions on nucleosome placement. *J. Mol. Biol.* 216:69–84.

Shui, X., L. McFail-Isom, G. G. Hu, and L. D. William. 1998a. The B-DNA dodecamer at high resolution reveals a spine of water on sodium. *Biochemistry.* 37:8341–8355.

Shui, X., C. C. Sines, L. McFail-Isom, D. VanDerveer, and L. D. William. 1998b. Structure of the potassium form of CGCGAATTCGCG: DNA deformation by electrostatic collapse around inorganic cations. *Biochemistry.* 37:16877–16887.

Spiegel, M. R. 1974. Fourier Analysis. McGraw-Hill Book Company, New York.

Sprous, D., W. Zacharias, Z. A. Wood, and S. C. Harvey. 1995. Dehydrating agents sharply reduce curvature in DNAs containing A tracts. *Nucleic Acids Res.* 23:1816–1821.

Sugimoto, N., S. Nakano, M. Yoneyama, and K. Honda. 1996. Improved thermodynamic parameters and helix initiation factor to predict stability of DNA duplexes. *Nucleic Acids Res.* 24:4501–4505.

Travers, A. A., and A. Klug. 1987. Nucleoprotein complexes. DNA wrapping and writhing. *Nature.* 327:280–281.

van Holde, K. E. 1988. Chromatin. Springer-Verlag, New York.

Wang, Y., R. Gellibolian, M. Shimizu, R. D. Wells, and J. Griffith. 1996. Long CCG triplet repeat blocks exclude nucleosomes: a possible mechanism for the nature of fragile sites in chromosomes. *J. Mol. Biol.* 263:511–516.

Wang, Y., and J. D. Griffith. 1996. The $[(G/C)_3NN]_n$ motif: a common DNA repeat that excludes nucleosomes. *Proc. Natl. Acad. Sci. USA.* 93:8863–8867.

Widlund, H. R., H. Cao, S. Simonsson, E. Magnusson, T. Simonsson, P. E. Nielsen, J. D. Kahn, D. M. Crothers, and M. Kubista. 1997. Identification and characterization of genomic nucleosome-positioning sequences. *J. Mol. Biol.* 267:807–817.

Widom, J. 1989. Toward a unified model of chromatin folding. *Annu. Rev. Biophys. Biophys. Chem.* 18:365–395.

Woda, J., B. Schneider, K. Patel, K. Mistry, and H. M. Berman. 1998. An analysis of the relationship between hydration and protein-DNA interactions. *Biophys. J.* 75:2170–2177.