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Energetic coupling between DNA bending and base pair opening

(conformational energy calculations/proton exchange/curvature/nucleic acid deformation)

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ABSTRACT **The pathway for base pair opening within a B-DNA duplex is investigated by theoretical molecular modeling. The results show that the disruption of a single base pair is energetically compatible with the deductions made from hydrogen exchange measurements. In addition, it is found that the opening process is greatly facilitated by DNA bending and that, conversely, once a base pair is disrupted, DNA can bend very easily. It appears that the energetic coupling between these two processes may play an important role in many biological reactions involving nucleic acid distortion.**

Although the crystal structures of double-stranded nucleic acids deduced from x-ray data show the base pairs to be stacked and hydrogen bonded, there is now ample experimental evidence for the existence of transiently opened base pairs with disrupted hydrogen bonds. Such open states have essentially been inferred from hydrogen exchange to explain how protons deeply buried inside the double helix and, moreover, involved in hydrogen bonds can exchange with water protons. Measurement of hydrogen exchange kinetics by several techniques (tritium labeling, deuterium labeling, and NMR) has been extensively used to investigate this base-opening reaction.

Despite the accumulation of a wealth of information leading to a better knowledge of the chemical mechanism and of the corresponding time range (1–4), the conformational and energetic details of the pathway for opening and the effect of changes in the tertiary structure of DNA remain unclear. Most notably, the mechanism by which a given base pair manages to accumulate enough energy to spontaneously open and disrupt its hydrogen bonds is still fully unknown. These questions are addressed here by theoretical calculations on a B-DNA oligomer. Using the JUMNA (junction minimization of nucleic acids) molecular modeling program (5), we study the energetics and the conformational route for opening one base within a short DNA fragment and then demonstrate how base pair opening and bending of the DNA double helix are related to one another.

METHODS

All of the conformational energy calculations presently described were carried out by using the FLEX parameterization (6, 7) developed over several years in our laboratory and already exploited in a large number of studies of nucleic acids and other biological molecules (see, for example, refs. 8–10).

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This energy expression consists of a series of pairwise additive terms as shown below.

$$E = \sum \frac{Q_i Q_j}{\varepsilon(R) R_{ij}} + \sum \left(\frac{-A_{ij}}{R_{ij}^6} + \frac{B_{ij}}{R_{ij}^{12}} \right)$$
$$+ \sum \left[\cos\theta \left(\frac{-A_{ij}^{HB}}{R_{ij}^6} + \frac{B_{ij}^{HB}}{R_{ij}^{12}} \right) + (1 - \cos\theta) \left(\frac{-A_{ij}}{R_{ij}^6} + \frac{B_{ij}}{R_{ij}^{12}} \right) \right]$$
$$+ \sum \frac{V_s}{2} (1 \pm \cos N_s \tau_s) + \sum V_a (\sigma_a - \sigma_a^\circ)^2.$$

The first term of this formula represents the electrostatic energy, calculated as a sum of interactions between atomic monopoles Q_i, obtained from a specially reparameterized Hückel–Del Re procedure (11, 12) and damped by a dielectric function $\varepsilon(R)$ having a sigmoidal form, based on that developed by Hingerty *et al.* (13). We have reformulated this function as shown below so that it is possible to vary both the plateau value of the dielectric reached at long distance (D) and the slope of the sigmoidal segment of the function (S).

$$\varepsilon(R) = D - \frac{(D-1)}{2} [(RS)^2 + 2RS + 2] \exp(-RS).$$

The calculations described here were performed under dielectric damping conditions corresponding to aqueous solution ($S = 0.16$, $D = 78$). The next three energy terms represent dispersion–repulsion interactions calculated with a 6–12 dependence using, in part, the parameter set developed by Zhurkin *et al.* (14). Hydrogen bonds (HB) are dealt with by the latter two of these terms, which take into account their angular dependence. All of these terms are summed over pairs of atoms separated by at least three chemical bonds. The last two terms represent the distortion energy associated with torsion angles τ_s (including anomeric effects) and valence angles σ_a.

To study the base-opening or bending processes within a DNA fragment it is necessary to have control over the helicoidal parameters describing the position of the bases in space as well as parameters indicating the path of the helical axis so that a given "open" or "bent" state can be maintained while energy optimizing the rest of the DNA conformation. This possibility, which is unavailable in normal molecular mechanics procedures, can be achieved with the recently developed JUMNA procedure (5).

The aim of the JUMNA approach is to combine the control over the helicoidal parameters of DNA with the rapidity and ease of treating internal conformation changes, including sugar repuckering, derived from our earlier Cinflex (constrained internal flexibility) procedure (7). To achieve this we divide the nucleic acid fragment to be studied into 3′-monophosphate nucleotides. These nucleotides are then positioned with respect to a helical axis by using a set of commuting helicoidal parameters, which consequently be-

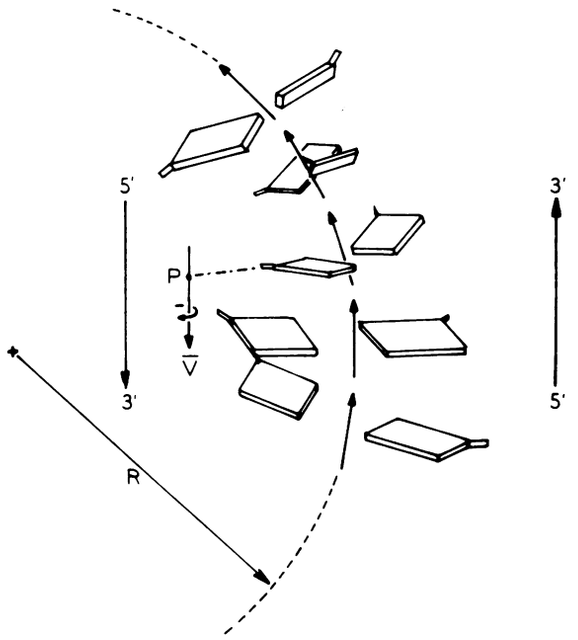

FIG. 1. Schematic diagram of the DNA model used for the present calculations. The segment studied, $(dA)_5 \cdot (dT)_5$, is represented by rectangles denoting the positions of its bases and small attached bars indicating the position of the glycosidic bonds. The bending of the segment is defined by a circle of radius R and the first and last helical axis segments are required to be tangential to this circle. Base opening is achieved by rotating a chosen base around a vector V passing through a point P.

come direct variables of the minimization. In addition there are a total of eight independent backbone variables per nucleotide (one glycosidic angle, five sugar variables, and two backbone torsions, C3'—O3' and 03'—P). While these parameters are sufficient to describe the relative position and conformation of any number of nucleotides with respect to a linear helical axis, four additional parameters must be added to allow helical axis kinks or dislocations to be described easily. These parameters involve two further translations and two rotations, which position successive helical axis segments with respect to one another. Finally, the task of maintaining the chemical continuity of the nucleic acid backbone (mathematically equivalent to satisfying a total of four constraints per nucleotide: the sugar ring closure, the backbone closure distance between O5' and C5', and two valence angles, P—O5'—C5' and O5'—C5'—C4') is achieved through the use of a sophisticated constraint minimization algorithm.‡

The results that are presented in the following section were obtained in two stages. First, one of the bases of the central pair of a 5-base-pair fragment was opened to a chosen extent and held rigidly (with the exception of internal tilt and propeller changes). Opening was achieved, as shown in Fig. 1, by rotating the nucleotide concerned around a vector V passing through a point P. Preliminary calculations that forced opening with the use of distance constraints between the bases of the central base pair showed that the energetically optimal position of P was roughly in the center of the sugar attached to the opening base and that the optimal orientation of V was perpendicular to the plane of the opening base. (The reader is referred to similar studies using energy penalty functions to force opening in refs. 17 and 18.) The energy of the fragment was then optimized by allowing the remaining variables (helicoidal parameters and backbone variables) to vary freely, with the exception of the geometry of the terminal base pairs, which were also fixed to restrict the effects of the opening perturbation to the fragment studied. Second, bending of the DNA segment was achieved by using analytically formulated constraints that forced the terminal helical axis segments to be tangential to a circle corresponding to a chosen radius of curvature, R (see Fig. 1). It should be stressed that this radius of curvature applies only locally to the oligomeric segment studied. From a more global point of view it may rather be interpreted as the presence of a relatively sharp kink within an otherwise linear nucleic acid. (Note that the direction of bending discussed in the following section is measured as the angle between the dyad axis of the lowest base pair, pointing into the minor groove, and the projection of the uppermost helical axis segment onto the plane perpendicular to the lowest helical axis segment.)

‡This variable subroutine metric algorithm was developed by I. Parker in collaboration with J. Kendrick and R.L. from refs. 15 and 16. A related algorithm (VA13F) may be found in the Harwell library (Atomic Energy Research Establishment, Harwell, U.K.).

RESULTS

The base-opening pathway presently studied is the swinging out of thymine toward the major groove at the center of a $(dA)_5 \cdot (dT)_5$ fragment. Rotation toward the minor groove has been found to be much less favorable energetically and leads to steric conflicts between atoms involved in the base pair hydrogen bonding. In the case of a straight DNA where no bending is allowed the energetic profile of thymine opening is shown by the uppermost curve of Fig. 2. The energy of the DNA fragment increases monotonically with the opening angle and, for example, shows a destabilization of roughly 30 kcal/mol at an opening angle of 50°. It can be noted that at this angle the thymine imino proton becomes fully accessible and thus is available for exchange with solvent protons.

To discover the energy relationship between this opening reaction and bending of the DNA fragment we have subsequently enforced a curvature on the DNA fragment, taking into account both the radius of curvature adopted and the orientation of the bending. The results presented in Fig. 3 show the bending energy (for two radii of curvature of 30 and 15 Å) of the oligonucleotide with closed base pairs together with the total distortion energy for the oligonucleotide having its central thymine opened by 50° as well as a bend of 15-Å radius (the latter energy thus includes the energetic contribu-

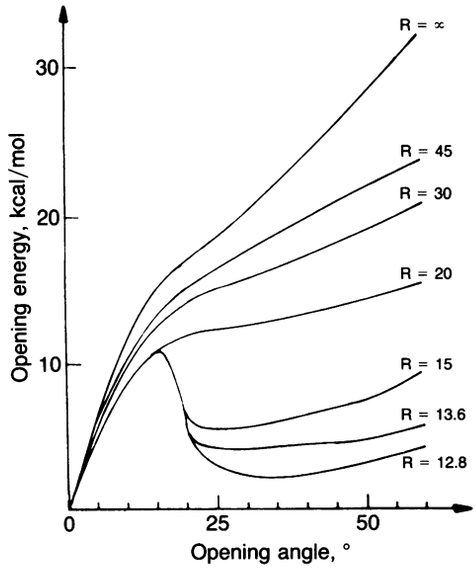

FIG. 2. Energy of opening the central thymine toward the major groove in $(dA)_5 \cdot (dT)_5$ as a function of the opening angle and for different degrees of DNA bending. One kilocalorie = 4.18 kJ.

tions for opening the central thymine toward the major groove as well as the energy necessary for bending the DNA). Each of these results is shown as a function of the orientation of bending, measured in degrees and defined as in the previous section.

The total distortion energy curve shows that the most stable conformation for the open state is obtained for an angle of 85°, which corresponds to bending into the minor groove at the level of the central base pair and also to bending away from the direction of the opening base motion. In the case of the closed DNA, the bending energy as a function of orientation has a sinusoidal shape, showing that bending toward the grooves of the helix is easier than toward the phosphodiester backbones, the amplitude of the curve being dependent on the radius of curvature imposed. The opening energy as a function of bending orientation can be obtained simply by subtracting the bending energy of the closed DNA from the total distortion energy of the DNA in the open and bent state. For the optimal bending direction, this results in an opening energy of about 7 kcal/mol for 50° rotation of the thymine, which may be compared with 28 kcal/mol needed to open within the straight DNA. Thus the bending of DNA leads to an important decrease of the opening energy.

This coupling between bending and opening energies is further clarified in Fig. 2, where the opening energy (δE_o) is shown as a function of the thymine opening angle for radii of curvature down to 12.8 Å (note that the bending orientation corresponds to the optimal value of 85° found for the segment with an open pair and discussed above). Examination of these curves clearly reveals the important reduction in the energy of opening as the bending becomes more pronounced. It can be seen that for the highest curvature studied, the 50° opened state is only 3 kcal/mol less stable than the closed state. It should be noted however that, at high curvature, an activation barrier appears at roughly 15° opening, which analysis shows to be due to the breaking of the hydrogen bonds of the central base pair. The corresponding values of the bending energy for the closed oligonucleotide at the different radii of curvature studied are given in Fig. 4. In light of recent interest

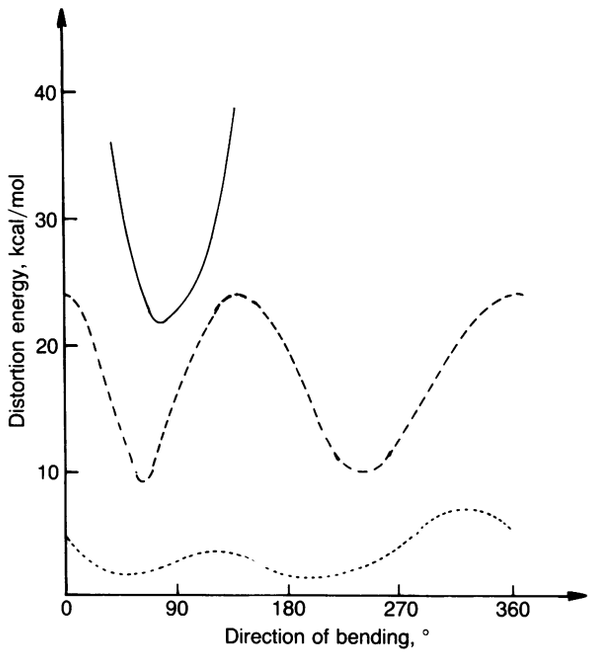

FIG. 3. The bending energy of the $(dA)_5$·$(dT)_5$ fragment as a function of bending orientation for two radii of curvature, 30 Å (---) and 15Å (- - -). The upper curve (—) gives the total distortion energy as a function of the bending orientation for a fragment with the central thymine opened by 50° and having a 15-Å radius of curvature.

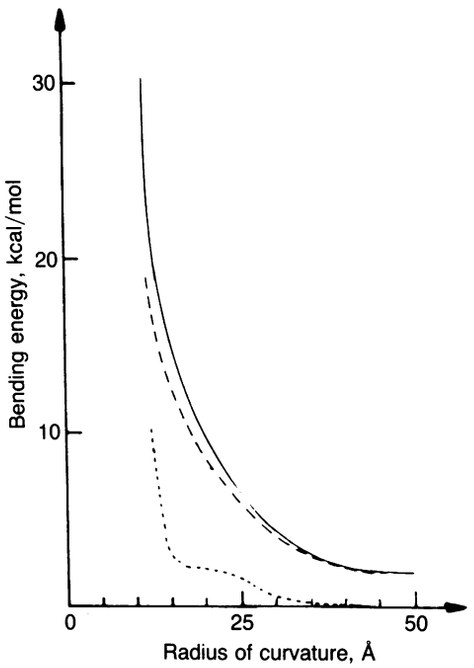

FIG. 4. The bending energy, δE_b (—), of the $(dA)_5$·$(dT)_5$ segment and its decomposition into the base–base component δE_{bb} (- - -), including inter- and intrastrand stacking and interstrand pairing energies, and the sugar-phosphate backbone component δE_{bs} (---), including inter- and intrastrand interactions as well as the torsional energies associated with the given backbone conformation.

in the natural curvature of DNA containing A·T tracts (19–21), it should be remarked that the segment presently studied showed no signs of intrinsic bending.

DISCUSSION

This theoretical study shows that the swinging out of a thymine, to an open state where it is fully accessible to the solvent, requires an activation energy similar to that determined experimentally (3, 22, 23). In addition it is found that the disruption of the central base pair in the oligomeric DNA fragment studied does not affect the hydrogen bonding of the neighboring pairs and in fact results in only negligible changes in their geometry. This can be seen in the molecular graphic shown in Fig. 5, where the conformations of the straight oligomer at various base-opening angles have been superposed. This pathway for thymine opening strongly suggests that an opening mechanism involving a single base pair might well account for the DNA distortion underlying the measured exchange rate for the protons in duplex DNA.

A further interesting result of this study is the clearly demonstrated energetic coupling between DNA bending and base pair opening. DNA bending facilitates subsequent base pair opening, as shown by the opening energy profiles of Fig. 2. A better understanding of the underlying physical process leading to the opening energy curves of Fig. 2 can be obtained by decomposing δE_o into two parts: δE_{ob}, due to base–base interactions, and δE_{os}, due to the sugar-phosphate backbones of the double helix. This separation, shown in Fig. 6, indicates that these two components have very different behaviors as a function of bending. The δE_{ob} values are always positive, increase as a function of the thymine opening angle, and decrease as a function of bending, with the exception of the activation energy barrier at roughly 15° opening due to hydrogen bond breakage and mentioned above. In contrast, the values of δE_{os} become increasingly negative as the DNA segment is bent and as the base pair is opened.

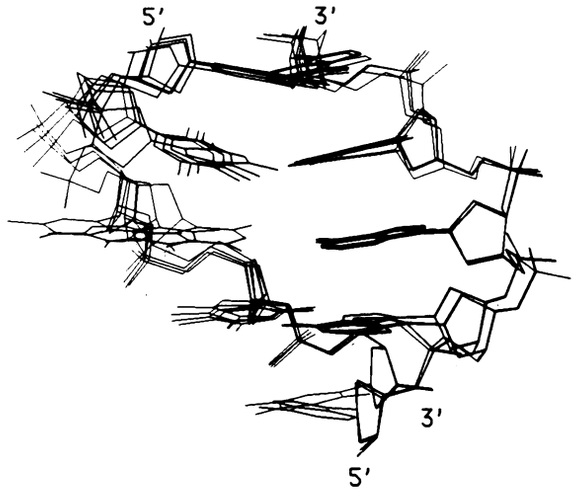

FIG. 5. Molecular graphics of $(dA)_5 \cdot (dT)_5$ with the central thymine opened by various angles up to 60°. Four states, superposed at the level of the first base pair, are shown. The opening thymine is visible in the center of the left-hand strand, and it can be seen that only minor perturbation of its neighboring pairs occurs. The majority of the deformation is located in the sugar-phosphate backbone on the 5' side of the opening nucleotide.

Thus the bending of the oligonucleotide has two major effects on base pair opening. First, bending reduces the energy necessary to overcome base–base interactions, or, more explicitly, base stacking, and second, and most remarkably, bending accumulates energy in the form of backbone strain, which can subsequently be retrieved by base pair opening.

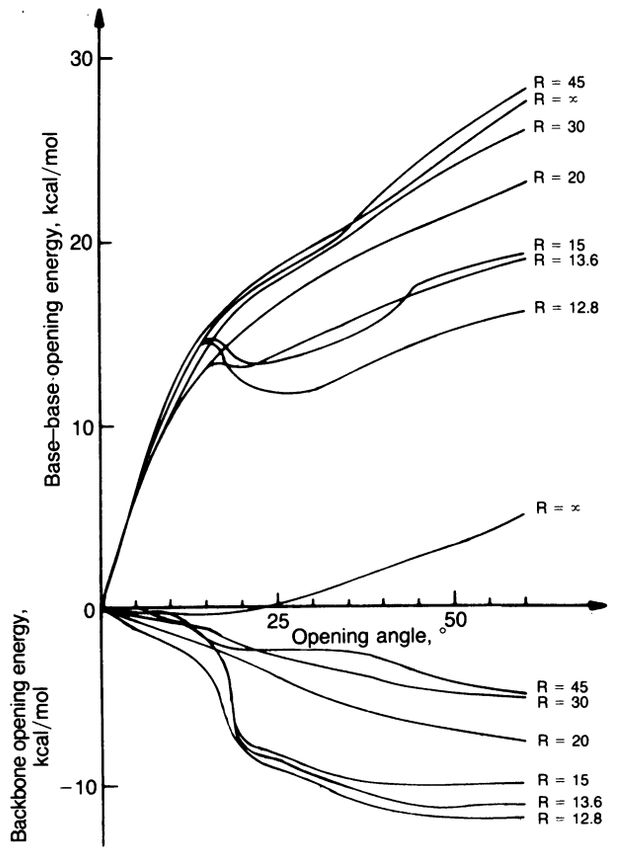

FIG. 6. Decomposition of the base opening energy δE_o into the base–base component δE_{ob} (upper curves) and the sugar-phosphate backbone component δE_{os} (lower curves) expressed as a function of the opening angle and for different degrees of DNA bending. The energy components are defined as in the legend to Fig. 4.

This buildup of strain energy can be clearly seen in Fig. 4, where the bending energy has also been decomposed into base–base (δE_{bb}) and sugar-phosphate backbone (δE_{bs}) components. Whereas, up to a radius of curvature of 30Å, the energy is almost entirely due to the base–base interactions, beyond this value the backbone energy increases rapidly. We are thus led to the far-reaching conclusion that for bent DNA the backbone acts as a spring that promotes opening and, for important bends, can lead to high equilibrium populations of open base pairs.

The discussion so far has considered only DNA bending followed by the subsequent opening of a base pair. A complete thermodynamic diagram describing the coupling between the opening and the bending process requires us to take into account an additional pathway, namely, base pair opening followed by DNA bending. The complete diagram, deduced from the results shown in Figs. 2 and 4, is presented in Fig. 7. The energies listed for each step correspond to thymine opening to 50° and bending to a radius of curvature of 15Å. It is interesting to note that, besides the low opening energy (7 kcal/mol) in the case of bent DNA, the bending energy of an already opened DNA is negative. This indicates that once a base pair is disrupted DNA has a natural tendency to bend. [It should be remarked here that Manning (24), using a very different theoretical approach, has also arrived at this conclusion.] In connection with this finding it should be noted that the overall distortion energy, which in the present case equals 22 kcal/mol is almost constant for any radius of curvature between roughly 50 and 15Å, showing that DNA also becomes very flexible once a single base pair has been disrupted.

In conclusion, this study brings to light two important facts. First, the disruption of a single base pair is characterized by a relatively low energy barrier, which is of the same order as the activation energies determined from hydrogen exchange experiments. Thus the hydrogen exchange process in double-stranded DNA may well follow such a pathway. Second, the energetics of base pair opening and the energetics of DNA bending are strongly coupled. Energy invested in bending can be used to facilitate base opening and, conversely, once a base pair is open, DNA will bend and, further, becomes very flexible over a wide range of radii of curvature. This coupling would appear to have important consequences not only for the interpretation of proton exchange but also for the chemical reactivity of sterically hindered base sites, where it implies that local curvature or kinking should

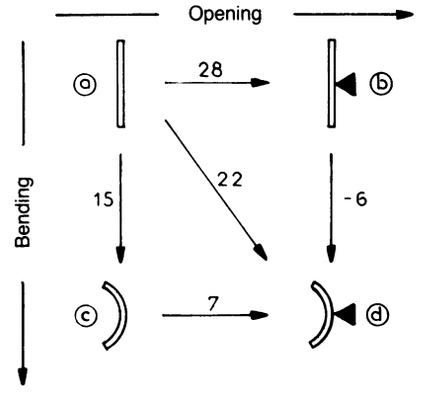

FIG. 7. Thermodynamic diagram showing the energetic coupling between the bending of DNA and base pair opening. The four states shown correspond to (*a*) relaxed straight DNA with intact base pairs, (*b*) a straight DNA segment with an open central base pair, (*c*) a bent DNA segment with intact base pairs, and (*d*) a bent DNA segment with an open central base pair. The energies given (in kcal/mol) correspond to 50° opening and bending to a radius of curvature of 15 Å for the $(dA)_5 \cdot (dT)_5$ segment.

facilitate reaction. It should be remarked that although we have employed strong kinking to reduce opening energy to roughly thermal values, smaller kinks and perhaps even natural curvature could lead to visible changes in the facility of reaction of occluded base sites. Finally, concerning predictions of the mechanism for the bending of long segments of DNA, our calculations would suggest that occasional opened base pairs could allow tight kinks to develop.

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