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Conformations of 145 base pair length poly (dG-dC)·poly (dG-dC) in solution and in association with histones

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ABSTRACT

We have studied the conformation of poly(dG-dC)·poly(dG-dC) in three conditions; i) associated with histone octamers, ii) alone at ionic strength 0.1, and iii) in solutions of over 2.5 M NaCl. The circular dichroism spectrum for the polymer bound to histones differs from that for the free polymer; the difference spectrum is similar to those for native and poly(dA-dT)·poly(dA-dT) core particles. Under the first two conditions, the ^{31}P NMR spectrum is symmetric with line widths of 91 and 41 Hz, respectively, at 109.3 MHz. In high salt, two ^{31}P peaks of equal intensity are observed, confirming recent results of Patel et al. (1) and indicating an alternating geometry for the phosphodiester backbone. Using this highly homogeneous DNA, we confirm that the Pohl-Jovin transition (2) is an intramolecular rearrangement, not requiring complete strand separation.

INTRODUCTION

Synthetic polydeoxyribonucleotides offer a unique advantage to those interested in nucleic acid structure; the synthetic molecules amplify features of a particular sequence, often enabling detection of structures enforced on the nucleic acid by such sequence (for review, see 3). The structural features may be masked in sequence heterogeneous native DNA, which probably has the entire spectrum of such allowable conformations. Two recently presented examples of such experimental virtues of these synthetic molecules follow. Using ^{31}P NMR, we have been able to detect an alternating conformation for the phosphodiester backbone of poly(dA-dT)·poly(dA-dT) both in solution and in the fiber state (4,5) and also, Patel and Canuel noted sequence-dependent variations in phosphodiester torsion angles for the octanucleotide dG-dG-dA-dA-dT-dT-dC-dC duplex in solution (6). Furthermore, Patel et al. noted an alternating B-DNA conformation for short poly(dG-dC) oligomers under high salt conditions (1), where the Pohl-Jovin transition (2) had occurred. These experimental data support a proposal offered at about the same time by Klug et al. (7) that such

alternating conformations might exist for alternating deoxypurine-deoxypyrimidine polymers. Second, we and others have studied semi-synthetic chromatin core particles formed from the inner histone octamer and poly(dA-dT)·poly(dA-dT) (8-10) or poly(dG-dC)·poly(dG-dC) (8). Particularly for the particles containing the AT polymer, removal of the effects of DNA sequence heterogeneity on physical and biochemical properties enabled refinement in the resolution of experiments on nucleic acid-protein interactions in these chromosomal particles (8-12).

A serendipitous offshoot of these latter experiments has been the ability to produce, by nuclease digestion of such semi-synthetic chromatins, highly homogeneous preparations of the alternating copolymers in double stranded form with length about 145 base pairs (bp). High molecular weight synthetic DNA is readily available from unprimed, DNA polymerase I-mediated synthesis (13,14) and small oligomers can be obtained synthetically or by fractionation of partial enzymatic digests of higher molecular weight material (15); preparations from semi-synthetic chromatins fill a gap between these two size groups. This size DNA has advantages for several types of physical study of structure in being small enough to tumble rapidly, enhancing resolution in NMR experiments, yet large enough that end effects on properties are expected to be small.

We have previously reported results of studies of the conformation of 145 bp poly(dA-dT)·poly(dA-dT) both in solution and in the core particle, using several spectroscopic methods (4,8,11,12). Here we describe analogous investigations of poly(dG-dC)·poly(dG-dC) structure.

EXPERIMENTAL SECTION

Poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) were products of P-L Biochemicals. Chicken erythrocyte inner histones (H2A, H2B, H3 and H4) were prepared as previously described and associated with the synthetic polynucleotides by salt step dialysis (8). Digestions with micrococcal nuclease (Worthington Biochemical Corp.) and fractionation of digests by isokinetic sucrose gradient centrifugation were done exactly as before (8). DNA was isolated from core particle preparations by phenol extraction of a solution containing 1% sodium dodecyl sulfate and 25 mM EDTA, pH 7, and precipitation at -20° with 2.5 volumes ethanol.

Conditions and methods for measurement of circular dichroism (16) and ^{31}P NMR (4,12) spectra have been detailed previously. Electrophoresis of DNA samples was performed on 5% polyacrylamide gels using a tris/borate/

EDTA buffer system (17). Approximate DNA sizes are based on calibrations of the gel using the mobilities of bromphenol blue and xylene cyanol FF (18).

RESULTS AND DISCUSSION

Circular Dichroism Spectra

Figure 1 shows the CD spectra for core particles containing poly(dG-dC)·poly(dG-dC) and the 145 base pair polynucleotide in the absence of proteins. The core particle spectrum is dissimilar to both that of native core particles (19) and that of nucleosomes containing poly(dA-dT)·poly(dA-dT) (9,11). A broad positive ellipticity band with maxima at 298 nm (800°) and 275 nm

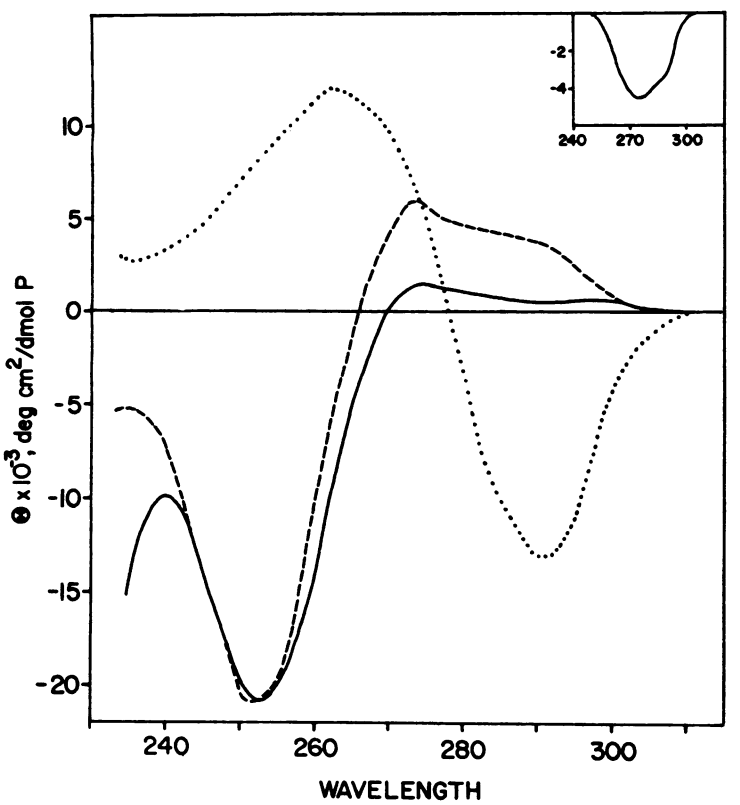

Figure 1 Circular dichroism spectra of 145 bp poly(dG-dC)·poly(dG-dC). Spectra were determined for the synthetic polynucleotide associated with inner histones as a semi-synthetic core particle (——), alone in solution at ionic strength 0.1M (---), and alone in solution in approximately 3 M NaCl (....). Inset: the difference CD spectrum for the core particle - polynucleotide at ionic strength 0.1 M.

(1400°) is present. Crossover to negative ellipticities is at 270 nm and a very large negative maximum occurs at 252 nm (-21000°). This large negative band is very similar to that present for the polymer alone; above 270 nm the protein free poly(dG-dC)·poly(dG-dC) spectrum is qualitatively similar to that for the core particle, but of greater ellipticity. The difference spectrum between particle and DNA (Figure 1, inset) is a broad negative ellipticity band with maximum at about 273 nm (-4600°). In form, this difference spectrum is similar to those observed for native core particles (20) or poly(dA-dT)·poly(dA-dT) core particles vs. their constituent DNAs, although the ellipticity is significantly smaller for the current case (4600° vs. 8000°). Cowman and Fasman (20) have suggested that the altered CD spectrum of nucleosomes derives from a contribution similar to the spectrum of psi-form DNA. Observations of similar difference (core particle - DNA) spectra for DNA samples with individual spectra as different as poly(dA-dT)·poly(dA-dT), on the one hand, and poly(dG-dC)·poly(dG-dC), on the other, provides strong support for their hypothesis. The quantitative difference between the difference spectra for 1) native or poly(dA-dT)·poly(dA-dT) particles (11,20) and 2) the poly(dG-dC)·poly(dG-dC) particles (Figure 1) may reflect an altered wrapping of the DNA in the latter case; unusual features of the DNAase I cutting sites in these particles have been noted previously (8).

^{31}P Nuclear Magnetic Resonance Spectra

The ^{31}P NMR spectra of core particles containing poly(dG-dC)·poly(dG-dC) and the protein-free nucleic acid were recorded at 20° (not shown). The two spectra are symmetrical and fit well by single Lorenzian distributions. Chemical shifts are virtually identical for phosphorus in either case. The spectra do differ in line width, 41 Hz for the DNA and 91 Hz for the nucleoprotein particle at an observing frequency of 109.3 MHz. Line width for the GC-containing particle is significantly less than that for native particles with random sequence DNA, 130 Hz (21), or semi-synthetic particles containing poly(dA-dT)·poly(dA-dT), 110 Hz (12). The differences between the line width for the GC-containing DNA and the same when in a core particle (91-41=50 Hz) and between the overall line width for AT-containing DNA and the same when in a core particle (110-64=46 Hz) are similar. The additional broadening of the signal for DNA and poly(dA-dT)·poly(dA-dT) that occurred when the nucleic acid was complexed with histones was previously attributed to possible regional differences in the geometry of the phosphodiester backbone in the nucleosome or variations in interactions of histones with the

nucleic acid along the length of nucleosomal DNA (12,21), based on an estimated relaxational line broadening of about 45 Hz for both particles and DNA (21,26). The current observation, that nearly identical broadening occurs for the poly(dG-dC)·poly(dG-dC) particle, provides further support for this contention. In the case of the GC-containing particle, as for all native and the other semi-synthetic particles studies by this method, (12, 21-23), the absence of peak splitting or asymmetry of the signal argues against the occurrence of kinks (24,25) in the DNA; the argument is limited by the requirement that the kink, if it occurred, would have to alter the geometry around the phosphorus sufficiently to allow a detectable chemical shift from the broad averaged peak for B-form DNA.

Figure 2 compares high resolution ^{31}P NMR spectra at 109.3 MHz of three 145 base pair, double stranded DNA samples in the same buffer at 20°± 2°; DNA samples containing poly(dA-dT)·poly(dA-dT), poly(dG-dC)·poly(dG-dC) or random sequence chicken DNA were all obtained from nucleosome core particles. Significant differences exist among the three spectra. Those for poly(dG-dC)·poly(dG-dC) and native DNA are both symmetrical single peaks, however, the line width for the random sequence DNA (103 Hz) is larger than that for the synthetic polymer (41 Hz). The overall line width for the poly(dA-dT)·poly-(dA-dT) sample (64 Hz) is intermediate between the other two, but the signal obviously consists of two partially resolved resonances of nearly equal intensity, separated by 24 Hz (4, Figure 2).

In consideration of the bases for these differences, it is important to note that the molecular dimensions of these three DNA samples are very likely identical; they are the same length, B-form in conformation, and double stranded linear molecules. Therefore we make the reasonable assumption that macromolecular dynamic properties, e.g. correlation times, are the same for all three samples. The poly(dG-dC)·poly(dG-dC) sample has the narrowest symmetrical signal. Making the assumption that all phosphorus atoms in this polymer are magnetically equivalent, the line width, 41 Hz, sets an upper limit to the line broadening that can be attributed to relaxation mechanisms such as dipole-dipole interactions and chemical shift anisotropy. The average line width for the two partially resolved peaks in the NMR spectrum of the poly(dA-dT)·poly(dA-dT) sample is 40 Hz (4), a value nearly identical to the line width for the poly(dG-dC)·poly(dG-dC) sample. Agreement of these two values supports the contention that this line width characterizes the relaxational line broadening for homogeneous phosphorus geometries in DNA of this length, although small variations in

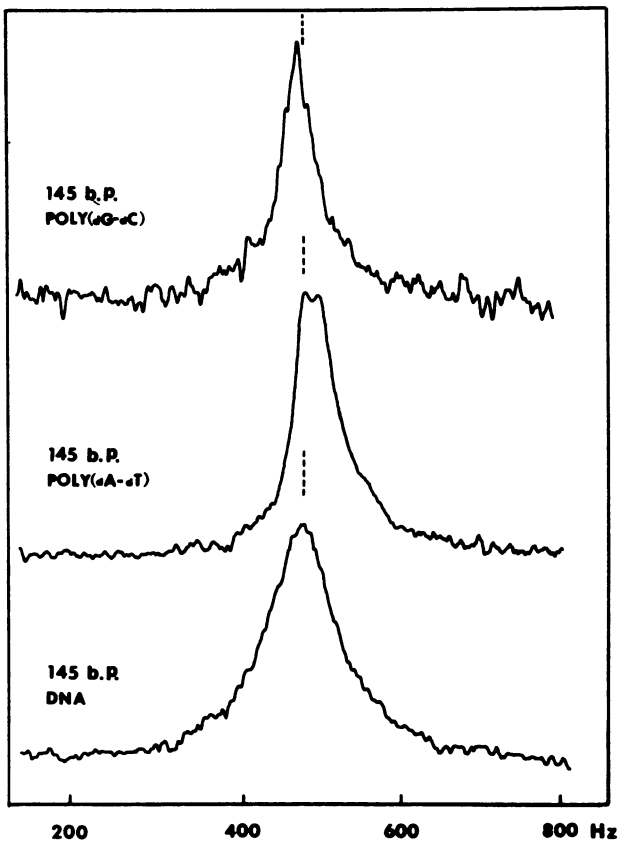

Figure 2 ^{31}P NMR spectra of 145 bp length DNA samples. Spectra for the indicated samples were recorded at about 20° in 10 mM Tris/Cl, pH 8, 1 mM EDTA at an observing frequency of 109.3 MHz. Chemical shift in Hz is measured upfield positive from trimethylphosphate as an internal reference as shown in Figure 3.

line width will occur due to chemical shift tensor orientation and anisotropic rotation of DNA in solution. In poly(dA-dT)·poly(dA-dT), an alternating conformation for the DNA leads to two equal populations of phosphorus atoms, differing in geometry (4,5,7).

In contrast to the two above discussed samples, random sequence DNA of identical size exhibits a much broader symmetrical NMR signal (Figure 2). Based on considerations outlined above, it seems apparent that the additional broadening (103-41=62 Hz) observed for native DNA vs. poly(dG-dC)·

poly(dG-dC) must be assigned to mechanisms other than relaxational ones. We previously suggested that native DNA might exhibit a broad signal due to a distribution of chemical shifts for the phosphorus atoms (4-6,12,21,26). The current results strongly support the idea that chemical shift dispersion, likely reflecting a spectrum of phosphodiester conformations imposed by particular base sequences, leads to a portion of the line width observed in NMR studies of native DNA samples. A similar conclusion has been made from solid-state ^{31}P NMR measurements on fibers of B-form DNA (28) and poly(dA-dT)·poly(dA-dT)(5).

Pohl-Jovin transition for 145 bp poly(dG-dC)·poly(dG-dC)

Pohl and Jovin have reported a striking conformational transition for poly(dG-dC) induced by increased ionic strength, using NaCl, $NaClO_4$ or $MgCl_2$ (2). Although not symmetrically, the circular dichroism spectrum inverted when polymer in 1 M NaCl was compared to polymer in about 3 M NaCl (2), as we show for the current double stranded poly(dG-dC)·poly(dG-dC) sample in Figure 1. Very recently, Patel et al. have studied the Pohl-Jovin transition with poly(dG-dC) samples of chain length about 8-16 using high resolution ^{31}P and ^{1}H NMR spectroscopy (1). The ^{31}P spectra showed a single peak with a small shoulder in 0.2 M NaCl and two well separated peaks of about equal intensity in 4 M NaCl (1). In these spectra, individual line widths in high salt were about three-fold narrower than that at low salt (1). Also, a definite shoulder was present in the upfield resonance in high salt (1). The sample we have used for studies of poly(dG-dC)·poly(dG-dC), of much greater chain length, also develops a split ^{31}P NMR signal during the Pohl-Jovin transition (Figure 3). The single, symmetrical peak at -4.16 ppm in 1.74 M NaCl splits into two peaks of about equal intensity, separated by 1.5 ppm, in salt concentrations over 2.5 M. The midpoint for the transition for this poly(dG-dC)·poly(dG-dC) is between 2 and 2.5 M NaCl. In contrast to spectra of the short GC oligomer (1), ^{31}P NMR line widths for 145 bp poly-(dG-dC)·poly(dG-dC) are similar in low and high salt (Figure 4). This suggests that the hydrodynamic properties of the polymer are not altered in major fashion during the transition. Based on similar ^{31}P NMR data plus ^{1}H NMR studies, Patel et al. assigned the origin of this split signal as an alternating B-DNA conformation for the polynucleotide in high salt concentrations (1); phosphorus geometry differs for dG(3'-5')dC and dC(3'-5')dG.

Drew et al. (28) have detected different structures by X-ray diffraction methods for d(CpGpCpG) crystals formed at low and high salt concentra-

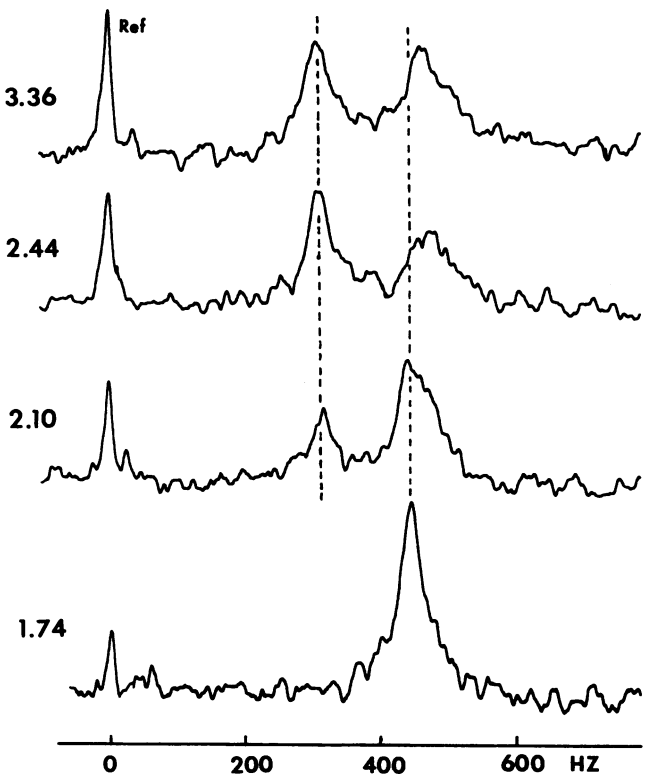

Figure 3 ^{31}P NMR spectra of poly(dG-dC)·poly(dG-dC) at various salt concentrations. Spectra were recorded for the 145 bp synthetic polydeoxyribonucleotide at 25° and 109.3 MHz in the indicated molarities of NaCl.

tions. Wang et al. (29) have established that d(CpGpCpGpCpG) crystals formed in the presence of 10 mM spermine and 15 mM $MgCl_2$ have as a structure a left-handed helix of anti-parallel phosphodiester chains with Watson-Crick base pairs. This Z-DNA has an alternating geometry for the phosphodiester backbone (29), consistent with the ^{31}P NMR studies which demonstrate two phosphorus environments in samples which have undergone the Pohl-Jovin transition (1, Figure 3). Thus, while not proven, it seems likely that the Pohl-Jovin transition involves conversion of a right-handed B-form DNA double helix into a left-handed alternating B-form (or Z-form) double helix.

In their original paper, Pohl and Jovin (2) suggested that the transition was an intramolecular rearrangement. Problematic in this interpretation

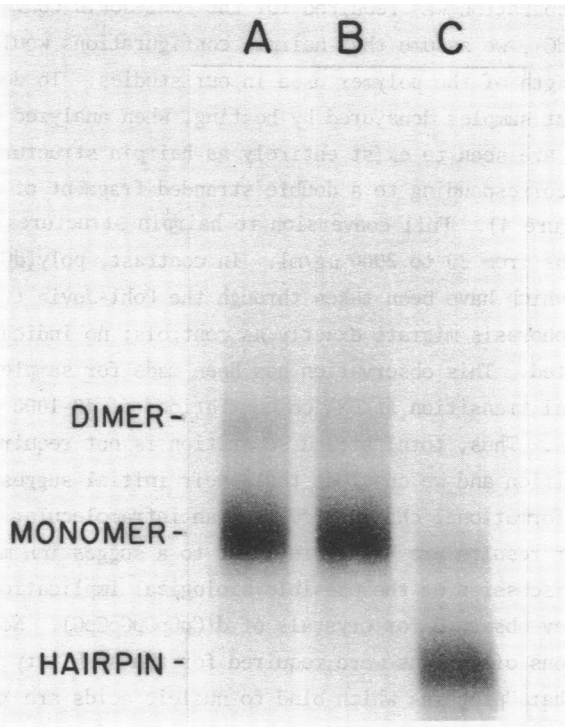

Figure 4 Gel electrophoretic analysis of DNA conformations of poly(dG-dC)·poly(dG-dC) samples. Gel electrophoresis was performed under non-denaturing conditions on a 5% polyacrylamide gel. (A) control sample. (B) sample which was incubated in 3 M NaCl at 0.04 mg/ml and then diluted to 0.75 M NaCl prior to precipitation and electrophoresis. (C) sample heated to 100° for 2 min at a DNA concentration of 1 mg/ml and an ionic strength of about 80 mM prior to electrophoresis.

are the dispersity of sizes for the synthetic poly(dG-dC) sample studied and difficulties in accurate size determination. Thus, the weight average molecular weights determined by sedimentation equilibrium measurements were 19 and 26 equivalent base pair units in alkali and at neutral pH, respectively (2). In 2.93 M NaCl, the weight average size was about 32 equivalent base pairs (2). Enzymatic chain length determinations led to a number average size of 49 bases (2), suggesting the possibility that some of the molecules were in hairpinned structures. With the availability of quite homogeneous polymers of known double stranded conformation (8), we now can test directly the nature of the rearrangement in the Pohl-Jovin transition.

If strand separation was required for the Pohl-Jovin conversion of poly-(dG-dC)·poly(dG-dC), we assume that hairpin configurations would form for molecules the length of the polymer used in our studies. To demonstrate this, we show that samples denatured by heating, when analyzed by gel electrophoresis, are seen to exist entirely as hairpin structures, migrating with a mobility corresponding to a double stranded fragment of about 80 base pairs length (Figure 4). Full conversion to hairpin structures occurs at DNA concentrations from 20 to 2000 μg/ml. In contrast, poly(dG-dC)·poly-(dG-dC) samples which have been taken through the Pohl-Jovin transition prior to electrophoresis migrate exactly as controls; no indication of hair-pinning is detected. This observation has been made for samples put through the conformational transition at DNA concentrations of 40-1000 μg/ml with identical results. Thus, total strand separation is not required for the Pohl-Jovin transition and we conclude that their initial suggestion (2) was correct, the conformational change occurs as an intramolecular rearrangement.

Finally, our results are highly relevant to a suggestion made by Wang et al. (29) in discussion of the possible biological implications of the Z-DNA structure they observed for crystals of d(CpGpCpGpCpG). Noting that high concentrations of cations were required for the stability of Z-DNA, they suggested that "proteins which bind to nucleic acids are known to contain large numbers of basic residues so that some of these proteins may convert segments of B-DNA into Z-DNA in vivo" (29). While the suggestion may be true for some proteins, the ^{31}P NMR results we have obtained in this study document that the highly basic core histones do not convert poly(dG-dC)·poly(dG-dC) into a structure which has alternating geometry for the phosphorus atoms. Since the two ^{31}P NMR peaks for poly(dG-dC)·poly(dG-dC) in high salt are separated by 163 Hz (1.50 ppm) at 109.3 MHz and the line width for the single symmetrical peak observed for the semi-synthetic core particle is only 91 Hz, any phosphorus atoms in the core particle having the conformation characteristic of Z-DNA should have been readily detected. No second peak was detected (limit of detection less than 5%), demonstrating that the complex of histones and poly(dG-dC)·poly(dG-dC) does not contain Z-DNA.

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REFERENCES

1. Patel, D.J., Canuel, L.L. and Pohl, F.M. (1979) Proc. Natl. Acad. Sci. USA 76, 2508-2511
2. Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396
3. Wells, R.D., Blakesley, R.W., Hardies, S.C., Horn, G.T., Larson, J.E., Selsing, E., Burd, J.F., Chan, H.W., Dodgson, J.F., Jensen, K.F., Nes, I.F. and Wartell, R.M. (1977) CRC Critical Reviews in Biochemistry 4, 305-340
4. Shindo, H., Simpson, R.T. and Cohen, J.S. (1979) J. Biol. Chem. 254, 8125-8128
5. Shindo, H. and Zimmerman, S.B. (1979) Nature 283, 690-691
6. Patel, D.J. and Canuel, L.L. (1979) Eur. J. Biochem. 96, 267-276
7. Klug, A., Jack, A., Viswamitra, M.A., Kennard, O., Shakked, Z. and Steitz, T.A. (1979) J. Mol. Biol. 131, 669-680
8. Simpson, R.T. and Kunzler, P. (1979) Nucleic Acids Res. 6, 1387-1415
9. Bryan, P.N., Wright, E.B. and Olins, D.E. (1979) Nucleic Acids Res. 6, 1509-1520
10. Rhodes, D. (1979) Nucleic Acids Res. 6, 1805-1816
11. Simpson, R.T. (1979) J. Biol. Chem. 254, 10123-10127
12. Simpson, R.T. and Shindo, H. (1979) Nucleic Acids Res. 7, 481-492
13. Schachman, H.K., Adler, J., Radding, C.M., Lehman, I.R. and Kornberg, A. (1960) J. Biol. Chem. 235, 3242-3252
14. Burd, J.F. and Wells, R.D. (1970) J. Mol. Biol. 53, 435-459
15. Pohl, F.M. (1974) Eur. J. Biochem. 42, 495-504.
16. Simpson, R.T. and Sober, H.A. (1970) Biochemistry 9, 3103-3109
17. Peacock, A.C. and Dingman, C.W. (1967) Biochemistry 6, 1818-1827
18. Maniatis, T., Jeffrey, A. and van deSande, H. (1975) Biochemistry 14, 3787-3794
19. Whitlock, J.P., Jr. and Simpson, R.T. (1976) Nucleic Acids Res. 3, 2255-2266
20. Cowman, M. and Fasman, G. (1978) Proc. Natl. Acad. Sci. USA 75, 4759-4763
21. Shindo, H., McGhee, J.D. and Cohen, J.S. (1980) Biopolymers 19, 523-537
22. Kallenbach, N.R., Appleby, D.W. and Bradley, C.H. (1978) Nature 272, 134-138
23. Klevan, L., Armitage, I.M. and Crothers, D.M. (1979) Nucleic Acids Res. 6, 1607-1616
24. Crick, F.H.C. and Klug, A. (1975) Nature 255, 530-533
25. Sobell, H.M., Tsai, C., Gilbert, S.G., Hain, S.C. and Sakore, T.D. (1976) Proc. Natl. Acad. Sci. USA 73, 3068-3072
26. Shindo, H. (1980) Biopolymers 19, 509-522
27. Shindo, H., Wooten, J.B., Pheiffer, B.H. and Zimmerman, S.B. (1980) Biochemistry 19, 518-526
28. Drew, H.R., Dickerson, R.E. and Itakura, K. (1978) J. Mol. Biol. 125, 535-543
29. Wang, A. H-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H., van der Marel, G. and Rich, A. (1979) Nature, 282, 680-686