Infrared linear dichroism of oriented DNA-ligand complexes prepared with the wet-spinning method

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Received 6 September 1984; Accepted 4 October 1984

ABSTRACT

Oriented DNA films prepared by the wet-spinning technique have been complexed with several ligands: the anthracycline antibiotic violamycin BI, the dipeptide L-carnosine, and the oligopeptide antibiotic netropsin. The formation of the DNA-ligand complexes is accompanied by dramatic changes of the conformational flexibility of DNA. The B-A transition which occurs usually between 80% and 70% relative humidity (RH) is more or less suppressed by the ligands. Violamycin BI at a total ligand per DNA base pair ratio, r_t, of ∿0.03 and L-carnosine at r_t ∿ 1.5 inhibit the B-A transition of ∿18 and ∿0.25 base pairs per ligand molecule, respectively. Netropsin at r_t = 0.2 induces a very stable B-DNA even at rather low RH (23%). The total hydration of this complex is significantly higher than for a drug-free DNA film. Netropsin-DNA complexes at r_t of 0.02 and 0.01 result in an inhibition of ∿45 base pairs per drug molecule with respect to the B-A transition.

INTRODUCTION

It is well known that DNA exists in several defined families of structures, among them the B and the A family (1). The transition between these two families is a cooperative process regulated by the water activity. In films or fibers the B-A transition usually occurs when the relative humidity (RH) the film is exposed to drops from high values (85-96%) to medium values (80-70%). The B-A transition can be inhibited by insufficient milieu conditions, e.g., type of cations, salt concentration etc. (2-4) or by the interaction of DNA with ligands (5-8a). Then, the DNA exists at medium RH values in a less-defined dehydrated form within the B family designated as C-DNA (3). In DNA-ligand complexes a mixture of C and A forms of DNA may exist at medium RH in dependence of the ligand-DNA base pair ratio (5,6). The study of DNA-ligand complexes with respect to

the effectiveness of different ligands to suppress the B-A transition can give information about the distortion of the conformational flexibility of DNA by the ligands.

Two methods have been used to investigate the conformational transitions of DNA-ligand complexes in the solid or gel-like state in the form of fibers or films, namely X-ray fiber diffraction (6,9) and infrared linear dichroism (IR LD) (5,7,8,8a, 10). From IR LD the geometry of the DNA phosphate group can be obtained which is characterized by the two angles θ_{00} defining the angle between the DNA helix axis and the $O_2 \ldots O_3$ line and the angle θ_{OPO} defining the $O_2\text{-}P\text{-}O_3$ bisector of the $(O_2\text{-}P\text{-}O_3)^-$ unit of the DNA phosphate group, respectively (11,10). The different DNA conformations (B, A, C, Z) can be discriminated by the values of the phosphate angles θ_{00} and θ_{OPO} obtained from IR LD as was shown by several investigators (10-14).

Based upon oriented films obtained by the wet-spinning method (15-17) we have prepared DNA films as well as DNA-ligand films suitable for infrared spectroscopy. The technique of pre-

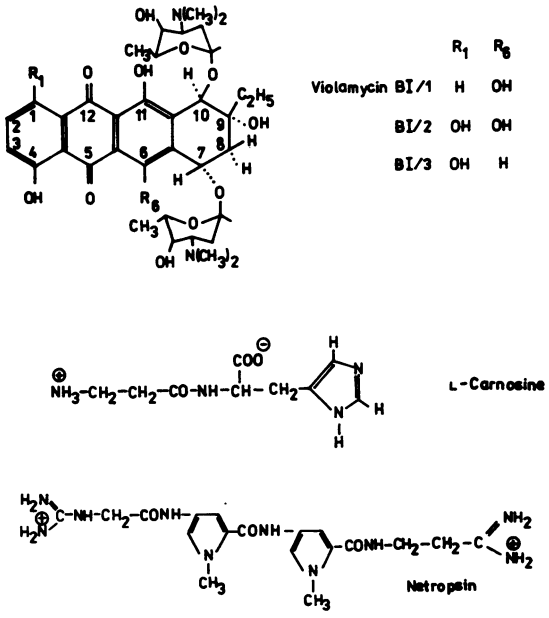

Figure 1. Chemical structure of the three ligands violamycin BI (three components), L-carnosine and netropsin.

paration of the films and of their transfer on infrared transparent material has been described elsewhere (18). We have studied changes of the phosphate geometry of DNA, characterized by the two phosphate angles Θ_{OO} and Θ_{OPO}, in dependence of the RH of DNA and DNA complexes with several ligands, the dipeptide L-carnosine, the anthracycline antibiotic violamycin BI, and the oligopeptide antibiotic netropsin. Their chemical structures are shown in Fig. 1. The results demonstrate dramatic differences of the ligand-induced reduction of the conformational flexibility of DNA in these three ligand complexes.

MATERIALS AND METHODS

Calf thymus DNA was from Sigma Chem. Corp. (USA). Netropsin and violamycin BI were isolated in the ZIMET Jena (GDR) and were kindly provided to us by Drs. H. Thrum and D. Strauss, respectively. L-carnosine (β-alanyl-L-histidine) was from Sigma Chem. Corp. (USA).

The wet-spinning method and equipment used to prepare highly oriented DNA films have been described elsewhere (15-17), as well as the transfer of thin oriented films on infrared transparent materials and the preparation of DNA-ligand complexes (18). The DNA complexes with L-carnosine, violamycin BI and with netropsin at r_t = 0.2 were prepared by bathing the DNA film in a solution of the ligand in 80% ethanol. The DNA-netropsin complexes with r_t = 0.02 and 0.01 were prepared by dropping an appropriate amount of the drug dissolved in 80% ethanol directly on the oriented DNA film. The IR LD spectra have been recorded in dependence of the relative humidity (RH) by a Fourier-transform infrared spectrophotometer Digilab FTS-14 and an infrared grating spectrophotometer Perkin-Elmer 325, both equipped with a wire-grid polarizer. Details of measurement and calculation of the DNA phosphate angles are described elsewhere (10).

The DNA-ligand films were checked by X-ray fiber diffraction. The salt content of the films was determined gravimetrically to be 3.5% NaCl. The ligand per base pair input ratio, r_t, was determined by different methods. By estimation of a weak infrared band at 1025 cm^{-1} of violamycin BI (W. Pohle, private communication), the r_t of its DNA complex was obtained to 0.03

(± 0.01). For the DNA-netropsin complexes, we obtained r_t spectrophotometrically (19). Finally, r_t of the L-carnosine - DNA complex was determined colorimetrically to be 1.5 (± 0.2) taking into account the hydration of DNA at ambient RH (20).

RESULTS

The phosphate angles θ_{OO} and θ_{OPO} which describe the geometry of the PO_2^- groups of DNA with respect to the helix axis are plotted versus the relative humidity (RH) in Fig. 2. The results of drug-free calf thymus DNA are presented in Fig. 2a. We found previously $\theta_{OO} \sim 55°$ and $\theta_{OPO} \sim 64°$ for B-DNA, and $\theta_{OO} \sim 64°$ and $\theta_{OPO} \sim 47°$ for A-DNA, respectively (10). For C-DNA, Brahms et al. found $\theta_{OO} \sim 48°$ and $\theta_{OPO} \sim 67°$ (13); we obtained $\theta_{OO} \sim 49°$ and

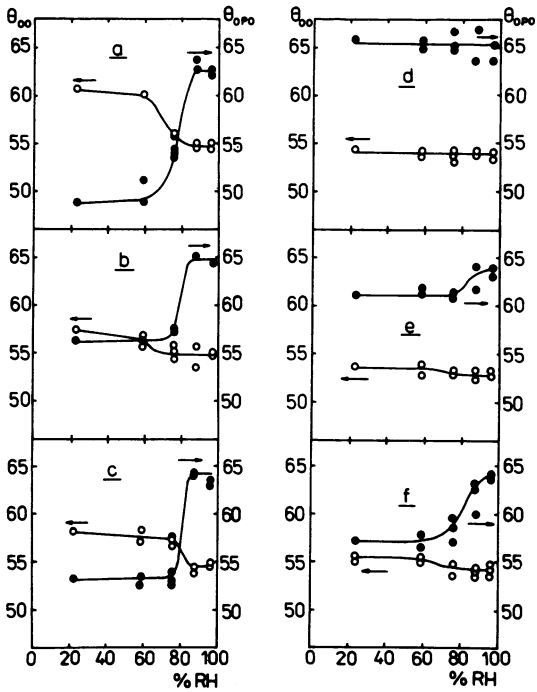

Figure 2. Phosphate angles θ_{OO} and θ_{OPO} of DNA obtained from infrared linear dichroism (cf. text) as a function of RH, the relative humidity. (a) Calf thymus DNA; (b) DNA-violamycin BI complex, r_t = 0.03; (c) DNA-L-carnosine complex, r_t = 1.5; (d) DNA-netropsin complex, r_t = 0.2; (e) DNA-netropsin complex, r_t = 0.02; (f) DNA-netropsin complex, r_t = 0.01.

$\theta_{OPO} \sim 63°$ (10). Inspection of Fig. 2a shows a good coincidence of the experimental values of uncomplexed DNA with those expected for a B-A transition of DNA. The amount of C-DNA at lower RH may be negligible since θ_{OO} does not drop significantly beneath 64°, and θ_{OPO} does not rise significantly beyond 47°.

In contrast, the same plots of θ_{OO} and θ_{OPO} versus RH of the three ligand-DNA complexes show the absence of a well-defined B-A transition (Fig. 2, b-f). At medium RH, DNA complexes with L-carnosine and violamycin BI (Fig. 2, c and b) result in values of the phosphate angles which are in between those of A-DNA and C-DNA; this holds also for the netropsin complex with $r_t = 0.02$ and 0.01 (Fig. 2, e and f). The data of netropsin-DNA with $r_t = 0.2$, however, indicate the existence of a B-like DNA to 100% even at medium and low RH values (Fig. 2d). No contribution was found of either C-DNA or A-DNA.

The fraction of A-DNA at medium RH values, f_A, can be esti-

Table 1. Characterization of DNA-ligand films by infrared linear dichroism. r_t is the total input ratio of ligand per DNA base pair. θ_{OO} and θ_{OPO} are the DNA phosphate angles as obtained from the infrared linear dichroism of the antisymmetric and symmetric PO_2^- stretching vibration of DNA at 60% RH, respectively. The angles are accurate within 1.5°. f_i is the fraction of DNA which does not adopt the A form at 60% or lower values of RH and is obtained from the phosphate angle θ_{OPO} of the DNA-ligand complexes by linear interpolation with respect to the values of the same angles of B- and A-DNA, respectively. n_i is the number of DNA base pairs involved in the loss of conformational flexibility of DNA induced by one ligand molecule.

Ligand	r_t	θ_{OO} (°)	θ_{OPO} (°)	f_i (%)	n_i (b.p.)
L-carnosine	1.5 (±0.2)	57	53	35	0.25 (±0.05)
violamycin BI	0.03 (±0.01)	56	56	53	18 (± 8)
netropsin	0.20 (±0.02)	54	65	100	> 5
netropsin	0.02	54	61	86	43
netropsin	0.01	55	57	47	47

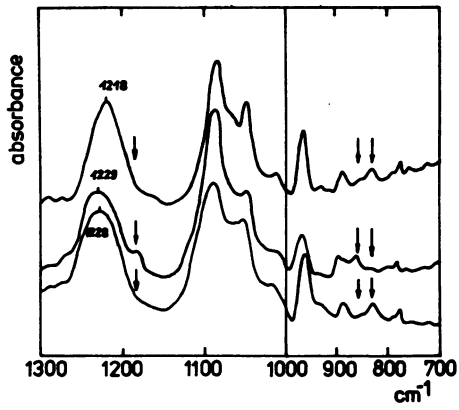

Figure 3. Infrared spectrum of (a) B-DNA, oriented film at 96% RH, (b) A-DNA, the same film at 59% RH, and (c) the netropsin-DNA complex, r_t = 0.2, at 59% RH, from top to bottom. The arrows indicate infrared bands indicative of B-DNA and A-DNA, respectively.

mated from the values of Θ_{OPO} by linear interpolation (7). The corresponding values of drug-free DNA at 96% and 59% RH were assumed to represent the B and A form of DNA, respectively. Then, the fraction f_i of DNA which lost the conformational flexibility to adopt the A form is given by $f_i = (1 - f_A)$. We obtain f_A = 65% (f_i = 35%) for DNA-carnosine and f_A = 47% (f_i = 53%) for DNA-violamycin BI (Tab. 1). The number n_i of DNA base pairs which are restricted in their conformational flexibility by one ligand molecule can be obtained from f_i by the simple relation $n_i = f_i / r_t$. The results are summarized in Tab. 1.

Independent of the results of IR LD, the fraction of A-DNA can be estimated independently either from the intensity ratio of bands at 835 cm^{-1} and 860 cm^{-1} (8a), assigned to B- and A-DNA respectively (21,22), or from the intensity of a band at 1185 cm^{-1} which is indicative of A-DNA (23). The results are less precise than the linear interpolation of Θ_{OPO} values but confirm the IR LD results. Especially for the netropsin complex at r_t = 0.2, all three methods indicate the absence of A-DNA at 59% RH where the A or C form of DNA should be established (Fig. 3).

DISCUSSION

L-Carnosine and violamycin BI affect the conformational flexibility of DNA very differently

As was shown in a previous communication (6) peptides have a moderate effect on the suppression of the B-A transition of DNA films. The dipeptide L-carnosine suppresses the B-A transition by f_i = 35% at an extremely high input ratio of r_t = 1.5. This is equivalent to the restriction of only 0.25 DNA base pairs per dipeptide molecule (Tab. 1). Obviously, there is no long-range effect of the bound dipeptide. The electrostatic attraction between DNA phosphates and the dipeptide is apparently screened by counterions reducing the influence on the DNA conformational flexibility.

In contrast, violamycin BI has a tremendeous effect on the suppression of the B-A transition of DNA. Despite the very small input ratio of r_t = 0.03, the B-A transition is suppressed for f_i = 53% of the DNA in the complex which corresponds to an inhibition of $n_i \simeq 18$ DNA base pairs by one drug molecule (Tab. 1). Violamycin BI (24) is a mixture of three anthracycline antibiotics (W. Koch, private communication) (Fig. 1) and intercalates between DNA base pairs in solution (25,26). The binding site of anthracyclines covers 3-4 DNA base pairs (27). The high value of $n_i = 18 \pm 8$ indicates a strong suppression of the conformational flexibility of DNA by the drug. On each side of the bound drug are ≃7 additional base pairs restricted in their flexibility to adopt the A form. This long-range effect on the DNA conformation may be important for a better knowledge of the molecular mechanism of action of intercalating drugs. The film of the DNA-violamycin BI complex was dichroic in visible light when observed through a polarizer. This finding is indicative at least of a highly ordered perpendicular arrangement of the drug chromophore with respect to the helix axis of DNA as expected for an intercalated drug, but does not conclusively proof intercalation.

Netropsin at high concentration prevents B-DNA from collapse even under conditions of substantial dehydration

The films of a DNA-netropsin-complex at r_t = 0.2 is in a B-like conformation independent of variation of the RH between

96% and 23% (Fig. 2d). The B form of DNA usually collapses under conditions of dehydration and adopts the A or C form. Netropsin is known to bind preferentially in the small groove of AT-rich regions of B-DNA by a non-intercalative mechanism via hydrogen bonds (19). From solution studies is known that netropsin spans 4-5 base pairs of DNA (19,28). Thus, r_t = 0.2 is equivalent to saturation of all DNA binding sites. In this "saturated" complex the infrared spectrum at 59% RH indicates the complete absence of A-DNA (Fig. 3). Our results agree with previous findings that netropsin stabilizes B-DNA (19) even reverting A-DNA (29) and Z-DNA (30) back to B-DNA. Similarly, the L-arginine-DNA complex at r_t = 0.9 exists only in the B form even at very low RH values as found recently by X-ray fiber diffraction (6). This points to structural similarity; both netropsin and arginine have a positively charged guanidinium group at their end. The water spine found to exist in the small groove of B-DNA (31) is replaced by the ligand (netropsin or arginine) which explains the unusual stability of the B form in these complexes against dehydration (6).

45 base pairs of DNA are restricted by one netropsin molecule

The complete suppression of any B-A or B-C transition in films of the "saturated" DNA-netropsin complex (r_t = 0.2) permits the calculation only of the lower limit of the number of restricted base pairs, n_i, which is 5 (Tab. 1). Lower input ratios, however, result in dichroic spectra which indicate a partial B-A transition (Fig. 2, e and f). From the experimental values of Θ_{OPO} (Tab. 1) we obtain by linear interpolation with respect to the values of Θ_{OPO} of uncomplexed DNA inhibited fractions of f_i = 86% and 47% for r_t = 0.02 and 0.01, respectively. These values result in the restriction of the conformational flexibility of DNA of a segment of 45 $\pm$ 2 base pairs by one netropsin molecule. Subtracting the 4-5 base pairs covered by bound netropsin (19,28) we obtain a long-range effect of netropsin affecting 20 base pairs on each side of the bound drug. This extremely large effect is paralleled by findings in a solution study of the DNA interaction with distamycin A (32), which structure is similar to netropsin, where one drug molecule was found to affect the conformational behavior of a 30

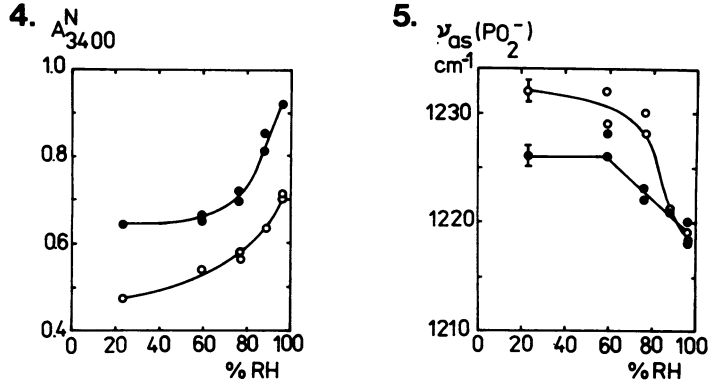

Figure 4. Hydration of DNA films obtained from the infrared absorption of water at ∿3400 cm^{-1}, A^N_{3400}, in dependence of the relative humidity, RH. Open circles: DNA; black circles: DNA-netropsin complex with r_t = 0.2.

Figure 5. Position of the antisymmetric stretching vibration, $\nu_a(PO_2^-)$, of the DNA phosphate groups as a function of the relative humidity, RH. Open circles: DNA; black circles: DNA-netropsin complex with r_t = 0.2.

base pair segment of DNA. One may speculate that processes in the course of gene expression or gene regulation which rely on the conformational flexibility of small DNA segments are heavily interfered by drugs like netropsin and distamycin A.

Hydration of DNA in the saturated netropsin complex is increased

The hydration of DNA films can be easily monitored by the intensity of the water stretching vibration band at 3400 cm^{-1} (10). The hydration of both drug-free DNA and the "saturated" DNA-netropsin complex at r_t = 0.2 drops monotonously with decreasing RH (Fig. 4). The overall hydration of the DNA-netropsin film, however, is increased by 25-35% with respect to uncomplexed DNA throughout the investigated RH interval. This seems contradictory to the interpretation given above that the substitution of the water spine in the small groove of DNA by netropsin may be responsible for the stabilization of the B form even down to 23% RH. The complex, on the other hand, is characterized by a more ample hydration than drug-free DNA (Fig. 4). This resembles the hydration behavior of films and solutions of the DNA-caffeine complex we studied previously by NMR and infrared

spectroscopy (33,34). In both cases, the complexation is virtually accompanied by an increase of the overall water content of the complex, despite a decrease of the number of water molecules bound in the first hydration shell which is supported for the DNA-netropsin complex by recent theoretical calculations (35).

The position of the $\nu_a(PO_2^-)$ is regulated by the hydration and not by the DNA conformation

The strong infrared band at ~1225 cm^{-1} of DNA was assigned to the antisymmetric stretching vibration, $\nu_a(PO_2^-)$, of the PO_2^- unit (36). The position of this vibration shifts as a function of RH (37,38). The wavenumber shift can be interpreted as a consequence either of conformational changes of DNA or of hydration of the phosphate groups of DNA. In both cases the shift would be controlled by the RH of the DNA film. The wavenumber of uncomplexed DNA is characterized by a strong positive shift when RH of the film is reduced from 96% to 76% followed by a moderate positive shift when RH is furthermore reduced to 23% (Fig. 5, open circles). The total shift amounts to 14 cm^{-1} in the drug-free DNA. The saturated DNA-netropsin film, on the other hand, shows a total wavenumber shift of only 9 cm^{-1} with a linear increase between 96% and 59% RH followed by a plateau (Fig. 5, black circles). This curve parallels the decrease of hydration of the complex with decreasing RH (Fig. 4). The strong parallelism of hydration and wavenumber shift of the $\nu_a(PO_2^-)$ suggests that the wavenumber shift is regulated primarily by the hydration of the DNA phosphate groups and not by a conformational change of DNA. This conclusion is strongly supported by the fact that the conformation of DNA in the saturated netropsin complex is B-like over the total investigated range of RH (96-23%) as demonstrated in Figs. 2 and 3. Nevertheless, the position of the $\nu_a(PO_2^-)$ shifts by 9 cm^{-1}. Our findings contradict the very early result of Falk et al. (37) that the hydration of the DNA phosphates is completed at ~60% RH and that the $\nu_a(PO_2^-)$ does not shift at higher values of RH. In contrast, we find the major effect just at values of RH beyond 60% RH (Fig. 5). However, contrary to previous assumptions (38), the wavenumber of the $\nu_a(PO_2^-)$ cannot be used to determine the conformation of DNA in films.

CONCLUSIONS

We want to point out the advantage of the application of wet-spun DNA films for infrared linear dichroism studies of DNA as well as DNA-ligand complexes. The DNA films obtained by wet-spinning have a high degree of orientation, homogeneous thickness and dichroism properties, and can be reproduced with the same degree of orientation, necessary for parallel studies.

The conformational flexibility of calf thymus DNA in wet-spun oriented films is affected by different ligands to different extents. The number of DNA base pairs restricted in their conformational flexibility by one ligand molecule increases from 0.25 for the dipeptide L-carnosine over 18 ($\pm$ 8) for the anthracycline antibiotic violamycin BI up to 45 ($\pm$ 2) for the oligopeptide antibiotic netropsin. The long-range effect of the two antibiotics on the conformational flexibility of DNA may be responsible for their biological activity by interference with processes during gene expression which rely on conformational changes of the involved DNA sections.

The "saturated" DNA-netropsin complex is characterized by a B-like DNA conformation independent of RH. Nevertheless, the wavenumber of the antisymmetric phosphate vibration shifts as a function of RH and cannot be used as an indication of the DNA conformation. The total hydration of this complex is increased by 25-35% respective to the uncomplexed DNA film despite a most probable reduction of water in the first shell of hydration of the complex.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. W. Pohle, Dr. J. Flemming and Prof. Ch. Zimmer for helpful discussions. The work was supported by the Swedish Natural Science Research Council.

The paper is dedicated to Prof. Dr. H. Berg on occasion of his 60th birthday.

REFERENCES

1. Ivanov, V.I., Minchenkova, L.E., Schyolkina, A.K., and Poletayev, A.I. (1973) Biopolymers 12, 89-110.
2. Cooper, P.J., and Hamilton, L.D. (1966) J. Mol. Biol. 16, 562-563.
3. Marvin, D.A., Spencer, M., Wilkins, M.H.F., and Hamilton, L.D. (1961) J. Mol. Biol. 3, 547-565.

4. Rhodes, N.J., Mahendrasingam, A., Pigram, W.J., Fuller, W., Brahms, J., Vergne, J., and Warren, R.A.J. (1982) Nature 296, 267-268.
5. Pohle, W., Fritzsche, H., and Richter, M. (1980) Stud. Biophys. 81, 127-128.
6. Fita, I., Campos, J.L., Puigjaner, L.C., and Subirana, J.A. (1983) J. Mol. Biol. 167, 157-177.
7. Liquier, J., Taboury, J., Taillandier, E., and Brahms, J. (1977) Biochemistry 16, 3262-3266.
8. Liquier, J., Gadenne, M.C., Taillandier, E., Defer, N., Faratier, F., and Kruh, J. (1979) Nucleic Acids Res. 6, 1479-1493.
8a. Taillandier, E., Fort, L., Liquier, J., Couppez, M., and Sautiere, P. (1984) Biochemistry 23, 2644-2650.
9. Campos, J.L., Subirana, J.A., Ayami, J., Mayer, R., Giralt, E., and Pedroso, E. (1980) Stud. Biophys. 81, 3-14.
10. Pohle, W., Zhurkin, V.B., and Fritzsche, H., Biopolymers, in press.
11. Pilet, J., and Brahms, J. (1972) Nature NB 236, 99-100.
12. Pilet, J., and Brahms, J. (1973) Biopolymers 12, 387-403.
13. Brahms, J., Pilet, J., Phuong Lan, T.-T., and Hill, L.R. (1973) Proc. Natl. Acad. Sci. USA 70, 3352-3355.
14. Pilet, J., and Leng, M. (1982) Proc. Natl. Acad. Sci. USA 79, 25-30.
15. Rupprecht, A. (1963) Biochem. Biophys. Res. Commun. 12, 163-168.
16. Rupprecht, A., Acta Chem. Scand. (1966) 20, 494-504.
17. Rupprecht, A. (1970) Biotechnol. Bioeng. 12, 93-121.
18. Rupprecht, A., and Fritzsche, H., submitted
19. Zimmer, Ch. (1975) Progr. Nucl. Acids Res. Mol. Biol. 15, 285-318.
20. Alden, C.J., and Kim, S.-H. (1979) J. Mol. Biol. 132, 411-434.
21. Champeil, P., Tran, T.P.L., and Brahms, J. (1973) Biochem. Biophys. Res. Commun. 55, 881-887.
22. Brahms, J., and Pilet, J. (1974) Israel J. Chem. 12, 153-163.
23. Pohle, W., and Fritzsche, H. (1980) Nucleic Acids Res. 8, 2527-2535.
24. Fleck, W., Strauss, D.G., Koch, W., and Prauser, H. (1974) Z. Allg. Mikrobiol. 14, 551-558.
25. Fritzsche, H., Dattagupta, N., Chaires, J.B., and Crothers, D.M. (1982) Stud. Biophys. 92, 67-68.
26. Fritzsche, H., Dattagupta, N., Chaires, J.B., and Crothers, D.M. (1983) Stud. Biophys. 94, 123-124.
27. Walter, A., Schütz, H., and Stutter, E. (1983) Int. J. Biol. Macromol. 5, 351-355.
28. Wartell, R.M., Larson, J.E., and Wells, R.D. (1974) J. Biol. Chem. 249, 6719-6731.
29. Ivanov, V.I., Minchenkova, L.E., Minyat, E.E., Frank-Kamenetskii, M.D., and Schyolkina, A.K. (1974) J. Mol. Biol. 87, 817-833.
30. Zimmer, Ch., Marck, Ch., and Guschlbauer, W. (1983) FEBS Letters 154, 156-160.
31. Dickerson, R.E., Drew, H.R., Conner, B.N., Wing, R.M., Fratini, A.V., and Kopka, M.L. (1982) Science 216, 475-485.

32. Hogan, M., Dattagupta, N., and Crothers, D.M. (1979) Nature 278, 521-524.
33. Fritzsche, H., Lang, H., Sprinz, H., and Pohle, W. (1980) Biophys. Chem. 11, 121-131.
34. Pohle, W., and Fritzsche, H. (1981) Stud. Biophys. 82, 81-96.
35. Zakrzewska, K., Lavery, R., and Pullman, B. (1984) Nucleic Acids Res., in press.
36. Tsuboi, M. (1953) J. Am. Chem. Soc. 79, 1351-1354.
37. Falk, M., Hartman, K.A., and Lord, R.C. (1963) J. Am. Chem. Soc. 85, 387-391.
38. Bradbury, E.M., Price, W.C., and Wilkinson, G.R. (1961) J. Mol. Biol. 3, 301-317.