Volume 10 Number 5 1982 Nucleic Acids Research

Differential stabilization by netropsin of inducible B-like conformations in deoxyribo-, ribo- and 2'-deoxy-2'-fluororibo-adenosine containing duplexes of $(dA)_n \cdot (dT)_n$ and $(dA)_n \cdot (dU)_n$[a]

Christoph Zimmer[b], Nobuko Kakiuchi[c] and Wilhelm Guschlbauer*

Service de Biochimie, Bat. 142, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif-sur-Yvette, Cedex, France

Received 19 January 1982; Revised and Accepted 15 February 1982

ABSTRACT: Six polynucleotide duplexes containing polydeoxyadenylic acid, polyadenylic acid or poly-2'-deoxy-2'-fluoro-adenylic acid in one strand, and polydeoxyuridylic acid or polydeoxythymidylic acid in the other strand have been studied by circular dichroism, ionic strength dependence of melting temperatures and binding of the DNA specific antibiotic netropsin. Circular dichroism spectra of $(dA)_n \cdot (dT)_n$ and $(dA)_n \cdot (dU)_n$ indicated the presence of the B-form of DNA, while those of $(dAfl)_n \cdot (dT)_n$ and $(rA)_n \cdot (dT)_n$ (and the corresponding $(dU)_n$ hybrids) indicated the presence of the A-form. $(dAfl)_n \cdot (dT)_n$ and $(dAfl)_n \cdot (dU)_n$ bound netropsin only slightly less than the $(dA)_n$ containing duplexes, while replacement by $(rA)_n$ decreased netropsin binding to a large degree. Since netropsin requires B-DNA for binding, it is concluded that the A to B transition is facilitated in the case of fluorine substitution in the sugar moiety, while the 2'-OH group greatly limits this conformational change.

The conformation of DNA-RNA hybrids is important for the understanding of transcription. While RNA always exists in the A-form, characterized by the 3'-endo puckering of the ribose (1), DNA is normally in the B-form, which is 2'-endo puckered, but may assume the A-form under certain conditions of low water activity, e.g. by dehydration of fibres (1-3) or in ethanolic solutions (4,5). The transition between the B- and A-forms of DNA are accomplished by a change of sugar puckering (1,6,7). RNA-DNA hybrids are believed to be in the A-form, as concluded from x-ray fibre diffraction studies (1,8,9). Solution studies on DNA-RNA hybrid polynucleotides (10-12) have established their differences in thermal stability. CD studies of synthetic hybrids in solution (13,14) indicated that certain sequences may adopt a B-like conformation. Recently, Zimmerman and Pheiffer (15) showed that $(rA)_n \cdot (dT)_n$ is able to adopt a B-type helical structure under conditions of high humidity.

The DNA binding antibiotic netropsin (fig. 1) has an absolute requirement for the B-form. This was established in studies with DNA's and polynucleotides (16-19). Netropsin binds specifically to A-T and I-C pairs of

0305-1048/82/1005-1721$2.00/0

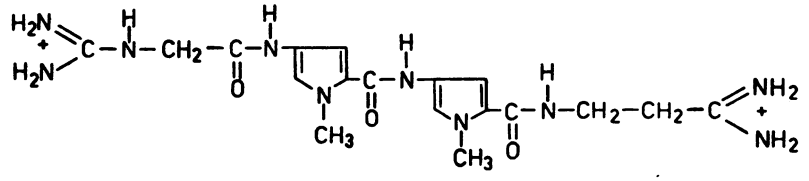

Figure 1: Chemical structure of netropsin.

B-DNA through electrostatic interactions of the guanidinium groups with the phosphates and via hydrogen bonds between the peptide NH groups and the O^2 of the pyrimidines (16-24). Netropsin may therefore be used as a probe for B-like conformations in polynucleotide duplexes. In the present work we have used this conformational specificity of netropsin to monitor the presence or induction of B-like structures in solution of duplexes containing $(dA)_n$, $(rA)_n$ or $(dAfl)_n$ in one strand, and $(dU)_n$ or $(dT)_n$ in the other strand.

MATERIAL AND METHODS

$(dA)_n$, $(dU)_n$ and $(dT)_n$ were purchased from P.L. Biochemicals Inc. (Milwaukee, WI), $(rA)_n$ from Choay (Paris). Poly-2'-deoxy-2'-fluoro-adenylic acid $(dAfl)_n$ was synthesized as previously described (25). Extinction coefficients from the literature were used (11,12,25). Netropsin hydrochloride was a gift of H. Trum (Jena); ε_{296}=21500 L. M^{-1} (26,27).

CD spectra were recorded on a Jobin-Yvon dichrographe III, absorption spectra on a Zeiss DMR 10 spectrophotometer; melting curves were recorded on an X-Y recorder branched to the Zeiss spectrophotometer (14). Netropsin binding was followed by measuring the induced Cotton effect at 315 nm as a function of r' = [netropsin] / [phosphate].

RESULTS

CD spectra: The CD spectra of the six duplexes studied were measured at three temperatures, 5°, 25° and 50°C (fig. 2). There are apparent similarities between some of the spectra. Those of $(dAfl)_n{\cdot}(dU)_n$ and $(rA)_n{\cdot}(dU)_n$ (but not of $(dA)_n{\cdot}(dU)_n$) resemble each other and are very similar to that of $(rA)_n{\cdot}(rU)_n$ (28), which is in the A-form. CD spectra of $(dAfl)_n{\cdot}(dT)_n$ and $(rA)_n{\cdot}(dT)_n$ (but not $(dA)_n{\cdot}(dT)_n$) are again very similar to that of $(rA)_n{\cdot}(rT)_n$ (not shown), which is very probably again in the A-form; these

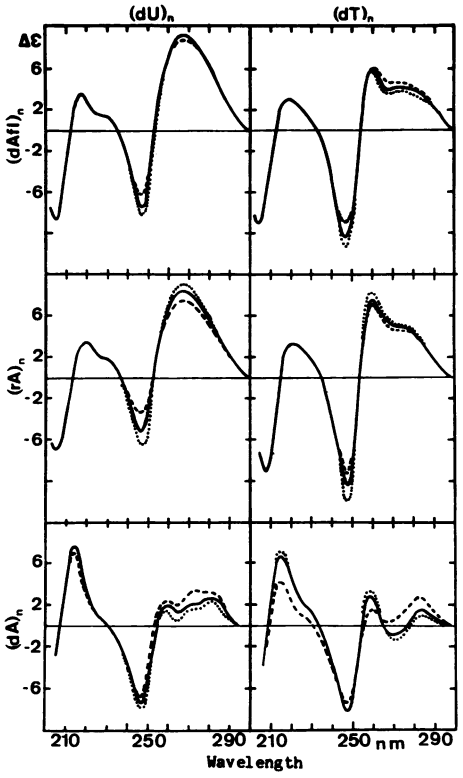

Figure 2: CD spectra of the six homopolymer duplexes studied in 0.1 M NaCl, 0.01 M sodium cacodylate. (·····) 5°C, (------) 25°, (- - -) 50°C.

spectra are, however, different from those of the $(dU)_n$ hybrids, especially in the higher wavelength range, which can be accounted for by the different electronic properties of U and T. On the other hand, the CD spectra of $(dA)_n{\cdot}(dU)_n$ and $(dA)_n{\cdot}(dT)_n$ show similarities, particularly in the intensity and position of the negative peak at 248 nm and the positive band at 212 nm. The two latter duplexes show also relatively large premelting changes, particularly in the higher wavelength region (29). The four other duplexes - of the A-form kind - show similar small premelting changes among each other, (except $(rA)_n{\cdot}(dU)_n$, which, however, begins to melt at 55°C (see fig. 3)). All premelting changes were fully reversible and are indicative of a certain, although variable dynamics of the duplex structures, the two $(dA)_n$ containing duplexes in the B-form apparently being the most flexible ones.

Melting profiles: In fig. 3 are summarized the T_m values of the six duplexes studied over the ionic strength range 0.01 M to 1.0 M Na^+. Transitions involving triple stranded helices (11,12) have been omitted for clarity's sake. The rather small differences between the various duplexes in both groups are striking. The order $(dAfl)_n \cdot (dU)_n > (rA)_n \cdot (dU)_n > (dA)_n \cdot (dU)_n$ is similar to the analogous $(dI)_n \cdot (dC)_n$ series (14). The increasing electronegativity of the 2'-substituent in the purine strand has a stabilizing effect (6,14,25,30).

The two duplexes $(dAfl)_n \cdot (dT)_n$ and $(dA)_n \cdot (dT)_n$ have the same thermal stability over the whole ionic strange range studied, while $(rA)_n \cdot (dT)_n$ has T_m's about 6° lower. This inversion had been noted before (11). In view of the CD spectra (fig. 2) this result is less surprising, since $(dA)_n \cdot (dT)_n$ is quite certainly in the B-form, while the two other $(dT)_n$ containing hybrids are probably an A-like form (see Discussion).

Netropsin binding: The interaction of the DNA binding ligand netropsin with B-DNA in solution is conveniently monitored by the extrinsic Cotton effect at 315 nm (16,18-21). Figure 4 shows the CD spectra of the duplexes $(dAfl)_n \cdot (dT)_n$ and $(rA)_n \cdot (dT)_n$ at increasing netropsin concentrations. The corresponding $(dU)_n$ hybrids showed similar, but lower changes (not shown) on netropsin binding and resembled those observed with the DNA duplexes

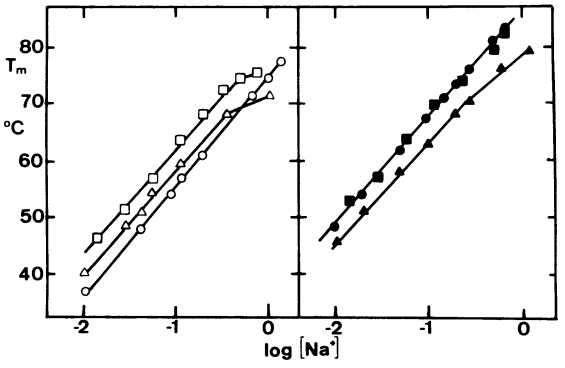

Figure 3: Melting temperatures (T_m) of the six duplexes studied as a function of ionic strength. Left: $(dU)_n$ duplexes (open symbols); right: $(dT)_n$ duplexes (closed symbols). Squares: $(dAfl)_n$ duplexes; triangles: $(rA)_n$ duplexes; circles: $(dA)_n$ duplexes.

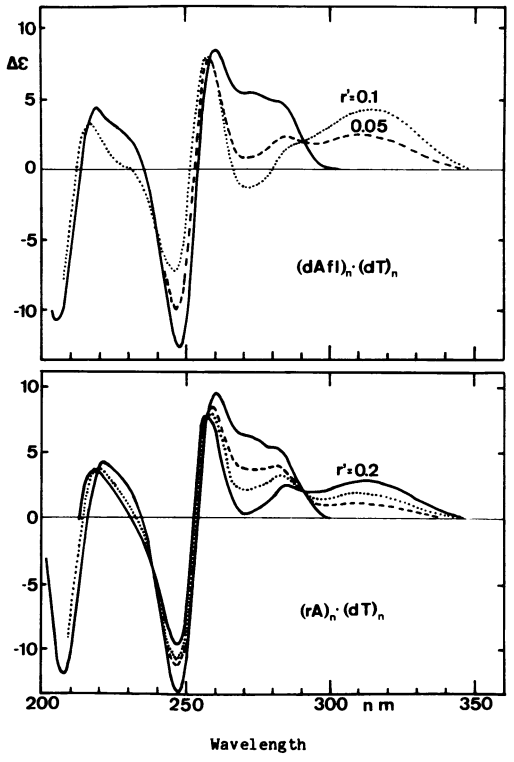

Figure 4: CD spectra of $(dAfl)_n \cdot (dT)_n$ (top) and $(rA)_n \cdot (dT)_n$ (bottom) (———) and in presence of netropsin: (- - -) r'=0.05; (·····) r'=0.1; (———) r'=0.2. All measurements were made in 0.1 M NaCl, 0.01 M sodium cacodylate.

$(dA)_n \cdot (dT)_n$ (18,20) and $(dA)_n \cdot (dU)_n$ (not shown), demonstrating netropsin binding to all six duplexes. Only one set of clear-cut isodichroic points was observed over the entire range of titration.

This shows that the isohelical binding of netropsin to a B-conformation takes place in the same way over the whole range of concentrations. The similarity of the shape of the difference spectra (fig. 5) for the six duplexes confirms this interpretation, although a variation in difference spectra may also reflect some conformational changes, particularly below 300 nm (27).

In fig. 6 the titration curves of the six duplexes are compared: the two DNA duplexes show the largest signals, approaching saturation at r' = 0.1, while the hybrid sequences show smaller and different binding curves. Although $(dAfl)_n \cdot (dT)_n$ and $(dAfl)_n \cdot (dU)_n$ show lower binding signals at saturation, the

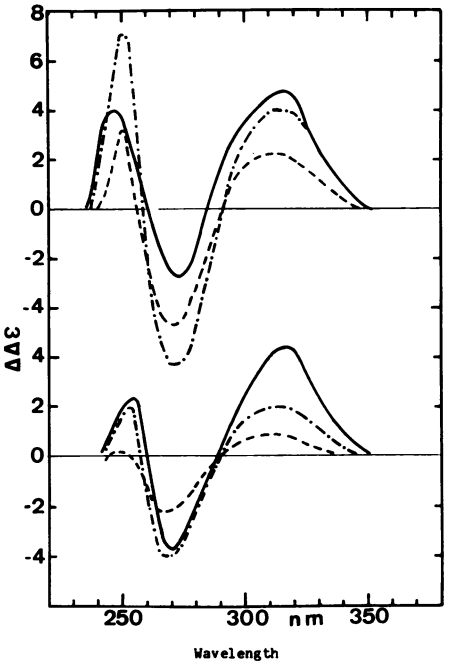

Figure 5: Interaction difference CD spectra of the six duplexes with netropsin (r'=0.2). Top: $(dT)_n$ duplexes; bottom: $(dU)_n$ duplexes. (- • -) $(dAfl)_n$ duplexes; (- - -) $(rA)_n$ duplexes; (------) $(dA)_n$ duplexes.

initial slope of the binding curve is nearly as steep as that of the two DNA duplexes. (The slope $\Xi = \partial\Delta\varepsilon_{315}/\partial r'$ was determined as the average of the points below r'=0.05). In contrast the two $(rA)_n$ containing duplexes show much lower slopes and saturation is reached at much higher r' values (fig. 6). The binding tendency in both series decreased in the order $(dA)_n$ > $(dAfl)_n$ > $(rA)_n$, but binding to the $(dT)_n$ duplexes was always stronger than to the $(dU)_n$ duplexes.

Although the neat isodichroic points indicate a similar geometry of chromophore binding and the similarity of the difference spectra is strong support for the existence of this geometry in all six duplexes, these data do not furnish any precision on the mechanism of binding. The precision of the CD measurements at very low r' values precludes, however, a detailed quantitative analysis of netropsin binding to these duplexes at present. Although the elucidation of the binding mechanism - and possible differences between the netropsin-duplex complexes - may offer valuable information on the details of

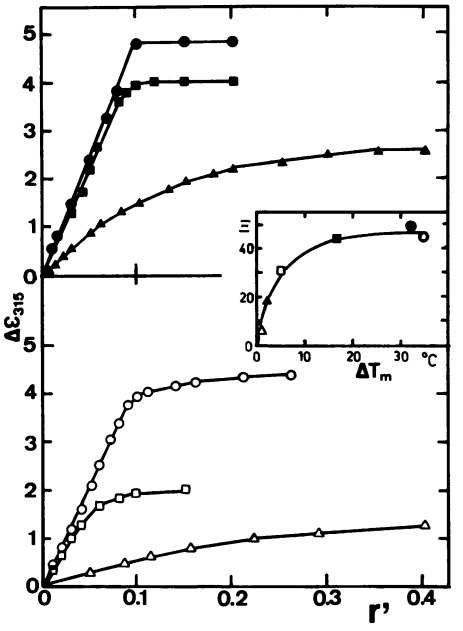

Figure 6: Binding curves for the six duplexes as a function of increasing amounts of netropsin. Top: $(dT)_n$ duplexes; bottom: $(dU)_n$ duplexes. Symbols as in fig. 3. Insert: Initial slope of binding curves $\Xi = (\partial \Delta\varepsilon_{315}/\partial r')$ as a function of the increase in T_m at r' = 0.2 (ΔT_m). Symbols as in fig. 3.

the A to B transition, its lack does not interfere with the observation of this transition, nor the interpretation.

It is worth mentioning that weak binding of netropsin to $(rA)_n \cdot (dT)_n$ was observed previously (17), but no evident explanation could be offered.

Netropsin binding stabilizes DNA structure (16-22). The increase in T_m is most pronounced for the two DNA duplexes, similar to A-T rich DNA's (16-20). In both series, the T_m at saturation (r' = 0.2) followed the CD signal (fig. 6, insert). Neither thermal stabilization, nor CD effects by netropsin were observed with $(rA)_n \cdot (rU)_n$ (17-20).

DISCUSSION

In discussing the results of this work two aspects of polynucleotide structure have to be considered: the thermodynamic stability and conformational preference of the duplexes for a given conformation is reflected in the two families of CD spectra (fig. 2) and the thermal stability

of the duplexes as a function of ionic strength (fig. 3); the kinetic flexibility of the duplexes is indicated by the premelting changes (fig. 2) and, particularly, by the modulation of their geometry on interaction with netropsin (figs. 4 to 6). The latter data clearly demonstrate the capacity of all duplexes to adopt a B-like conformation upon binding with netropsin, although to a different degree.

The importance of the sugar conformation on the stability of the duplexes has been demonstrated before (6,14,25,30) and is corroborated here (fig. 3). The strong preference of the 2'-fluorinated nucleosides for the 3'-endo conformation in solution (31,32) is apparently maintained in oligo- and polymers (33). This conformational preference does not preclude a certain flexibility, especially under constraint. dIfl (34) and dUfl (35) can assume the intermediate conformations 3'-endo-4'-exo and 4'-exo-O'-endo, respectively, in solid state, suggesting that the energy barrier between the canonical forms 3'-endo and 2'-endo may be much smaller in 2'-fluorinated nucleosides than in ribosides. Recent potential energy calculations appear to agree with this observation (36).

The conformational preference of the $(dAfl)_n$ strand is demonstrated in the CD spectra of the two hybrids: the CD spectrum of $(dAfl)_n \cdot (dU)_n$ is very similar to that of $(rA)_n \cdot (dU)_n$ and that of $(rA)_n \cdot (rU)_n$ (28), which is a typical A-helix, but distinctly different from $(dA)_n \cdot (dU)_n$. Similarly, the corresponding spectra in the $(dT)_n$ hybrids indicate an A-form, while $(dA)_n \cdot (dT)_n$ is a B-helix (3).

Binding of netropsin in the two $(dAfl)_n$ duplexes is, however, much closer to that of the $(dA)_n$ duplexes (fig. 6). This is direct indication that the $(dAfl)_n$ hybrids can adopt a B-like conformation more easily than the $(rA)_n$ duplexes. This again points to the higher conformational flexibility of these duplexes compared with the ribo-hybrids. Also, these results strengthen our previously expressed view (14) that the conformational preference of the pyrimidine carrying strand delimits that of the duplex, while the purine carrying strand has a modulating role. Netropsin - which binds to the pyrimidines - accentuates this conformational preference and thus induces a A to B transition, as had also been shown to take place in ethanolic solutions of DNA (37). Binding of netropsin and distamycines to $(dI)_n \cdot (dC)_n$ and $(dIfl)_n \cdot (dC)_n$ (38) is in good agreement with the present findings. The importance of the conformational flexibility of A-T clusters in the binding of

netropsin to DNA also follows from recent viscosity data (39).

The $(rA)_n$ containing duplexes may adopt a B-like conformation upon netropsin binding, but much higher concentrations of netropsin are needed and much flatter and lower binding curves are observed (fig. 6). Without going into the details of the binding mechanism, the binding of the drug is in these cases not complete and binding constants are much lower. The difference with the other duplexes can be viewed either as an expression of a different degree of binding and/or as a difference in the geometry of the minor groove. The recent observation (15) that $(rA)_n \cdot (dT)_n$ may adopt a B-like form with a 10-fold helix in highly hydrated fibers favours the second hypothesis. Because of the 2'-exo puckering of the $(rA)_n$ strand (15), this helix is different from the canonical B-form: the base pairs are shifted away from the helix axis and the backbone conformation is naturally changed too. Besides, a hydrogen bond between the 2'-OH and the neighbouring O_1, has been postulated for the stabilization of the helix in the minor groove. One expects that the recognition of the bases by netropsin in the small, but more shallow groove in such a model of the ribo-hybrid would be altered in comparison with binding to the canonical B-helix with a much deeper minor groove. A looser binding of netropsin, more on the surface of the shallow minor groove of $(rA)_n \cdot (dT)_n$ should result in a lower degree of isohelical orientation of the drug, giving rise to smaller CD amplitudes, as observed. The interpretation of our netropsin binding data thus accords well with the conclusions of Zimmerman and Pheiffer (15).

The recent work of Drew et al. (40) evidenced the existence of various intermediate conformations, including 1'-exo and 0'-endo in a DNA dodecamer duplex. The suggestion of anticorrelation of sugar conformations in paired nucleotides, i.e. that $\bar{\delta}$ (the mean of the conformational angle δ around the 3'-4' bond) is 123° in B-DNA (with spreads of δ=75° to δ=155°) may well apply to the present duplexes. For instance, a conformational change from a 3'-endo:3'-endo A-form (δ_A=83°, δ_T=83°) (41) through the $(rA)_n \cdot (dT)_n$ B-form (15) (2'-exo:3'-exo, δ_A=97°, δ_T=158°, $\bar{\delta}$=127.5°) to an 0'-endo:2'-endo pair (δ_A=97°, δ_T=144°, $\bar{\delta}$=120.5°) would be feasible. Probably, this would be sufficient to accommodate the $(dAfl)_n \cdot (dT)_n$ and $(dAfl)_n \cdot (dU)$ duplexes into a form to account for better netropsin binding than the $(rA)_n \cdot (dT)_n$ helix, with the $(rA)_n$ strand apparently blocked in the 2'-exo pucker.

The marked differences between the $(dU)_n$ and $(dT)_n$ containing duplexes

is worth considering. The presence of the additional 5-methyl group in the pyrimidine strand has been shown to increase the thermal stability of various polynucleotide duplexes (11,12,42) and is further confirmed here for three pairs of different hybrids. It also gives a rationale for the considerably higher T_m's of $(dA)_n \cdot (dT)_n$ (fig. 3). The more pronounced binding of netropsin to $(dT)_n$ containing duplexes (fig. 6) shows also that the formation of B-like structures is favoured by the presence of the 5-methyl group which is stabilized to a greater extent by the greater overlap of this group with the neighbouring base (6). The absence of this methyl group in $(dU)_n$ favours the A-form (fig. 2), which will change less readily into a B-like structure.

Our data are in favour of the recent conclusions of Zimmerman and Pheiffer (15) that $(rA)_n \cdot (dT)_n$ has the potential to undergo a transition from the A-form to a B-like structure. This phenomenon could to be more generally true for hybrids, the pyrimidine carrying strand of which is the deoxypolymer (14,38). Netropsin binding tests the possibility of RNA•DNA hybrids to adopt the B-type structure and suggests a possible modulation between A and B-forms under the influence of an effector (here netropsin). The conformational variations expected during transcription may well be modulated in a similar manner.

The role of sugar puckering and the energy barriers between the extreme puckerings have been correlated to motions between the internucleotide phosphodiester and bases and have been suggested to be responsible for the transmission of conformational changes (43). One would expect that such a conformational flexibility could be dependent on the nucleotide sequence and the geometry of the sugar-phosphate backbone. The findings on sequential hybrids by Gray and Ratliff (13) corroborate these considerations.

Acknowledgments: We wish to thank Dr. Christian Marck for many useful suggestions, help in some of the experiments and stimulating discussions. C.Z. acknowledges gratefully a short-term fellowship from the Commissariat à l'Energie Atomique (France) during the course of this work. The authors are grateful to Profs. W.K. Olson and L.-S. Kan for preprints of their unpublished work.

[a]Part 3 in the series "Polynucleotide helix geometry and stability"; part 2 is ref. 14. Unusual abbreviations: $(dAfl)_n$: poly-2'-deoxy-2'-fluoro-adenylic acid; CD: circular dichroism.

[b]Present Address: Forschungszentrum für Molekularbiologie und experimentelle

Therapie, Akademie der Wissenschaften der DDR, DDR-69-JENA, G.D.R.

[c]Present Address: Division of Biophysics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

REFERENCES

1) Arnott, S. (1970) Progr. Biophys. Mol. Biol. 21, 267-319.
2) Langridge, R.D., Marvin, D.A., Seeds, W.E., Wilson, H.R., Hooper, C.W., Wilkins, M.H.F. & Hamilton, D.C. (1960) J. Mol. Biol. 2, 38-64.
3) Leslie, A.G.W., Arnott, S., Chandrasekaran, R. & Ratliff, R.L. (1980) J. Mol. Biol. 143, 49-72.
4) Brahms, J. & Mommaerts, W.F.H.M. (1964) J. Mol. Biol. 10, 73-88.
5) Ivanov, V.I., Minchenkova, L.E., Schyolkina, A.K. & Poletayev, A.I. (1973) Biopolymers 12, 89-110.
6) Guschlbauer, W. (1981) in "Biomolecular Structure, Conformation, Function and Evolution", R. Srinivasan, Ed., Pergamon Press, vol. 2, p. 273-284.
7) Arnott, S., Chandrasekaran, R. & Selsing, E. (1975) in "Structure and Conformation of Nucleic Acids & Protein and Nucleic Acid Interactions," M. Sundaralingam & S.T. Rao, Eds., University Park Press, p. 577-597.
8) Milman, G., Langridge, R.D. & Chamberlin, M.J. (1967) Proc. Natl. Acad. Sc. USA 57, 1804-1810.
9) O'Brien, E.J. & MacEwan, A.W. (1970) J. Mol. Biol. 48, 243-261.
10) Chamberlin, M.J. & Patterson, D.J. (1965) J. Mol. Biol. 12, 410-428.
11) Riley, M., Maling, B. & Chamberlin, J.M. (1966) J. Mol. Biol. 20, 359-389.
12) Zmudzka, B., Bollum, F.J. & Shugar, D. (1969) J. Mol. Biol. 46, 169-183.
13) Gray, D.M. & Ratliff, R.L. (1975) Biopolymers 14, 487-498.
14) Kakiuchi, N., Marck, Ch., Rousseau, N., Leng, M., DeClercq, E. & Guschlbauer, W. (1982) J. Biol. Chem. 257, in press.
15) Zimmerman, S.B. & Pheiffer, B.H. (1981) Proc. Natl. Acad. Sc. USA 78, 78-82.
16) Zimmer, Ch., Reinert, K.E., Luck, G., Wähnert, U., Löber, G. & Thrum, H. (1971) J. Mol. Biol. 72, 329-348.
17) Wartell, R.M., Larson, J.E. & Wells, R.D. (1974) J. Biol. Chem. 249, 6719-6731.
18) Zimmer, Ch. (1975) Progr. Nucleic Acid Res. Mol. Biol. 15, 285-318.
19) Zimmer, Ch., Luck, G., Lang, G. & Burckhardt, G. (1979) Proc. 12th FEBS Meeting (S. Rosenthal, H. Bielka, Ch. Coutelle & Ch. Zimmer, Eds.), Pergamon Press, vol. 51, p. 83-94.
20) Luck, G. Triebel, H., Waring, M. & Zimmer, Ch. (1974) Nucl. Acids Res. 1, 503-530.
21) Zasedatelev, A.S., Gursky, G.V., Zimmer, Ch. & Thrum, H. (1974) Mol. Biol. Rep. 1, 337-342.
22) Patel, D.J. & Canuel L.L. (1977) Proc. Natl. Acad. Sc. USA 74, 5207-5211.
23) Martin, J.C., Wartell, R.M. & O'Shea, D.C. (1978) Proc. Natl. Acad. Sc. USA 75, 5483-5487.
24) Zimmer, Ch., Marck, Ch., Schneider, Ch. & Guschlbauer, W. (1979) Nucl. Acids Res. 6, 2831-2837.
25) Ikehara, M., Fukui, T. & Kakiuchi, N. (1978) Nucl. Acids Res. 5, 1877-1887.
26) Zimmer, Ch., Marck, Ch. & Guschlbauer, W. (1980) Spectrosc. Lett. 13, 543-554.
27) Zimmer, Ch., Marck, Ch., Schneider, Ch., Thiele, D., Luck, G. & Guschlbauer (1980) Biochim. Biophys. Acta 607, 232-246.
28) Brahms, J. (1965) J. Mol. Biol. 11, 785-801.

29) Sarocchi, M.Th. & Guschlbauer, W. (1973) Eur. J. Biochem. 34, 232-240.
39) Guschlbauer, W., Blandin, M., Drocourt, J.L. & Thang, M.N. (1977) Nucl. Acids Res. 4, 1933-1943.
31) Uesugi, S., Miki, H., Ikehara, M., Iwashi, H. & Kyogoku, Y. (1979) Tetrahedron Letters 42, 4073-4076.
32) Guschlbauer, W. & Jankowski, K. (1980) Nucl. Acids Res. 8, 1421-1433.
33) Cheng, D., Kan, L.S., Ts'o, P.O.P., Uesugi, S., Takatsuka, T. & Ikehara, M. (1982) Biochemistry, in press.
34) Hakoshima, T., Omori, H., Tomita, K.I., Miki, H. & Ikehara, M., (1981) Nucl. Acids Res. 9, 711-729.
35) Marck, Ch., Lesyng, B. & Saenger, W. (1982) J. Mol. Struc., in press.
36) Olson, W.K. (1982) J. Am. Chem. Soc., in press.
37) Minchenkova, L. & Zimmer, Ch. (1980) Biopolymers 19, 823-831.
38) Marck, Ch., Kakiuchi, N. & Guschlbauer, W. (1982), in preparation.
39) Reinert, K.E., Geller, D. & Stutter, E. (1981) Nucl. Acids Res. 9, 2335-2349.
40) Drew, H.R., Wing, R.M., Tanako, T., Broka, C., Tanaka, S., Itakura, K. & Dickerson, R.E. (1981) Proc. Natl. Acad. Sc. USA 78, 2179-2183.
41) Arnott, S., Hukins, D.W.L. & Dover, S.D. (1972) Biochem. Biophys. Res. Comm. 48, 1392-1390.
42) Zmudzka, B., Bollum, F.J. & Shugar, D. (1969) Biochemistry 8, 3049-3059.
43) Sundaralingam, M. & Westhof, E. (1980) Biophys. J. 32, 250-252.