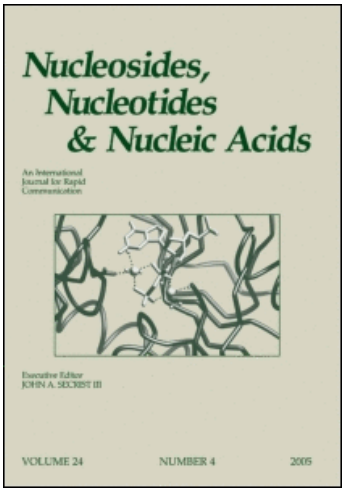


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# Conformational Transitions in Poly d(CGCGCGTTAATT)

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# CONFORMATIONAL TRANSITIONS IN POLY d(CGCGCGTTAATT)*

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**ABSTRACT** : Conformational studies on poly d(CGCGCGTTAATT) in solution by circular dichroism spectroscopy are reported. The polynucleotide exhibits B conformation in sodium chloride solution and on addition of $NiCl_2$ a B-Z transition is observed. $NiCl_2$ titrations carried out in the presence of 5M NaCl show a midpoint of transition at 2.25 mM $NiCl_2$ and a complete (maximum conversion to Z form) transition at 16 mM $NiCl_2$. In 60% alcohol the polynucleotide remains in B conformation. The polynucleotide isomerizes into $\psi$ and A conformations in the presence of spermidine and spermine respectively. The thermodynamic parameters calculated from the melting profiles using a two state model show that the polynucleotide is almost equally stable in its B and Z conformations.



## INTRODUCTION

Natural DNA of heterogeneous sequences as well as synthetic DNA are capable of adopting more than one conformation. The conformation of DNA depends upon its base sequence , and also on its environmental conditions. Since the discovery of a left-handed conformation in (CGCGCG) (1) several workers using various physico-chemical techniques,

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* Poly d(CGCGCGTTAATT) means here poly d $\begin{bmatrix}\text{CGCGCGTTAATT}\\ \text{GCGCGCAATTAA}\end{bmatrix}_5$

have demonstrated that the left - handed DNA conformation is favored by alternating purine-pyrimidine sequences, chemical modification of bases and by negative supercoiling (2). However, using Raman Spectroscopy, it has been shown that the left - handed conformation is not restricted to (CpG) sequences and that the alternating pyrimidine-purine is not necessary (3). In solution , 5-methylated or 5-brominated , $(dC\text{-}dG)_n$ . (dG-dC) has been shown to undergo the B-Z transition under various conditions (4-13). It has been reported that spermine isomerized (dG-dmC) into the Z form and aggergated $(dG\text{-}dC)_n$ without any change in its conformation (43, 40). The B-Z transformation of poly (dG-dC) has also been observed with spermine at a 4-5 micromolar level in aqueous solution (46, 47) and also in the presence of a small amount of ethanol , dioxane and cobalt hexaamine (45). Poly d(AC).poly d(GT) has been shown to adopt the Z conformation in solution only when all the C-5 positions of the cytosine are methylated (14). Interestingly , it has also been shown by IR Spectroscopy that poly d(AC) poly (GT) is stabilized in the Z form by $Ni^{2+}$ (15). Sequences like d(CGCGCGTG) (16) and d(CGCATGCG) (17) have been shown to be in the Z conformation in the solid state. Sequences such as d(TGCGCGCA) and d(CACGCGTG) have been shown to adopt a Z conformation in concentrated solution by Laser Raman Spectroscopy (18). The sequences containing mixtures of chemically modified AT and GC base pairs have also been shown to undergo a B-Z transition in solution (19). Recently it has been shown that the oligonucleotides d(CGCGCGGCGCGC) and d(CGTGCGCACG) undergo a B-Z transition when millimolar amout of $NiCl_2$ is added to their 5M NaCl solution (20). The conditions required to bring about a B-Z transition are more stringent in oligonucleotides in which AT base pair interruptions are present. Poly (dA-dT).poly (dA-dT) has been shown to adopt a Z conformation on addition of millimolar amounts of nickel chloride to its 5M NaCl solution (21). It has also been shown that supercoiling stabilized an $(dA\text{-}dT)_{16}$ insert into a plasmid in the Z form in the presence of $Ni^{2+}$ (21a). The oligonucleotide d(AAAAATTTTT) crystallized in the B conformation and remained in the B conformation in saturated salt solution (22, 23). The only known oligonucleotide (4-12 in length) that contains AA or TT and that exists in any other form than the B conformation is the octamer d(CGTTAACG), which showed a slight amount of A form in saturared salt solution but which crystallized in the B form (24). The dodecamer d(CGCGCGTATATA) exhibited the Z form in 5M NaCl. We have earlier shown that the presence of d(TT), d(TTAA) and d(TTAATT) adjacent to d(CGCGCG) in the oligonucleotide do not prevent a B-Z conformational transition on addition of millimolar amount of $NiCl_2$ to their 5M NaCl solution (39). It has been found that long runs of

$d(CG)_n$ in the sequence that forms Z - DNA are generally uncommon in naturally occurring sequences (25). If Z - DNA has a role in the regulation of cellular processes there must be other sequences than $d(CG)_n$ with the potential to adopt the Z conformation. It has now been recognized that some sequences of native DNA exist in the Z conformation , and there are others with a potential to adopt the Z conformation (44). Here in I describe the solution conformational studies on poly d(CGCGCGTTAATT). Our circular dichroism spectroscopy results shows that the polynucleotide remains in the B form in sodium chloride solution, and on addition of $NiCl_2$ it undergoes a B-Z conformational transition. The polynucleotide reveals $\psi$ and A conformations in the presence of spermidine and spermine respectively.

## EXPERIMENTAL

### Materials

Phosphoramidites and tetrazole were from Pharmacia, dimethylaminopyridine and sodium cacodylate were from Sigma. All other solvents and reagents used in the syhthesis were purchased from Qualigen India Ltd. and were of analytical grade. Acetonitrile and dichloromethane were refluxed with calcium hydride for 12 hrs , distilled and store over $3^oA$ and $4^oA$ molecular seives.

### Synthesis Of Polynucleotides

The two polynucleotides, 60mers, $d(CGCGCGTTAATT)_n$ and $d(AATTAACGCGCG)_n$ were synthesised using $\beta$ cyanoethylphosphoramidite (26, 27) chemistry on a Pharmacia Gene Assembler. The polynucleotide linked to the polymer support by succinate linkage , benzyl and isobutyryl groups protecting the reactive amino groups on the bases and the phosphorous protecting group i.e. $\beta$ cyanoethyl , were deblocked by treatment with 29% ammonia solution at $60^oC$ for 16 hrs. The polymer support was filtered and ammonia solution was evaporated. The crude polynucleotides thus obtained were purified by running a preparative polyacrylamide gel electrophoresis (PAGE) containing 7M urea (28). The bands of polynucleotides were visualized on a gel with the help of a fluorescent TLC plate. The area of slowest migrating spot containing the desired product was cut. The gel was then crushed and soaked in water : methanol (80:20 v/v) overnight. Finally , the polynucleotide were purified on Sephadex G-15 column to remove the

urea. After purification both the oligonucleotides , checked by autoradiography , were found to be homogeneous and of equal size.

### $T_m$ Measurements

The solutions for $T_m$ were prepared by heating equimolar amounts of both the strands at 95°C for 2 min and allowing them to cool slowely to room temperature in a buffer ( 10 mM sodium cacodylate, 1mM EDTA and 100 mM sodium chloride). About 0.5 A 260 /1 ml was used. The melting curves were recorded on Beckman 5260 spectrophotometer fitted with a 7035 B X-Y recorder (Hewlett Packard). The change in absorbance at 260 nm was recorded as a function of temperature. The cell temperature was changed continuously by means of a thermocouple with a heating rate of 1°C/ min. The percentage of random coil was plotted against temperature.

### CD Measurments

The buffer (10 mM sodium cacodylate , 1 mM EDTA) containing an equimolar mixture of complementary strands was heated at 95°C for two minutes and allowed to reanneal slowly. CD spectra of the polynucleotide were recorded on a Jasco J-20 spectropolarimeter at 22°C with a 1 cm path length cell. Solid sodium chloride was added to increase the concentration of sodium chloride in solution. The concentration of the polynucleotide solution was determine with the absorbance at 260 nm using the extinction coefficient of the duplex d(CGCGCGTTAATT) 5 , 1296 ml/μ mole. This extinction coefficient value was determine as per reference 38. A polynucleotide concentration of 37.5 μmole phosphate was used.

## RESULTS

### $T_m$ Measurents

The melting profiles of the polynucleotide plotted as % random coil vs temperature are shown in Fig.1. Using this melting profile , ln K was determined at various temperatures. The thermodynamics parameters entropy , Δ S and enthalpy , Δ H and Gibbs free energy , Δ G were calculated using a two state model , by plotting ln K vs 1/T°K (48). From the plot of ln K vs 1/T°K , the ΔH and ΔS were determined by the following equation

$$\ln K = \frac{\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R}$$

where K in the equilibrium constant.

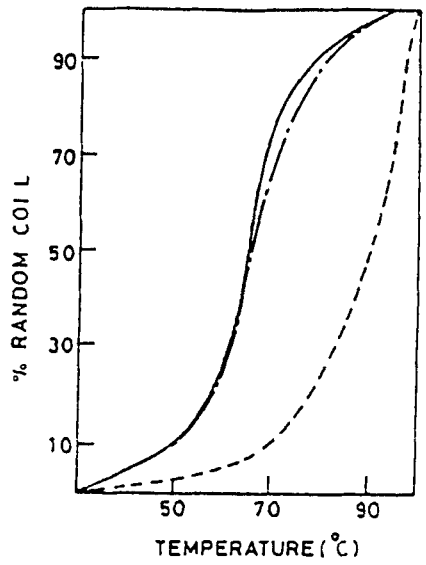


Fig.1 Melting profiles of poly d(CGCGCGTTCCTT) in 10 mM Na cacodylate , 1 mM EDTA (i) (-) in 100 mM NaCl , (ii) (---) in 5 M NaCl and (iii) (- · -) in 5M NaCl +16 mM NiCl 2.



From the valves of Δ S and Δ H , using the equation Δ G = Δ H –T Δ S the values for Δ G at 25°C were calculated. Thermal hyperchromicities at various salt concentrations are tabulated in Table 1. The $T_m$ data suggest that the polynucleotide is a duplex at room tremperature.

## CD Measurements

Circular dichroism specroscopy has been used extensively to study the conformation of DNA (29, 30). The CD spectra of the polynucleotide are shown in Fig.3,4,5. The polynucleotide remained in B conformation in NaCl solution (Fig.3). On addition of millimolar amounts of $NiCl_2$ to the polynucleotide B-Z transition was observed. The $NiCl_2$ titration carried out in the presence of 5M NaCl (Fig. 2) showed that the mid point of transition was 2.25 mM $NiCl_2$ and the complete transition (maximum Z) was at 16 mM $NiCl_2$. On further addition of $NiCl_2$ no increase in ellipticity at 293 nm was observed. In 5M NaCl +16mM $NiCl_2$ the polynucleotide showed a small trough at 243 nm , a intense trough at 293 and a peak at 270 nm. The analysis of this spectrum was carried out by plotting spectrum of various compositions of standard B and Z forms , suggested that the polynucleotide in 5M NaCl + 16 $NiCl_2$ contents 80% Z form and 20% B form.

Table 1. Summary of $T_m$ , $\Delta G$ and % H (percent hyperchromicity is based on the difference in A-260 at 30°C and 90°C) data of poly d(CGCGCGTTAATT)

| Salt Concentration | Conformation | Tm°C | %H | ΔG Kcal/mol (25°C) |
|---|---|---|---|---|
| 100 mM NaCl | B | 65 | 19.40 | -37.05 |
| 5 M NaCl | B | 90 | 39.97 | -38.37 |
| 5 NaCl+16 mM $NiCl_2$ | 80%Z+20%B | 67 | 10.77 | -36.75 |
| 60% EtOH (not shown) | B | | | |
| 60% EtOH+16 mM $NiCl_2$ | B (small amount of Z component) | | | |
| Poly d(CGCGCGTTAATT)/ spermidine ratio= 10 | ψ | | | |
| Poly d(CGCGCGTTAATT)/ spermine ratio =3.33 | A+ modified aggregated DNA | | | |



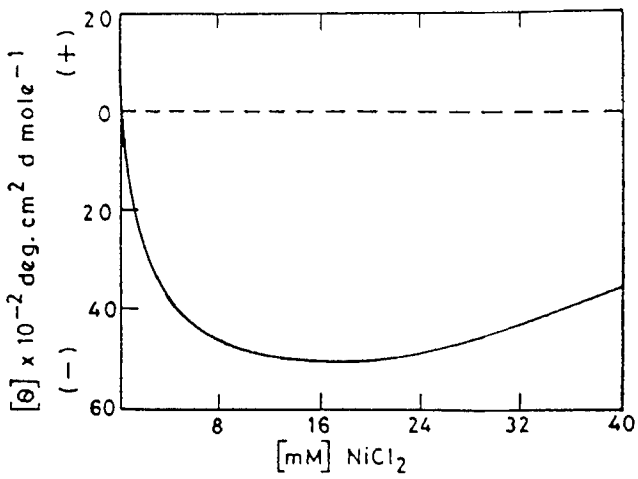


Fig.2 $NiCl_2$ titration in the presence of 5M NaCl. Change in ellipticity at 293 nm vs millimolar amount of $NiCl_2$.

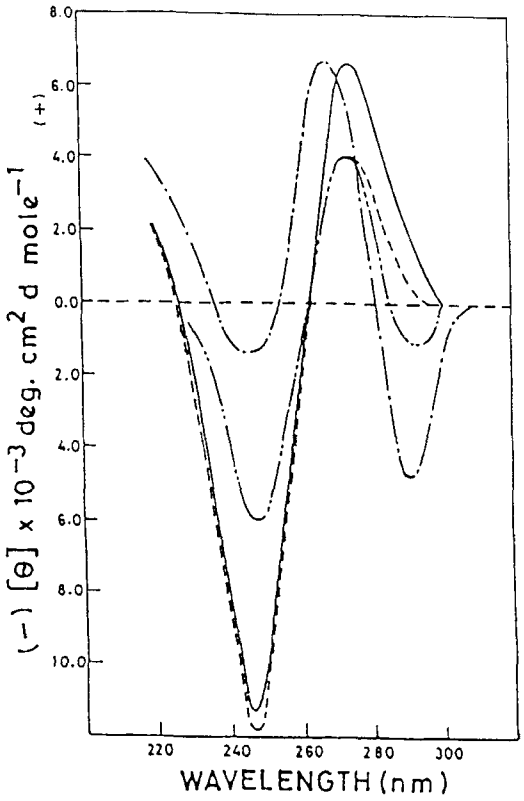


Fig.3 CD spectra of poly d (CGCGCGTTAATT) in 10 mM Na cacodylate , 1 mM EDTA (i) in 100 mM NaCl (-), (ii) 5M NaCl (- - -),(iii) in 5M NaCl, 16 mM $NiCl_2$ (- · -), (iv) in 60% ethanol , 16 mM $NiCl_2$ (- ·· -).

The polynucleotide remained in the B conformation in 60% ethanol. On addition of $NiCl_2$ to the alcohol solution a small trough at 293 nm was observed (Fig.3). CD spectra of poly d(CGCGCGTTAATT) in 10 mM Na cacodylate , 1 mM EDTA, 100 mM NaCl solution as a function of spermidine are shown in Fig.4. On addition of spermidine to the polynucleotide ( spermidine /N=0.1) an intense negative band at 250 nm (16 X 10 $^{-3}$ deg cm $^2$ d mole $^{-1}$) was observed. Such a spectrum is called a $\psi$ spectrum. It was believed that there was no change in the conformation of the duplex, but aggregation gave rise to intense CD signals (49). This type of spectrum has been observed in DNA on addition of protamine (50). On further addition of spermidine the width of the spectrum and the intensity of the trough was increased.

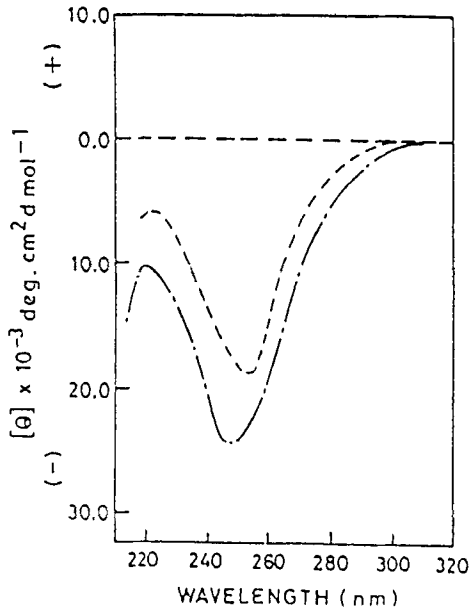


Fig. 4 CD spectra of poly d(CGCGCGTTAATT) (N) in 10 mM Na cacodylate , 1mM EDTA in presence of spermidine (SP) (i) SP/N=0.1 (- - - ), (ii) SP/N =0.33 (- · - - -).



CD spectra of poly d(CGCGCGTTAATT) in 10mM Na cacodylate , 1mM EDTA, 100 mM NaCl solution as a function of spermine are shown in Fig. 5. Initially on addition of spermine (spermine/N =0.1) no change was observed. At spermine /N =0.33, the CD of the polynucleotide showed a positive band at 305 nm , unlike the band observed at 305 nm previously for the A form of DNA. This spectrum may correspond to the sum of the spectra of the A form and the modified aggergated form (13). On further addition of spermine no change in the spectrum of the polynucleotide was observed.

## DISCUSSION

$(CG)_3$ is the smallest oligonucleotide which undergoes a B-Z transition (35). The oligonucleotides d(CGCGCGTATACGCGCG) (34) and d(CGCGCGTATATA) (35) have been shown to undergo a B-Z transition under the influence of high salt. It is well know that at high salt (2.5 M NaCl) , divalent cations like $Mg^{2+}$ (0.6 M $MgCl_2$) transform poly d(G-C) into the Z form (40). The B-Z transition in high salt,i.e.5M NaCl solution,

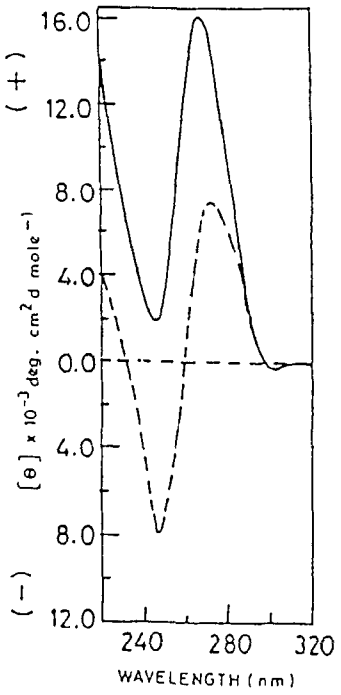


Fig.5 CD spectra of poly d(CGCGCGTTAATT) (N) in 10 mM Na cacodylate , 1 mM EDTA in presence of spermine (S) (i) S/N =0.1 (---), (ii) S/N =0.33 (-).



occurs due to the combination of both dehydrating and electrostatic screening effects. Ethanol acts synergistically with $Mg^{2+}$ in bringing about the B-Z transition (41). Vande Sande and Jovin in fact showed that in 60% ethanol, requirement of $Mg^{2+}$ in bringing about the B-Z transition is only 10 mM at room temperature and 4 mM at 45°C. Poly (dA-dT). poly (dA-dT) (21) has been forced to under go a B-Z transition under the influence of $NiCl_2$ in the presence of sodium chloride. The TT and AA in d(CGCGTTAACGCG ) (36,37) and d(CGTTAACG) (24) appear to be responsible for the B conformation. The polynucleotide assumes a B conformation in 5M NaCl solution. However, on addition of millimolar amount of $NiCl_2$ to its 5M NaCl solution , the polynucleotide undergoes a B-Z transition. This fact suggests that $NiCl_2$ has a strong influence on the conformation of this molecules in the presence of high NaCl concentration , since $NiCl_2$ itself could not bring about B-Z transition. The lack of Z conformation in the absence of $NiCl_2$ appear to be due the additional hydration of the A/T bases . The $Ni^{2+}$ ions in buffer or

unbuffered 5M NaCl is in complex state $Ni(H_2O)_6^{2+}$ (21). The interaction of hexaaquonickel ions with the N-7 site of the purine residues organize the water molecules in the complex. Therefore the binding of nickel with its coordinated water molecules to the N-7 sites of the purines may favor the syn geometry of the purines and favor the Z conformation of these oligonucleotides. The $NiCl_2$ titration in the presence of 5M NaCl (Fig.2) shows that the mid point of the B-Z transition for poly d(CGCGCGTTAATT) is at 2.25 mM $NiCl_2$ and the complete transition is at 16mM $NiCl_2$. Thus it appears that if a non alternating block such as TTAATT is present adjacent to d(CGCGCG) in a polynucleotide the B-Z transition is not as facile as in the case of a strictly alternating purine and pyrimidine sequences such as $d(CG)_n$. This not only requires low water activity but also the binding of $Ni^{2+}$ ions as in the case of d(CGCGCGGCGCGC) and d(CGTGCGCACG). The amount of $NiCl_2$ required is much higher for this polynucleotide (present work) than d(CGCGCGGCGCGC) and d(CGTGCGCACG) but is less than required for poly(dA-dT).poly(dA-dT). However, the mechanism for isomerization of poly(dA-dT).poly(dA-dT) is different from $d(CG)_n$ . The addition of $NiCl_2$ to 60% ethanol could not bring about B-Z transition into the polynucleotide indicating that the mode of dehydration caused by ethanol is different from that of 5M NaCl. Spermine also induces a B-Z transition in poly d(G-C). However , poly d(CGCGCGTTAATT) isomerized into the A conformation suggesting that the non alternating block d(TTAATT) of poly d(CGCGCGTTAATT) restricts the isomerization into the Z conformation. Interestingly, spermidine induces a B - $\psi$ transition to this polynucleotide, suggesting that the mode of interaction of spermidine and spermine are different.

Examination of the thermodynamic parameters i.e. $\Delta$ G (Table 1) inticates that there is not much difference in stability of the polynucleotide in its B and Z form. However, from the difference in $T_m$ of the B form (5M NaCl) and Z form (5M NaCl + 16 mM $NiCl_2$) it appears that the difference in stability of the B and Z form is of enthalpic origin. The $T_m$ profiles of the polynucleotide indicates that the melting is cooperative under various salt conditions. The melting of the polynucleotide was not completed in 5M NaCl solution.

## REFERENCES

1. Wang , A.H.J.; Quigley, G.J.; Kolpak, F.J.; Crawford, J.L. ; Van Boom, J.H.; Vander Marel, G.A. and Rich, A (1979) Nature **282**, 680.
2. Rich, A.; Nordheim, A. and Wang, A.H.J. (1984) Ann. Rev. Biochem. **53** , 791.

3. Benevides, J.M.; Wang, A.H. ; Vander Marel, G.A.; Van Boom, J.H.; Rich, A. and Thomas, G.J. (1984) Nucleic Acids Res. **12**, 5913.
4. Holak, T.A. ; Borer, P.N.; Levy, G.C.; Van Boom, J.H. and Wang, A.H.J. (1984) Nucleic Acids Res. **12**, 4625.
5. Tran-Dind, S.; Taboury,J.; Neumann, J.M.; Huynh-Dinh, T.; Genissel,B.; d'Estaintot, L.B. and Igolen, J. (1984) Biochemistry **23**, 1362.
6. Giessner-Prettre,C.; Pullman, B.; Tran-Dinh, S.; Neumann, J.M.; Huynh-Dinh,T. and Ingolen, J. (1984) Nucleic Acids Res. **12** , 3271.
7. Feigon, J.; Wang, A.H.; Vander Marel, G.A.; Van Boom, J.H. and Rich, A. (1984) Nucleic Acids Res. **12**, 1243.
8. Genest, D.; Hartmann, B.; Thuong, N.T.; Ptak,M. and Leng, M. (1984) Biochem. Biophys. Res. Commun. **125**, 803.
9. Fazakerley, G.V. (1984) Nucleic Acids Res. **12**, 3543.
10. Taboury, J.A; Adam, S.; Taillander, E. ; Neumann, J. M. ; Tran-Dinh, S.; Huynh-Dinh, T.; d'Estaintot, L.B.; Conti, M. and Igolen, J. (1984) Nucleic Acids Res. **12**, 6291.
11. Ivanov,I.V. and Minyat, E.E. (1981) Nucleic Acids Res. **9**, 4783.
12. Woisard, A. and Fazakerley, G.V. and Guschebauer, W.(1985) J. Biomol. Strct. Dyn. **2** , 1205.
13. Woisard, A. and Fazakerley, G.V. (1986) Biochemistry **25**, 2672.
14. McIntosh, L.P.; Grieger, I.; Eskstein, F.; Zarling, D.A.; Vande Sande,M. J.H. and Jovin, T.M. (1983) Nature **294** , 83.
15. Taillandier, E.; Tabour, J.A. ; Adam, S.and Liquier, J. (1984) Biochemistry **23** , 5703.
16. Ho, P.S.; Frederick, C.A. ; Quigley, G.J. Vandre Marel, G.A.; Van Boom, J.H.; Wang, A.H.J. and Rich, A. (1985) EMBO J. **4**, 3617.
17. Fugi, S.; Wang,A.H.J.; Quigley, G.J.; Westerink.H. Vander Marel, G.A.; Van Boom, J.H. and Rich, A. (1985) Biopolymers **24**, 243.
18. Wang, Y.;Thomas, G.A. and Peticolas, W.L. (1987) Biochemistry **26**,5178.
19. Feigon, J. Wang, A.H.J.; Vander Marel, G.A. ; Van Boom, J.H. and Rich, A. (1985) Science **230** , 82.
20. Mishra, R.K. ; Latha, P.K. and Brahmachari, S.K. (1986) Nucleic Acids Res. **10**, 4651.
21. Taillander,E.; Liquier, J. And Tabour, T.A. (1985) in Advances in Infrared and Raman Spectroscopy, eds. clark, R.J.H. and Hester, R,H. (Heyden , New York) 65.
21a. Nejedly, K.; Klysik, J. and Palecek, E. (1989) FEBS **243**, 313.
22. Patapoff, T.M.; Thomas, G.A. ; Wang,Y. and Peticols, W.L. (1988) Biopolymers **27** , 493.
23. Taillander, E.; Ridoux, J.; Liquier, J.; Leupin, W.; Denny, W.A.; Wang, Y.; Thomas, G.A. and Petticols, W.L. (1987) Biochemistry **26** , 3361.

24. Peticols, W.L.; Wang, Y. and Thomas, G.A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85** , 2579.
25. Trifonov, E.N.; Konopka, A.K. and Jovin, T.M. (1985) FEBS Lett.**185** , 197.
26. Beaucage, S.L. and Caruthers, M.H. (1981) Tetrahedron Lett. **22**, 1859.
27. Sinha, N.D. ; Biernat, T. and Koster, H. (1983) Tetrahedron Lett. **24**, 5843.
28. Ansorge, W. and Barker, R.J. (1984) Biochem. Biophys. Methods **9**, 3347.
29. Rich, A.; Nordheim, A. and Wang, A.H.J. (1984) Ann.Rev. Biochem. **53** , 791.
30. Latha, P.K. and Brahmachari, S.K. (1986) J.Sci. Indust.Res. **45**, 521.
31. Quadrifoglio,F. ; Manzini, G.; Vasser, M.; Dinkelspiel,K. and Cear,R. (1981) Nucleic Acids Res. **9** , 265.
32. Tabour, J.A. and Taillandier, E. (1985) Nucleic Acids Res. **13**, 4469.
33. Pohl, F.M. (1976) Nature **260** , 365.
34. Shouche, Y.S. ; Latha, P.K. ; Ramesh, K.; Mandyan, V. and Brahmacha -ri, S.K. (1985) Proc. Int. Symp.Biomol. Str. Interaction , J.Bio.Sci. Supply. **8**, 563.
35. Quardrifoglio, F.; Manzini, G.; Dinkelspiel, K . and Cear, R. (1982) Nucleic Acids Res. **10** 3759.
36. Wing, R.; Drew, H.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K. and Dickerson, R.E. (1980) Nature, **287**, 755.
37. Drew, H.R. and Dickerson, R.E. (1981) J.Mol. Biol. **157**, 536.
38. Gait, M.J. (1984) Oligonucleotide Synthesis :A Practical Approach, IRL Press, Oxford.
39. Kumar, A. (1991) Biochemistry International **23**, 467.
40. Behe, M. and Felesenfeld, G. (1981) Proc. Natl. Acad. Sci. (USA) **78**, 1619.
41. Van de Sande, J.H. and Jovin, T.M. (1982) EMBO J. **1**, 115.
42. Thomas, T.J. and Bloomfield, V.A. (1988) Biopolymers **24**, 725.
43. Thomas, T.J.; Baarch, M.J. and Messemer, R.P.(1988) Anal. Biochem. **168**, 358.
44. McLean, M.J. and Well, R.D. (1988) Biochem. Biophys. Acta, **950**, 243.
45. Rao, M.V.R.; Atreyi, M. And Saxena, S. (1990) Biopolymers **29** , 1495.
46. Ivanov, V.I. and Minyat, E. (1981) Nucleic Acids Res. **9**, 4783.
47. Rao, M.V.R. ; Atreyi, M. Saxena, S. (1991) FEBS Letts. **278** , 63.
48. Albergo, D.D.; Marky, L.A.; Breslauer, K.J. and Turner, D.H. (1981) Biochemistry **20**, 1409.

49. Dameschun, H.; Damaschun,G.; Becker.; Bude, E. ; Misselwitz, R. and Zirwer, D. (1978) Nucleic Acids Res. **10** ,3801.
50. Carroll,D. (1972) Biochemistry **11**, 421.

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