Thermochimica Acta, 119 (1987) 103–112
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

ENERGETICS OF LEFT-HANDED HELIX FORMATION OF NUCLEIC ACIDS

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SUMMARY

The inversion of canonical right-handed double helical conformations of nucleic acids (B-DNA, A-RNA) into the left-handed form, due to changes in the polymer environment, can be followed by a variety of physical methods such as UV-, CD-, or NMR-spectroscopy. The only quantitative approach to the evaluation of the energy parameters in based on adiabatic scanning microcalorimetry. The results presented in this paper demonstrate that the inversion process is accompanied by an enthalpy change of about 5 KJ per mole basepairs and an entropy change of about 14 J per degree and mole basepairs. The inversion temperature for the thermally induced transition is about 45° C in almost all cases. This holds both for DNA- as well as RNA-sequences.

INTRODUCTION

Mixes sequences of DNA and, as was shown recently by two groups (1,2)also of RNA, exist alternatively in a right-handed (B-DNA, A-RNA) and a left-handed double helical conformation.(3-5)Under conditions of high water activity the B-DNA conformation (A-RNA-)prevails.(6) If the water activity is reduced on addition of certain amounts of salt or organic solvents and/or the temperature is increased above a particular threshold value transconformation occurs in DNAs or RNAs with alternating purine/pyrimidine sequences into the left-handed double helix.The quantitative analysis of the equilibria involved and the elaboration of predictive algorithms (10)for estimating the "Z-forming-potential" of given sequences in specified environments requires the knowledge of central thermodynamic quantities (7-9) such as the intrinsic stability constants of basepairs in the two conformations. In this paper the quantitative informations derived to date from spectroscopic and calorimetric data and from related theoretical calculations are presented in a comprehensive way.

THEORETICAL PART

Statistical model: Following a procedure outlined extensively by Soumpasis and Jovin (10) a theoretical model for the helix inversion can be developed.
The Z structure is characterized by a dinucleotide repeat.Thus it is convenient to consider sequences of 2n basepairs (bp) as present in the canonical alternating polynucleotides poly d(G-C) e.g.to adopt the Z form under certain experimental conditions.This

0040-6031/87/$03.50

is demonstrated by cristallographic and solution studies. The state with all bp's in B form is taken as the reference state. The transition free energy difference equals

$$\Delta G(k,m,n) = -RT \ln U(k,m,n) \qquad (1)$$

where U(k,m,n) is the statistical weight (relative to the B state) of a microstate with k units (2 bp's each) in the Z form and m B/Z junctions.The associated free energy difference can be further decomposed into the form

$$\Delta G(k,m,n,) = 2k\,\delta g + 2m\,Fj + \Delta Gs(k,m,n) \qquad (2)$$

where δg (in kcal mole-1) is the intrinsic free energy difference between the Z and the B forms for one mole bp's, Fj is an effective positive junction free energy for one mole B/Z junctions, and ΔGs is the change in total free energy of supercoiling due to the relaxation of the whole circular plasmid molecule accompanying the B-Z transition of k units in an inserted stretch of alternating pu/py sequence.(We consider a particular sequence optionally embedded in a closed circular DNA carrier which can be placed under topological stress, because this is an experimentally observable situation). Studies of sufficiently short DNA oligomer transitions are very convenient for obtaining energetic parameters because of the validity of an all-or-none formalism to a first aproximation. One can define an apparent equilibrium constant K by the help of some spectroscopically observable "degree of transition" ,i.e. the average fraction of units in the Z conformation .Thus

$$K = \theta / (1-\theta) \quad \text{and} \quad \ln K = -\Delta G/RT \qquad (3),(4)$$

Assuming that the solution is dilute with respect to the DNA component,

$$\Delta G = G_a^1 - G_b^1 = G_a^1(n,p,T) - G_b^1(n,p,T) \qquad (5)$$

where G_a^1 is the total Gibbs free energy of the system comprising just one mole of noninteracting DNA molecules all in conformation Z and $n_1 \ldots . n_r$ moles of solvent components constituting the composition vector n with 1:water,2:cosolvent,3:first electrolyte; p is the pressure ; and T is the temperature.G_b^1 is similarly defined. With this formulation, all thermodynamic derivatives of lnK have a clear meaning.For example,

$$(d \ln K / dT) = \Delta H(n,p,T)/RT^2 \qquad (6)$$

$$(d \ln K / dnj) = -\Delta \mu j(n,p,T)/RT \qquad (7)$$

$$(d \ln K / dp) = -\Delta V(n,p,T)/RT \qquad (8)$$

where ΔH, μj,and ΔV are the differences in enthalpy, chemical potential of the j-th component and solution volume of the two systems.In practice the derivatives of lnK have a clearcut meaning and describe variations in lnK with respect to precisely those quantities that are experimentally varied,namely the amounts of

the different components (salts,cosolvents) present in solution ,T and p.
A logarithmic dependence of the apparent K on NaCl concentration was observed in the initial study of Pohl and Jovin in 1972 (11). Cooperative transitions were also observed with poly d (G-C) undergoing B-Z transitions in the presence of organic solvents (12)either alone or as a cosolvent at very low salt concentrations.(13) It is noticable that the NaCl-induced B-Z transition of poly d(G-C) is essentially isoenergetic; i.e. the enthalpy change is less than 0.3 kcal/mole bp from calorimetric and from spectroscopic equilibrium-kinetic studies.The reaction has to be entropically driven and probably dominated by interionic interactions. In all other situations ,i.e. in mixed solvents, or under the influence of bound di- or oligovalent cations and other conformationally selective ligands, the B-Z transition of poly d(G-C) can also be induced by temperature variations at constant solvent composition.The latter is also true for DNA sequences methylated or halogenated at the 5'position of the cytosine moiety (14).Such substitutions lead to a selective stabilization of the left-handed conformation of the two canonical alternating purine-pyrimidine families,d(G-C) and d(A-C)d(G-T).With temperature dependent transitions, the apparent spectroscopically determined van't Hoff transition enthalpy change (for an unknown size of the cooperative unit)

$$\Delta HvH = 4R*T2(d/dT) \qquad (9)$$

is related to the intrinsic transition enthalpy per base pair (determined by calorimetry). In the case of long polymers described by means of the infinite length Ising model

$$\Delta HvH = l_o \, \$H_{\bullet,1} \qquad (10)$$

where l_o is the observed cooperative unit length.

EXPERIMENTAL PART

MATERIALS AND METHODS

Poly d(G-m^{5}C) and poly d(G-C) was purchased from Pharmacia P-L Biochemicals . Poly r(G-C) , poly r(G-m^{5}C) , and poly r(G-br^{5}C) were prepared according to minor modifications of the method of Hall et al (15). The polynucleotide samples were thawed and exhaustively dialyzed against several changes of the appropriate buffer solutions.All chemicals used were of analytical reagent grade and the water was double distilled. The stock solutions were eventually diluted with the standard buffers.

Spectroscopic measurements: UV spectra and transition curves were either recorded on a Hitachi Perkin Elmer Mod. 124 spectrophotometer at atmospheric pressure or with a Pye Unicam Model 1800 spectrophotometer, equipped with a suitable pressurable (10 bar) cell holder (16). The latter instrument permits the registration of absorbance changes to transition temperatures above 100°C without disturbance from boiling of the solvent water.

Differential scanning microcalorimetry: The concentrated polynucleotide solutions were applied to the adiabatic scanning microcalorimeter ,Type DASM-1 from Mashpriborintorg (Moscow,USSR), which is essencially based on a dsign of P.Privalov(17). In a standard experiment ,1mL of the polymer solution was degased and inserted into the sample cell and heated simultaneously with 1 mL of a buffer solution of identical composition ,only lacking the biopolymer, contained in the identical reference cell. Details of the measuring procedure are given in the literature. The enthalpy of the transition was calculated by comparing the experimentally observed peak areas to an electrically induced calibration area of known enthalpy equivalents (18).

RESULTS AND DISCUSSION

Fig.1 shows the optical properties of poly d(G-m^{5}C), left, and of poly r(G-m^{5}C) ,right,as a function of the temperature. The ultraviolet absorbance spectra under appropriate buffer conditions are shown at room temperature (full line) below the transition at room temperature, and at elevated temperature above the transition (dashed line) .

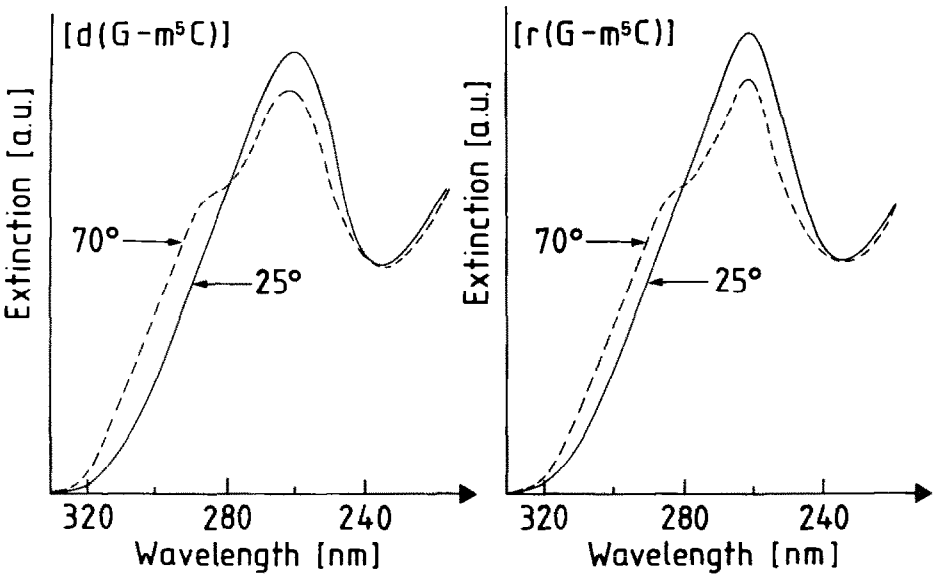

Fig.1a/b.Absorption spectra of poly d(G-m^{5}C) (a) at 2 mM Mg^{2+},50 mM Na^+ and of poly r(G-m^{5}C) at 5 M $NaClO_4$.

The two series of spectra are remarcable similar. The occurance of a shoulder at about 275 nm and the decrease of the maximum absorbance at 260 nm are hints for the conformational change induced by the temperature rise.The thermal behavior of the system is reversible , i.e. after cooling the sample to 20°C the helix inversion is cancelled. In principle it is now possible to record the change of the optical density at a fixed wave length as a function of the temperature either as a hypochromic effect at 255 nm or as a hyperchromic effect at 295 nm.(16). In analogy to the helix coil transition one can term these plots transition curves. (cf. Fig.2)

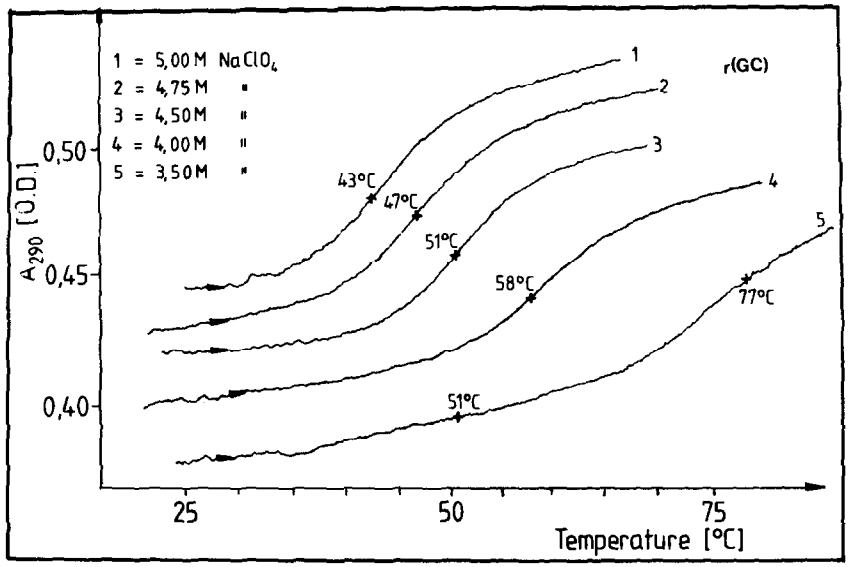

Fig.2 A-RNA-Z-RNA transition of poly r(G-C) at different $NaClO_4$ concentrations(5M,4.75M,4.5M,4.0M,3.5M) vs. temperature.

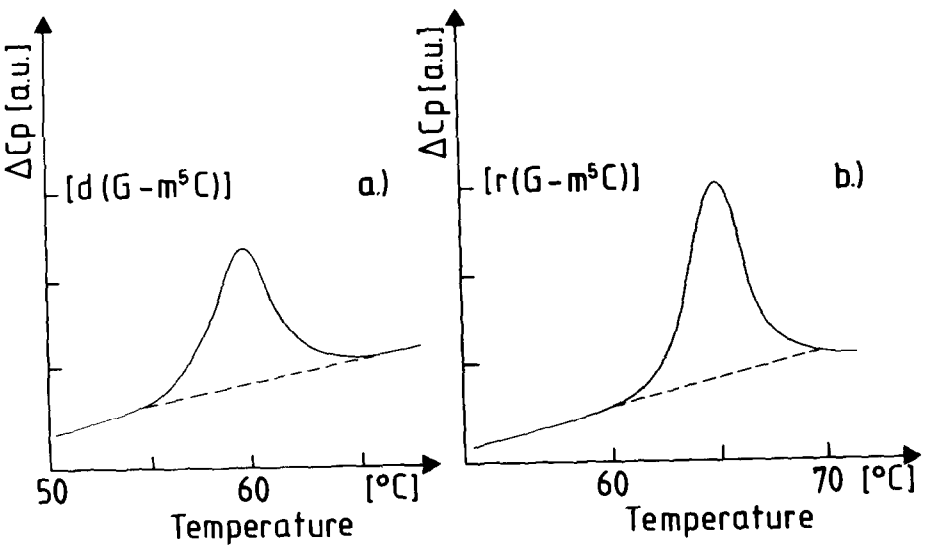

Fig 3a/b Apparent change in heat capacity due to the helix inversion for poly d(G-m^{5}C) (a) and for poly r(G-m^{5}C) (b).Cation concentrations as in Fig.1.

The true thermodynamic parameters (ΔH,ΔG, ΔS) for one mole of subunits can only be deduced from direct calorimetric measurements. Scans of DNA and of RNA solutions under appropriate conditions are shown in Fig. 3a for the poly d(G-m^{5}C) and in Fig. 3b for the poly r(G-m^{5}C) double helix. The areas under the peaks correspond to the total enthalpy changes which accompany the inversion of the handedness of the helices.

The entire set of the experimentally obtained data is given in Table I for the DNA analogues and in Table II for the RNA analogues.

The thermodynamic parameters for the temperature dependent B-Z transitions in DNA polymers obtained from van't Hoff analysis of spectroscopic data and of some directly obtained calorimetric data are presented in Table I.

TABLE I

Thermodynamic parameters for B-Z transitions in linear DNA

Polynucleotid	T °C	ΔHvH Kcal/mole	ΔS cal K-1mol	ΔHcal Kcal/mbp
poly d(G-C)	38	o	o	o
poly d(G-m5C)	40			1.0
poly d(G-m5C)	42			1.0
poly d(G-m5C)	43			o.5
poly d(A-m5C)d(G-T)	57	220	670	-
poly d(A-brC)d(G-T)	40	195	620	0.9
poly d(A-mC)d(G-brU)	40	480	1500	1.1
poly d(A-brC)d(G-brU)	40	410	1600	1.2

Several conclusions can be derived from this information:(i) DNAs with 5' cytidine substitutions (methyl,halogen) in both sequence families show a similar apparent transition enthalpy ΔH per base-pair of ca 4.2 Kj per mole base pairs, derived from spectroscopic measurements, which is very close to the directly determined value from adiabatic scanning microcalorimetry.(4.2 Kj/mbp for poly d(G-m5C))(ii)the halogen substituents are more effective than a methyl group in stabilizing the Z form. Poly d(G-br5C) and poly d(G-io5C) are constitutively left-handed at all ionic strengths;(iii) the cooperative lengths,i.e. junctional free energies, vary greatly from one polymer to another,i.e.are DNA-specific as well as a function of conditions. Thus reported values for l. for d(G-C) duplexes range from 25 bp's to 100-1000 bp's.Three recent estimates for the methylated polymer are 180,226,and 110 bp's. and (iv)the van't Hoff plots for oligomers are linear.

Other data are available under more complex solution conditions.Szu and Charney(19) have reported the thermodynamic parameters of the thermal transition of poly d(G-m5C) in 0.5M

$MgCl_2$,50mM NaCl from least square fitting of experimental degrees of transition vs. temperature curves to the Ising model expression.Using their data one obtains an apparent transition enthalpy of 1.4 Kcal mole -1, and an entropy value of 4.5 cal K^{-1} $mole^{-1}$ at Tm = 39.3 °C, values which compare favourable with those derived from a recent calorimetric study of the same DNA in 10mM MgCl2,50mM NaCl(8). A smaller value was published in a very recent study for the conditions 1 mM $MgCl_2$,50 mM NaCl, namely 0.6 Kcal for the transition enthalpy per base pair,and 2.0 cal per degree and mole base pairs for the transition entropy.(9)
In mixed solvents under certain extreme conditions, the B-Z transition can be rendered endothermic, i.e. such that the B conformation is favored upon heating.
Only a very short and incomplete basic formalism is presented here and the reader is kindly refered to the original literature,but it was worthwhile to understand the few pertinent thermodynamic data related to the B-Z transition.It is apparent that one is in a position to make preliminary yet quantitative predictions and to separate the contributions of intrinsic sequence-dependent from more global effects. However, additional informations are required in order to construct more precisely and extensively phase diagrams e.g..

LEFT-HANDED RNAs

Very recently it was successfully demonstrated that corresponding transitions of helical sense occour also in doublestranded RNAs, contradicting a statement of Dickerson, which in an apodicting way states that it is immediately clear that there is nothing like a Z-RNA due to the 2'OH-group of the ribose ring.This statement seems to be premature in the light of the results presented here for a series of poly(G-C) and the methylated and halogenated analogs.(cf Fig.2)
UV spectroscopic studies have shown that poly(G-C) in aqueous solution containing 4 M NaClO4 can reversibly be converted into a left-handed Z-RNA by rising the temperature to 60oC. This conversion temperature will linearly increase when the perchlorate concentration is lowered in a logarithmic way, up to 110oC .Above this temperature only the helix coil transition can be followed by the established experimental techniques.The reversibility and cooperativity of the helix/helix conversion facilitates the investigation and the quantitative evaluation of the transition enthalpy per base pair by the help of scanning microcalorimetry.As will be discussed in more detail in the following paragraph in a 5 M NaClO4 solution the conversion temperature drops to 41°C ,the conversion enthalpy per mole bp's yields 4.17 KJ, the corresponding entropy change amounts to 13.4 J K-1 .The van't Hoff enthalpy , which can be derived from the temperature dependence of the degree of transition, i.e. the fraction of polymer strands which populates the left-handed conformational state, is calculated to 820 KJ per mole of the cooperative unit. From the ratio of the two enthalpies the size of the cooperative length can be determined to amount to about 200 bps.Very similar are the results for the poly (G-m^5C) and for poly (G-br^5C).cf Table II.
For the first time the quantitative approach to investigate the order/order transitions was made . Instead of looking for the traditional order/disorder transitions in the form of the helix/random coil transition the three possible order/order transitions are viewed in a comprehensive maner, namely (i)the disproportionation,i.e. the rearangement of two identical double

helices to a triple helix and a partly stacked polypurine single strand;(ii) the displacement reactions ,i.e. the exchange of one of the two strands within the double helix to a single strand from the solution to form a new double helix with an inhanced thermal stability (this displacement reaction can even lead to a crosswise exchange of one strand each between two double helical complexes),and (iii) the helix inversion reactions, where the handedness of a double helix is inverted by an intramolecular mechanism as outlined above for the deoxypolynucleotides.(5)
As it turned out there is no fundamental difference in the occupation of conformational space of secondary structures between DNAs and RNAs. As a rule RNA helices are more stable than DNA helices.So at least in principle it should be possible to induce the analogue to the Z-DNA structure in a RNA double helix for strictly alternating purine/pyrimidine sequences in a suitable environment. With the help of ^{31}P-NMR and CD spectroscopy it was demonstrated that poly (G-C) can in fact invert its handedness from the canonical A-RNA conformation into the left-handed Z-RNA conformation(1).This statement is based on the assumption that spectroscopical similarities reflect a conformational similarity. The prove of the existence of a left-handed RNA structure by X-ray analysis is still lacking.A continuous scan of the inversion process by NMR spectroscopy is not possible and the registration of the change of a CD signal at a fixed wave length due to the order/order transition allows only to scan the course of the reaction (degree of transition as function of the changing environmental conditions) to yield the van't Hoff enthalpy.These methods are short of giving quantitative results for the conformational unit (e.g. bp).The registration of the CD signal can be replaced by the registration of the UV absorbance at 290 nm because the Z-RNA structure is characterized by a higher extinction coefficient at this wave lenth as compared to the A-RNA conformation .The recorded UV-melting curves are symmetrical with respect to the transition temperature Tm ,i.e. the temperature at which the absortion increased to half of its final value. This shape reflects the presence of only one inversion center per polymer strand.The change of the handedness can best be described as an intramolecular process.This observation is supported by a moderate inversion rate which seems to be independent of the temperature.
The heat capacity changes ,due to the structural changes within the helical structure in the limited temperature range of the thermal transition,is a measure for the transition enthalpy of the polymer sample under investigation in the calorimeter vessel.Knowing the actual polymer concentration through a colorimetric phosphorus analysis allows to compute the true transiton enthalpy per base pair.(4.2 KJ/mole bps).It is remarcable close to the experimentally observed value for the B-DNA to Z-DNA transition of poly d(G-m5C) in 10 mM $MgCl_2$,50 mM NaCl.cf Table I. The positive sign of ΔH is acceptable due to the fact that the right-handed conformation is stable at room temperature even at very high counter ion concentrations.This is different from the analogeous deoxypolymers.From the experimental observations it is obviously justified to treat the inversion process as an true equilibrium.Consequently the standard thermodynamic equations hold to calculate the other thermodynamic parameters, such as the true transition enthalpy as well as the true free enthalpy change at any given temperature, taken the transition enthalpy as temperature-independent.From the Gibbs-Helmholtz equation at T=Tm follows that the transition entropy is the ratio of the enthalpy and the temperature, yielding an entropy

value of 13.4 J K^{-1} for the inversion reaction. This value is very small as compared to the helix/coil transitions (ca. 90 J K^{-1}),but it is not unreasonable for an order/order transition, for we start from a helix conformation and we end up in a helix conformation.Hence there is no contribution from chain entropie from a backbone which is freely rotating around the single bonds along the chains. But only contributions from changes in solvation and from changes in surface charge densities among the two conformers, but it is impossible to give any detailled description of the various contributions to the entropy changeSince both the transition enthalpy and the transition temperature are small it is not surprising that the free enthalpy of the transition, calculated for standard conditions, is not bigger than 0.27 KJ per mole bps.There is some caution necessary in the application of the standard formalism to calculate the cooperative length for the order/order process. There are always three destinct regions in each double strand, namely the left-handed helix, the right-handed helix , and the A-Z junction in between. There is only one center of inversion possible in each polymer molecule. Thus it is formally applicable to determine the cooperative length by deviding the van't Hoff enthalpy by the calorimetrically determined enthalpy of inversion to yield the "size of a mole" in also for this case ,but this does not mean that the number of base pairs which undergo the conversion simultaneously are located in a loop of this calculated magnitude (200 bps).
Table II gives an compilation of the experimentally obtained thermodynamic data for the A-RNA to Z-RNA transition.

TABLE II

Energetics of A-RNA to Z-RNA Transition

Polynucleotide	Tm	ΔHcal	ΔScal	ΔHvH	l.
	oC	KJ mbp^{-1} J	$K^{-1}mbp^{-1}$	KJ m^{-1}	
Poly r(G-C)	41	4.1	13.o	236	240
Poly r(G-m^5C)	52	4.6	14.1	253	230
Poly r(G-br^5C)	46	4.4	13.9	244	230

CONCLUDING REMARKS

We have presented a brief outline of the basic formalism and the corresponding thermodynamic data for the inversion of the handedness for DNAs and, for the first time in the literature also for a family of double stranded RNAs. The experimentally observed transition enthalpies are rather small and in the range of the thermal energies available from the environment. Thus , local structure fluctuations may be key elements in mediating biological regulatory functions through the inversion of the handedness, i.e. the B-Z transition in DNAs and the A-RNA to Z-RNA transition in RNAs.

ACKNOWLEDGEMENTS

This investigation was supported by the Deutsche Forschungsgemeinschaft(DFG, Schwerpunkt Biophysik der Organisation der Zelle) and by the Fonds der Chemischen Industrie. The author thanks Dr.T.Jovin ,MPI für Biophysikalische Chemie, for supplying the RNA samples.

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