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Circular Dichroism as a Tool of Investigation in the B-Z Transition of DNA

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Some useful CD applications aiming to investigate the B-Z conformational change in nucleic acids as well as their interactions with a ruthenium complex of potential terapeutic interest are described in this paper.

The results presented here regard:

a) the energetics of the conformational transition from the B right-handed to the Z left-handed helix in synthetic oligodeoxynucleotides with a cytosine-guanine alternating sequence;

b) The B to Z transition for sequences containing all four canonical bases and the role of nickel ions and sodium perchlorate in promoting this tranformation;

c) the interaction of $Ru(II)(DMSO)_4Cl_2$, a compound exhibiting a good antitumor activity in animals, with both mononucleosides and polynucleotides.

INTRODUCTION

Nucleic acids share with other classes of biopolymers, as well as with many smaller biomolecules, the property of being chiral and of frequently inducing optical activity in symmetrical molecules upon interaction with them. Hence the important role that circular dichroism (CD) has assumed in a wide spectrum of biophysical investigations on nucleic acids, ranging from the study of the various chain conformations in solution and of their interconversion to the interactions of the chains with drugs, metal ions and their complexes, leading to non-covalent as well as to covalent adducts.

In this paper we wish to present a brief survey of the results recently obtained in our laboratory by using CD and regarding few different topics, namely: 1) the energetic of the B-Z interconversion in $(dCdG)_n$ oligodeoxynucleotides[1–5]; ii) the stabilization of the left-handed Z double helix in DNA oligomers of different sequence and composition by means on Ni^{2+}; iii) the covalent interaction of some promising antineoplastic ruthenium complexes with DNA chains and with nucleotides[6].

i) The B-Z Interconversion in Oligodeoxynucleotides

After the discovery of the novel left-handed helical structure (the Z DNA) in 1979[7,8], a wide spectrum of biophysical and biochemical studies was developed with the aim of clarifying its structural details, the effects of the base sequence and of the solvent conditions on its thermodynamic stability as well as the energetics of the interconversion of this helix with the more familiar B one, and moreover to assess its presence in natural DNA, at least *in vitro* if not yet definitely *in vivo*, in search of an appealing biological role of this conformation in the mechanisms of gene activity regulation (see reviews 9—11).

In the investigations of the ability of oligo- and poly-deoxynucleotides of different sequences to assume this conformation in solution, CD soon gained wide popularity due to the relevant and typical changes observed in the CD spectrum of B DNA as a consequence of its transition to Z (in fact an approximate inversion of the bands ranging from about 300 down to less than 180 nm takes place). As to the Z inducing agents in aqueous solution, the most widely used were initially concentrated NaCl, up to saturation, and ethanol, up to 60%; soon after the effects of multivalent cations as Mg^{2+}, Mn^{2+}, $Co(NH_3)_6^{3+}$, spermine, etc., and of different anions, as in the case of $NaClO_4$, and organic solvents were explored, providing the evidence that cations of higher charge were generally more effective and that at high concentration the nature of the anion was not irrelevant ($NaClO_4$ is a better Z inducer than NaCl).

One of the investigations undertaken in our laboratory concerns the energetics of the B-Z interconversion in alternating cytosine-guanine oligodeoxynucleotides induced by concentrated NaCl. In this case CD has proved to be well suited for determining the equilibrium amounts of B-double helix, Z-double helix and coil conformations of the oligomers in aqueous solution as a function of temperature and NaCl content. Figure 1 shows a sample case for d(CGCGCG) in 2.5 M NaCl: the CD spectra recorded at different temperatures show that the effect of increasing temperature is a shifting of the equilibrium from Z to B and then to coil. A knowledge of the CD spectra of the pure B form (low temperature, low NaCl content), pure Z form (low temperature, saturated NaCl) and coil (high temperature) allows one to determine, from a CD spectrum recorded at equilibrium in each given condition, the amounts of the three conformations satisfying the following system of equations:

$$\Delta\varepsilon_{295} = \Delta\varepsilon^{B}_{295} f_B + \Delta\varepsilon^{Z}_{295} f_Z + \Delta\varepsilon^{C}_{295} f_C$$

$$\Delta\varepsilon_{255} = \Delta\varepsilon^{B}_{255} f_B + \Delta\varepsilon^{Z}_{255} f_Z + \Delta\varepsilon^{C}_{255} f_C$$

$$1 = f_B + f_Z + f_C$$

The enthalpy changes relative to the B-Z transition have been obtained by plotting $\ln K = \ln (f_B/f_Z)$ versus $1/T$. It was, therefore, possible to estimate the thermodynamic parameters of the B-Z interconversion which show that for short $d(CG)_n$ oligomers the Z double helix has a lower enthalpic content, whereas the B form has a higher entropy. Increasing NaCl promotes the B to Z transition since it affects more the enthalpy difference between the

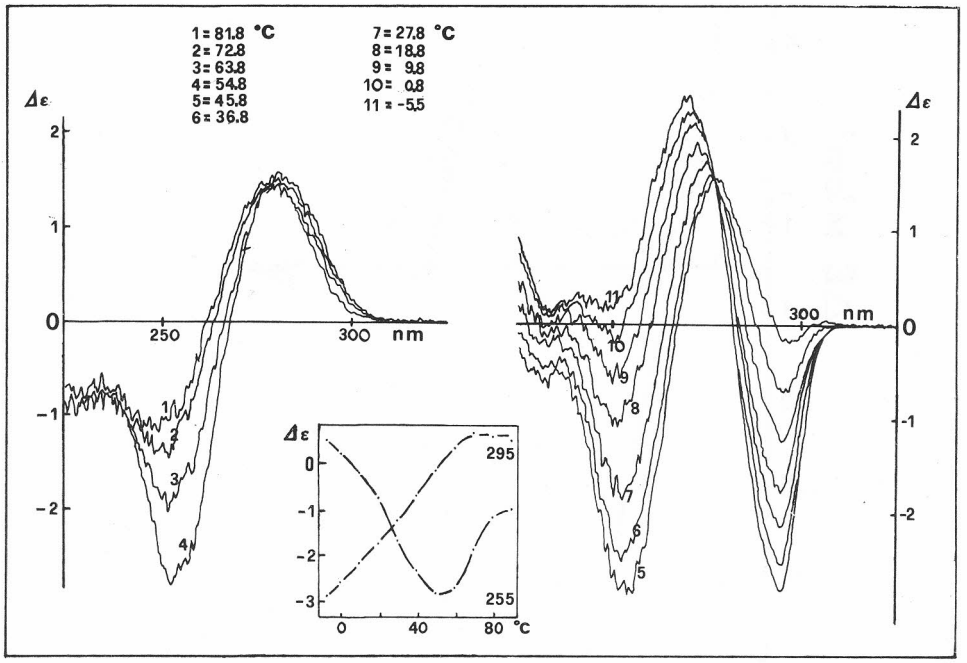

Figure 1. CD spectra for d(CGCGCG) in 0.1 M Tris. HCl (pH = 7.5), 2.5 M NaCl, as a function of temperature. The temperature values from 1 to 8 are as follows: 72.8, 54.8, 45.8, 36.8, 29.8, 18.8, 9.8, 0.8 °C.

two forms, favouring the Z one, than the entropy. However, the balance between enthalpy and entropy changes is rather subtle and thus easily affected by both the nature of Z promoting agent and the length of the oligomers.

ii) Z Stabilizing Effect of Nickel Ions in High Salt Solution

Regarding the effect of base composition and sequence on the stability of the Z structure, the general view is that: a) pyrimidine-purine alternation is a compelling, if not absolute, requirement to get this left-handed helix, b) the higher the percentage of G/C base pairs, the easier is the formation of Z DNA. However, the correlation between Z stability and base sequence is not yet known in sufficient detail. In linear DNA oligomers indeed the usual high salt conditions, generated with either NaCl or $MgCl_2$, are capable of inducing the Z conformation only in a very limited number of sequences, i. e. almost only in d $(C—G)_n$ and some others chemically derived from these, as d $(G—Br^5C)_n$ and d $(G—m^5C)$, precluding the possibility of observing this conformation in linear fragments for a wider spectrum of sequences, including those capable of assuming the Z double helix under torsional stress in covalently closed circular DNAs.[12]

Taking advantage of the fact that the combined action of concentrated $NaClO_4$ and a few mM Ni^{2+} is highly effective in promoting the Z conformation[12], we have started a CD study of the relative Z propensity of a number of oligomers of different sequence and composition, finding that most of

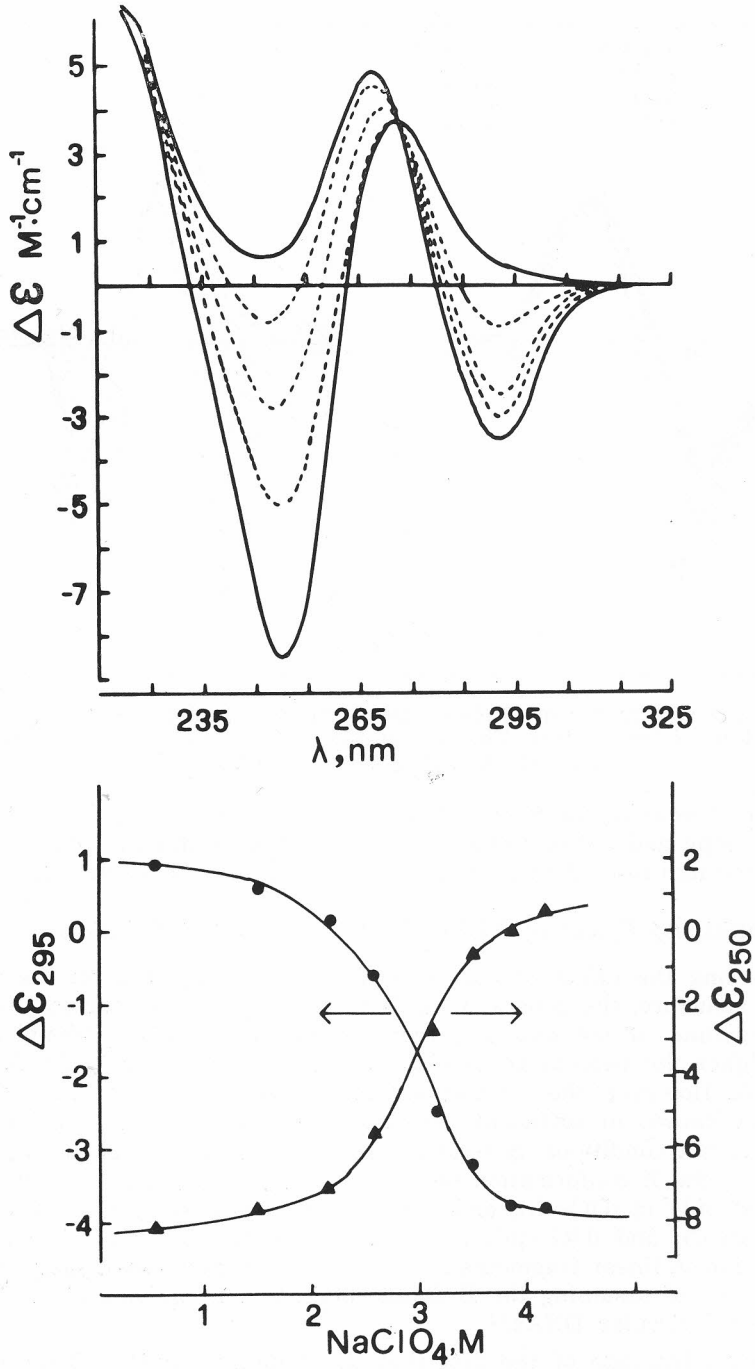

Figure 2. CD spectra of d(ATACGCGCGTAT) relative to the $NaClO_4$ titration in 10 mM Tris · HCl (pH = 7.4), 100 mM $NaClO_4$ and in the presence of 5 mM $Ni(ClO_4)_2$. Solid lines represent the initial and final spectra, while broken lines are the intermediate one. The insert shows the ellipticity values at 295 nm as a function of $NaClO_4$.

the sequences considered do assume the left-handed helix in these conditions (as shown for a sample case in Figure 2). Table I summarizes the first results obtained, and shows that even without the support of torsional stresses: a) sequences with AT content up to 50% can afford the B—Z transition in the presence of a central $(C—G)_n$ tract; b) a perfect pyrimidine-purine alternation is not a very stringent requirement for the left-handed helix. However, a more extended and quantitative investigation is in progress in our laboratory and will further contribute to a better characterization of this phenomenon.

TABLE I

Summary of Titration Data for the B-Z Interconversion. The Numbers Indicate the Added Salt Concentrations at the Semitransition Points, when Measured. B-Z Indicates the Presence of not Negligible Amounts of Both Conformations Near Salt Saturation. No Z Indicates the Absence of Appreciable Amounts of Z Even Near Salt Saturation — not Tested

Oligomer	NaCl (M)	$NaClO_4$ (M)	$NaClO_4$* (M)
d(CGCGCGCGCGCG)	2.7	1.8	—
d(CGCACGCGCGTGCG)	3.3	2.9	2.1
d(ATACGCGCGTAT)	no Z	B-Z	2.9
d(CATACGCGCGTATG)°	no Z	B-Z	2.0
d(CGACGCGCGTCG)	no Z	B-Z	4
d(GGTCGCGCGACC)	no Z	—	2.5
d(ACACACGCGTGTGT)	—	—	2.7
d(ACACACATGTGTGT)	—	—	no Z

* titrations carried out in the presence of 5 mM Ni^{2+}
° titration carried out in the presence of 10 mM Ni^{2+}

iii) Reaction of $Ru(II)(DMSO)_4Cl_2$ with DNA Chains and With Nucleotides

Previous studies have shown that $Ru(II)(DMSO)_4Cl_2$ exhibits a good antitumor activity in experimental animals[13]. Both *cis* and *trans* isomers compare well with the properties of cisplatin, a well known and widely used antiblastic, but show an apparently lower toxicity. Their mutagenic properties strongly suggest that the target *in vivo* for these drugs is DNA[14].

We have performed a study of the reaction between the drugs and natural and synthetic DNA and have demonstrated that both complexes bind irreversibly to the DNA chain. CD spectra obtained on DNA-complex adducts showed that whereas the *cis* isomer does not change the B conformation of the chain, even at a high drug/DNA ratio, the *trans* isomer does change the CD bands of the polymer beyond a certain ratio, thus suggesting at least a local modification of the conformation, similar to that already found following the reaction between cisplatin and DNA.[15]

When the reaction with the drug is performed with a synthetic polynucleotide, poly(dGdC), at very low ionic strength and is followed by CD measurements as a function of time, the circular dichroism spectrum of the original B conformation is gradually changed to that typical of the left-handed Z form. The rate at which this conversion is brought about is higher for the *trans* isomer than for the *cis* form (Figure 3). These measurements allowed

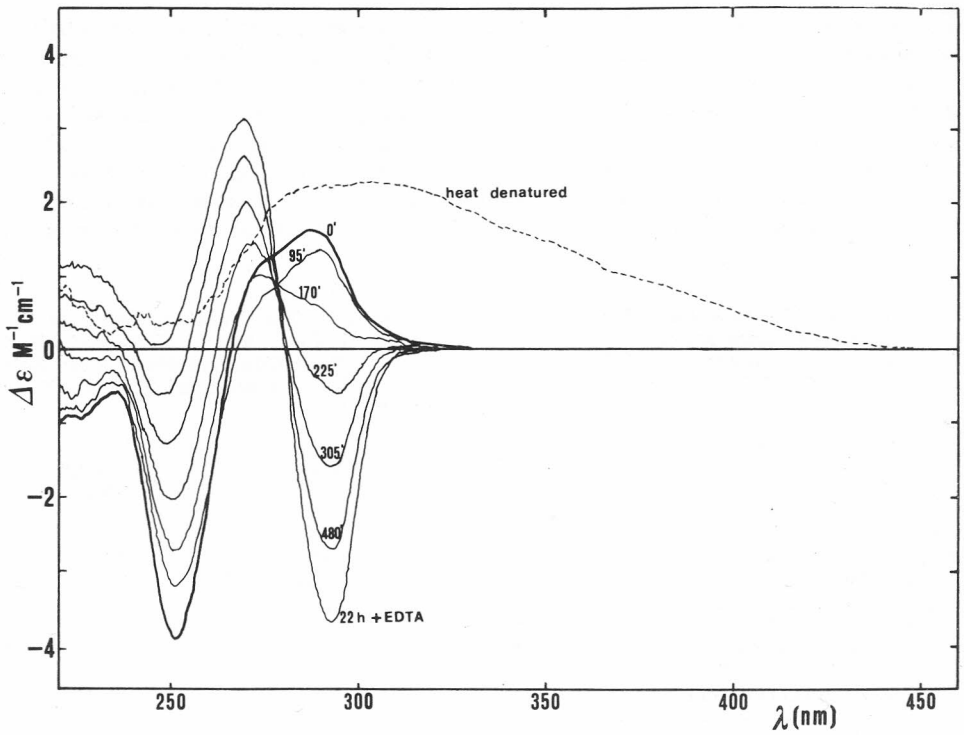

Figure 3. CD spectra of poly(dGdC) : poly(dGdC), incubated with an equimolar amount of Ru(II)DMSO)$_4$Cl$_2$ at 37 °C in 0.2 mM $NaClO_4$, taken at different reaction times. The transition is completed within 10 hours.

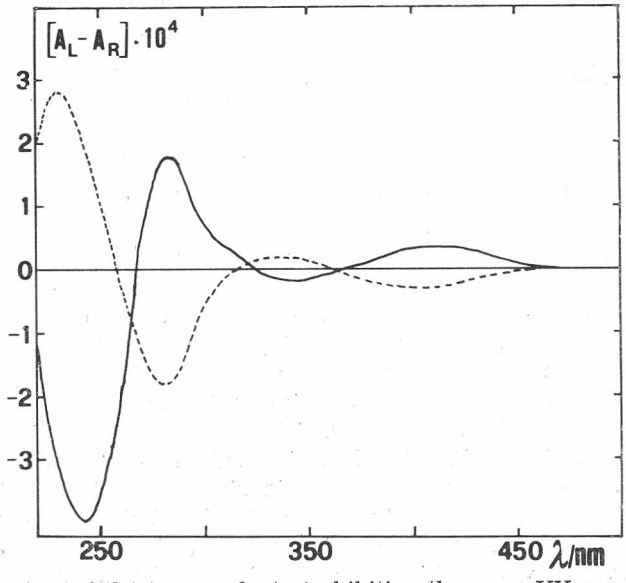

Figure 4. CD spectra of the two products (exhibiting the same UV spectrum) obtained from the reaction of *trans* Ru(II)(DMSO)$_4$Cl$_2$ with equimolar amounts of 5'dGMP in aqueous solution.

us to conclude that the main target of reaction of both isomers on the DNA chain is the N7 of guanine residue. The bulky complexation at this site, in fact, pushes the nucleotide residue to assume the *syn* conformation at the N-glycosidic bond which, in turn, stabilizes the left-handed form of the alternating chain.

When *trans*-Ru(II)$(DMSO)_4Cl_2$ is incubated with 5′dGMP, only two products are formed and can be separated with gel filtration HPLC. The relative amount of the two species is dependent on the temperature, thus showing that the two compounds are interconvertible. The UV spectra are nearly identical whereas the CD spectra taken in aqueous solutions of the two species are almost mirror images of one another at high wavelengths (Figure 4), thus suggesting that the compounds are enantiomers with respect to the metal center. (Actually, they are diastereoisomers as deoxyribose is a chiral compound and for this reason the CD spectra are not exact mirror images at low wavelengths where the transitions of the nucleotide are located). Subsequent high resolution proton and phosphorus NMR measurements showed that 5′dGMP reacts with the metal complex with a 1 : 1 stoichiometry at neutral pH's and chelates the octahedral ruthenium atom *via* N7 and phosphate group in two different steric arrangements[16]. At the moment it is not clear which coordination sites are occupied by the chelating ligand in the two isomers.

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SAŽETAK

Cirkularni dikroizam kao oruđe u istraživanju B-Z prijelaza DNA

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Opisane su neke korisne primjene CD u istraživanju konformacijske izmjene B-Z u nukleinskih kiselina, kao i njihova interakcija s rutenijevim kompleksima od terapeutskog interesa.

a. energijâ konformacijskih prijelaza iz B-desnoruke u Z-lijevoruku uzvojnicu sintetskih oligodeoksi-nukleotida s alternirajućom sekvencijom citozin-guanin.

b. prijelaza iz B- u Z-konformaciju za sekvencije koje sadrže sve četiri kanonske baze, kao i utjecaj nikal-iona i natrij-perklorata na poticanje ovih prijelaza.

c. interakcije $Ru(II)(DMSO)_4Cl_2$ spoja koji pokazuje dobru antitumorsku aktivnost na životinjama, s mono- i polinukleotidima.