CIRCULAR DICHROISM STUDIES ON THE ECHINOMYCIN–DNA COMPLEX

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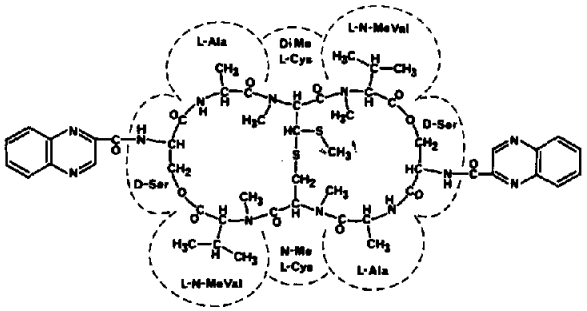

Fig.1. Structure of echinomycin [11,12].

1. Introduction

Echinomycin (fig.1) is a peptide antibiotic which inhibits the template activity of DNA [1,2]; its biological effects are directly related to its ability to interact with double-stranded DNA by a peculiar mechanism involving bifunctional intercalation [3,4]. Conformational energy calculations [5] on the isolated molecule have shown that the octapeptide lactone ring of echinomycin is restricted to adopt 2 alternative structures characterized by comparable conformational energy values, differing in the disposition of the Ala–Cys peptide bonds. One of the structures has been found to fit detailed NMR data satisfactorily [6]. Major changes in the conformation of the peptide portion of the molecule are not expected to occur in different chemical environments because of the rigidity of the lactone ring revealed by NMR. However, there is a large degree of rotational freedom around the amide linkages connecting the quinoxaline chromophores to the peptide lactone ring: as a consequence the chromophores can change their mutual orientation, particularly as regards the free and DNA-bound states.

In order to gain information about the structure of the antibiotic in the DNA-bound state as compared to its state in free solution we have studied circular dichroism spectra of echinomycin and of its complex with DNA. Circular dichroism (CD) represents a technique particularly suitable for detecting changes in conformational parameters involving chromophores or chromophoric environments in optically active substances.

2. Materials and methods

Echinomycin was a product of CIBA-Geigy Ltd, Basel. Calf thymus DNA was purchased from Worthington and sheared as in [7]. Antibiotic–DNA

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complexes were prepared by adding a small volume of echinomycin dissolved in acetone to a stirred solution of DNA in SHE buffer (2 mM Hepes, 10 μM EDTA, 9.4 mM NaCl, pH 7.0; total ionic strength 0.01) [3]. The acetone was subsequently removed by evaporation. The DNA concentration was 260 μM in nucleotides. Formation of the complex was checked by comparing the absorption spectra of the mixture around 320 nm before and after addition of an equal volume of dimethylsulphoxide (DMSO) to dissociate the complex [3,8]. From the A_{325} after dissociation the concentration of echinomycin was determined to be 21 μM utilizing $\epsilon_{325} = 1.24 \times 10^4$ cm^2 mol^{-1} in 50% (v/v) DMSO–SHE buffer [3,8]. CD spectra were recorded on a Cary 60 spectropolarimeter with a model 6002 CD attachment, calibrated with d-10-camphorsulphonic acid. For the curves shown in fig.2 the concentration of echinomycin was 100 μM determined by ultraviolet absorption utilizing $\epsilon_{315} = 1.15 \times 10^4$ cm^2 mol^{-1} [3,8]. The optical path length was 1 cm for the region 375–275 nm and 1 mm for the region 275–205 nm.

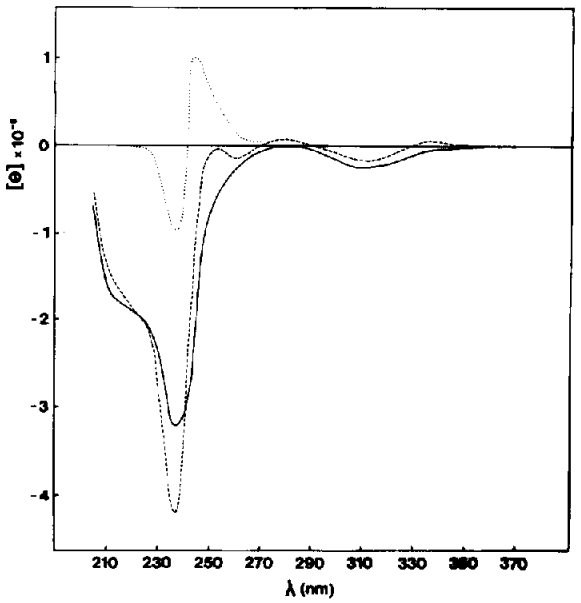

Fig.2. Circular dichroism (CD) spectra of echinomycin in various solvents. (——) In methanol. (– – –) In mixture of 60% water, 40% methanol (v/v). (· · ·) Difference between the spectra in water–methanol and pure methanol in the 270–220 nm region. The molar ellipticity [θ] is given in deg·cm^2/dmol echinomycin.

3. Results and discussion

Figure 2 shows the CD spectra of echinomycin in methanol and in a 40/60 (v/v) methanol–water mixed solvent. The solubility of echinomycin in water (5 μM [3]) is too low to permit direct analysis of CD spectra in purely aqueous solvents. In both solutions a very strong band at about 240 nm characterizes the π–π^* electronic transition of the quinoxaline chromophores, with a dissymmetric factor $\Delta\epsilon/\epsilon$ as high as $\sim 3 \times 10^{-3}$. Other bands with smaller dissymmetric factors are observed in the higher wavelength region of the spectra. The very high values of the Cotton effects suggest that the electronic transitions of the quinoxaline moieties are strongly perturbed. The dominant contribution to this effect can be assigned, as in the case of actinomycin D [9], to local atropisomerism resulting from rotation of the molecular plane of the chromophores with respect to the peptide groups directly bonded to them. Furthermore a possible excition coupling of the electric transition moments of the quinoxaline chromophores with each other could give rise to an additional conservative contribution to the circular dichroism.

A contribution of this kind does indeed appear as the result of adding water to the methanolic solution of the antibiotic, as evidenced by the dotted curve in fig.2 which represents the difference between the spectra in methanol/water and pure methanol. The inflection point of the conservative Cotton effect is coincident with the A_{240}, as expected for coupling between identical chromophores. The presence of additional conservative contributions is suggested by comparison of the spectra at higher wavelengths. In agreement with the argument that there is association between quinoxaline chromophores is the hypochromic effect observed in the absorption spectrum in water–methanol as compared to that in pure methanol. A plausible explanation of this behaviour can be advanced on the basis that increasing proportions of water in solution will tend to favour self-association of hydrophobic groups; in particular quinoxaline groups would be expected to stack in either an inter- or intra-molecular fashion. Intermolecular stacking seems more likely on the basis of

the following observations: molecular associations were detected by NMR of chloroform solutions at higher concentrations, as evidenced by the concentration dependence of chemical shifts [6]; about 10% hypochromism in the absorption spectra was observed by a 10-fold reduction of the concentration of 50/50 (v/v) water–methanol solutions; echinomycin becomes progressively less soluble as the water content in the solution is increased; and finally, conformational calculations rule out the possibility of sterically allowed structures with an intramolecular stacking distance of the order of 3.4 Å which is expected for strong chromophore–chromophore interactions.

Figure 3 shows the CD spectrum of the echinomycin–DNA complex in aqueous solution at an antibiotic to nucleotide binding ratio $r \sim 0.08$, i.e., close to saturation of the available binding sites. The most striking feature is the strong negative peak at about 240 nm. The dominant contribution to this band very likely originates from coupling of the quinoxaline chromophores and the adjacent peptide groups of echinomycin, indicating that in the complex these groups are not coplanar. Additional minor effects are implied by the reduced amplitude of this band compared with that of pure antibiotic in fig.2, taking account of the binding ratio.

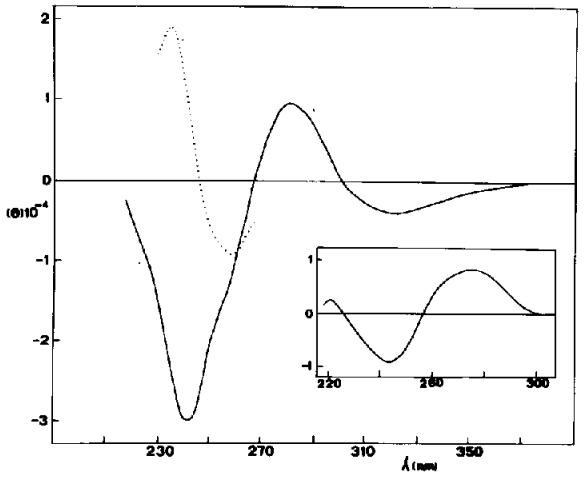

Fig.3. (——) CD spectrum of echinomycin–DNA complex in SHE buffer. (· · ·) Spectrum obtained by subtracting the CD of echinomycin monomer (see spectrum in methanol, fig.2) and the CD of DNA (inset) from (——) in the 270–220 region. The molar ellipticity [θ] is given in deg·cm²/dmol of DNA nucleotide residues.

Interpretation of this spectrum is simplified if we subtract the CD spectrum of free, non-associated echinomycin in methanol (fig.2), as well as the DNA contribution (see inset, fig.3) by making the reasonable assumption that for the sequences not involved in the binding the CD spectrum of DNA itself is not substantially altered. As a result we obtain a semiconservative Cotton effect (dotted line in fig.3) whose point of inflection occurs about half way between the absorption maxima of echinomycin and DNA. This effect can readily be assigned to coupling between different chromophores as expected for intercalation of the quinoxaline moieties between the DNA base pairs.

Although these conclusions should be treated with caution it is noteworthy that the sign of the Cotton effect is reversed as compared with that due to self-association of chromophores in the free antibiotic shown in fig.2. This indicates that the chirality resulting from the interaction of the quinoxaline groups with the DNA base pairs is opposite to what is found for free echinomycin (i.e., in the self-associated state). This in turn can be interpreted on the basis that different chiralities arise as a consequence of rotation around the amide linkages connecting the quinolaxine moieties to the peptide lactone ring. In the previously proposed structures these groups are disposed in different ways. In the first model [5], based on potential energy calculations, the quinoxaline rings are out of plane with respect to the adjacent peptide linkages. In the

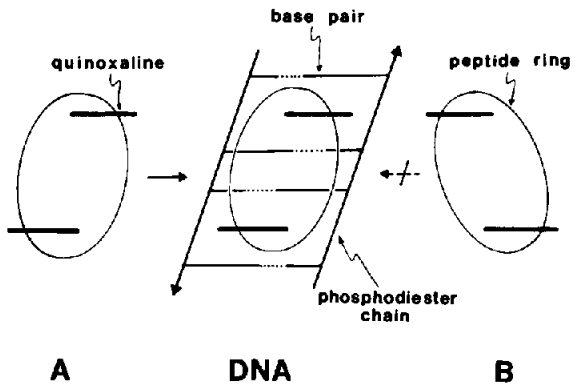

Fig.4. Schematic representation of the chirality of DNA and of intercalating (A) and non-intercalating (B) echinomycin conformations [5,6].

second one [6], which fits the NMR data in $CDCl_3$ better, the peptide groups are coplanar with the quinoxaline rings. The chirality deriving from the arrangement of the chromophores in the first model appears most suitable for interaction with DNA, while the chirality in the second model is opposite to that of DNA, as schematically illustrated in fig.4.

4. Discussion

On the basis of the present analysis we suggest that in the complex between echinomycin and DNA the quinoxaline chromophores are rotated with respect to the plane of the adjacent peptide groups, thus making possible additional interactions with DNA (e.g., hydrogen bonds) as in the case of actinomycin D [10]. Moreover, in order to facilitate interaction with DNA the chirality of echinomycin appears to undergo reversal with respect to the chirality of the molecule in the free or self-associated states.

Acknowledgements

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