CYCLOLINOPEPTIDE – AN ANTAMANIDE ANALOG

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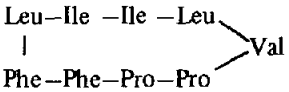
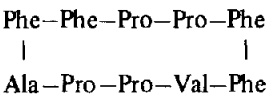
1. Introduction

Cyclolinopeptide (CLP) is a homodetic cyclic nonapeptide occurring in linseed [1], and has the structure I. Its structural homology (i.e., the Phe–Phe–Pro–Pro sequence) with the ionophore antamanide (structure II) has generated some interest [2,3] in its physicochemical and complexing properties. A synthetic sample of CLP [4] has been studied earlier for its conformational properties [3,5,6], but no comparative study of CLP with antamanide has been reported. Antamanide has been studied in some detail recently [7,8]. With this comparison in view, we have studied the conformational and complexing properties of CLP and have found that (i) it has considerable conformational flexibility in solution, (ii) it possesses little ion-complexing ability, and (iii) despite its homology with antamanide it possesses no ionophoric character.



(I) CLP



(II) Antamanide

Our studies were conducted with a sample of CLP isolated from linseed cake (by Soxhlet extraction from acetone and recrystallized form acetone–ether, m.p. 243°C), and we observed that the spectroscopic properties of the synthetic sample and ours agree satisfactorily.

The conformational study on CLP was done in solution using infrared (IR) and circular dichroism (CD) spectroscopy. Special attention was given to the possibility of aggregation of CLP in solution, and also the determination of intramolecular hydrogen bonds in the peptide. IR spectral and CD spectral runs showed the band intensities to vary linearly with concentration in the range 10^{-4} to 10^{-1} M, so that the possibility of association of CLP in this region may be neglected. Peptide hydrogen–deuterium exchange studies (involving the rate of disappearance of the peptide NH vibrational bands upon the addition of D_2O to a solution of CLP in dioxan) revealed that all the CONH hydrogens in the peptide exchanged for deuterium within 10 min, suggesting the absence of any strong intramolecular hydrogen bonds in CLP. This result offers support to Tonelli's prediction [6] that CLP lacks interpeptide hydrogen bonds.

The CD spectral details of CLP in a variety of sol-

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vents are listed in table 1, and illustrated in fig.1. Firstly, we note only minor differences in the aromatic region (250–280 nm) Cotton effects of CLP in these media, thereby suggesting that the stereochemistry of the Phe residues is essentially unaltered in these solvents. Next we notice that the CD profiles fall into several families. In dioxan, acetonitrile, and alcohols one sees the peptide n – π* band near 218 nm with an intensity of about –70 000 to –90 000, and the longer component of the exciton-split π – π* band pair at 206 nm with an ellipticity of –80 000 to –100 000. The positive member of the split π – π* occurs below 200 nm. A second family of CD curves is discernible in trifluoroethanol (TFE), and in TFE–H_2O mixtures with the following features: $\lambda_{\eta\pi}$ 214 nm ($[\Theta] = -78 \times 10^3$), $\lambda_{\pi\pi}$ 205 nm ($[\Theta] = -80 \times 10^3$), and the positive π – π* band further blue-shifted. The main feature of this is the consistent blue-shifting of all the CD bands compared to those in the dioxane family. This is not explainable in terms of a simple solvent effect on the peptide n – π* and π – π* bands (since the solvent effects on these two bands are opposite of each other). In the absence of aggregation effects, one is led to infer that the conformation of CLP in TFE and in TFE–H_2O (called conformation A) is different from that in dioxane, CH_3CN, alcohols and in trimethyl phosphate (conformation B). In the solvent hexafluoroisopropanol (HFIP), the CD spectrum shows a mere shoulder at 215 nm (- 40 000) and a negative band at 200 nm (–80 000) with the positive band lying further down. In HFIP then, CLP takes on yet another different conformation (C). Tonelli [6] has predicted, based on potential energy calculations, that CLP may adopt several different conformations of approximately the same energy at room temperature, and that in none of these is the molecule intramolecularly hydrogen-bonded. Our spectral results on naturally occurring CLP seem to bear out these predictions. CLP thus appears to possess sufficient conformational flexibility in solution.

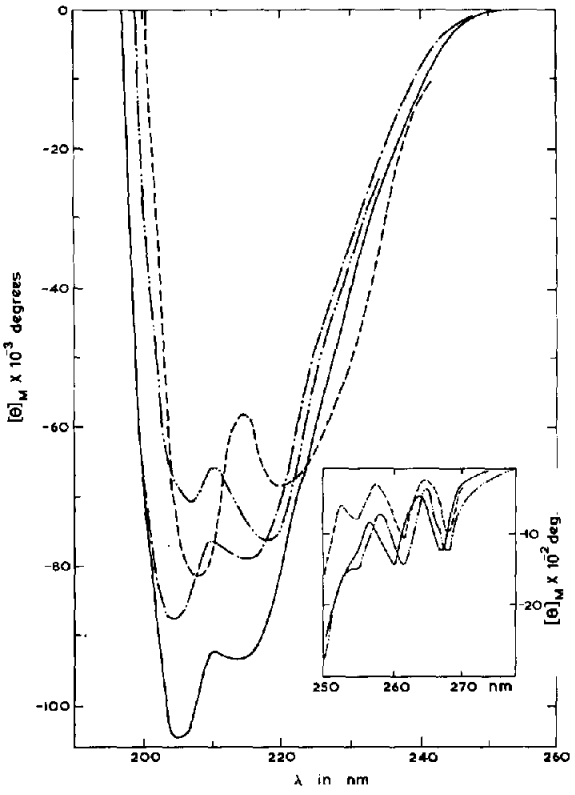

Fig.1. Circular dichroism spectra of CLP in various solvents. (———) In TFE–H_2O (9:1 v/v). (– . . – . . –) In TFE. (– . . – . . –) In methanol. (– – – –) In dioxane. Inset: the aromatic absorption region.

The partial homology between CLP and antamanide prompted us to study the ion-binding properties of CLP. Solvent extraction experiments (in which the uptake of alkali metal picrate salt from water to an organic layer containing the peptide [7] was monitored) revealed no preference by CLP to bind group IA or IIA metal cations. ^{13}C N.m.r. spectra of CLP in the presence of several salts produced shifts in the carbonyl C resonances of CLP by no more than a maximum of 0.1 ppm, far lower than those seen with ionophores such as antamanide [8]. Ion transport experiments wherein the transport of alkali picrate between two limbs of a U-tube via a central organic layer containing the peptide [9] was monitored, showed negative results with CLP while antamanide does indeed transport Na^+ ions. Thus CLP has little affinity for any of the group IA of IIA metal ions. Again, the effects of several salts on the CD spectra of CLP are minimal, quite contrary to what has been seen with antamanide [7]. The maximal effect is seen with Ca^{2+}, as shown in table 1 and fig.2, but the results seem to suggest that the effect is not one of ionophore-binding but rather that in the presence of Ca^{2+}, CLP undergoes a conformational shift from the form B to conformation A, perhaps due to a medium effect since the complexing ability of CLP has been shown above to be weak.

Table 1
CD spectral details of CLP

Solvent	λ_1 [a]	$[\Theta]_1$	λ_2	$[\Theta]_2$	λ_3	$[\Theta]_3$	λ_4	$[\Theta]_4$	λ_5	$[\Theta]_5$
Dioxane	268	−980	262	−1000	255	−750	220	-70.2×10^3	208	-81×10^3
Dioxane–H_2O (9:1)	268	−875	262	−980	255	−900	218	-88×10^3	207	-10×10^4
CH_3CN	268	−1000	261	−975			218	-88×10^3	207	-10.2×10^4
CH_3CN–H_2O (9:1)							217.5	-75×10^3	206	-85×10^3
							211	-82.5×10^3		
MeOH	268	−1200	261.5	−1400	255	−1450	218	-76×10^3	207	-71×10^3
							217.5			
EtOH	268	−1250	261.5	−1450	255	−1300	217.5	-72×10^3	207.5	-70×10^3
MeOH + 0.1 M $NaClO_4$	268	−925	261.5	−1125	255	−1000	217.5	-70×10^3	203	-79×10^3
MeOH + 0.1 M LiCl	268	−975	261	−1050	255	−1100	217.5	-82.5×10^3	207	-80×10^3
MeOH + 0.1 M $CaCl_2$	267.5	−1000	261	−1175	255	−1000	212	-82.5×10^3	203	-10×10^4
TFE + H_2O (9:1)	267	−1200	260.5	−1400	255	−1250	212	-92.5×10^3	205	-10.7×10^4
TFE	267	−1350	260	−1600	255	−1450	215	-79×10^3	205	-87.5×10^3
TFE (rough)[b]							214	-78×10^3	204	-83×10^3
TMP (rough)[b]							219	-45×10^3	204	-42×10^3
HIFP (rough)[b]							215(Sh)	-38×10^3	200	-78×10^3

[a] λ are in nm, $[\Theta]_M$ are in deg·cm²/decimole CLP
[b] Estimated from the paper by Naider et al [3].

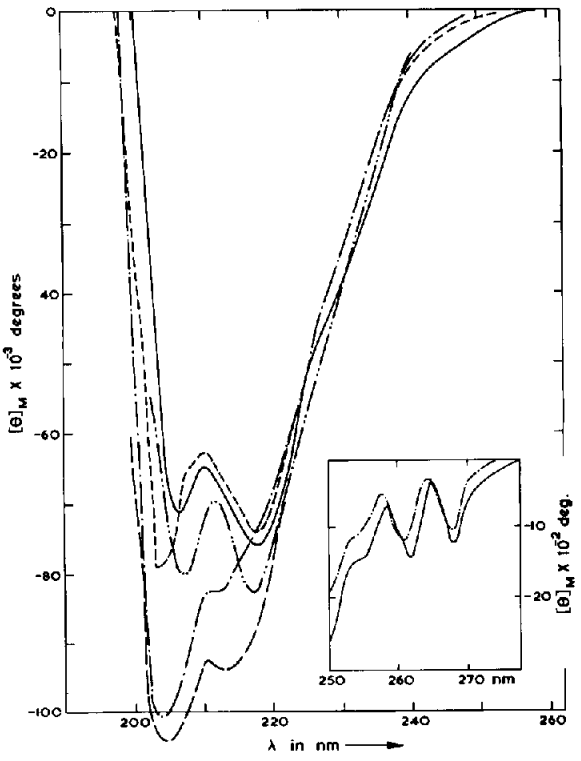

Fig.2. Circular dichroism spectra of CLP in the presence of several salts. (———) In methanol (no added salt). (– . . – . . –) In methanol + 0.1 M LiCl. (– – – – –) In methanol + 0.1 M $NaClO_4$. (– . – . –) In methanol + 0.1 M $CaCl_2$. (— — — —) In TFE–H_2O (9:1 v/v), no added salt.

Thus, while the structural homology of CLP and antamanide suggests the possibility of their behaviour being similar, our studies indicate that the structural differences between the two peptides are more significant in determining their properties than the homology. [10]

Acknowledgements

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