CONFORMATION OF POLYPEPTIDE ANTIBIOTICS VI CIRCULAR DICHROISM OF STENDOMYCIN

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SUMMARY: In trifluoroethanol stendomycin exhibits a CD pattern in which the extrema and ratios of peak magnitudes are virtually identical to α-helical poly-D-glutamic acid in water at pH 3.9. The results are presented to stimulate interest in the conformation of stendomycin as the most decisive test of the optical rotation approach to determine polypeptide backbone conformation.

INTRODUCTION: The antibiotic, stendomycin which was isolated by Thompson and Hughes (1963), is a tetradecapeptide lactone in which seven amino acid residues are of the D-configuration (Bodanszky et al. 1968). In the present communication the conformation of stendomycin is shown to be highly solvent dependent. Temperature studies in trifluoroethanol indicate a relatively stable structure. In water, however, the circular dichroism (CD) pattern exhibits an inverse temperature transition; at low temperature the CD pattern is that characteristic of disordered polypeptides; at 40°C and above, a stable CD pattern is observed which is more indicative of order. In trifluoroethanol the circular dichroism pattern, with the exception of being half amplitude, is almost identically that of left handed α-helical poly-D-glutamic acid in water at pH 3.9. The observation of a characteristic α-helix CD pattern raises the question, even more so than did gramicidin S, concerning the applicability of CD studies on homopolymers as models for interpreting the CD data on polypeptides and proteins. Should future studies show stendomycin in trifluoroethanol not to contain a substantial number of residues with ψ and ϕ angles characteristic of the α-helix, then the optical rotation approach as commonly used will have been shown to be severely ambiguous. Infrared (IR) measurements support the CD results in suggesting the presence of substantial α-helical structure.

EXPERIMENTAL: Stendomycin was obtained from Eli Lilly and Company, lot No. 382-431B-216-2. The primary structure as reported by Bodanszky et al. (1969) is given in Fig. 1. The amino acid analysis for a 90 hr hydrolysis in constant boiling hydrochloric acid at 108°C is given in Table I. The analysis indicated a clean sample with mole ratios in satisfactory agreement with the reported composition (Bodanszky et al. 1967).

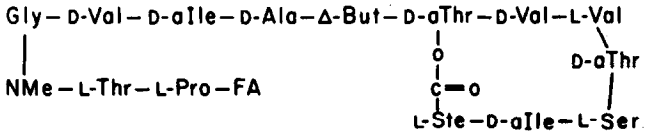

Fig. 1 Primary structure of stendomycin (Bodanszky et al. 1969).

TABLE I

AMINO ACID ANALYSIS OF STENDOMYCIN

Amino acid	Mole ratio
threonine	1.81
serine	0.88
proline	1.03
alanine	1.00
valine	2.96
isoleucine	1.32
leucine	0.10
basic component (stendomycidine)	0.88

Data were obtained on a Cary Model 60 with the Cary circular dichroism accessory. Temperature of the sample was monitored with the YSI model 42SC telethermometer equipped with a hypodermic probe. Infrared measurements were

taken on a Beckman Model IR-11 containing the IR-12 interchange. For the infrared studies films were cast on LiF windows from trifluoroethanol solutions. CD studies on the films resulted in spectra which were characteristic of trifluoroethanol solution.

RESULTS AND DISCUSSION: The CD spectra of stendomycin in several solvents at ambient temperature are given in Fig. 2. In trifluoroethanol and acetonitrile and to a lesser extent in trimethylphosphate the CD pattern is characteristic of that obtained on left-handed α-helical homopolymers of the D configuration. In trifluoroethanol (TFE), the first extremum is at 223 mμ, the follow-

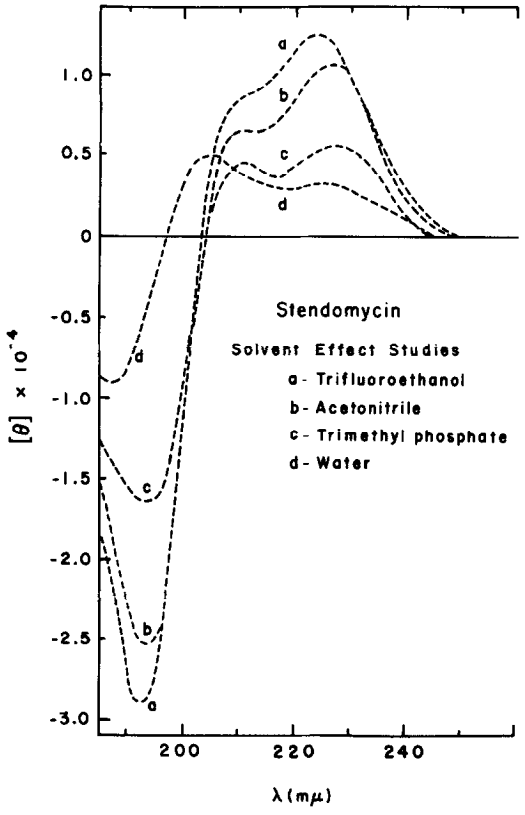

Fig. 2 Effect of solvent on the circular dichroism pattern of stendomycin. While the magnitude of the ellipticities in trifluoroethanol is one half that of left-handed α-helical poly-D-glutamic acid in water at pH 3.9, the positions and relative magnitudes of the bands are virtually identical.

ing shoulder is at 208-210 mμ and the negative band is at 192 mμ. These are the values obtained for aqueous poly-D-glutamic acid at pH 3.9. The extent of similarity between the positions and relative magnitudes of bands in stendomycin and poly-D-glutamic acid is greater than between different α-helical homopolymers. The ratio $[\theta]_{223}/[\theta]_{192}$ is 2.3 for stendomycin and 2.1 for poly-D-glutamic acid at pH 3.9. Thus these critical values are very close to those obtained on homopolymer model compounds. By contrast the band positions for gramicidin S are 217 mμ, 208 mμ and 186 mμ and the ratio $[\theta]_{217}/[\theta]_{186}$ is just

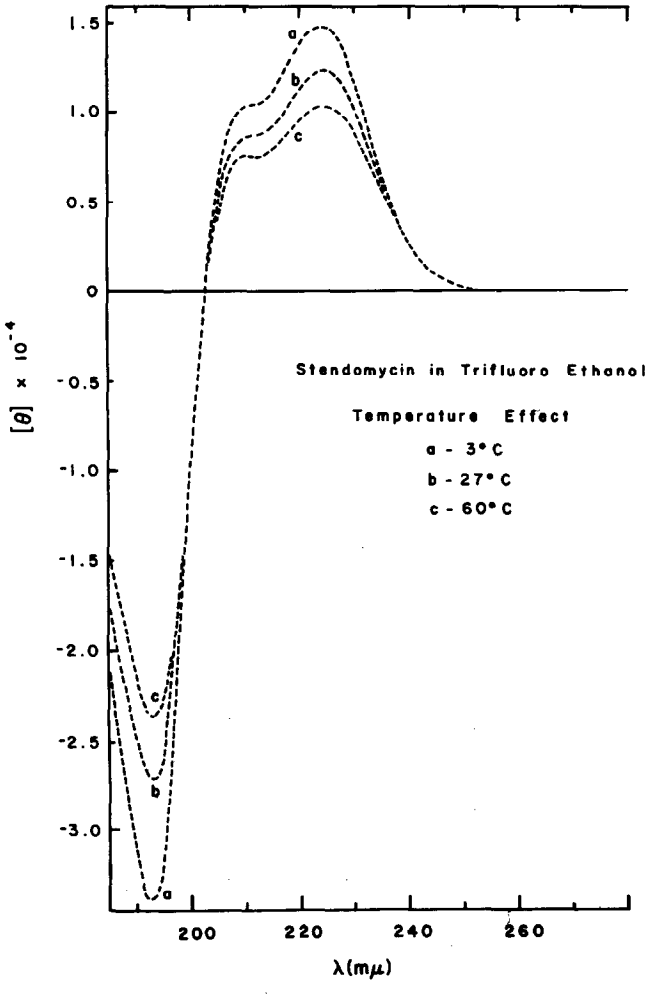

Fig. 3 Temperature effect on the circular dichroism spectrum of stendomycin in trifluoroethanol.

less than one (Balasubramanian, 1967; Quadrifoglio and Urry, 1967; Urry et al. 1968). If one were to calculate an α-helical content by the usual procedures a value of about 50% would be obtained for stendomycin.

Temperature studies on stendomycin in trifluoroethanol indicate a relatively stable structure (see Fig. 3). Increasing the temperature from 3°C to 60°C resulted in only about a 30% decrease in magnitude of the CD bands. By contrast in water the CD pattern is very temperature dependent, exhibiting an inverse transition. In the range from 40°C to 70°C the CD pattern (which is that of a disordered polypeptide of D amino acids) is essentially constant. On lowering the temperature a band appears at 223 mμ and the previous band near 200 mμ drops in magnitude and approaches 210 mμ. Seen in homopolymers and proteins this effect would be interpreted as an increase in order on raising the temperature.

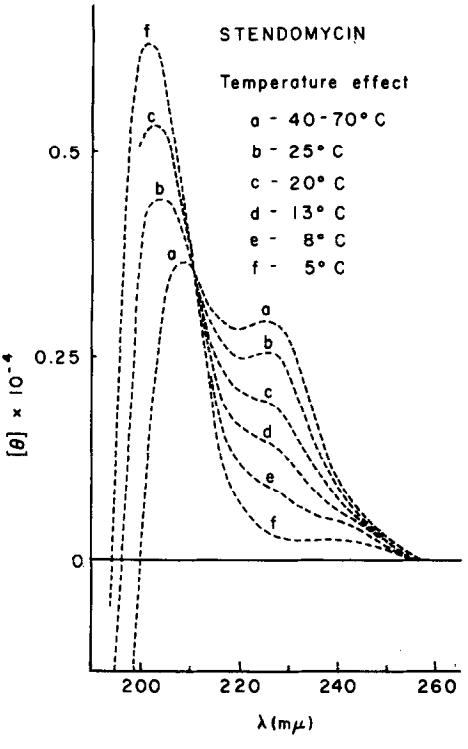

Fig. 4 Temperature effect on the circular dichroism spectrum of stendomycin in water at pH 5.6. At 40°C and above, the CD pattern is characteristic of disordered polypeptides. On lowering the temperature the CD pattern approaches that of ordered polypeptides.

This inverse transition in water is a reflection of the large number of hydrophobic residues and suggests that, in the ordered structure, hydrophobic residues are directed into the medium. Such differentiating conformational behavior of the antibiotic may prove of interest in its mode of action.

In an effort to further examine the conformation of stendomycin and to clarify the status of the optical rotation approach, IR studies were carried out on films cast from trifluoroethanol on lithium flouride windows. IR spectra on poly-L-alanine, stendomycin, gramicidin S and hydrogenated gramicidin S were obtained. CD spectra on all films confirmed the solution pattern. Poly-L-alanine in the α-helical conformation exhibits the amide I band at 1659 cm^{-1}. Stendomycin exhibits a strong band at 1659 cm^{-1} and a weak band, almost a shoulder, at 1632 cm^{-1}. In gramicidin S and its hydrogenated derivative the band is at 1637 cm^{-1}. Accordingly IR studies on the films would argue for the presence of α-helical conformation in stendomycin but for β-structure in gramicidin S. The latter is consistent with the structure of gramicidin S as deduced from nuclear magnetic resonance studies (Stern et al. 1968; Ohnishi and Urry 1969).

The tone of the foregoing discussion is intended to convey the present uncertainties in interpreting optical rotation spectra. Determining the conformation of stendomycin provides a critical test in resolving those uncertainties. Should future studies using other physical methods show stendomycin in trifluoroethanol not to contain appreciable α-helix, then conclusions derived from CD studies on the conformation of naturally occurring polypeptides and of globular proteins will reasonably be viewed with heightened skepticism. Nuclear magnetic resonance studies are in progress in order to determine what information this method can give.

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