

AMINO ACIDS AND PEPTIDES. CXII.*

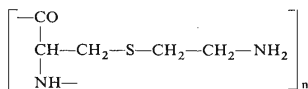
THE CONFORMATION OF POLY-L-THIALYSINE IN SOLUTIONS:
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Polymerisation of N-carboxy-anhydride was used to prepare poly-L-thialysine, and the circular dichroism of the latter was measured in water at various pH's and in mixtures of water and 2-propanol, water and methanol, with varying alcohol contents. The results show that poly-L-thialysine preferentially assumes a β -conformation even under conditions in which poly-L-lysine occurs in an α -helix.

In recent years some attention has been given to studies of the conformation of poly-L-lysine in relation to the dissociation of the ϵ -amino group and the conformational transition from an α -helix to a β -conformation which occurs in an alkaline medium under moderate heating¹⁻⁴. In addition to conformational changes conditioned by a change in the charge of the ϵ -amino group, transitions were also observed from a random-coil conformation to a helix in media in which the poly-L-lysine molecule keeps its charge. Epand et Scheraga⁵ have studied the behaviour of poly-L-lysine in a water-methanol environment, and reported that at certain alcohol concentrations an α -helix is formed.

The goal of the present work was to compare the behaviour of poly-L-thialysine (poly(S- β -aminoethyl)cysteine) (I) with poly-L-lysine (II) in terms of their ability to form an ordered conformation. Thus we could follow the effect of a S atom in the side chain from this aspect. It is known⁶⁻⁸ that exchange of an S atom for a methylene



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group, and *vice versa*, can influence the biological activity of the amino acid and its derivatives. In addition to electronic effects, stereochemistry can also play a role here^{9,10} particularly with the change of CH₂ for S in the side chain. Poly-L-thialysine seemed to be a suitable model for the study of such factors.

Poly-L-thialysine was prepared by polymerisation of N^ε-benzyloxycarbonyl-L-thialysine N^α-carboxy-anhydride in dioxane, with trimethylamine as initiator, according to a procedure used to prepare poly-L-lysine¹¹. Removal of the protecting benzyloxycarbonyl groups was carried out, because of the poor solubility of the blocked polymer in acetic acid, in a mixture of acetic acid and dichloroacetic acid.

EXPERIMENTAL

N^ε-Benzyloxycarbonyl-L-thialysine N^α-carboxy-anhydride: A suspension of 2.0 g (6.7 mmol) of N^ε-benzyloxycarbonyl-L-thialysine (prepared as previously described¹², dried over P₂O₅) in 50 ml of dioxane was bubbled with phosgene for 3 h. The solution was evaporated, the dried residue was dissolved in ethyl acetate and the product was precipitated with hexane; after several crystallisations from ethyl acetate-hexane the yield was 1.28 g (50%), m.p. 93–94°C. The literature¹³ presents 95°C for a sample prepared by another route.

Poly-(N^ε-benzyloxycarbonyl-L-thialysine): To a solution of 1.28 g (4 mmol) of the above N-carboxy-anhydride in 15 ml dioxane 0.04 mmol of triethylamine (0.1 ml of a solution of 0.55 ml triethylamine in 10 ml dioxane) were added. The mixture was mixed in dry-box for 48 h at room temperature, added to 200 ml water acidified with 1 drop of concentrated hydrochloric acid, the precipitate was collected, washed with water and dried over P₂O₅; the yield was 0.85 g (77%). The polymer was dissolved in dioxane (50 ml) in the cold, the solution filtered and diluted with acidified water (250 ml); the yield was 0.55 g (50%). For (C₁₃H₁₆N₂O₃S)_n (n . 280.3) calculated: 10.00% N; found: 10.14% N. The molecular weight (estimated by the sedimentation coefficient in dimethylformamide) ranged from 5000 to 10 000.

Poly(L-thialysine hydrochloride): To a suspension of protected polymer (150 mg) in dichloroacetic acid (2 ml) a 33% solution of HBr in acetic acid (2 ml) was added. After 15 min the mixture was degassed *in vacuo*, decanted 5 × with ether and dried over KOH *in vacuo*. After solution in water (8 ml) the product was placed on a column of Zerolite (OH⁻ cycle) and eluted with water. The first two fractions (10 ml each) contained ninhydrin positive substance. These fractions were acidified with hydrochloric acid to pH 3, the solution was evaporated *in vacuo*, the dried residue was dissolved again in a small volume of water and precipitated with ethanol and ether. The precipitate was decanted with ethanol and ether and dried *in vacuo*; yield 62 mg (64%). For (C₅H₁₁ClN₂OS)_n (n . 182.7) calculated: 32.87% C, 6.07% H, 15.34% N; found: 33.32% C, 5.82% H, 15.22% N.

Measurements: Circular dichroism spectra were measured on a Cary 61 instrument in the range 260–190 nm in cells with optical pathways 0.05 and 0.01 cm. Concentrations of measured solutions varied from 0.05 to 0.1% w/v. Solutions of the required pH were prepared by solution of the sample in water previously made alkaline with NaOH. A sample measured in 1M-NaF was dissolved in the salt solution and the pH was adjusted later. pH values were determined with glass and calomel electrodes on an electronic pH meter with a precision of 0.1 pH unit. Infrared spectra were measured on a Perkin-Elmer 621 instrument between Irtran windows. The concentrations of the measured solutions were about 1%. Before measurement the sample was deuterated by repeated evaporation with D₂O in a dessiccator over phosphorus pentoxide.

RESULTS AND DISCUSSION

We followed the CD spectra of poly-L-thialysine in relation to pH in aqueous solutions (Fig. 1) and in relation to concentration of 2-propanol in water (Fig. 2) and methanol in water (Fig. 3). It appears from Fig. 1 that with increasing pH values there is a gradual change in conformation of poly-L-thialysine from one arrangement to a second. The same appears to be the case with relation to the concentration of 2-propanol, even if the positive band at 200 nm is more marked. The negative maximum in water alone occurs at 224 nm, whereas in solutions of 2-propanol at about 221 nm. The isosbestic point in both cases lies at 215 nm. As opposed to both of the above systems, in methanol-water poly-L-thialysine reflects the alcohol concentration in the CD spectrum to a slight degree only, and there is no evidence of an appreciable conformational shift. The conformation in water at a relatively acid pH or in solutions with a low alcohol concentration can be considered as random, with the possible exception that we are dealing with a polyelectrolyte¹⁴. In comparison with poly-L-lysine in ionised form, the poly-L-thialysine spectrum differs in the intensity of the negative $\pi-\pi^*$ band and the sense and the position of the $n-\pi^*$ band. We do not consider it probable that the random configuration is markedly influenced by electrostatic forces in the molecule, since the spectrum in 1M-NaF at pH 6.5 falls into the data presented in Fig. 1 and has the same character as the other curves. On the other hand, the CD spectrum of polythialysine in alkaline solu-

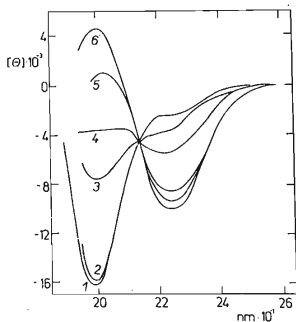


FIG. 1

Circular Dichroism of Poly-L-thialysine in Aqueous Solutions at Various pH's

1 H₂O, pH 3.3; 2 1M-NaF, pH 6.5; 3 H₂O, pH 8.3; 4 H₂O, pH 8.6; 5 H₂O, pH 10.7; 6 H₂O, pH 11.5.

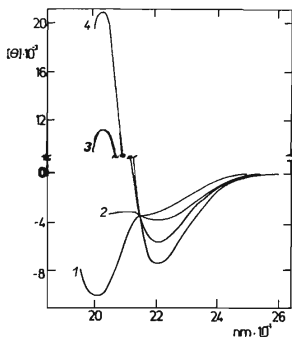


FIG. 2

Circular Dichroism of Poly-L-thialysine in Aqueous Solutions of 2-Propanol

1 71% (v/v) 2-propanol; 2 80%; 3 82%; 4 85%.

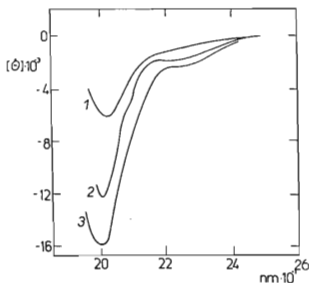
tion or in solutions with a high 2-propanol content correspond to a polypeptide chain in β -conformation. In comparison with the spectrum of poly-L-lysine in β -conformation, the negative band is shifted to longer wavelengths. This interpretation is possibly supported by the fact, among others, that solutions of poly-L-thialysine in the given solvents are opalescent and the opalescence continues to increase. This indicates intermolecular associations which are characteristic for β -conformation.

From the relation of the CD spectrum to pH in aqueous solutions one also comes to the qualitative conclusion that the S atom in the γ -position increases the dissociation constant of the ϵ -ammonium group in comparison with poly-L-lysine, as has been shown for the isolated amino acids¹⁵.

A similar behaviour has been found by Maeda and Ikeda¹⁶ with poly(S- β -carboxyethyl-L-cysteine), which has common structural features with poly-L-thialysine. The spectra of both substances are very similar even in terms of the positions of the individual maxima. The authors interpreted the conformation of poly(S-carboxyethyl-L-cysteine) also on the basis of infrared spectra measured both in solution and in solid phase. In order to reinforce our conclusions on poly-L-thialysine from the CD spectra, we characterised its conformation by analysis of infrared spectra in the region of the amide-I bands. The sample was measured in neutral medium (D_2O) and had an amide-I band at 1650 cm^{-1} . In the spectrum of a sample in alkaline medium (D_2O and NaOD, pH 11) there are two clear bands, the weaker at 1680 cm^{-1} and the stronger at 1620 cm^{-1} . These measurements confirmed that poly-L-thialysine in alkaline medium takes on a β -conformation.

Poly-L-thialysine, poly(S-carboxyethyl-L-cysteine), poly(S-carboxymethyl-L-cysteine) and poly-L-serine form a group of polyamino acids in which the β -conformation is preferred to the α -helical arrangement. Poly-L-lysine can form a β -conformation under certain conditions, but otherwise prefers the α -helix. The tendency to form stable helical structures can be expected in poly-L-thialysine because of the presence of a long side chain which can stabilise an α -helix (compare the low stability of the α -helix in poly-L-lysine with a clearly lower stability of poly-L-ornithine and particularly of poly(L-2,4-diaminobutyric acid)¹⁷). The reason for the abnormal behaviour

FIG. 3
Circular Dichroism of Poly-L-thialysine in
Aqueous Solutions of Methanol
1 97% (v/v) methanol; 2 50%; 3 H_2O ,
pH 3.3.



of poly-L-thialysine and other polyamino acids is undoubtedly related to the presence of the S atom in the γ -position of the side chain. The effect of the latter clearly changes the energy of the most advantageous torsion angles Φ and Ψ of the main peptide chain. It would appear that the presence of other heteroatoms in the same position would also prove disadvantageous to formation of an α -helix, e.g. O in poly-L-serine and N in poly(L-2,4-diaminobutyric acid). Unfortunately, sufficient attention has not yet been paid to calculations of conformational maps of the appropriate dipeptide units as has been given to amino acid residues with a hydrocarbon side chain (using empirical¹⁸ or quantum chemical¹⁹ approaches). The only calculation²⁰ concerning serine (and, without detailed data, cysteine as well) indicates that the α -helical arrangement should be more stable in this amino acid residue than in residues which have a C atom in the γ -position. In other words, the opposite from our result and the results of Japanese authors¹⁶. Conformational maps presented in the literature²⁰ result from an empirical approach to interatomic interactions. It would therefore be interesting to compare experimental data with data obtained from quantum chemical calculations which seems to reflect experimental facts in a more realistic manner.¹⁹

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