CIRCULAR DICHROIC SPECTRUM OF POLY-L-TYROSINE IN AQUEOUS SOLUTION

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SUMMARY: The UV and CD spectra of poly-L-tyrosine were investigated at pH 10.6 and pH 11.2. At pH 10.6 (μ=0.1), the CD spectrum exhibits a medium positive band at 230mμ, an extremely small negative band at 217mμ, and a large positive band at 200mμ. At pH 11.2 (μ=0.1), a new positive CD band appears at 277mμ while the bands at 230mμ and 217mμ are shifted to longer wavelengths by 15 and 10mμ respectively. These results, together with UV spectral data and a specific rotation- pH profile, suggest that at pH 10.6, poly-L-tyrosine exists in the helical conformation with only a small fraction of its side chains ionized; at pH 11.2, the polypeptide retains its helical structure but with a considerable increase in ionization.

As a result of our study of the interaction of poly-L-tyrosine, L-$(Tyr)_n$, with nucleic acids, we became primarily interested in the formation and properties of the 1:1 soluble complex between polyriboadenylic acid and L-$(Tyr)_n$ at neutral pH and low ionic strength (1). While investigating the physico-chemical properties of this complex, we assumed that the L-$(Tyr)_n$ was in the coil conformation, since the polypeptide was always exposed to high pH (0.1N NaOH) prior to adjusting the pH of the solution. According to a previous study, when solutions of L-$(Tyr)_n$ are first brought to pH 12 or higher and then back-titrated to pH 11.2, they appear to consist mainly of random coil molecules as judged by their ORD behaviour; however, L-$(Tyr)_n$, which is directly dissolved at pH 11.2 without prior exposure to high pH, is in the helical form (2,3). Subsequently, ORD studies as a function of pH indicated to us that the conformational state of L-$(Tyr)_n$ at pH 10.6 (and as a first approximation also at neutral pH) was most likely not the coil form but rather a more highly organized structure, most probably a helix. As the helical and coil forms of L-$(Tyr)_n$ in aqueous solution exhibit distinctly different spectra, (3) a CD study of the polypeptide was therefore undertaken to ascertain its conformational state at pH 10.6. Shiraki and Imahori (4) have reported a study of L-$(Tyr)_n$ in methanol and methanol + 6M LiCl, which includes UV, ORD, CD and IR measurements. Their CD spectrum for helical L-$(Tyr)_n$ in methanol is in general agreement with the one of the same conformation predicted by Chen and Woody (5) on theoretical grounds, and with that obtained by Damle (6) for he-

lical L-$(Tyr)_n$ in trimethyl phosphate; it is, however, different from the previously reported findings of Beychok and Fasman (3). We present in this paper the results of our CD study of L-$(Tyr)_n$ in aqueous solution. At pH 10.6, the CD spectrum presented agrees qualitatively with those of Shiraki and Imahori (methanol) (4) and Damle (6), while at pH 11.2, the results of Beychok and Fasman (3) are essentially confirmed.

MATERIALS AND METHODS

L-$(Tyr)_n$, M.W. 90,000, was purchased from Pilot Chemical Inc., Watertown, Mass. All the chemicals were of reagent grade. The polypeptide was treated as described in the literature (2) except that the dialysis was performed at 25°C instead of 40°C. L-$(Tyr)_n$ was dissolved in 0.1N NaOH, and the stock solution was stored at 4°C in the dark. The concentration of the solution was determined spectrophotometrically at pH >13 (1N NaOH) (2).

Preparation of L-$(Tyr)_n$ solutions: The L-$(Tyr)_n$ stock solution was diluted to an appropriate concentration with 0.1N NaOH and its pH was adjusted to the desired value by adding 6N HCl. A Radiometer pH meter, model r22, equipped with microelectrodes (glass electrode No. 2222B; with the latter, no sodium ion correction is required here) was used. After adjusting the pH, the concentration was verified spectrophotometrically. In order to compare our results with those of Beychok and Fasman's (3) at pH 11.2 and 0.2M NaCl, a L-$(Tyr)_n$ solution was prepared exactly as described by Fasman *et al.* (2).

UV and CD measurements: The UV spectra were taken at room temperature with a Cary 15 spectrophotometer. The CD measurements were done at 25°C using a Cary 60 spectropolarimeter equipped with a CD attachment (the accuracy of each measurement is shown by either a vertical bar or a circle). Blanks were run before and after recording each spectrum.

Effect of pH on the specific rotation at 350mμ. The specific rotation of L-$(Tyr)_n$ at 350mμ, $[\alpha]_{350}$, as a function of pH was measured with the Cary 60 (accuracy indicated by a vertical bar). The rotation of the polypeptide was first obtained in 0.1N NaOH (pH 12.75) and, afterwards, at pH 10.6. In order to verify the reversibility of the process, the pH was then raised in small increments by adding pulverized NaOH and the rotation was then determined for each pH.

RESULTS AND DISCUSSION

The variation of the specific rotation of L-$(Tyr)_n$ at 350mμ as a function of pH is shown in Fig. 1. At low polymer concentration and ionic strength, L-$(Tyr)_n$ is insoluble at pH < 10.3. The specific rotation is seen to change abruptly over a narrow pH range, as previously reported (2,7). The transition is completely reversible with respect to pH; the reversibility being also re-

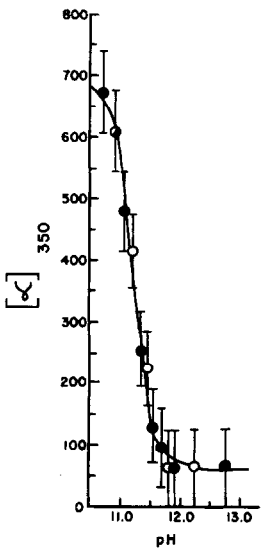

Fig. 1 Specific rotation of L-$(Tyr)_n$ at 350mμ as a function of pH; (o) and (●) indicate two separate experiments.

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cently reported for the pH range of 11.8 to 11.2 (7). An earlier study infers, however, that the transition is irreversible (2).

The UV spectra of L-$(Tyr)_n$ at pH 10.6 (μ=0.1), and pH 11.2 (μ=0.1 and 0.2) are shown in Fig. 2, and the CD spectra at these pH's are given in Fig. 3. The λmax of L-$(Tyr)_n$ at pH 10.6 is 277mμ, even though the solution was originally titrated from pH 12.75. According to the arguments of Fasman et al.(2) a L-$(Tyr)_n$ solution exposed to this sequence of steps should have its λmax at 275mμ. The apparent pKa of L-$(Tyr)_n$ in 0.2M NaCl is approximately 11.5 (2). The UV spectrum at pH 10.6 (Fig. 2) therefore indicates that the polypeptide has a low degree of ionization. Although L-$(Tyr)_n$ in known to aggregate at pH 11.25 (7), the concentrations used here are considerably lower than those at which aggregation is known to occur; for our solutions, no scattering was observed at 350mμ. As the pH is increased from pH 10.6 to 11.2 (μ=0.1 or 0.2), the UV spectrum shows a marked increase in the shoulder at 294mμ and,hence, in the degree of ionization since the absorption at 294mμ is characteristic of the ionized species.

The CD spectrum at pH 10.6 (μ=0.1), exhibits a medium positive band at 230mμ, an extremely small negative band at 217mμ and a very large positive band at 200mμ. At pH 11.2 (μ=0.1), a small broad band centered around 277mμ appears in the CD spectrum (Fig. 3), while the bands at 230 and 217mμ are

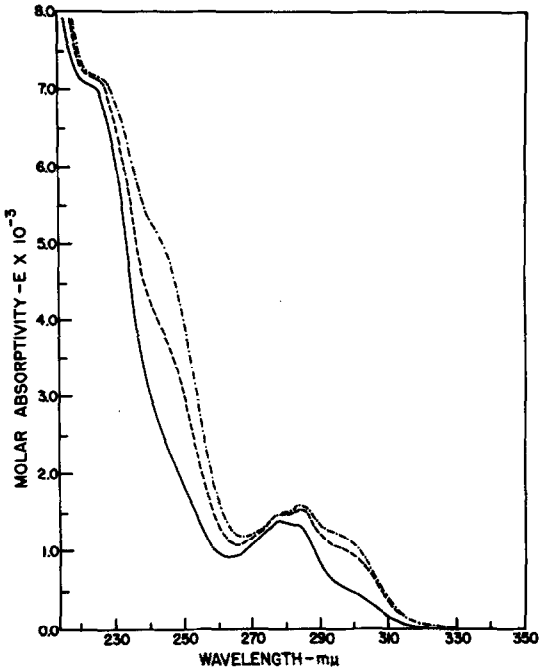

Fig. 2 UV spectra of L-(Tyr)$_n$, pH 10.6 (μ=0.1), ———; pH 11.2 (μ=0.1), -----; and pH 11.2 (μ=0.2), ·-·-·-·-·-

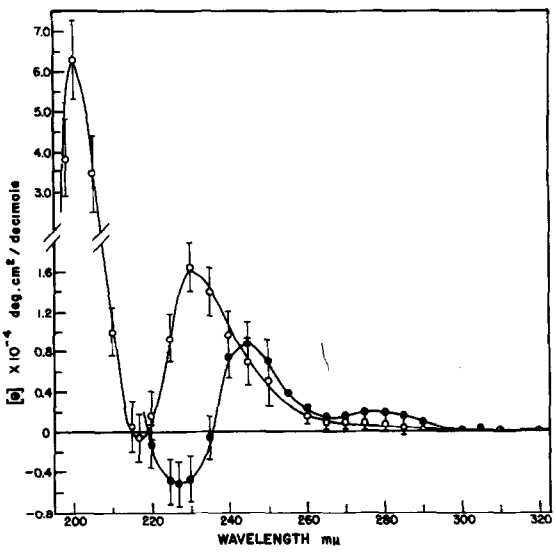

Fig. 3 CD spectra of L-(Tyr)$_n$, pH 10.6 (μ=0.1), (o) and pH 11.2 (μ=0.1), (●); concentrations used ranged from 0.078% to 0.018%.

shifted to 245 and 227mμ respectively. (The 200 mμ band was not investigated at this pH). While there is no change in the positions of the bands at 245μ

and 227 mμ at pH 11.2 (μ=0.2), (Beychok and Fasman's conditions), the amplitudes of these are increased by roughly 17% and 80% respectively compared to their values at pH 11.2 (μ=0.1). The positions of the bands at 245 and 227mμ agree essentially with those reported earlier (2), being displaced by ±3-4mμ; the 277mμ band is, however, displaced to the red by 7mμ. The UV spectrum at pH 11.2 (μ=0.2) is also similar but not identical to that previously reported (2)

On the basis of infrared measurements, Patrone et al. (7), have assigned a β antiparallel structure to poly-L-tyrosine at pH 11.25. The CD spectrum at pH 10.6 (Fig. 3) suggests however that the L-$(Tyr)_n$ is in the helical conformation with a small fraction of its residues ionized. Support of this is as follows; firstly, our CD spectrum at pH 10.6 is in qualitative agreement with the one reported by Shiraki and Imahori in methanol (4) and by Damle in trimethyl phosphate (6). The shape of the curve is approximately the same, and so are the positions of the bands. These authors have reported an extremely small negative band at 280mμ. However, in aqueous solution, we could not detect this band even with a cell of 5.0 cm path length. It can be noted that the UV spectra of L-$(Tyr)_n$ in methanol and in aqueous solution (pH 10.6) are very similar, both having a λmax at 277mμ. From their UV, CD, ORD and IR studies, Shiraki and Imahori (4) assigned an α-helical conformation to L-$(Tyr)_n$ in methanol (random coil in methanol + 6M LiCl).Also, on the basis of infrared dichroism and hydrodynamic measurements, Damle (6) assigned an α-helical conformation to L-$(Tyr)_n$ in trimethyl phosphate. Secondly, as previously discussed, there is a sharp and reversible transition of the specific rotation at 350mμ over a narrow pH range (Fig. 1). Thirdly, Chen and Woody (5) predicted on a theoretical basis that the CD spectrum of right-handed helical L-$(Tyr)_n$ in the un-ionized form should show a small positive band at 280mμ, a moderate positive band at 230mμ, a small negative band at 215mμ, and a large positive band at 250mμ. Of the four conformations considered by these authors (5), only the right-handed helix agrees with the CD spectra obtained in methanol (4) and in trimethyl phosphate (6), except in the area of 280mμ where a negative band is observed in these two solvents. Our CD spectrum in aqueous solution (pH 10.6) (Fig. 3) also agrees with the theoretical considerations of Chen and Woody (5), although a positive band was not found at 280mμ. The molar ellipticity in this region is slightly positive, as shown by a study using a 5.0cm cell. On the basis of these arguments, it appears that our study of the CD spectrum of L-$(Tyr)_n$ would provide the first experimental evidence for the existence of this polypeptide in the α-helical conformation in aqueous solution (pH 10.6). At pH 11.2 (μ=0.2), as studied previously by Beychok and Fasman (5), the polypeptide probably maintains the helical conformation (See Fig. 1), but with a considerable increase in ionization.

We will limit this discussion to the CD spectrum at pH 10.6. The 230mμ band has its origin in the π-π* transition of the absorption band of the phenol group (at 227mμ for L-$(Tyr)_n$) (4). The position of the 217mμ negative band results from the strong coupling of the phenolic absorption band (at 227mμ) with the peptide chromophore in the α-helix (4). As pointed out by Chen and Woody (5), in the case of the right-handed helical conformation of L-$(Tyr)_n$, the phenolic transition (227mμ) couples much more strongly with the amide π-π* transition than with the n-π* one. The 200mμ positive band can be assigned to the long wavelength component (parallel) of the peptide π-π* transition(5) An exact knowledge of the conformation of L-$(Tyr)_n$ in aqueous solutions is essential for a proper understanding of the interaction of this polypeptide with nucleic acids (1). In addition, CD studies of this polypeptide might provide valuable information for the assignment of the CD spectra of proteins which have a high content of tyrosine, such as T_1 ribonuclease (8).

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