Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Atreyi, M. , Rao, M. V. R. and Scaria, P. V.(1984) 'Conformation of Poly-(L-lysine) Containing Some Azoaromatic Side Chains', Journal of Macromolecular Science, Part A, 21: 1, 15 — 19
To link to this Article: DOI: 10.1080/00222338408065901
URL: http://dx.doi.org/10.1080/00222338408065901

J. MACROMOL. SCI.–CHEM., A21(1), pp. 15-19 (1984)

Conformation of Poly-(L-lysine) Containing Some Azoaromatic Side Chains

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ABSTRACT

The pH dependence of the conformation of poly-(L-lysine) with 4.6 mol% of its side chain amino groups attached to an azo dye, 4'-dimethyl aminoazobenzene-4-carboxylic acid, has been studied. Circular dichroic spectra showed that in acidic as well as in neutral media the polymer exists in the random coil conformation, like that of poly-(L-lysine). In basic medium the polypeptide acquires a β-structure, unlike poly-(L-lysine) which exists in an α-helical conformation.

Studies on the conformation of polypeptides containing azoaromatic groups attached to the side chains indicated that the conformation, in nonaqueous media, of the backbone of the polypeptide is fairly independent of the concentration and nature of the azoaromatic side chains [1-10]. The earlier work was mainly concerned with photoisomerization of the azoaromatic moieties in polymers of phenylalanine [1-5], aspartic acid [6-9], and glutamic acid [10]. Poly-L-lysine, unlike the above systems, is a highly water-soluble polypeptide. Results of studies on poly-L-lysine, with $\approx 5.0\%$ of its ϵ–NH_2 groups linked to 4'-dimethyl aminoazobenzene-4-carboxylic acid (DAAC) moieties, are reported.

0022-233X/84/2101-0015$3.50/0

EXPERIMENTAL

Materials and Methods

p-Aminobenzoic acid was obtained from Lobo Chem, India. Dimethylaniline was distilled under reduced pressure at 77°C, before use. Commercial grade solvents were all purified by reported methods [11]. Ultraviolet and visible spectra were taken with a Gilford 2600 UV/VIS Spectrophotometer, and the CD spectra were recorded with a JOBIN YVON Model III Dichrograph with cells of 0.5 mm path length.

Synthesis of Poly-(L-lysine)

Poly-ϵ-carbobenzoxy-L-lysine (PCBL) was prepared by the polymerization of a 4% solution of ϵ-carbobenzoxy-L-lysine-N-carboxy anhydride (A) in dioxane, using n-butylamine (I) as initiator with A/I = 200. After 48 h of reaction, the polypeptide was collected by precipitating with ether. The precipitate was washed free of any unreacted anhydride. The molecular weight of the sample was determined by intrinsic viscosity to be 40,000. The carbobenzoxy protecting group was removed with HBr/acetic acid (38%); the poly-L-lysine hydrobromide salt thus obtained was passed through an ion-exchange column (IRA-45), and the poly-L-lysine (PLL) obtained was freeze dried.

4'-Dimethyl Aminoazobenzene-4-carboxylic Acid (DAAC)

The procedure adopted for the preparation of DAAC was similar to the synthesis of Methyl Red (4'-dimethyl aminoazobenzene-2-carboxylic acid) [12]. 6.0 g of p-aminobenzoic acid was dissolved in a cold mixture of 5 mL of concentrated HCl and 15 mL of water, to which were added 25 g of ice and 7.5 mL of HCl with continuous stirring. 3.6 g of sodium nitrite in 7 mL of water was then added to the reaction mixture. The temperature of the reaction mixture was maintained between 0 and 5°C, and 8.8 mL of dimethylaniline was added and stirred. After an hour, 5 mL of a 57.8% solution of sodium acetate was added to the reaction mixture, and the stirring was continued for a further 4 h. The mixture was kept below 5°C overnight. Another aliquot of 5 mL of cold sodium acetate solution was added, and after 3 h the temperature of the reaction mixture was allowed to rise gradually to room temperature in 24 h.

24 mL of 4% sodium hydroxide solution was added to the reaction mixture with stirring. The mixture was allowed to stand at room temperature for 48 h and then filtered. The dye was washed with water, 10% acetic acid, and again with water and dried. The dye was recrys-

tallized from dimethylformamide (DMF). TLC in butanol:ethanol: ammonia (2 N) (6:2:2) gave a single spot. The melting point was 259-260°C.

Poly-(L-lysine)-DAAC Complex

70 mg (0.26 mmol) of DAAC was dissolved in 3 mL of DMF and 30 mg (0.26 mmol) of N-hydroxysuccinimide was added to it followed by the addition of 55 mg (0.26 mmol) of dicyclohexyl carbodiimide. The solution was kept at low temperature with stirring for about 5 h. The precipitated dicyclohexyl urea was filtered off and the resultant solution of active ester of DAAC was used in a later step.

500 mg of poly-(L-lysine) was taken in a 1:1 mixture of tetrahydrofuran (THF) and water, and 1.5 mL of DAAC active ester solution was added to it. Stirring at room temperature was maintained for 6 h. The solvent was evaporated and the solid polypeptide-dye complex was washed with small amounts of DMF and several parts of ether.

The polymer was highly soluble in water and was colored deep red. The amount of dye incorporated in the polymer was determined spectroscopically using the $E^{1\%}$ values of the dye at 280 and 450 nm in 11.9 pH solutions. The percentage incorporation of dye was found to be 9% (w/w), i.e., 4.6% (mol/mol of lysyl residues).

RESULTS AND DISCUSSION

The absorption spectra of DAAC shows three main absorption bands in the 191, 273, and 460 nm regions. The $\pi\pi^*$ transition of the azobenzene ($\simeq$ 325 nm) is shifted to the visible region (460 nm) on substitution of the $-N(CH_3)_2$ group at the 4' position. As the pH of the medium was lowered, the intensity of this absorption decreased and the half-width of the band significantly increased ($E^{1\%}$ (pH 6.9) = 1044 and $E^{1\%}$ (pH 2.8) = 476). This is probably due to protonation of the nitrogen, and also to the discharge of the carboxylate ion. An additional weak absorption around 540 nm appears at pH 2.8.

The absorption in the aromatic region is also dependent on pH. The absorption maximum at 273 nm, which is constant above pH 7.8, shows a red shift to 297 nm when the pH is brought to about 4.8 and the absorption band becomes complex with further lowering of pH to 2.8. This is also presumably due to the presence of a protonated species at the COO^- end and the $-N(CH_3)_2$ terminal.

A comparison of the absorption spectrum of the dye with that of the polylysine-dye complex revealed interesting differences in the pH-dependence characteristics.

The absorption spectrum of the polylysine-dye complex showed a red shift of the 460 nm absorption with an increase in intensity in

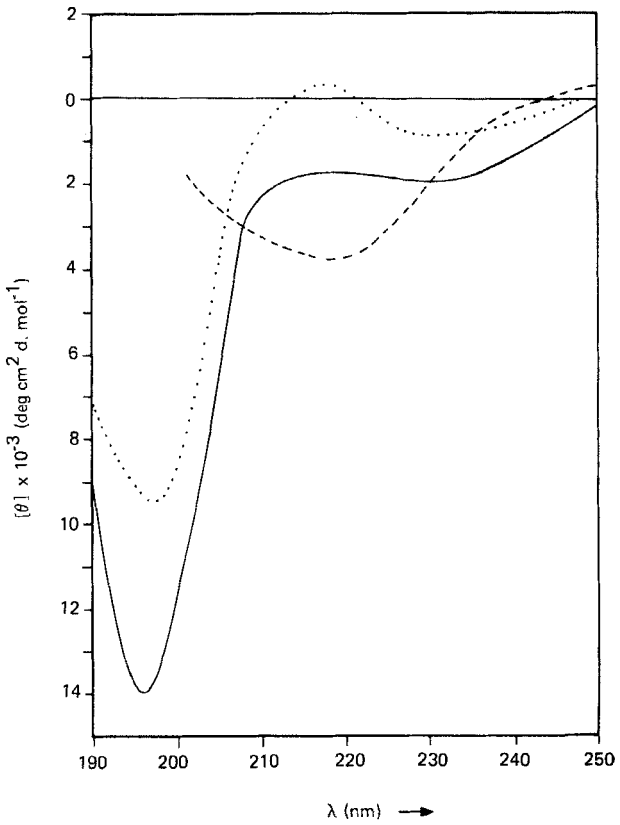

FIG. 1. CD spectra of aqueous solutions of poly-(L-lysine)-DAAC at various pHs: (···) pH 2.5, (—) pH 6.5, (--) pH 12.0.

acidic pH, unlike that of the free dye where a blue shift accompanied by a decrease in intensity was observed. The absorption near the 273 nm region remains more or less unaffected with pH while the band at 191 nm shows a red shift on increasing the pH.

Circular dichroic spectra of polylysine-dye complex, as a function of pH, are shown in Fig. 1. At acidic as well as neutral pH, the CD spectrum has the characteristics of a random coil/extended helical structure. This is similar to the behavior of PLL alone [13], suggesting that the dye component did not influence the conformation of the polypeptide backbone in the acidic pH range.

At higher pH (12.0), with the uncomplexed side chain ϵ–NH_2 groups discharged, the polypeptide is expected to be in the α-helical conformation [13]. Interestingly, however, the CD spectrum has features of

the beta conformation, with significantly reduced ellipticity at the band minimum. Obviously, the azoaromatic dye moieties in the side chains of polylysine hinder the formation of helical structure but support the beta structure. The rather low ellipticity indicates that even though the content of azoaromatic dye is only 4.6 mol%, the dye apparently sterically restricts formation of ordered structures. This observation is akin to that seen in the poly-L-glutamic acid–p-aminoazobenzene system; a system with 36% incorporation of the dye was found to have a beta conformation at acidic pH instead of the expected α-helical conformation [10].

ACKNOWLEDGMENT

Thanks are due to the Director, CDRI, Lucknow, for the circular dichroism measurements.

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Accepted by editor April 10, 1983
Received for publication May 18, 1983