Conformational Studies of Sequential Polypeptides Containing L-β-(3,4-Dihydroxyphenyl)-α-alanine (Dopa) and L-Lysine

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ABSTRACT: The conformations of five sequential polypeptides, poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa), poly(L-lysyl-L-lysyl-L-Dopa), poly(L-lysyl-L-Dopa), poly(L-Dopa-L-Dopa-L-lysine), and poly(L-Dopa-L-Dopa-L-Dopa-L-lysine), have been studied by means of circular dichroism. The conformations depend on the amino acid sequence, the ionization state of the amino and catechol side chains, the solvent, and temperature. When lysine content is greater than 67 mol %, the sequential polypeptides show a tendency to adopt a helical conformation in alkaline solution below 50 °C or in alcohol-rich solutions and a β-structure above 60 °C. The poly(dipeptide) assumes a β-structure. When aromatic Dopa content is greater than 67 mol %, sequential polypeptides fold into a helical conformation below pH 7. A discussion is presented that includes results on other aromatic sequential polypeptides.

Introduction

L-β-(3,4-Dihydroxyphenyl)-α-alanine (Dopa) is recognized as an intermediate in the biosynthesis of melanin pigments and catecholamine hormones and has been used in the treatment of the dopamine deficiency of Parkinson patients.[1,2] Recently, Waite et al. reported that some of nature's most powerful adhesives secreted by marine molluscs such as mussels, oysters, and barnacles, which must routinely cope with the force or surf and tides, are Dopa-containing proteins (up to 70 residues/1000).[3-7] The present conformational study deals with Dopa-containing synthetic polypeptides.

The conformational study of model sequential polypeptides continues to provide much valuable information about the conformations of natural proteins and synthetic polypeptides. The factors that influence the conformations are many and include the amino acid composition, the sequence, the solvents, pH, and temperature. Especially, in all sequential polypeptides containing aromatic side chains, the interpretation of circular dichroism (CD) data is not straightforward. Recently, conformational studies, based mainly on CD analysis, of some phenylalanine- or tyrosine-containing sequential polypeptides have been reported. Briefly, the CD spectra of poly(L-lysyl-L-phenylalanine),[8] poly(L-tyrosyl-L-lysine),[9] and poly(L-tyrosyl-L-glutamic acid)[10] showed negative ellipticities: $[\theta]_{217}$ = −14 000 at pH 9.6, $[\theta]_{220}$ = −8000 at pH 11.5, and $[\theta]_{215}$ = −5000 at pH 9.6, respectively. These three sequential polypeptides were assigned a β-structure. On the other hand, depending upon the pH and solvent composition, poly(L-tyrosyl-L-lysyl-L-lysine) and poly(L-tyrosyl-L-lysyl-L-lysyl-L-lysine) adopted α-helical or random coil conformations.[9] The CD values of the poly(tripeptide) were $[\theta]_{220}$ = −10 500 at pH 11.5 and $[\theta]_{218}$ = 0 at pH 7. The poly(tetrapeptide) gave the ellipticities $[\theta]_{220}$ = −12 000 at pH 11.5 and $[\theta]_{218}$ = 0 at pH 7.

In previous papers of this series we reported conformational studies of poly(L-Dopa) and a series of random and sequential copolypeptides of L-Dopa with L-glutamic acid.[11-13] Unfortunately, since most of these peptides were only slightly soluble in water (even at alkaline pH), the conformational studies were limited to the use of trimethyl phosphate (TMP), TMP–water mixed solvents, and dimethyl sulfoxide (Me_2SO) as solvents. To study poly(L-Dopa) further, we extended the conformational studies to include water-soluble sequential polypeptides containing L-lysine. Since the difference between phenylalanine, tyrosine, and Dopa is the number of hydroxyl groups in the benzene ring, the spectral data on poly(dipeptides), poly(tripeptides), and poly(tetrapeptides) containing L-Dopa are useful in elucidating their optical properties and in studying the conformations of aromatic sequential polypeptides.

Experimental Section

Materials. The synthesis of the five sequential polypeptides poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) (DP (as total amino acid residues) = 120), poly(L-lysyl-L-lysyl-L-Dopa) (DP = 100), poly(L-lysyl-L-Dopa) (DP = 110), poly(L-Dopa-L-Dopa-L-lysine) (DP = 80), and poly(L-Dopa-L-Dopa-L-Dopa-L-lysine) (DP = 110) has been reported elsewhere.[14] The molecular weights were estimated from the intrinsic viscosities for the corresponding precursor sequential polypeptides (see details in ref 14). All five sequential polypeptides are soluble in *N,N*-dimethylformamide (DMF), Me_2SO, hexafluoroacetone sesquihydrate, and dichloroacetic acid and insoluble in 1,1,1,3,3,3-hexafluoro-2-propanol and 2,2,2-trifluoroethanol (TFE). L-Lysine-rich sequential polypeptides are soluble in water, 0.1 M sodium chloride solution, and up to 98:2 (v/v) methanol–water and TFE–water mixtures at all pHs. L-Dopa-rich sequential polypeptides are soluble in additional organic solvents such as methanol, TMP, and trifluoroacetic acid but insoluble in water between pH 7.5 and pH 11.

Methods. CD spectra were obtained with a Jasco CD J-40A instrument, made by Japan Spectroscopic Co. Ltd., under constant nitrogen flush. Constant temperature was maintained by circulating water or chilled ethanol through the jacket of a specially designed cell holder from a Haake F3-K constant-temperature bath. The temperature of the solution in the cell was measured with a Takara thermistor. Cells with path lengths of 0.1–10 mm were used, and the concentrations of the sample were in the 0.043–0.0006% range. The experimental data were expressed in terms of mean residue ellipticity $[\theta]$ ((deg cm^2)/dmol).

Results

Charge-Induced Conformational Transitions. CD spectra of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa), poly(L-lysyl-L-Dopa), and poly(L-Dopa-L-Dopa-L-Dopa-L-lysine) in 0.1 M sodium chloride are shown in Figures 1–3, respectively. Below pH 8 (Figure 1, curves A and B), when the lysyl side chains are fully protonated and the Dopa side chains are un-ionized, the spectra show maxima at 280, 230, and 215 nm and troughs at 305 and 250 nm. At pH 13.1 (Figure 1, curve D), when the lysyl side chains are un-ionized and the Dopa side chains are fully ionized, the spectrum shows a maximum at 310 nm and troughs at 222 and 212 nm. At pH 10.1 (Figure 1, curve C), when both side chains are mostly un-ionized and partially zwitterionic, the spectrum shows maxima at 305 and 230 nm. CD spectra of poly(L-lysyl-L-Dopa) and poly(L-Dopa-L-Dopa-L-Dopa-L-lysine) in 0.1 M sodium chloride at acidic, neutral, and alkaline pH are shown in Figures 2 and 3 (see also Table I). However, CD spectra of Dopa-rich (more than 50 mol %) sequential polypeptides could not be observed

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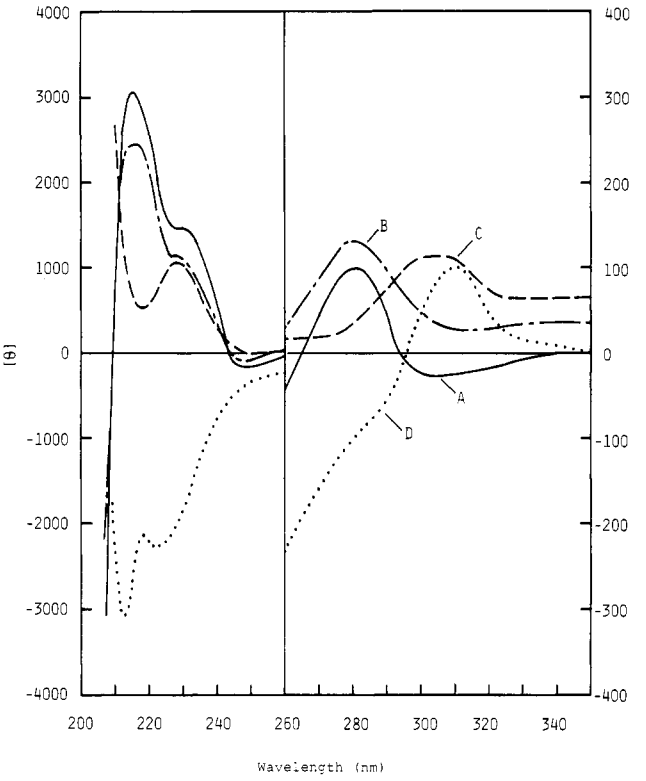


Figure 1. CD spectra of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) in 0.1 M sodium chloride as a function of pH at 25 °C: (A) pH 4.3; (B) pH 8.0; (C) pH 10.1; (D) pH 13.1.

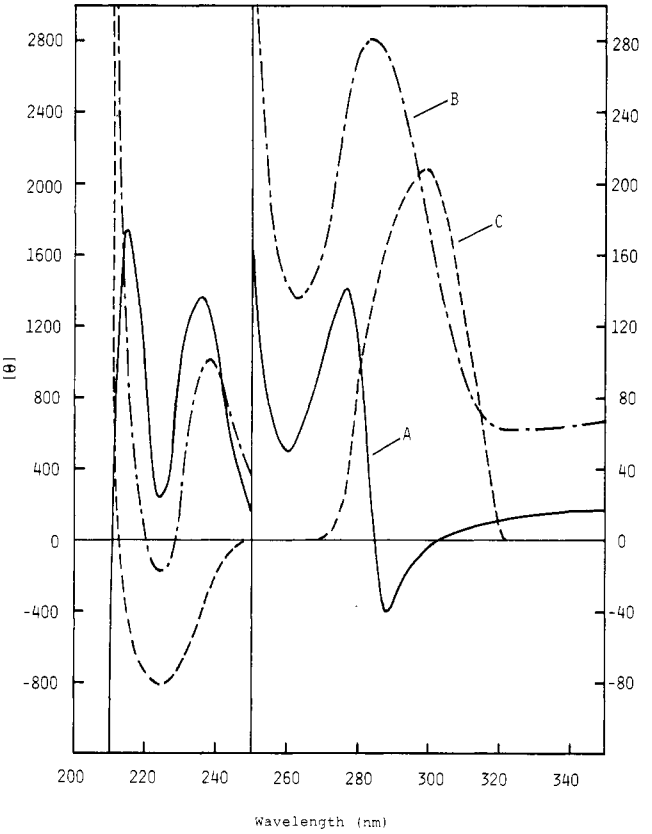


Figure 2. CD spectra of poly(L-lysyl-L-Dopa) in 0.1 M sodium chloride as a function of pH at 25 °C: (A) pH 4.4; (B) pH 8.4; (C) pH 12.9.

at intermediate pH (pH 8–11) because of insolubility.

Figures 4 and 5 show plots of ellipticities at 280 nm and of $[\Theta]_{max}$ wavelengths in the region 280–310 nm for the five sequential polypeptides as a function of pH. In the case

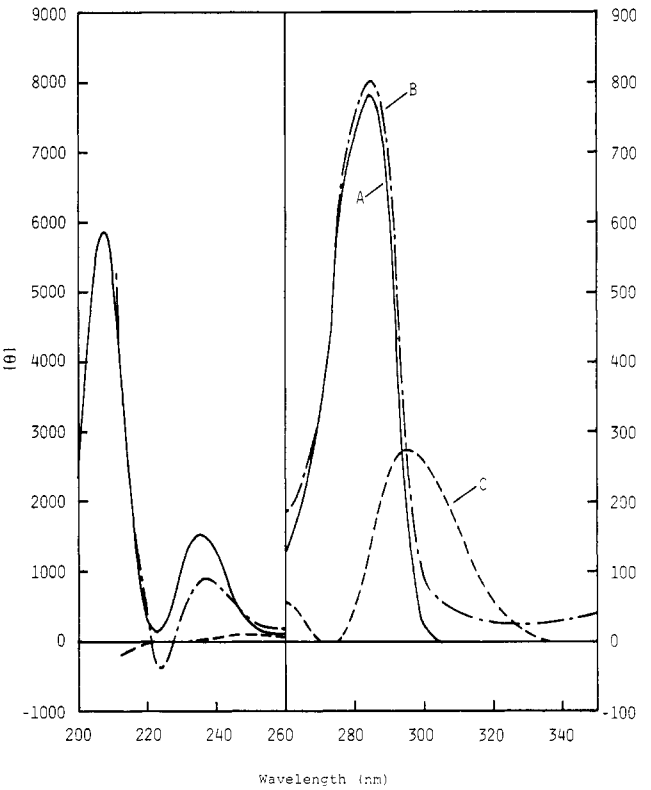


Figure 3. CD spectra of poly(L-Dopa-L-Dopa-L-Dopa-L-Lysine) in 0.1 M sodium chloride as a function of pH at 25 °C: (A) pH 4.4; (B) pH 7.0; (C) pH 12.6.

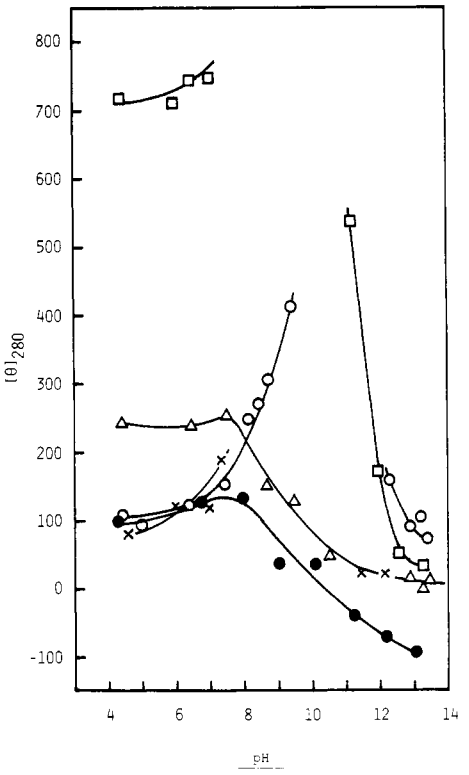


Figure 4. Plots of ellipticities at 280 nm of five sequential polypeptides as a function of pH at 25 °C: (●) poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa); (△) poly(L-Lysyl-L-lysyl-L-Dopa); (○) poly(L-lysyl-L-Dopa); (×) poly(L-Dopa-L-Dopa-L-lysine); (□) poly(L-Dopa-L-Dopa-L-Dopa-L-lysine).

of poly(L-Dopa) in 0.2 M sodium chloride–TMP (1:1 (v/v)), the bands at 280 nm with $[\Theta]_{280}$ = 2000 and at 230 nm with $[\Theta]_{230}$ = 3800 below pH 10.4 (un-ionized catechol side chains and helical) shifted to 295 nm with $[\Theta]_{295}$ = 1500

Table I
CD Data of the Polypeptides Presented in This Work

polypeptide	pH (conditions)	ellipticity (wavelength, nm), (deg cm²)/dmol
Lys-Lys-Lys-Dopa	4.3 (0.1 M NaCl)	100 (280), 1480 (230), 3080 (215)
	13.1 (0.1 M NaCl)	100 (310), −2280 (222), −3080 (213)
	6.8 (98% MeOH)	−9100 (222), −11000 (207)
	5.9 (98% TFE)	−8400 (222), −9000 (207)
Lys-Lys-Dopa	4.4 (0.1 M NaCl)	240 (280), 1560 (233), 1250 (225), 2800 (214)
	13.2 (0.1 M NaCl)	60 (300), −2400 (211)
	6.8 (98% MeOH)	−14100 (221), −15400 (207)
	5.9 (95% TFE)	−11200 (222), −13900 (207)
Lys-Dopa	4.4 (0.1 NaCl)	−40 (288), 140 (277), 1360 (236), 230 (224), 1740 (215)
	12.9 (0.1 M NaCl)	210 (299), −800 (225)
	4.4 (98% MeOH)	−4650 (224)
	3.3 (98% TFE)	−3300 (224), 6000 (202)
Dopa-Dopa-Lys	4.6 (0.1 M NaCl)	−50 (292), 120 (274), 600 (236), −370 (225), 1000 (215)
	12.2 (0.1 M NaCl)	65 (302), 26 (277)
	5.1 (98% MeOH)	−9100 (222)
	4.0 (98% TFE)	−7000 (223)
Dopa-Dopa-Dopa-Lys	4.4 (0.1 M NaCl)	780 (285), 1500 (235), 300 (223), 5900 (207)
	12.6 (0.1 M NaCl)	275 (295)
	3.8 (98% MeOH)	−3300 (224)
	3.1 (98% TFE)	−2500 (225)

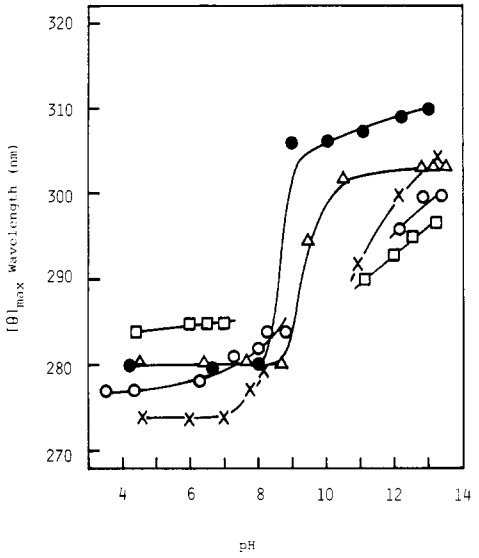


Figure 5. Plots of the $[\theta]_{max}$ wavelengths of five sequential polypeptides as a function of pH at 25 °C: (●) poly(L-lysyl-L-lysyl-L-lysyl-L-lysyl-L-Dopa); (△) poly(L-lysyl-L-lysyl-L-Dopa); (○) poly(L-lysyl-L-Dopa); (×) poly(L-Dopa-L-Dopa-L-lysine); (□) poly(L-Dopa-L-Dopa-L-Dopa-L-lysine).

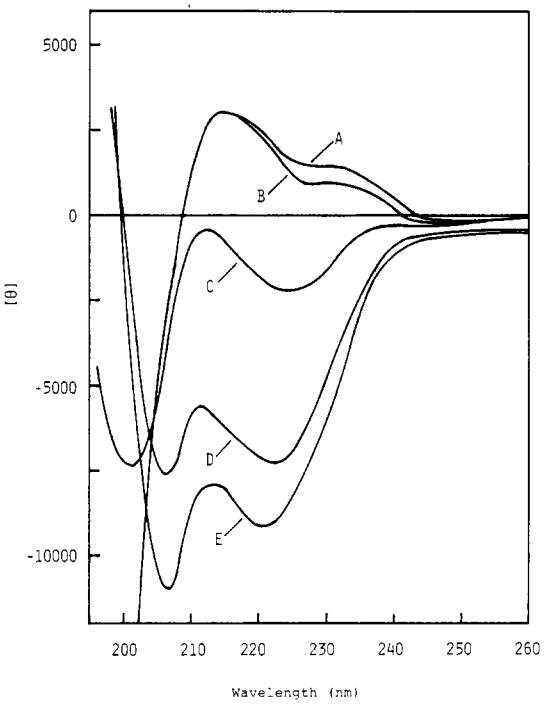


Figure 6. CD spectra of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) as a function of methanol concentration (v/v) in water at 25 °C: (A) 0–50% MeOH; (B) 75% MeOH; (C) 90% MeOH; (D) 95% MeOH; (E) 98% MeOH; pH 6.8.

and could not be measured below 260 nm above pH 11.5 owing to high absorption (ionized catechol and random coil).[11] Thus, the CD peak position near 280 nm is sensitive to the ionization state of the catechol ring and may reflect the conformational state of the Dopa side chains. On the other hand, poly(L-lysine) is known to give troughs at 222 and 206 nm with $[\theta] = -40\,000$ when in a right-handed helix above pH 10 (un-ionized) and a single trough at 195 nm with $[\theta]_{195} = -35\,000$ for the random coil below pH 10 (ionized). In the present sequential polypeptides containing Dopa and lysine the 274–284-nm peak below pH 7 shifted to 296–310 nm at pH 13. Simultaneously, changes due to the lysyl component occurred in the 200–240-nm region. The n–π^* transition at 230 nm and the parallel-polarized π–π^* exciton transition at 215 nm of the peptide group, which involves the contribution of the substituted benzenes, changed sign from positive (below pH 10) to negative (at pH 13) and increased the negative ellipticities from 1450 to −1800 (230 nm) and from 3100 to −2800 (215 nm). These increases in negative CD suggest a random coil to (partial) helical transition when the sequential polypeptide is rich in lysine (see Figure 1).

Solvent-Induced Conformational Transition. Figures 6 and 7 show the [θ] values for poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) in water–methanol and water–TFE mixed solvents (pH 6–7). This polypeptide shows transitions from a random coil to the α-helical conformation with increasing methanol or TFE concentrations.[15–17] Since poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) is α-helical in 75% TFE and is a random coil structure in 75% methanol, TFE is more effective in inducing the conformational transition. However, ellipticities at 222 and 206 nm in 98% alcohol seem low compared with the usual values of α-helical n–π^* and π–π^* peptide transitions.

Temperature-Induced Conformational Transition. The helical form of poly(L-lysine) can be converted into the β-form upon heating.[18–21] Likewise, mild heating of alkaline solutions of poly(L-lysyl-L-lysyl-L-Dopa) and

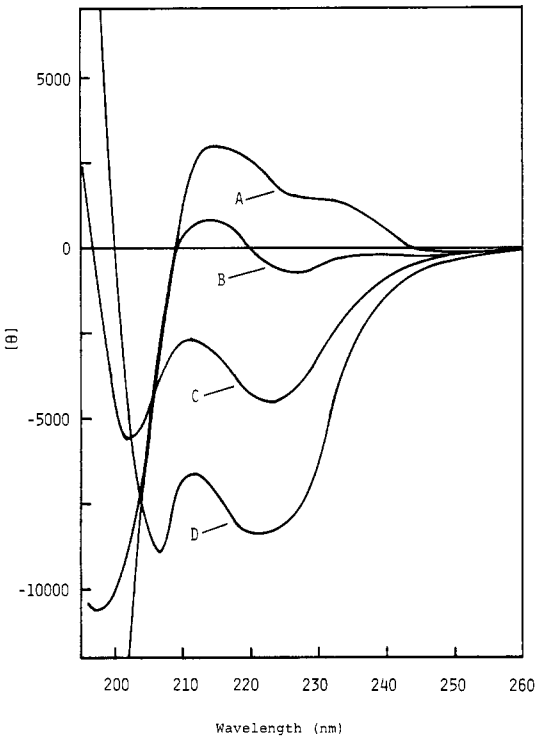


Figure 7. CD spectra of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) as a function of TFE concentration (v/v) in water at 25 °C: (A) 0% TFE; (B) 50% TFE; (C) 75% TFE; (D) 90–98% TFE; pH 5.9.

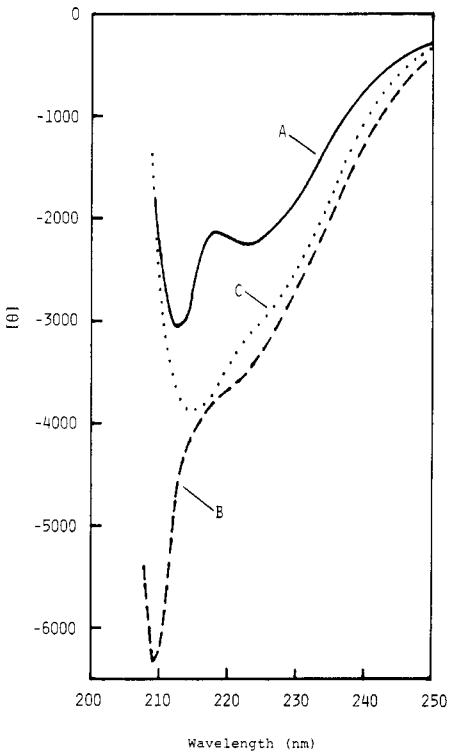
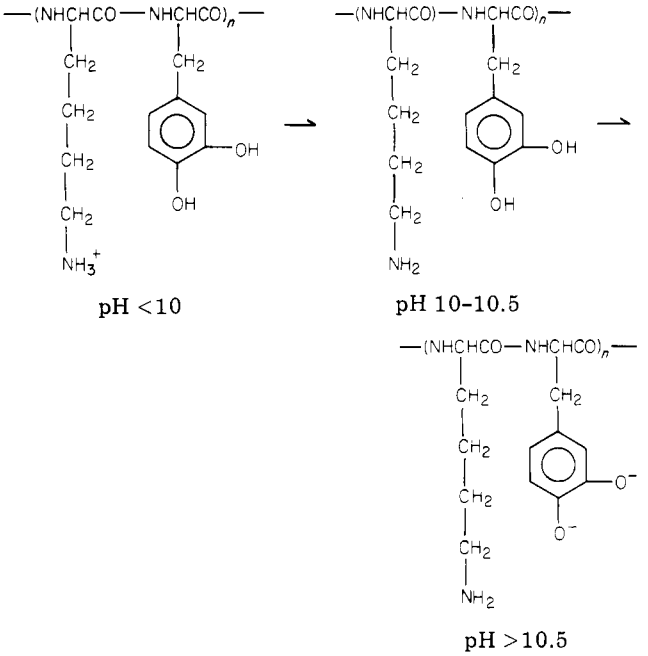


Figure 8. Temperature-induced conformational transition of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) at pH 12.8. After the CD spectrum of the sample was recorded at 25 °C (curve A), the sample was heated to 50 °C for about 30 min (curve B) and additionally heated to 68 °C for about 30 min (curve C).

poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) causes changes in CD spectra and ellipicities. Figure 8 shows the CD spectra of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) at three different temperatures. Increasing the temperature from 25 to 50 °C and then additionally to 68 °C first causes a blue shift in the π–π^* trough and then a red shift. The temperature-induced conformational transition based on $[\theta]_{222}$ is shown in Figure 9. Poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) enhances the negative ellipticity very much ($[\theta]_{222}$ = –960 and –3500 at 0.5 and 50 °C, respectively), and above 60 °C the double minimum of the helical CD spectrum at 25 °C changed into a single minimum near 215 nm with reduced magnitude.

Discussion

In aqueous solution at 25 °C un-ionized poly(L-lysine) above pH 10 is known to exist in a right-handed α-helix and ionized poly(L-lysine) is a random coil structure.[24] Likewise, in water–TMP (1:1 (v/v)) mixed solvents un-ionized poly(L-Dopa) below pH 10.5 assumes a right-handed helical structure and ionized poly(L-Dopa) a random coil.[11] To put it simply, sequential polypeptides containing L-Dopa and L-lysine are assumed to be mostly un-ionized and partially zwitterionic between pH 10 and pH 10.5; above pH 10.5 they have ionized hydroxyl groups and below pH 10 ionized amino groups, as shown below:



To understand the nature of aromatic polypeptides, we summarize briefly ellipticity values of random and sequential copolypeptides of Dopa and glutamic acid from our own work[13] and of tyrosine and glutamic acid from the work of Trudelle and Spach.[25] In random copolypeptides, ellipticity vs. Dopa or tyrosine content showed a smooth variation, without any sharp changes. On the other hand, in sequential polypeptides the ellipticities at 285 and 222 nm depended on the Dopa or tyrosine residue array.[13,25] The present sequential polypeptides containing Dopa and lysine showed a positive dichroic band at 280–310 nm, which has been assigned to the arrangement of the induced dipole moments of the stacked side chain.[26] Figures 4 and 5 show the CD spectra in the 280-nm region. Clearly, there is no simple relationship between Dopa content and the position or ellipticity of the 280-nm band. The band at 280 nm is overlapped by the contribution of the ^1L_a (210–220 nm) and ^1L_b (255–275 nm) trantisions of the substituted benzenes in the Platt notation.[27,28] Additionally, below 250 nm there is a strong contribution of the n–π^* and π–π^* peptide transitions.[29] Thus, the arrays of aromatic side chains influence not only the CD band positions but also the rotational strengths (see Table I).[25]

The addition of water-miscible organic solvents to aqueous solutions (pH 3–7) of sequential polypeptides

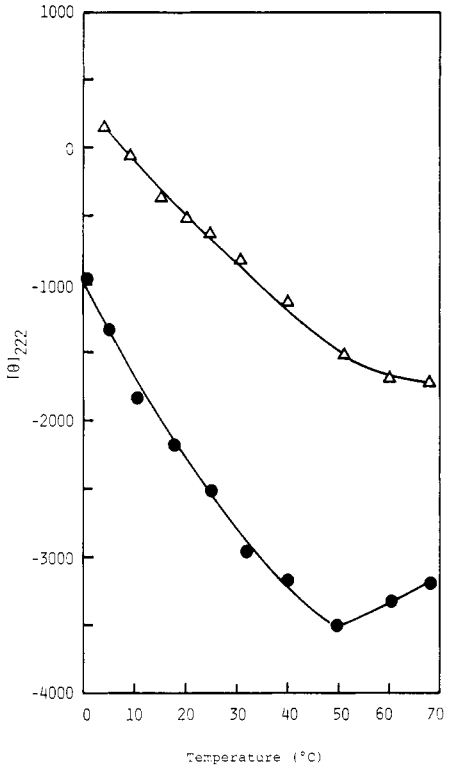


Figure 9. Changes in ellipticity with temperature for aqueous solutions of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) (●) and poly(L-lysyl-L-lysyl-L-Dopa) (△) at pH 12.8.

containing L-lysine and L-Dopa lowers the dielectric constant of the solvent and stabilizes ordered conformations of the polypeptides.[9,24] The effect of the addition of TFE or methanol on the CD spectrum of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) is illustrated in Figures 6 and 7. At 0–75% methanol concentrations, methanol shows no effect. At higher methanol concentrations (95–98%), the CD spectrum indicates a substantial degree of helicity (Figure 6). TFE is more effective than methanol since the former is more organic (hydrophobic) than the latter (Figure 7). Lysine-rich polypeptides increased their helicities with increasing alcohol concentration. Here again, the ellipticity values reflect the array of hydrophilic and hydrophobic amino acids. At 98% methanol the molar ellipticity values of poly(L-lysyl-L-lysyl-L-Dopa) are $[\theta]_{222} = -14\,000$ and $[\theta]_{207} = -15\,400$, and those of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) are $[\theta]_{222} = -9100$ and $[\theta]_{207} = -11\,000$. The reason is not clear, but this dichroic tendency surprisingly agrees with a similar finding by Pierre et al.,[9] who reported ellipticity values of $[\theta]_{222} = -19\,000$ and $[\theta]_{209} = -21\,000$ for poly(L-tyrosyl-L-lysyl-L-lysine) and $[\theta]_{222} = -14\,000$ and $[\theta]_{208} = -17\,000$ for poly(L-tyrosyl-L-lysyl-L-lysyl-L-lysine) in 90% methanol (note that the [θ] values in the figures and in the table in ref 9 are contradictory).

In aqueous solution at 25 °C un-ionized poly(L-lysine) exists in α-helical form and can be converted into the β-structure upon mild heating at 50 °C or higher temperatures.[18-21] Lowering the temperature from 25 to 10 °C enhanced the helicity of poly(L-lysine) from $[m]_{233} = -12\,700$ to $[m]_{233} = -13\,600$. In analogy to protein denaturation, poly(L-lysine) became helical at low temperature and disordered at high temperature.[24] At first glance, the temperature dependence of the ellipticities for L-lysine-rich sequential polypeptides showed the reverse results (Figure 8) and both poly(L-lysyl-L-lysyl-L-Dopa) and poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) displayed an "inverse" temperature-induced transition; i.e., higher temperature favors the helical form. However, from the π–π^* trough position, increasing the temperature first causes an increase in random coil content and then leads to β-association. Above 60 °C poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa) adopted the partial β-structure (Figure 8). We tested the reversibility of the α (partial)-to-β (partial) transition with the alkaline solution (pH 12.8) of the β-form of poly(L-lysyl-L-lysyl-L-lysyl-L-Dopa). In one cooling experiment after 17 h, the original spectrum (Figure 8, curve A) was regenerated at 25 °C. This finding means that the helical form is favored over the β-form at lower temperature (25–50 °C).

The results of our conformational studies of five sequential polypeptides containing L-lysine and L-Dopa are summarized as follows. First, from the CD data, when lysine content is greater than 67 mol %, sequential polypeptides composed of lysine and Dopa show a tendency to adopt a helical conformation in alkaline solution at room temperature or in alcohol-rich solutions and a β-structure above 60 °C. Sequential poly(L-lysyl-L-Dopa), with 50 mol % lysine content, takes a β-structure, in agreement with similar findings by Seipke et al.,[8,30] Pierre et al.,[9] and Trudelle.[10] When the Dopa content is greater than 67 mol %, sequential polypeptides assume the α-helical conformation below pH 7 at 25 °C based on the CD peak position at 274–285 nm, the IR frequencies for the amide I band, the NMR α-CH chemical shifts,[11] and a copolymer study to confirm the helical sense.[12] Next, the conformations of poly(L-lysyl-L-phenylalanine),[8,30] poly(L-tyrosyl-L-lysine),[9] and poly(L-lysyl-L-Dopa) (this study) were assigned a β-structure. The only difference between the three aromatic amino acids is that phenylalanine has no hydroxyl group, tyrosine one hydroxyl group at the 4-position, and Dopa two hydroxyl groups at the 3- and 4-positions in the benzene ring. However, the CD ellipticities and positions (peak and trough) considerably differed from each other. The ellipticities near 220 nm (215–225) decreased in magnitude in the order −14 000 (phenylalanine polypeptide), −8000 (tyrosine polypeptide), and −800 (Dopa polypeptide) with increasing hydrophilic properties in the side chains. Roughly, the less hydrophobic a polypeptide is, the smaller ellipticity it gives. Conformational studies of 3- or 4-monosubstituted and 3,4-disubstituted (amino, nitro, hydroxyl, methoxy, and carboxyl) aromatic amino acids are now in progress to gain further insight into the optical activity of aromatic sequential polypeptides.

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References and Notes

(1) Birkmayer, W.; Hornykiewicz, O. *Wien. Klin. Wochenschr.* **1961**, *73*, 787.
(2) Cotzias, G. C.; Van Woert, M. H.; Schiffer, L. M. *N. Engl. J. Med.* **1967**, *276*, 374.
(3) Waite, J. H.; Anderson, S. O. *Biochim. Biophys. Acta* **1978**, *541*, 107.
(4) Waite, J. H.; Saleuddin, A. S. M.; Anderson, S. O. *J. Comp. Physiol.* **1979**, *130B*, 301.
(5) Waite, J. H.; Anderson, S. O. *Biol. Bull.* **1980**, *158*, 164.
(6) Waite, J. H.; Tanzer, M. L. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 1554.
(7) Waite, J. H.; Tanzer, M. L. *Science (Washington, D.C.)* **1981**, *212*, 1038.
(8) Seipke, G.; Arfmann, H. A.; Wagner, K. G. *Biopolymers* **1974**, *13*, 1621.
(9) Pierre, S. S.; Ingwall, R. T.; Verlander, M. S.; Goodman, M. *Biopolymers* **1978**, *17*, 1837.
(10) Trudelle, Y. *Polymer* **1975**, *16*, 9.
(11) Yamamoto, H.; Hayakawa, T. *Polymer* **1977**, *18*, 979.
(12) Yamamoto, H.; Hayakawa, T. *Polymer* **1978**, *19*, 1115.
(13) Yamamoto, H.; Hayakawa, T. *Int. J. Biol. Macromol.* **1982**, *4*, 258.
(14) Yamamoto, H.; Hayakawa, T. *Biopolymers* **1982**, *21*, 1137.
(15) Epand, A. R. F.; Scheraga, H. A. *Biopolymers* **1968**, *6*, 1383.

(16) Liem, R. K. H.; Poland, D.; Scheraga, H. A. *J. Am. Chem. Soc.* **1970**, *92*, 5717.
(17) Yamamoto, H.; Hayakawa, T.; Yang, J. T. *Biopolymers* **1974**, *13*, 1117.
(18) Sarker, P. K.; Doty, P. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 981.
(19) Townend, R.; Kumosinski, T. F.; Timasheff, S. N.; Fasman, G. D.; Davidson, B. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 163.
(20) Snell, C. R.; Fasman, G. D. *Biochemistry* **1973**, *12*, 1017.
(21) Yamamoto, H.; Yang, J. T. *Biopolymers* **1974**, *13*, 1109.
(22) Davidson, B.; Fasman, G. D. *Biochemistry* **1967**, *6* 1616.
(23) Miyazawa, T. "Polyamino Acids, Polypeptides and Proteins"; University of Wisconsin Press: Madison, WI, 1962, p 201.
(24) Grourke, M. J.; Gibbs, J. H. *Biopolymers* **1971**, *10*, 795.
(25) Trudelle, Y.; Spach, G. *Polymer* **1975**, *16*, 16.
(26) Horie, H.; Toda, F. Proceedings of the 35th Annual Metting of the Chemical Society of Japan, 1976, p 69.
(27) Platt, J. R. *J. Chem. Phys.* **1949**, *17*, 484.
(28) Woody, R. W. *Biopolymers* **1978**, *17*, 1451.
(29) Beychok, S. "Poly-α-Amino Acids"; Marcel Dekker: New York, 1967; p 293.
(30) Seipke, G.; Arfmann, H. A.; Wagner, K. G. *Biopolymers* **1980**, *19*, 189.

On the Realization of the Helix–Coil Theory for Protein Chains in Solutions Containing Dodecyl Sulfate. Experiments on α-Tropomyosin and Bovine Serum Albumin

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ABSTRACT: Measurements are reported of α-helix content vs. temperature for reduced rabbit α-tropomyosin (α-Tm) and for reduced bovine serum albumin (BSA) in near-neutral aqueous solutions containing dodecyl sulfate anions (DS^-). The results for the two proteins are similar, α-Tm being somewhat more helical than BSA. The helix content for both is independent of substitution of Li^+ for Na^+, protein concentration (0.1–12 mg mL^{-1}), added salt concentration (33–500 mM), and DS^- concentration (18–30 mM). Increasing temperature reduces helix content, but in a rather noncooperative manner. In α-Tm, the results are almost the same whether or not each 284-residue protein chain is cross-linked to another at Cys 190. A recent prescription for the calculation of the helix content of proteins in DS^--added systems from the statistical mechanical theory of the helix–random coil transition is examined and found to be in serious disagreement with experiment, even when certain parameters are fixed by the experiments themselves. A detailed analysis of the prescription suggests the following: (1) The underlying idea, that only helix and random coil conformations need be considered for proteins in DS^-, may be correct; thus, the formal theory, which only accounts for short-range interactions (through parameters σ and $s(T)$), may be conceptually adequate. (2) The difficulties probably arise from flaws in the method for obtaining the input parameters appropriate to the DS^--added system from those presently available for the DS^--free system. The desirability of independent measurements of the input parameters for the DS^--added case is stressed.

I. Introduction

Over a period of some years, a statistical mechanical theory has been developed for treating the equilibrium between α-helical and randomly coiled conformations of single polypeptide chains in solution.[1] The theory assumes the equilibrium is dominated by "short-range" interactions, which are embodied in two types of parameters, the helix-initiation parameter (σ) and the helix-propagation parameter [$s(T)$], the latter being temperature dependent.

Each type of amino acid residue (e.g., alanine is expected to have its own characteristic σ and $s(T)$, and the values appropriate to aqueous medium have been determined and tabulated for virtually all the types of residues found in proteins, thus allowing the theory to be realized.[2-17] However, the theory's exclusion of long-range interactions makes it inapplicable to native protein molecules in aqueous media, since these display prominent tertiary and quaternary structural features. A beginning has been made in extending the theory to encompass those long-range interactions peculiar to two-chain, α-helical, coiled coils,[18-21] but these represent a very limited class of proteins, and, moreover, it is a bit early to judge the success of the endeavor.

Mattice et al. pointed out that the theory, even without such extension, might be applicable to single protein chains if all disulfide bonds are reduced and the chains dissolved in sodium dodecyl sulfate (SDS[22]) solutions, since there is evidence that the dodecyl sulfate anion (DS^-) in sufficient concentration disrupts tertiary and quaternary interactions, leaving only the short-range interactions to dictate the conformation.[23,24] In that work, Mattice et al. not only present this idea as a series of explicit assumptions bearing on the applicability of the formal theory but also give the further assumptions needed to realize the theory in DS^--added media (for which no experimental values of σ and $s(T)$ are available). Furthermore, they present enough calculations and experimental results for proteins near room temperature to demonstrate the promise of the approach.[23-25]

This is potentially a very germinal proposal not only because it bears on the question of limits of validity of the developing theory of polypeptide conformations but also because of the important role played by detergent solutions in the biochemical study of proteins. It is very common to study proteins in such media (for gel electrophoresis, for example), and, indeed, many important proteins (e.g., some membrane-bound proteins) have solubility properties that disallow solution studies in the absence of detergent. It would thus be highly desirable to have a theory that would allow calculation of the chain conformation in the DS^--added regime. Indeed, if such a reliable theory were available, it would allow one to calculate such things as local stabilities at specific chain sites[23]—information difficult or impossible to obtain by experiment.

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