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Conformation of β-endorphin and β-lipotropin: Formation of helical structure in methanol and sodium dodecyl sulfate solutions

(polypeptide hormone/opiate-like peptide/circular dichroism/intrinsic viscosity/sedimentation coefficient)

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ABSTRACT **Circular dichroic spectra of camel β-endorphin and ovine β-lipotropin in water show little, if any, secondary structure. Intrinsic viscosities and sedimentation coefficients of the two peptides also suggest that the molecules are not compact and globular. Methanol or sodium dodecyl sulfate promotes the formation of helical structure to an extent as much as one-half of either peptide molecule. The conformation of the complex between camel β-endorphin and dodecyl sulfate may be related to the opiate-like function of this peptide hormone.**

The presence of an endogenous ligand for the opiate receptor found in brain tissue has been demonstrated by Terenius, Snyder, Simon, and their coworkers (1–3), using a modification of a method suggested by Goldstein *et al.* (4). Two pentapeptides with opiate-like activity (Met-enkephalin, H-Tyr-Gly-Gly-Phe-Met-OH, and Leu-enkephalin, H-Tyr-Gly-Gly-Phe-Leu-OH) were isolated from pig brains by Hughes *et al.* (5). A polypeptide with potent opiate-like activity was isolated from camel pituitary glands by Li and Chung (6), and its amino acid sequence, identical to the COOH-terminal 31 amino acid residues of sheep β-lipotropin (7–9), was determined (6). This peptide was designated β-endorphin. The enkephalins (5) are related to the first five NH_2-terminal residues of β-endorphin (6). Both camel and human endorphins have been synthesized (10, 11). Their sequences (6, 12) differ in only two positions (see Fig. 1). These two molecules are equipotent in both *in vitro* (12) and *in vivo* (11) bioassays for opiate-like activity.

The conformations of the enkephalins, β-endorphin, and β-lipotropin have been investigated by several groups. Proton magnetic resonance studies have suggested that Met-enkephalin exists as a single β-turn in fully deuterated dimethyl sulfoxide (13–16), while circular dichroism (CD) studies indicate that the addition of salt to aqueous solutions of Met-enkephalin induces a "β-like" structure (17). Little is known about the conformations of the β-endorphins, although two preliminary reports have indicated that these molecules are low in helical content in water, but become more helical in trifluoroethanol (18, 19). Similarly, β-lipotropin is reported to contain about 12% α-helix in dilute salt solutions, which increases to 30% in 50% dioxane/water (20). The ability of nonpolar solvents to produce helical structures in β-lipotropin has also been observed by Makarov *et al.* (21).

We report here on the conformations of human β-endorphin (β_h-endorphin) and camel β-endorphin (β_c-endorphin) and sheep β-lipotropin (β_s-lipotropin) in water, methanol, and sodium dodecyl sulfate ($NaDodSO_4$) solutions as determined by CD and hydrodynamic studies. In addition, the limitations of predicting the secondary structure of these types of molecules from their primary structure, and of interpreting their CD spectra in terms of secondary structure, are discussed.

MATERIALS AND METHODS

β-Lipotropin was isolated from sheep pituitary glands according to the method of Li *et al.* (8). β_c-endorphin and β_h-endorphin were synthesized as previously described (10, 11). The concentration of the polypeptides in aqueous solution was determined spectrophotometrically. Absorbances, $A_{1\,cm}^{1\%}$, of 9.6 at 276 nm for β_s-lipotropin, 3.9 at 275 nm for β_c-endorphin, and 7.7 at 276 nm for β_h-endorphin were calculated from the respective amino acid compositions. $NaDodSO_4$, synthesized from dodecyl alcohol, was a gift from K. Shirahama. Methanol was of spectral grade; other chemicals were of reagent grade. Water was double-distilled in glass.

CD spectra were measured with a Jasco J-10 spectropolarimeter for wavelengths below 250 nm and a Cary 60 spectropolarimeter equipped with a 6002 CD attachment for the near-ultraviolet region. Both instruments were standardized with a *d*-10-camphorsulfonic acid. The temperature was maintained at 25°, unless stated otherwise. The data are expressed in terms of mean residue ellipticity, $[\theta]$, molar ellipticity, $[\Theta]$, or both. A mean residue weight of 109 was used for β_s-lipotropin and 111 for β_c-endorphin.

Viscosities were measured in a suspension-type Ubbelohde viscometer at 5.0°. The flow time for 0.1 M KCl was about 2000 sec. The intrinsic viscosity, $[\eta]$, was determined from the Huggins equation; the concentrations of the polypeptides varied from 0.2 to 0.8%. Sedimentation velocity experiments were done on a Spinco model E analytical ultracentrifuge at 5.0°. A synthetic boundary cell was used because of the low sedimentation coefficient, *s*, of the polypeptides. The *s* values were extrapolated to zero concentrations (s^0).

RESULTS

Conformation in Water. Fig. 2 shows the CD spectra of β_s-lipotropin and β_c-endorphin in the region dominated by amide bond absorption. In water at pH 5.9 both polypeptides appear to have little, if any, secondary structure. The absence of the double minimum at 210 and 222 nm characteristic of an α-helix and the appearance of a large negative band near 200 nm suggest that the structure is mostly nonperiodic, although a small amount of helix and β-form cannot be completely ruled out. The two polypeptides appear to be denatured in 6 M guanidine hydrochloride. The CD spectra of β_c-endorphin in water were run at 1.2, 0.12 and 0.012 mg/ml. The slight re-

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Abbreviations: subscripts $_c$, $_h$, and $_s$ indicate camel, human, and sheep, respectively; $NaDodSO_4$, sodium dodecyl sulfate; CD, circular dichroism.

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                5                  10
H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
               15                  20
Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
           25                      31
Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
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FIG. 1. Amino acid sequence of human β-endorphin. Camel β-endorphin is identical except it has histidine-27 and glutamine-31.

duction in the magnitude of the CD signal at all wavelengths upon dilution suggests a small amount of adsorption of the peptides on the cell wall rather than a concentration dependence in the conformation.

pH Effect. The β_c-endorphin molecule (see Fig. 1) has 7 positive and 2 negative charges at neutral pH (including the α-NH_3+ and α-COO^- at the NH_2- and COOH-terminals). Residues 27–29 carry 3 positive charges (see Fig. 1). Raising the pH of the polypeptide solution to 12 did not change the CD spectra of either β_c-endorphin or β_s-lipotropin, suggesting that changes in electrostatic interactions do not cause detectable changes in polypeptide conformation.

Thermal Effect. The changes in CD spectra with temperatures are small and reversible. Raising the temperature of the polypeptide solution reduced the magnitude of the negative CD band near 200 nm. For β_s-lipotropin the $[\theta]$ minimum at 202 nm was −14,400, −13,600, −11,300, and −11,000 deg cm^2 $dmol^{-1}$ at 2°, 25°, 54°, and 78°, respectively. For β_c-endorphin the $[\theta]$ minimum was −15,500, −13,500, −11,000, and −9,000 at 3°, 25°, 53°, and 76°, respectively; the position of the minimum red-shifted from 200 to 202 nm on going from low to high temperature. In contrast, the spectra for β_c-endorphin in the 210–240 nm region showed increasingly negative ellipticities at the four temperatures. Thus, $[\theta]_{220}$ was −800, −1,700, −2,500, and −3,000 at 3°, 25°, 53°, and 76°, respectively. The corresponding CD for β_s-lipotropin is somewhat complicated; the magnitude of $[\theta]_{220}$ was −3,700 at 3°, which dropped to −3,500 at 25° and rose to −3,600 and −3,800 at 54° and 78°.

Side-Chain CD. The CD spectra of β_s-lipotropin and β_c-endorphin in the region of side-chain absorption are presented in Fig. 3. In aqueous solution both polypeptides exhibit only weak optical activity. The spectra in water and in 6 M guanidine hydrochloride are either equivalent (for β_c-endorphin) or nearly the same (for β_s-lipotropin), indicating that neither polypeptide has a rigid tertiary structure with aromatic groups buried within asymmetric local environments. Both hormones show negative bands at 269 and 261–262 nm characteristic of phenylalanine residues (22). The negative bands at 283 and 276 nm for β_s-lipotropin and the corresponding positive bands for β_c-endorphin can be assigned to tyrosine residues (23). The side chain CD of β_h-endorphin (not shown) contained no discernible bands above 270 nm, but exhibited the two negative phenylalanine bands as seen in the β_c-endorphin spectrum. The effects of pH and temperature on the side-chain CD were not determined.

Hydrodynamic Properties. The intrinsic viscosity, $[\eta]$, and sedimentation coefficient, s^0, of β_s-lipotropin and β_h-endorphin have been measured in 0.1 M KCl at 5°. β_s-lipotropin had an $[\eta]$ of 0.15 dl/g and β_h-endorphin had an $[\eta]$ of 0.10 dl/g, indicating that both polypeptides are not compact, globular molecules. The corresponding $s^0_{20,w}$ (converted from data at 5°) was 1.3 S for β_s-lipotropin and 0.8 S for β_h-endorphin.

Conformation in Methanol and $NaDodSO_4$ Solutions. Addition of methanol to an aqueous solution of β_s-lipotropin and β_c-endorphin induces an α-helical conformation (Fig. 2). Both polypeptides increase in helicity with increasing concentrations of methanol. β_c-Endorphin has virtually the same CD spectrum in 100% methanol as in 90% methanol; β_s-lipotropin precipitates in 90% methanol. β_h-Endorphin is equivalent to β_c-endorphin. Equally striking is the helix-promoting effect of $NaDodSO_4$ on the two polypeptides; almost one-half of either molecule becomes helical in $NaDodSO_4$ solution. We have used an excess of $NaDodSO_4$ (about 3 mM), which is approximately six times the mean residue molarity of the polypeptides in these solutions. By cooling the solutions to 4°, most of the excess $NaDodSO_4$ could be precipitated; the supernatant solutions at 25° still gave the same spectra as those shown in Fig. 2.

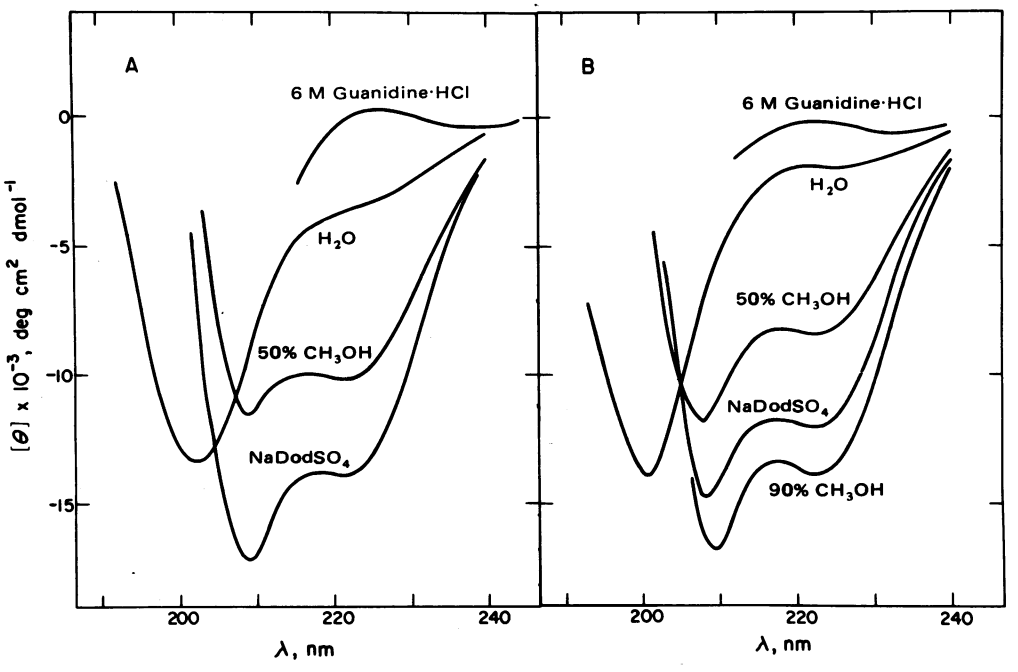

FIG. 2. Circular dichroism of (*A*) ovine β-lipotropin and (*B*) camel β-endorphin in the ultraviolet region.

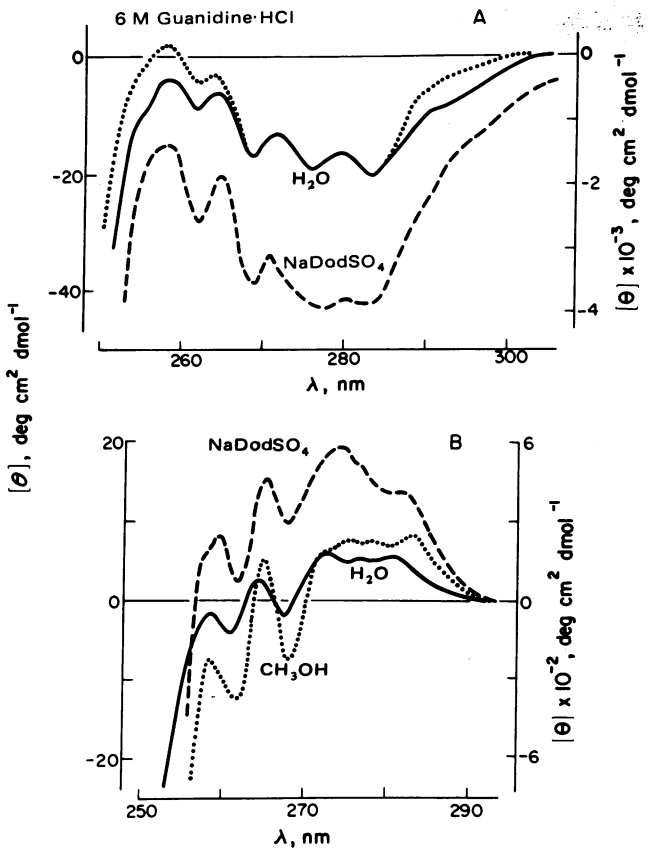

FIG. 3. Circular dichroism of (*A*) ovine β-lipotropin and (*B*) camel β-endorphin in the near ultraviolet region. Ordinates: left, mean residue ellipticity; right, molar ellipticity.

The addition of either methanol or $NaDodSO_4$ increases the CD intensity of both the phenylalanine and tyrosine bands in these molecules (Fig. 3), suggesting the production of somewhat more rigid, asymmetric environments for these chromophores in the presence of solvents less polar than water. However, even in $NaDodSO_4$ solutions no distinct band can be assigned to the tryptophan residue in β_s-lipotropin, suggesting that some areas of conformational flexibility still exist. Similarly, in $NaDodSO_4$ solution the two tyrosine residues in β_h-endorphin still give no signal although this may be due to a cancellation of positive and negative bands. Precipitation of most of the excess $NaDodSO_4$ had no effect and did not return the CD spectra to those found in water.

DISCUSSION

The CD spectrum below 250 nm can be used to estimate the secondary structure of proteins (24, 25). This method of analysis is best for proteins containing a moderate amount of α-helix, but the estimates of the β-form are still uncertain in many cases. In order to give reliable estimates the CD of the amide bonds must dominate the spectrum below 240 nm and overshadow the contributions of the nonpeptide chromophores (at present the CD bands due to aromatic groups and cystine residues cannot be resolved below 250 nm). Because both β_s-lipotropin and β_c-endorphin appear to lack any appreciable amount of secondary structure, the method of Chen *et al.* (24, 25) is not applied to the data in Fig. 2. With data similar to ours, St-Pierre *et al.* (20) have reported 12% α-helix and 16% β-form for β_s-lipotropin in water based on the method of Chen *et al.* (24). These authors also observed a gradual decrease in the CD magnitude throughout the wavelength region from 190 to 240 nm as the temperature was increased from 15 to 50°, whereas our results between 220 and 230 nm showed a minimum at room temperature which increased with increasing temperature. The thermally induced changes in our spectra could be due to overlapping between the peptide and nonpeptide CD bands.

Because the reference $[\theta]$ values for the helical conformation in methanol and $NaDodSO_4$ solutions might differ in magnitude from those in water or dilute salt solutions, we again find it unwise to attempt any quantitative analysis of the CD data for both β_s-lipotropin and β_c-endorphin in Fig. 2. For these reasons the value of 29% α-helix previously reported for β_s-lipotropin in 50% dioxane/water (20) should be considered only tentative. Nevertheless, it is apparent from our data that both polypeptides, in either methanol or $NaDodSO_4$ solutions, contain an appreciable amount of helical conformation, perhaps as much as one-half of the molecules. Removal of most of the excess $NaDodSO_4$ does not reverse the original effect, suggesting that the helical conformation is stabilized by the formation of a complex between the $NaDodSO_4$ and the polypeptide. This observation may be of considerable interest, because it has been proposed (26, 27) that cerebroside sulfate, a molecule that shares many chemical properties with $NaDodSO_4$, is an active component of the opiate receptor in brain tissue. This suggests the possibility that the functional conformation of these opiate-like peptides is achieved only after binding to the receptor complex.

In the absence of x-ray diffraction studies it has become fashionable to predict the secondary structure of proteins from analysis of the amino acid sequence as has been done by Chou and Fasman (28, 29), who claim 70–80% accuracy. The rules for such predictions are empirical and the conformational parameters are based on probabilities of a residue appearing in a particular conformation for globular proteins. Therefore it is doubtful that the rules can be applied to polypeptides such as β_c-endorphin and even β_s-lipotropin. Nevertheless, it is useful to illustrate that this empirical method has limitations, just as the CD analysis of protein conformations has its limitations and should be used with caution. Using the parameters of Fasman *et al.* based on 29 proteins (30), we calculated that β_s-lipotropin and β_c-endorphin would have 65 and 55% helix, 5 and 16% β-form, and 19 and 13% β-turn, respectively. Had the old parameters based on 15 proteins (28, 29) been used, the helical content of β_s-lipotropin and β_c-endorphin would have been 46 and 0%, respectively. Using the old parameters, St-Pierre *et al.* (20) calculated 30% helix for β_s-lipotropin (according to their tabulation, β_c-endorphin should have no helix). As the conformational parameters change, so will the predicted secondary structure. In addition, the rules and predictions are equivocal among different users. More important, these sequence predictions bear no relation to the actual conformation of β_s-lipotropin and β_c-endorphin in solution because the two polypeptides are neither compact nor rigid (see discussion below). If β_s-lipotropin and β_c-endorphin can be crystallized and studied by x-ray diffraction methods, it will quite possibly be found that both polypeptides have an appreciable amount of helix in crystalline form, just as they do in methanol or $NaDodSO_4$ solution.

From denatured proteins and polypeptides in 5–7.5 M guanidine hydrochloride at 25° under conditions where —S—S— linkages cannot exist, Tanford (31) found that

$$[\eta](\mathrm{ml/g})M_0 = 77n^{0.666} \qquad [1]$$

in which M_0 is the mean residue weight and n is the number of residues in the polypeptide molecule. Substituting 91 and 31 into Eq. 1, we found that β_s-lipotropin should have an $[\eta]$ of 0.14 dl/g and β_h-endorphin, 0.07 dl/g. These are close to our experimental values, but such coincidence might be fortuitous, because the experimental conditions were different (our experiments were at 5° and without guanidine hydrochloride). Nevertheless, the two polypeptides appear to be flexible, although they are not "random coils." Using Flory's equation for flexible polymers (32)

$$\Phi^{1/3}p^{-1} = N_A s^0[\eta]^{1/3}\eta_0/M^{2/3}(1 - \bar{v}\rho) \qquad [2]$$

we found that the coefficient $\Phi^{1/3}p^{-1}$ was 3.6×10^6 for β_s-lipotropin and 3.9×10^6 for β_h-endorphin, both of which were much larger than Flory's 2.5×10^6 (experimental). This is probably due to some degree of inflexibility of the two polypeptides. On the other hand, if the two polypeptides were regarded as rigid particles, Scheraga–Mandelkern's β-function (33), which replaces $\Phi^{1/3}p^{-1}$ in Eq. 2 by a function of axial ratio of an equivalent hydrodynamic ellipsoid, can be used. Assuming a prolate ellipsoid of revolution, both polypeptides would have an axial ratio greater than 300, which is simply out of the question. Thus, the hydrodynamic properties of β_s-lipotropin and β_h-endorphin indicate that the molecules are partially extended and may contain a small amount of secondary structure. This conclusion is reasonably consistent with the CD results in water (Fig. 2).

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1. Terenius, L. (1973) *Acta Pharmacol. Toxicol.* **32,** 317–320.
2. Pert, C. B. & Snyder, S. H. (1973) *Science* **179,** 1011–1014.
3. Simon, E. J., Hiller, J. M., & Edelman, I. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1947–1949.
4. Goldstein, A., Lowney, L. I. & Pel, B. K. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 1742–1747.
5. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. & Morris, H. R. (1975) *Nature* **258,** 577–579.
6. Li, C. H. & Chung, D. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1145–1148.
7. Li, C. H., Barnafi, L., Chrétien, M. & Chung, D. (1965) *Nature* **208,** 1093–1094.
8. Li, C. H., Barnafi, L., Chrétien, M. & Chung, D. (1966) *Excerpta Med. Found. Int. Congr. Ser.* **112,** 349–364.
9. Gráf, L. & Li, C. H. (1973) *Biochem. Biophys. Res. Commun.* **53,** 1304–1309.
10. Li, C. H., Lemaire, S., Yamashiro, D. & Doneen, B. A. (1976) *Biochem. Biophys. Res. Commun.* **71,** 19–25.
11. Li, C. H., Yamashiro, D., Tseng, L.-F. & Loh, H. H. (1977) *J. Med. Chem.* **20,** 325–328.
12. Li, C. H., Chung, D. & Doneen, B. A. (1976) *Biochem. Biophys. Res. Commun.* **72,** 1542–1547.
13. Roques, B. P., Garbay-Jaureguiberry, C., Oberlin, R., Anteunis, M. & Lala, A. K. (1976) *Nature* **262,** 778–779.
14. Jones, C. R., Gibbons, W. A. & Garsky, V. (1976) *Nature* **262,** 779–782.
15. Garbay-Jaureguiberry, C., Roques, B. Q., Oberlin, R., Anteunis, M. & Lala, A. K. (1976) *Biochem. Biophys. Res. Commun.* **71,** 558–565.
16. Anteunis, M., Lala, A. K., Garbay-Haurequiberry, C. & Roques, B. P. (1977) *Biochemistry* **16,** 1462–1466.
17. Poupaert, J. H. & Portoghese, P. S. (1976) *J. Med. Chem.* **19,** 1354–1356.
18. Gráf, L. (1976) *Proc. N.Y. Acad. Sci. Symp.*, Nov. 1976.
19. Bayley, P., Snell, C. & Smyth, D. (1977) *Meeting of the Biochemical Society March, 1977,* University of Aberdeen, Abstract no. 16.
20. St-Pierre, S., Gilardeau, C. & Chrétien, M. (1976) *Can. J. Biochem.* **54,** 992–998.
21. Makarov, A. A., Esipova, N. A., Pankov, Yu. A., Brishkousky, B. A., Lobachev, U. M. & Sukhomudrenko, A. G. (1976) *Mol. Biol. (Moscow),* **10,** 704–711.
22. Horwitz, J., Strickland, E. H. & Billups, C. (1969) *J. Am. Chem. Soc.* **91,** 184–190.
23. Horwitz, J., Strickland, E. H. & Billups, C. (1970) *J. Am. Chem. Soc.* **92,** 2119–2129.
24. Chen, Y. H., Yang, J. T. & Martinez, H. M. (1972) *Biochemistry* **11,** 4120–4131.
25. Chen, Y. H., Yang, J. T. & Chou, K. H. (1974) *Biochemistry* **13,** 3350–3359.
26. Loh, H. H., Cho, T. M., Wu, Y. C. & Way, E. L. (1975) *Life Sci.* **14,** 2231–2245.
27. Loh, H. H., Cho, T. M., Wu, Y. C., Harris, R. A. & Way, E. L. (1977) *Life Sci.* **16,** 1811–1818.
28. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13,** 211–221.
29. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13,** 222–245.
30. Fasman, G. D., Chou, P. Y. & Adler, A. J. (1976) *Biophys. J.* **16,** 1201–1238.
31. Tanford, C. (1968) *Adv. Protein. Chem.* **23,** 121–282.
32. Flory, P. J. (1953) *Principles of Polymer Chemistry* (Cornell University Press, Ithaca, NY), pp. 595–639.
33. Scheraga, H. A. & Mandelkern, L. (1953) *J. Am. Chem. Soc.* **75,** 179–184.