Tomita, M., Furthmayr, H., & Marchesi, V. T. (1978) *Biochemistry 17*, 4756-4770.
Tsuji, T., Irimura, T., & Osawa, T. (1980) *Biochem. J. 187*, 677-686.
Winzler, R. J., Harris, E. D., Pekas, D. J., Jackson, C. A., & Weber, P. (1967) *Biochemistry 6*, 2195-2202.
Yamashita, K., Tachibana, Y., & Kobata, A. (1978) *J. Biol. Chem. 253*, 3862-3869.

# Ordered Conformation of Polypeptides and Proteins in Acidic Dodecyl Sulfate Solution†

Chuen-Shang C. Wu, Kiyoshi Ikeda,‡ and Jen Tsi Yang*

ABSTRACT: The conformation of some polypeptides and proteins in sodium dodecyl sulfate ($NaDodSO_4$) solutions was studied by circular dichroism. The type and extent of induced structure depend on their helix- and $\beta$-forming potential. Anionic side groups in segments of helix or $\beta$ form tend to destabilize the ordered structure unless they are protonated. $\beta$-Endorphin has one Glu inside a predicted helical segment; its helicity in a $NaDodSO_4$ solution is enhanced at pH below 4. $\alpha$-Melanocyte-stimulating hormone having a Glu in a $\beta$ segment undergoes a pH-induced coil to $\beta$ transition in 1.25 mM $NaDodSO_4$ (excess surfactant will disrupt the $\beta$ form). Reduced somatostatin assumes a $\beta$ form in 2 mM $NaDodSO_4$ and a partial helix in 25 mM $NaDodSO_4$, both of which are unchanged in acidic pH because it lacks -COOH groups. The unordered gastrin with five consecutive Glu's becomes helical in a $NaDodSO_4$ solution at pH 4. Neurotensin with one Glu has no structure-forming potential and is unordered in both neutral and acidic $NaDodSO_4$ solutions. This charge effect also manifests in segments of ordered structure for polypeptides and proteins such as glucagon, cytochrome *c*, parvalbumin, ribonuclease A, and lysozyme. The effect is especially predominant in tropomyosin that is rich in clusters of anionic side groups. Its more than 90% helicity is reduced to about one-half in a neutral $NaDodSO_4$ solution, but most of it can be restored by lowering the pH to 2-4.

$NaDodSO_4$[1] is known to alter the helicity of many proteins in aqueous solution (Reynolds & Tanford, 1970; Visser & Blout, 1971; Jirgensons, 1976; Mattice et al., 1976; Su & Jirgensons, 1977). Proteins of low helicity often become more ordered and those having more than 50% helix less ordered. Some proteins that are rich in $\beta$ form can actually be converted into helices in excess $NaDodSO_4$ solutions; notable examples are concanavalin A (Kay, 1970) and elastase (Visser & Blout, 1971). These findings make it difficult to interpret the change in conformation of proteins in surfactant solutions.

Previously, we reported that the conformation of oligo- and polypeptides in surfactant solutions depends on the amino acid sequence which in turn dictates the structure-forming potentials (Wu & Yang, 1978; Yang & Wu, 1978). In a $NaDodSO_4$ solution the anionic surfactant first binds to the cationic side groups of polypeptides. Additional surfactant ions then cluster around the polypeptide chain, segments of which are induced to adopt an ordered structure. At low molar surfactant/peptide ratio a $\beta$ form can exist if a segment has the $\beta$-forming potential, but excess surfactant usually disrupts the $\beta$ form and may convert it into a helix if the segment also has the helix-forming potential. Polypeptides without any structure-forming potential remain unordered regardless of the surfactant concentration used. We report herein the charge effect on the conformation of oligopeptides and proteins in $NaDodSO_4$ solution. Anionic Glu and Asp residues can destabilize the induced conformation if they are located in the structure-forming segments of the polypeptide chain. While these like-charged residues will repel each other, thus weakening an ordered structure, it is the electrostatic repulsion between the negatively charged Glu and Asp and $DodSO_4^-$ that will hinder the clustering of the latter anions onto the polypeptide chain. Thus, even a lone charged Glu or Asp strategically located can interfere with the formation of an induced helix or $\beta$ segment. The pH effect on the conformation of protein-surfactant complexes has been studied by Jirgensons (1976) and Su & Jirgensons (1977), but the quantitative aspect of such effect has not yet been established.

## Experimental Section

*Materials.* Synthetic $\alpha$-MSH (Lot C 0126), bovine neurotensin (Lot A 1024), and somatostatin (Lot D 1226) were purchased from Beckman and horse heart cytochrome *c* and bovine pancreas RNase A from Schwarz/Mann. Synthetic human $\beta$-endorphin was obtained from Peninsula Laboratories and egg white lysozyme from Sigma Chemical Co. Parvalbumin B from carp muscle was prepared by the method of Pecherer et al. (1971) and tropomyosin by the method of Cummins & Perry (1973).

Polypeptides and proteins were dissolved in water, except that tropomyosin was in buffers of 0.1 ionic strength (Conway, 1952). The concentrations of parvalbumin and tropomyosin were determined by micro-Kjeldahl analysis, using 15.8% and 16.6% as the nitrogen contents, respectively. Because of limited supplies the concentrations of $\alpha$-melanocyte-stimulating hormone, neurotensin, and somatostatin were based on the

† From the Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143. *Received June 6, 1980.* This work was aided by U.S. Public Health Service Grant GM-10880.
‡ Present address: Department of Biology, Faculty of Science, Osaka University, Osaka, Japan.

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; $\alpha$-MSH, $\alpha$-melanocyte-stimulating hormone; RNase, ribonuclease; CD, circular dichroism.

0006-2960/81/0420-0566$01.00/0 

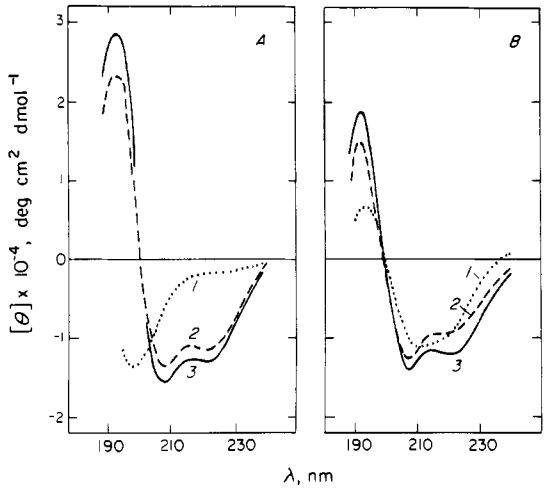


FIGURE 1: CD of (A) β-endorphin and (B) ribonuclease A. (A) curves: (1) 0.089 mg/mL in $H_2O$ (pH 6.4); (2) 0.036 mg/mL in 25 mM $NaDodSO_4$ (pH 6.4); (3) same as (2) but at pH 2.0. (B) curves: (1) 0.42 mg/mL in 10 mM sodium phosphate (pH 7.0); (2) 0.40 mg/mL in 10 mM $NaDodSO_4$ and 5 mM dithiothreitol (pH 8.5); (3) same as (2) but at pH 2.4.

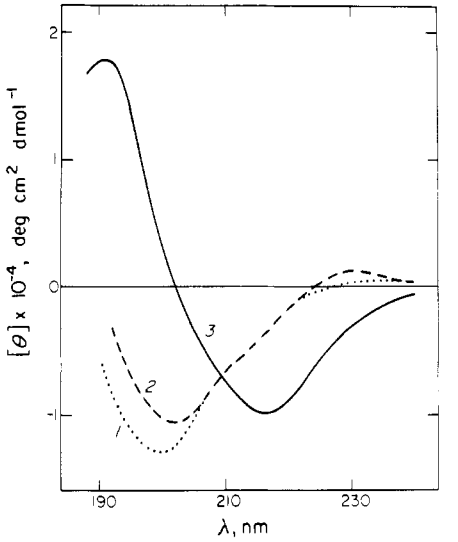


FIGURE 2: CD of α-melanocyte-stimulating hormone. Solvents: (1) water (pH 6.6); (2) 25 mM $NaDodSO_4$ (pH 3.6 and 7.0); (3) 1.25 mM $NaDodSO_4$ (pH 3.1). Concentration of α-MSH: 0.055 mg/mL.

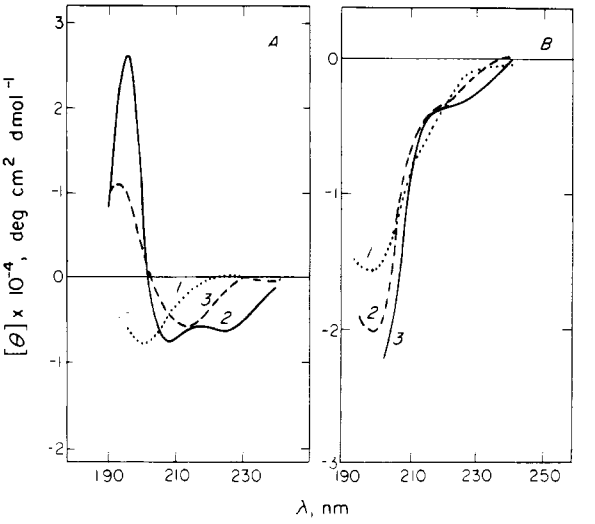


FIGURE 3: CD of (A) somatostatin and (B) neurotensin. (A) curves: (1) 0.20 mg/mL in $H_2O$ (pH 6.9); (2) 0.056 mg/mL in 25 mM $NaDodSO_4$ (pH 2.1 and 6.9); (3) 0.12 mg/mL in 2.2 mM $NaDodSO_4$ (pH 7.0). Curves 2 and 3 contain 0.9 mM dithiothreitol. (B) curves: (1) 0.25 mg/mL in $H_2O$ (pH 7.1); (2) 0.13 mg/mL in 25 mM $NaDodSO_4$ (pH 6.7); (3) same as (2) but at pH 2.3.

weights supplied by the manufacturer (0.5–1 mg). The concentrations of other peptides and proteins were measured spectrophotometrically with the following absorption coefficients: $E_{1cm}^{1\%} = 7.7$ at 276 nm for β-endorphin (Yang et al., 1977), 7.16 at 277.5 nm for RNase A (Bigelow, 1961), and 26.4 at 280 nm for lysozyme (Sophianopoulas et al., 1962) and $E_{1cm}^{1mM} = 106$ at 410 nm for cytochrome *c* (Margoliash & Frohwirt, 1969).

The acidic pH of the solutions was adjusted by adding 1 N HCl (~10 μL for a 10-mL solution). The buffers used for tropomyosin were glycine (pH 2–3.5 and 9.5-12), acetate (pH 4–5.5), and phosphate (pH 6–7.5); NaCl was added to make up a total ionic strength of 0.1.

*Methods.* CD was measured with a Jasco J-500A spectropolarimeter under constant nitrogen flush, which had been calibrated with a purified *d*-10-camphorsulfonic acid solution (Chen & Yang, 1977). A specially designed aluminum block with a jacket served as the cell holder which was attached to a Haake constant-temperature regulator. Fused silica cylindrical cells of various lengths were used to cover the wavelength range of 185–240 nm. The cells were calibrated with a sucrose solution of known optical rotations (National Bureau of Standards grade) on a Cary 60 spectropolarimeter. The CD spectra of fresh polypeptide and protein solutions were recorded within 2 h at 25 °C. No time dependence was observed for all samples in $NaDodSO_4$ solutions. The data were expressed in terms of mean residue ellipticity, [Θ], in deg $cm^2$ $dmol^{-1}$. The mean residue weights are 129 for neurotensin, 117 for somatostatin, 128 for α-MSH, 112 for β-endorphin, 113 for cytochrome *c*, 107 for parvalbumin, 111 for RNase A, 111 for lysozyme, and 115 for tropomyosin.

## Results

Protonation of Glu and Asp residues often enhances the ordered structures of polypeptides and proteins in $NaDodSO_4$ solutions. But the type of the induced conformation is unaltered by the pH adjustment; that is, an induced helix (or β form) remains in a helical form (or β form) when the pH is lowered from neutral to acidic. The CD spectra in Figures 1–3 illustrate four such examples.

In Figure 1A β-endorphin in water shows a strong negative CD band near 198 nm that is typical of an unordered form (curve 1). This polypeptide forms a partial helix in 25 mM $NaDodSO_4$ as evidenced by the appearance of a double minimum at 222 and 208 nm and a stronger positive band at 192 nm (curve 2). Lowering the pH of the solution to 2.0 increases the ellipticities by ~15% (curve 3), suggesting an increase in helicity by several percent.

In Figure 1B the CD spectrum of RNase does not show a distinctive double minimum, and the positive 193-nm band is small (curve 1), probably because its CD due to the 40% β form and 23% helix overlaps. Addition of $NaDodSO_4$ alters the spectrum with a minimum at 205 nm, a shoulder near 222 nm, and a positive maximum at 191 nm; the crossover is blue shifted by ~3 nm (not shown). But the charge effect is present; for instance, $-[\Theta]_{222}$ is raised from 6000 at pH 6.4 to 8000 at pH 2.4, both of which are smaller than 9000 for native RNase. The $[\Theta]_{222}$ of the reduced RNase in $NaDodSO_4$ solution does not change much as compared to that of the native protein, but the intensities of both the 208- and 192-nm bands are considerably enhanced when the disulfide bonds are broken (curve 2). Lowering the pH of the solution to 2.4 increases the ellipticities at 222, 208, and 192 nm by about

Table I: Effect of pH on CD of Polypeptides and Proteins in $NaDodSO_4$ Solutions

| compounds (no. of residues) | pH | [Θ] (deg cm² dmol⁻¹) 222 nm | 208 nm[b] | 192 nm | $f_H$[a] (X-ray) | $f_H$ (CD) |
|---|---|---|---|---|---|---|
| α-melanocyte-stimulating hormone (13) | 3.1 | −10 000[c] | | 17 800 | | |
| | 6.0 | −1 000 | | | | |
| somatostatin, reduced (14) | 2.1 | −6 100 | −7 700 | 26 000 | (0.43) | 0.21 |
| | 6.9 | −6 100 | −7 600 | 26 000 | | 0.21 |
| gastrin (17) | 3.7 | −16 400 | −16 000 | 33 500 | (0.53) | 0.55 |
| | 7.6 | −300 | | | | 0 |
| glucagon (29) | 2.0 | −13 800 | −15 800 | 38 000 | 0.76[d] | 0.45 |
| | 7.8 | −10 900 | −13 200 | 30 000 | | 0.34 |
| β-endorphin (31) | 2.0 | −13 000 | −15 600 | 28 400 | (0.55) | 0.42 |
| | 6.4 | −11 500 | −13 600 | 23 800 | | 0.36 |
| cytochrome *c* (104) | 2.0 | −12 200 | −15 800 | 24 800 | 0.39 | 0.39 |
| | 8.2 | −9 700 | −12 400 | 18 000 | | 0.29 |
| parvalbumin (108) | 2.2 | −16 900 | −19 700 | 39 500 | 0.62 | 0.56 |
| | 7.0 | −14 700 | −18 300 | 32 200 | | 0.48 |
| ribonuclease A, reduced (124) | 2.4 | −12 100 | −14 000 | 18 800 | 0.23[e] | 0.38 |
| | 8.5 | −9 300 | −12 800 | 14 800 | | 0.28 |
| lysozyme, reduced (129) | 2.1 | −11 400 | −15 000 | 16 500 | 0.41[f] | 0.36 |
| | 6.1 | −10 800 | −13 400 | 14 200 | | 0.33 |
| tropomyosin (284) | 3.2 | −32 300 | −34 400 | 74 800 | (0.98) | 0.86 |
| | 7.4 | −17 400 | −21 700 | 33 800 | | 0.44 |

[a] X-ray results were taken from the references listed by Chang et al. (1978); figures in parentheses are based on the Chou–Fasman prediction (Chou & Fasman, 1974). [b] The minimum varies between 205 and 210 nm. [c] $[\Theta]_{216}$ instead of $[\Theta]_{222}$. [d] Glucagon has 55% regular helix. [e] The value refers to native RNase which also contains 40% β form. [f] The value refers to native lysozyme which also contains 16% β form.

30, 10, and 13%, respectively (Figure 1B, curve 3).

The CD spectrum of the unordered α-MSH in water remains unaffected by the addition of 25 mM $NaDodSO_4$ (molar $NaDodSO_4$/residue ratio = 60), nor does it change much when the pH of the solution is lowered from 7 to 3.6 (Figure 2, curves 1 and 2). However, in 1.25 mM $NaDodSO_4$ (molar $NaDodSO_4$/residue ratio = 3) this hormone adopts a β-form at pH 3.1 (curve 3), but not in neutral solution.

Acidic pH has no effect on two classes of peptides in $NaDodSO_4$ solution: peptides without anionic side groups and those having no structure-forming potential. The tetradecapeptide somatostatin with one –S–S– bond belongs to the former class. Its CD does not show any ordered structure in water (Figure 3A, curve 1). In $NaDodSO_4$ solution somatostatin does form a small amount of the helix, which is independent of pH (not shown). With the disulfide bond broken the reduced somatostatin adopts a β form at ~2 mM $NaDodSO_4$ (molar $NaDodSO_4$/residue ratio = 2) (Figure 3A, curve 3). Further increase in the $NaDodSO_4$ concentration to above 10 mM transforms the β form into a partial helix (Figure 3A, curve 2). Both the induced helix and β form are unaffected by the lowering of pH from 6.9 to 2.1.

Neurotensin represents the class of peptides that has no helix or β-form potential. It is unordered in both water and $NaDodSO_4$ solution regardless of the pH used (Figure 3B).

Table I lists the numerical values of [Θ]'s at the extrema of several polypeptides and proteins in addition to those shown in Figures 1–3. Gastrin undergoes a pH-induced coil to helix transition in acidic $NaDodSO_4$ solution (Wu & Yang, 1978). The charge effect is also observed for glucagon (Wu & Yang, 1980), cytochrome *c*, and parvalbumin in $NaDodSO_4$ solution, although the increase in helicity at low pH is not as drastic as that of gastrin. The change in the ellipticities for the reduced lysozyme–$NaDodSO_4$ complex is even less when the pH is lowered from 6.1 to 2.1.

The fibrous protein tropomyosin with more than 90% helix is stable against acid denaturation. Its secondary structure appears to be slightly enhanced at pH below 5, as is evident from the variation of $[\Theta]_{222}$ with pH (Figure 4). But tropomyosin does denature in alkaline solution (above pH 10).

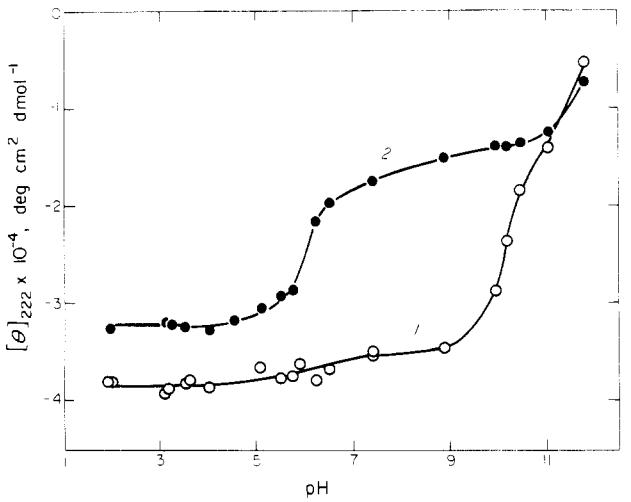


FIGURE 4: Effect of pH on the ellipticity of tropomyosin at 222 nm. Solvents: (1) no $NaDodSO_4$; (2) 20 mM $NaDodSO_4$. Protein concentration: 0.095 mg/mL. Dithiothreitol (0.5 mM) is added to all solutions.

Addition of $NaDodSO_4$ at neutral pH reduces the ellipticities and thereby the helicity by about half, most of which can be restored by lowering the pH to 6 or less (Figure 4 and Table I). Titration of Glu and Asp residues in $NaDodSO_4$ solution shows an apparent p$K$ of ~6 (due to the highly negatively charged environment), which is similar to that found for gastrin in $NaDodSO_4$ solution (Wu & Yang, 1978).

## Discussion

The charge effect can be explained by inspecting the structure of polypeptides and proteins based on X-ray studies or, in the absence of them, the empirical sequence-predictive methods. Because the predictive parameters are derived from the sequences of compact, rigid proteins, their applications to polypeptide– or protein–surfactant complexes should be viewed with caution. We chose the Chou–Fasman method (Chou & Fasman, 1974, 1978) for its simplicity, although other methods can also be used with different degrees of sucess. The very

nature of these empirical methods warrants against a too literal interpretation (Matthews, 1975).

$\alpha$-MSH is a tridecapeptide with one Glu in the segment of

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-$NH_2$

residues 4–9, which has a strong helical potential (underlined) and none for the $\beta$ form. [The Kabat–Wu method (Kabat & Wu, 1973) predicts a helical segment for residues 4–7 and also no $\beta$-forming potential.] Our CD results fail to detect any helical conformation for this hormone in 25 mM $NaDodSO_4$ at pH 7.0 or 3.6 (Figure 2). In 1.25 mM $NaDodSO_4$ this peptide adopts a $\beta$ form at pH 3.1 but not at pH 7.0. This is an exception to the sequence prediction. Because excess surfactant will disrupt the $\beta$ form, we suspect that $\alpha$-MSH may exist in an intermolecularly aggregated form. It is relevant to mention that poly(L-glutamic acid) undergoes a helix–coil transition in acidic solution, but completely protonated $(Glu)_n$ is aggregated and exists in a $\beta$ form (Zimmerman et al., 1975). Poly(L-benzylglutamate) is completely helical in a poor solvent, but oligo(L-benzylglutamate) is unordered in the same solvent at low peptide concentrations and takes up a $\beta$-like structure at high concentrations (Yang & Doty, 1957).

The tetradecapeptide somatostatin has no Glu and Asp

Ala-Gly-Cys-Lys-Asn-Phe-Phe
Cys-Ser-Thr-Phe-Thr-Lys-Trp

residues and therefore shows no charge effect on its conformation when the pH of the solution is lowered from 6.9 to 2.1. Here the two $Lys^+$ residues are neutralized by $DodSO_4^-$. (The $Ala^+$ and $Cys^-$ contain the terminal $\alpha$-$NH_3^+$ and $\alpha$-$COO^-$, respectively.) This polypeptide has a helix-forming potential (underlined), but the helical conformation is constrained by the presence of a single disulfide bond. Once the –S–S– linkage is broken, this peptide immediately becomes partially helical in 25 mM $NaDodSO_4$. According to the Chou–Fasman method (Chou & Fasman, 1978), somatostatin also has a $\beta$-forming potential for residues 6–12. Thus, in a low $NaDodSO_4$ solution (2 mM) it adopts a $\beta$ form instead of a helix (Figure 3A). We also believe that cationic side groups, e.g., Lys-4 and –9, are essential for the formation of a $\beta$ structure (Wu & Yang, 1980).

The tridecapeptide neurotensin has no helix- or $\beta$-forming

pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu

potential according to the Chou–Fasman method. It remains unordered in both neutral and acidic $NaDodSO_4$ solutions despite the binding of two Arg's and one Lys with dodecyl sulfate ions. While Pro-containing residues 6–9 and 9–12 can form $\beta$ turns according to the Chou–Fasman prediction, we have not been able to observe the CD of $\beta$ turns in a $NaDodSO_4$ solution.

Human Leu-15 gastrin I with 17 residues has five consec-

pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe-$NH_2$

utive Glu residues, which make the formation of the predicted helix (underlined) intangible. This effect of clustered like-charged residues in weakening helix formation has been discussed by Lewis & Bradbury (1974). Once these Glu's are protonated, this peptide in a $NaDodSO_4$ solution immediately adopts a helical conformation (Wu & Yang, 1978).

Glucagon with 29 residues is highly helical (underlined) in crystals but has little secondary structure in water. About half of the molecule adopts a helical conformation in $NaDodSO_4$

His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr

solution (Wu & Yang, 1980). Protonation of Asp-9, -15, and -21 further enhances the helicity.

Human $\beta$-endorphin with 31 residues and no disulfide bond

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu

has two predicted helical segments (underlined; the exact number of residues in each segment is of course uncertain). Residues 14–18 also have the $\beta$-forming potential according to the Chou–Fasman method (Chou & Fasman, 1978). In a $NaDodSO_4$ solution about half of the molecule becomes helical (Yang et al., 1977). Glu-8 in the first segment can destabilize an induced helix. (Glu-31 at the COOH terminus is unlikely to influence the ordered conformation of this polypeptide.) Thus, the helicity of $\beta$-endorphin in $NaDodSO_4$ solution is increased by lowering the pH from 6.4 to 2.0 (Table I and Figure 1A).

The charge effect on proteins is more difficult to evaluate than on oligo- and polypeptides because of the size of proteins. But the increase in ellipticities in acidic $NaDodSO_4$ solution appears to correlate well with the positions of carboxylate ions in the peptide chains. Table I lists four proteins of known three-dimensional structure. Cytochrome *c* has five helical segments for residues 9–18, 49–54 (Asp-50), 62–70 (Glu-62, -66, and -69), 71–75, and 91–101 (Glu-92, Asp-93); parvalbumin has six for residues 8–18 (Asp-8 and -10, Glu-16), 26–33, 40–53 (Asp-41, -42, -51, and -53, Glu-52), 60–70 (Asp-61, Glu-60 and -62), 78–89 (Asp-79, Glu-81), and 98–108 (Asp-100, Glu-101); RNase A has three for residues 3–13 (Glu-9), 24–34, and 51–57 (Asp-53); lysozyme has six for residues 5–15 (Glu-7), 25–35 (Glu-35), 79–84, 88–99, 108–115, and 119–124 (Asp-119). If anionic side groups are located near the N- and C-terminal boundaries of helical segments, the charge effect is usually small. Lysozyme has two out of the three carboxylate groups in helical segments located at either the C- or N-terminal boundary. Thus, lowering the pH does not increase its helicity very much (Table I). If Glu and Asp residues are found in the middle of helical segments, in particular, when the number of helical residues on both sides of an anionic residue is less than four, the charge effect is often quite large. At least one such helical segment or more is present in cytochrome *c*, parvalbumin, and RNase A. Tropomyosin, whose three-dimensional structure is not yet known, is unusual in the sense that about one-third of its residues are ionic. The Glu and Asp residues are evenly distributed along the polypeptide chain, but at least in seven places the carboxylate groups are clustered in three consecutive residues, and in many instances the anionic side groups are separated by less than four residues (Stone et al., 1974). This can account for the drastic reduction in helicity when $NaDodSO_4$ is added to a neutral solution of tropomyosin.

The results in Table I should enable us to estimate the fractions of the helix, $f_H$, of polypeptides and proteins in $NaDodSO_4$ solutions and compare them with those from X-ray studies or by the sequence-predictive method. We assume that the helix is the only induced structure in excess $NaDodSO_4$ solution because the $\beta$ form only exists at low molar surfactant/peptide ratios and is converted into the helix or unordered form at high ratios [according to Chou & Fasman (1974) many predicted $\beta$ segments in proteins also possess the helix-forming potential.] The choice of reference CD spectra for a helix and an unordered form in a surfactant solution is

still problematic. It is well-known that CD values for the helical conformation in water and in organic solvents are often different. This could be due to solvent effect or because the polypeptide is not completely helical in certain solvents. To resolve this uncertainty, we remeasured the CD spectrum of poly(L-glutamic acid) at pH 4, which is known to be helical. Addition of $NaDodSO_4$ at the same pH did not affect the CD magnitudes within experimental errors; for instance, $[\theta]_{222}$ was about $-40\,000$ deg cm$^2$ dmol$^{-1}$ in both cases (K. Ikeda and J. T. Yang, unpublished data). It appears that the solvent effect is essentially identical in water and in an aqueous $NaDodSO_4$ solution. On the other hand, the helical conformation of poly(L-ornithine)–$NaDodSO_4$ complex in neutral solution only had an $[\theta]_{222}$ close to $-30\,000$ deg cm$^2$ dmol$^{-1}$ at room temperature; this magnitude gradually increased (or decreased) upon lowering (or raising) the temperature of the solution (Satake & Yang, 1973), suggesting that the low values may represent a <100% helix. The CD values of the unordered form of a polypeptide in the 222-nm region does not seem to be much affected by the presence of surfactants; in any case their magnitude is much smaller than that of the helical form. Therefore, we assume that the reference CD values for the helix and unordered form in $NaDodSO_4$ solution are the same as those in water. Thus, $[\theta]_{222}$ is taken as $-21\,800$ and $-28\,400$ deg cm$^2$ dmol$^{-1}$ for a helix having an average length of 6 and 10 residues, respectively, and $-37\,400$ deg cm$^2$ dmol$^{-1}$ for an infinite helix based on the CD analysis of proteins (Chen et al., 1974; Chang et al., 1978). The CD for the unordered form in the 222-nm region is also uncertain, but it does not affect our calculations too much because of its relatively small magnitude. We choose $-2000$ deg cm$^2$ dmol$^{-1}$ at 222 nm for the unordered form, which is close to that shown by most denatured proteins (Chen et al., 1974). Thus, we have for the fraction of helix

$$f_H = ([\theta]_{222} + 2000)/([\theta]_H + 2000)$$

The polypeptides and proteins (Table I) are assumed to have an average helical length of 10 residues, except that somatostatin has 6 and tropomyosin is regarded as an infinite helix. In general, the estimated $f_H$'s in acidic $NaDodSO_4$ solution are close to those found by X-ray studies or estimated by the sequence-predictive method. A notable exception is reduced somatostatin; its estimated $f_H$ is only about half of that obtained by the Chou–Fasman method, probably because a short helical segment is quite unstable. Reduced RNase in acidic $NaDodSO_4$ solution appears to be more helical than that found in the native molecule. Very likely some of its disrupted $\beta$ form can be converted into an helix in the presence of excess $NaDodSO_4$. These semiquantitative estimates should be viewed with reservation, but they do support the concept that the induced conformation of polypeptides and proteins in $NaDodSO_4$ solution is related to their amino acid sequences.

References


Bigelow, C. C. (1961) *J. Biol. Chem. 236*, 1706–1710.

Chang, C. T., Wu, C.-S. C., & Yang, J. T. (1978) *Anal. Biochem. 91*, 13–31.

Chen, G. C., & Yang, J. T. (1977) *Anal. Lett. 10*, 1195–1207.

Chen, Y. H., Yang, J. T., & Chau, K. H. (1974) *Biochemistry 13*, 3350–3359.

Chou, P. Y., & Fasman, G. D. (1974) *Biochemistry 13*, 222–245.

Chou, P. Y., & Fasman, G. D. (1978) *Annu. Rev. Biochem. 47*, 251–276.

Conway, B. E. (1952) in *Electrochemical Data*, pp 214, Elsevier, New York.

Cummins, P., & Perry, S. V. (1973) *Biochem. J. 133*, 765–777.

Jirgensons, B. (1976) *Biochim. Biophys. Acta 434*, 58–68.

Kabat, E. A., & Wu, T. T. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1473–1477.

Kay, C. M. (1970) *FEBS Lett. 9*, 78–80.

Lewis, P. N., & Bradbury, E. M. (1974) *Biochim. Biophys. Acta 336*, 153–164.

Margoliash, E., & Frohwirt, N. (1969) *Biochem. J. 71*, 570–572.

Matthews, B. W. (1975) *Biochim. Biophys. Acta 405*, 442–451.

Mattice, W. L., Riser, J. M., & Clerk, D. S. (1976) *Biochemistry 15*, 4264–4272.

Pecherer, G. F., Demaille, J., & Capony, J. P. (1971) *Biochim. Biophys. Acta 236*, 391–408.

Reynolds, J. A., & Tanford, C. (1970) *J. Biol. Chem. 245*, 5161–5165.

Satake, I., & Yang, J. T. (1973) *Biochem. Biophys. Res. Commun. 54*, 930–936.

Sophianopoulas, A. J., Rhodes, C. K., Holcomb, D. N., & van Holde, K. E. (1962) *J. Biol. Chem. 237*, 1107–1112.

Stone, D., Sodek, J., Johnson, P., & Smillie, L. B. (1974) *Proc. FEBS Meet. 31*, 125–136.

Su, Y.-Y. T., & Jirgensons, B. (1977) *Arch. Biochem. Biophys. 181*, 137–146.

Visser, L., & Blout, E. R. (1971) *Biochemistry 10*, 743–752.

Wu, C.-S. C., & Yang, J. T. (1978) *Biochem. Biophys. Res. Commun. 82*, 85–91.

Wu, C.-S. C., & Yang, J. T. (1980) *Biochemistry 19*, 2117–2122.

Yang, J. T., & Doty, P. (1957) *J. Am. Chem. Soc. 79*, 761–775.

Yang, J. T., & Wu, C.-S. C. (1978) *Versatility Proteins*, [*Proc. Int. Symp. Proteins*], 99–118.

Yang, J. T., Bewley, T. A., Chen, G. C., & Li, C. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3235–3238.

Zimmerman, S. S., Clark, J. C., & Mendelkern, L. (1975) *Biopolymers 14*, 585–596.