8-amino hydrogen and the sugar 5′-oxygen (Neidle et al., 1979).

Acknowledgments

We thank Dr. Nathan O. Kaplan for discussions concerning this work and also for his support by grants from the National Institutes of Health and the American Cancer Society.

References

Altona, C., & Sundaralingam, M. (1973) *J. Am. Chem. Soc. 95*, 2333–2344.
Davies, D. B., & Danyluk, S. S. (1974) *Biochemistry 13*, 4417–4434.
DeLeeuw, H. P. M., DeJager, J. R., Koeners, H. J., VanBoom, J. H., & Altona, C. (1977) *Eur. J. Biochem. 76*, 209–217.
Evans, F. E. (1979) *Arch. Biochem. Biophys. 193*, 63–75.
Evans, F. E., & Sarma, R. H. (1974) *J. Biol. Chem. 249*, 4754–4759.
Evans, F. E., & Kaplan, N. O. (1976) *J. Biol. Chem. 251*, 6791–6797.
Evans, F. E., & Sarma, R. H. (1976) *Nature (London) 263*, 567–572.
Evans, F. E., & Kaplan, N. O. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4909–4913.
Evans, F. E., & Kaplan, N. O. (1979) *FEBS Lett. 105*, 11–14.
Evans, F. E., Lee, C. H., Kapmeyer, H., & Kaplan, N. O. (1978) *Bioorg. Chem. 7*, 57–67.
Giessner-Prettre, C., & Pullman, B. (1977a) *J. Theor. Biol. 65*, 171–188.
Giessner-Prettre, C., & Pullman, B. (1977b) *J. Theor. Biol. 65*, 189–201.
Hruska, F. E., Wood, D. J., McCaig, T. N., & Smith, A. A. (1974) *Can. J. Chem. 52*, 497–508.
Hruska, F. E., Wood, D. J., & Singh, H. (1977) *Biochim. Biophys. Acta 474*, 129–140.
Ikehara, M., Uesugi, S., & Yoshida, K. (1972) *Biochemistry 11*, 830–842.
Lee, C. H., & Sarma, R. H. (1976) *J. Am. Chem. Soc. 98*, 3541–3548.
Lee, C. H., & Tinoco, I. (1977) *Biochemistry 16*, 5403–5414.
Neidle, S., et al. (1979) *Biochim. Biophys. Acta 565*, 379–386.
Pless, R., Dudycz, L., Stolarski, R., & Shugar, D. (1978) *Z. Naturforsch., C 33*, 902–907.
Pohorille, A., Perahia, D., & Pullman, B. (1978) *Biochim. Biophys. Acta 517*, 511–516.
Prado, F. R., Giessner-Prettre, C., & Pullman, B. (1978) *J. Theor. Biol. 74*, 259–277.
Remin, H., & Shugar, D., (1972) *Biochem. Biophys. Res. Commun. 48*, 636–642.
Sarma, R. H., Lee, C. H., Evans, F. E., Yathindra, N., & Sundaralingam, M. (1974) *J. Am. Chem. Soc. 96*, 7337–7348.
Schweizer, M. P., & Robins, R. K. (1973) in *Conformation of Biological Molecules and Polymers* (Bergman, E. E., & Pullman, B., Eds.) Vol. 5, pp 329–343, Israel Academy of Sciences and Humanities, Jerusalem.
Tavale, S. S., & Sobell, H. M. (1970) *J. Mol. Biol. 48*, 109–123.
Westhof, E., Roder, O., Croneiss, I., & Ludemann, H. D. (1975) *Z. Naturforsch. C 30*, 131–140.
Westhof, E., Plach, H., Cuno, I., & Ludemann, H. D. (1977) *Nucleic Acids Res. 4*, 939–953.
Wood, D. J., Hruska, F. E., Mynott, R. J., & Sarma, R. H. (1973) *Can. J. Chem. 51*, 2571–2577.

Helical Conformation of Glucagon in Surfactant Solutions†

Chuen-Shang C. Wu and Jen Tsi Yang*

ABSTRACT: On the basis of circular dichroism (CD), glucagon in dilute acidic or alkaline solutions (e.g., less than 1 mg/mL) has little secondary structure (the polypeptide is sparingly soluble between pH 4 and 9). High peptide concentration, low temperature, and high ionic strength slightly enhance the helical conformation. In a 25 mM sodium dodecyl sulfate ($NaDodSO_4$) solution at pH below 4, about one-half of the glucagon molecule adopts a helical conformation that is independent of the polymer concentration. Sodium decyl sulfate is equally effective, but dodecylammonium chloride, dodecyltrimethylammonium chloride, and dodecyl heptaoxyethylene ether are slightly less effective in promoting the helicity of glucagon. Of the trypsin-digested glucagon fragments, only the C-terminal fragment (residues 19–29) can adopt a partially helical conformation in both ionic and nonionic surfactant solutions, but the N-terminal fragment (residues 1–12) and the middle fragment (residues 13–17) remain unordered in the presence of surfactant micelles. The results support our working hypothesis that surfactant micelles cluster around the polypeptide chain and hydrophobic interaction among the amphiphiles stabilizes the induced conformation that is related to the structure-forming potentials of the amino acid sequence. Charged side groups can interact with the amphiphile heads having charges of opposite sign, but charges of the same sign can destabilize the induced ordered conformation.

The anionic surfactant $NaDodSO_4$[1] has been widely used for studying protein subunit structures and for determining the molecular weights of proteins by gel electrophoresis (Weber & Osborn, 1969). It is known to alter the helical content of many proteins (Visser & Blout, 1971; Jirgensons, 1973; Mattice et al., 1976), but the mechanism of such interaction is not fully understood. The binding of $NaDodSO_4$ by proteins is believed to be stoichiometric on a weight basis (Reynolds & Tanford, 1970).

† From the Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143. *Received November 14, 1979.* This work was aided by U.S. Public Health Service Grant GM-10880. Presented at the 11th International Congress of Biochemistry, Toronto, Canada, July 1979.

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; $NaDecSO_4$, sodium decyl sulfate; $DodNH_3Cl$, dodecylammonium chloride; $Dod(CH_3)_3NCl$, dodecyltrimethylammonium chloride; $C_{12}E_7$, dodecyl heptaoxyethylene ether; T-1, T-2, and T-3, glucagon fragments of residues 1–12, 13–17, and 19–29, respectively; CD, circular dichroism.

0006-2960/80/0419-2117$01.00/0

Previously, we found that the conformation of oligo- and polypeptides in surfactant solutions depended on their amino acid sequence (Wu & Yang, 1978; Yang & Wu, 1978). Our working hypothesis is that micelles of the amphiphiles can cluster around a polypeptide chain and hydrophobic interaction among the nonpolar tails stabilizes any ordered structure that would have otherwise been broken up because of peptide backbone-water interaction. Such induced conformation is related to the helix- and β-forming potentials of the amino acid residues. Charged side groups can interact with each other or bind with the polar heads of the amphiphiles having charges of opposite sign, but charges of the same sign may destabilize the induced polypeptide conformation.

We report herein the conformation of glucagon and its trypsin-digested fragments in surfactant solutions. Glucagon is a polypeptide with 29 residues without disulfide bonds:

```
++             5               -  10
His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-
                  T-1

                      15
     +                -        +   +      20
Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-
       ↑           T-2       ↑   ↑

     -                25                 -
Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr
                  T-3
```

(where the arrows indicate the breaking up of the covalent bonds by trypsin digestion and T-1, T-2, and T-3 refer to the three fragments). X-ray diffraction studies of glucagon indicate that residues 10–25 are in a regular α-helical form, which can be extended to include residues 6–9 and 26–27 as less regular helixes. Thus, this hormone in crystals has 55% regular helix or 76% helix including the distorted segments (Sasaki et al., 1975; Bedarkar et al., 1978). Yet in dilute aqueous solution glucagon is essentially in an unordered form (Gratzer & Beaven, 1969; Srere & Brooks, 1969). Addition of cationic surfactant (Bornet & Edelhoch, 1971), organic solvents (Gratzer & Beaven, 1969; Epand, 1972; Contaxis & Epand, 1974), and certain lipids (Schneider & Edelhoch, 1972; Epand et al., 1977) can induce the helical conformation in glucagon. We will show that glucagon can become partially helical in both ionic and nonionic surfactants. The induced ordered structure is mostly located in, but not confined to, fragment T-3. Our results fully support the above-mentioned hypothesis.

Experimental Section

Materials. The commercial crystalline glucagon (Elanco) has the same amino acid composition as that of porcine, bovine, or human glucagon (Bromer et al., 1957a,b; Thomsen et al., 1972). The hormone moved as a single band by gel electrophoresis. $NaDodSO_4$, and $NaDecSO_4$ were gifts of Dr. K. Shirahama. $DodNH_3Cl$ and $Dod(CH_3)_3NCl$ (Eastman) were recrystallized twice from ethanol. $C_{12}E_7$ (Nikko Chemical, Japan) was used without further purification. All other chemicals were of analytical grade.

Preparation of Glucagon Fragments. The three glucagon fragments T-1, T-2, and T-3 were prepared by trypsin digestion (Bromer et al., 1957a,b). Fragment T-3 was precipitated at pH 2. Fragments T-1 and T-2 were separated on a Bio-Rad P-2 column (2 × 90 cm) with 20 mM glycine–0.1 M NaCl (pH 2) as the solvent. Each of the three fragments was desalted on a Bio-Rad AG 501-X8 column and eluted with 0.5 M NH_4HCO_3 before lyophilization. By amino acid analyses, fragments T-1 and T-2 were slightly cross contaminated but fragment T-3 was relatively pure. All peptide concentrations were determined spectrophotometrically by assuming molar absorption coefficients of 1200 and 5500 at 280 nm for tyrosine and tryptophan.

Buffers of glycine (pH 2–3.5 and 9.5–12), acetate (pH 4–5.5), and phosphate (pH 6–7.5) and their combinations were used (Conway, 1952); NaCl was added to yield an ionic strength of 0.1. Addition of $NaDodSO_4$ was assumed not to affect the pH of the peptide solutions.

Potentiometric Titration. The method of Satake & Yang (1976) was used, but the reference solution contained 1 mM $NaDecSO_4$ in a glycine–NaCl buffer (pH 2; ionic strength 0.1). $NaDecSO_4$ instead of $NaDodSO_4$ was used to improve the precision of measurements. The degree of binding of the surfactant ion by the peptide, x, is expressed as

$$x = (C_t - C_f)/C_p$$

where C_t and C_f are the total and free surfactant concentrations and C_p is the peptide concentration.

CD Spectra. CD was measured with a Jasco SS-10 spectropolarimeter (modified by Sproul Scientific Instruments). Cells of various paths were used: 0.1 mm for C_p = 2–10 mg/mL at 222 nm and C_p < 1 mg/mL below 200 nm; 0.5, 1, and 2 mm for C_p = 0.1–2 mg/mL between 200 and 250 nm; 10 mm for C_p < 0.1 mg/mL above 200 nm. The absorbance of the solutions was kept under 2. All cells were calibrated with a sucrose solution of known optical rotation (National Bureau of Standards grade) on a Cary 60 spectropolarimeter. Each spectrum was recorded within 2 h; in $NaDodSO_4$ solution it showed no time dependence even after 5 to 8 days. The data were expressed in terms of mean residue ellipticity, $[\Theta]$, with mean residue weights of 120, 113, 130, and 123 for glucagon and its fragments T-1, T-2, and T-3, respectively.

Results

Glucagon. On the basis of CD spectra, glucagon in a dilute solution (85 μM) at pH 2 or 10 is essentially unordered as evidenced by the large negative band near 200 nm (Figure 1, curve 1) (glucagon is sparingly soluble at neutral pH). Addition of $NaDodSO_4$ (25 mM) promotes a partially helical conformation; its CD is typified by a double minimum near 209 and 222 nm and a maximum at ~192 nm (Figure 1, curve 3). Judged from the CD magnitudes, nearly one-half of the polypeptide molecule is estimated to be helical (the chain length dependence of the CD for a helix is taken into consideration; Chen et al., 1974). $NaDecSO_4$ is equally effective, but $DodNH_3Cl$ and nonionic $C_{12}E_7$ are less effective in inducing the helical conformation of glucagon (Figure 1, curve 2).

Figure 2 illustrates the three factors that affect the glucagon conformation. In the absence of $NaDodSO_4$, increasing the glucagon or salt concentration enhances the helicity but high temperature destabilizes it, as evidenced by the CD magnitude at 222 nm (curve 1). In 25 mM $NaDodSO_4$ solution the CD of glucagon is concentration independent (Figure 2A, curve 2). The salt effect is small, and the peptide–surfactant complex precipitates at 1 M NaCl (Figure 2B, curve 2). High temperature does not completely destroy the helical conformation; at 68 °C more than one-third of the glucagon molecule is still helical (Figure 2C, curve 2).

Figure 3 shows the pH dependence of the CD of glucagon. In the absence of $NaDodSO_4$ the magnitude of $[\Theta]_{222}$ slightly decreases at both extreme pH values (curve 1). With $NaDodSO_4$ $[\Theta]_{222}$ is most negative, that is, most helical, at pH

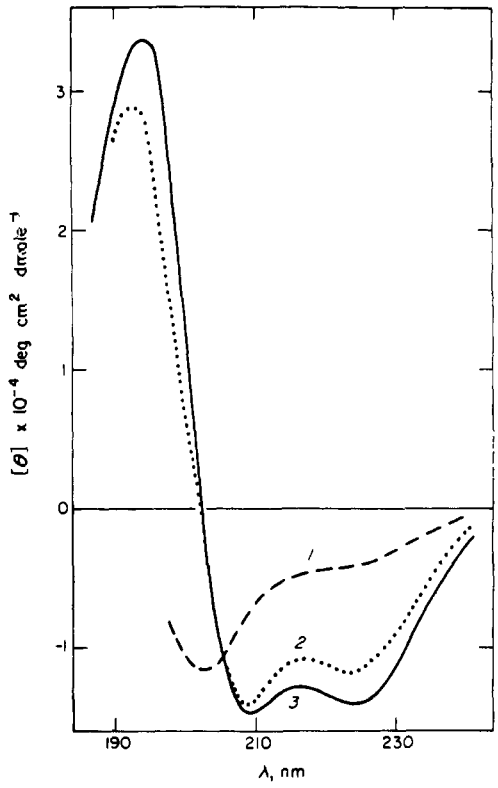

FIGURE 1: CD spectra of glucagon in NaCl–glycine buffer (pH 2; ionic strength 0.1) at 25 °C. Curves: (1) no surfactant; (2) 20 mM $DodNH_3Cl$ or dodecyl heptaoxyethylene ether; (3) 25 mM $NaDodSO_4$. Peptide concentration: 85 μM.

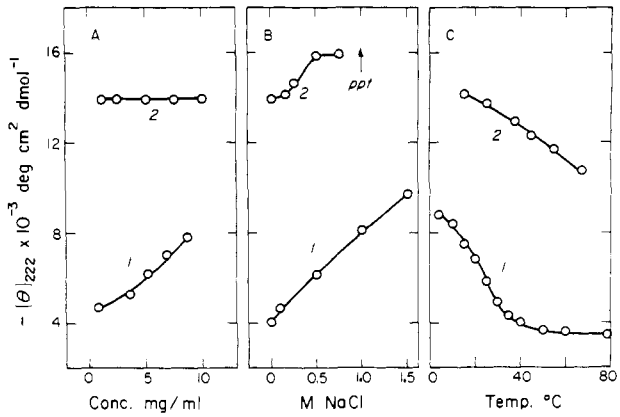

FIGURE 2: Effects of peptide and salt concentrations and temperature on the CD of glucagon in glycine buffer (pH 2). Curves: (1) no surfactant; (2) 25 mM $NaDodSO_4$. (A) Curves 1 and 2 in 20 and 10 mM glycine at 25 °C. (B) Curves 1 and 2, 83 μM glucagon in 5 mM glycine and 174 μM glucagon in 10 mM glycine at 25 °C. (C) Curves 1 and 2, 1 mM glucagon in 10 mM glycine and 50 mM NaCl and 0.17 mM glucagon in 10 mM glycine.

below 4 (curve 2). There appears to be two transitions, one at pH 5.2 and the other at pH 12. The ellipticity at pH 13 is ~3 times that in the absence of $NaDodSO_4$.

Glucagon Fragments. As expected, all three glucagon fragments, T-1, T-2, and T-3, are unordered in aqueous solution (Figure 4, curve 1). In 25 mM $NaDodSO_4$ solution the dodecapeptide T-1 may have a trace of helical conformation. Although many CD spectra for unordered proteins also have such shoulders near 220 nm, the negative CD band below 200 nm is red-shifted to 204 nm in the presence of $NaDodSO_4$. Both $DodNH_3Cl$ and $C_{12}E_7$ seem to have little effect on the T-1 conformation (curve 2). Not surprisingly, the pentapeptide T-2 lacks any ordered structure in all three surfactant solutions

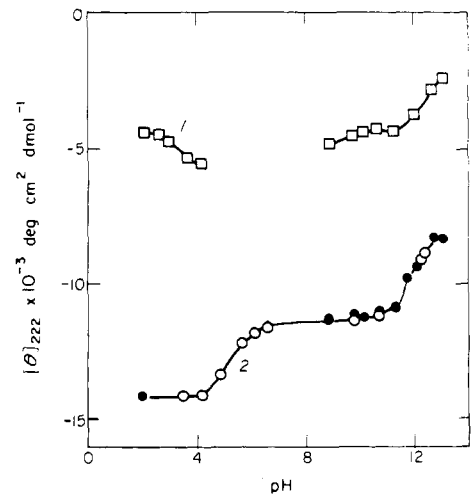

FIGURE 3: CD of glucagon at various pH values. Curves: (1) 78 μM peptide with no surfactant; (2) 90 μM peptide in 25 mM $NaDodSO_4$. All solutions contained NaCl–glycine, –acetate, or –phosphate buffer (ionic strength, 0.1).

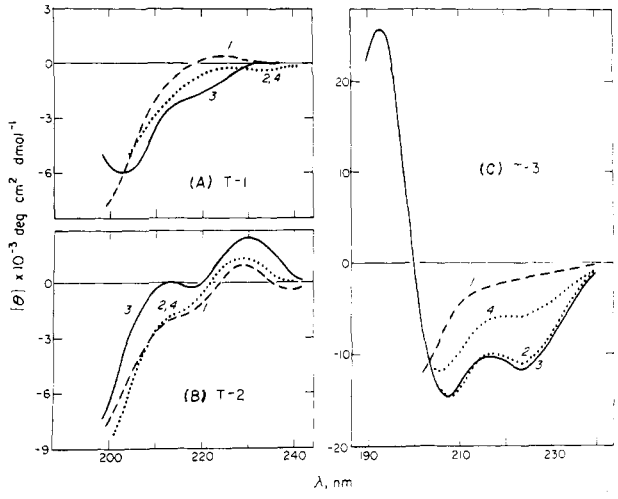

FIGURE 4: CD of trypsin-digested fragments of glucagon. Curves: (1) no surfactant; (2) 20 mM $DodNH_3Cl$; (3) 25 mM $NaDodSO_4$; (4) 20 mM dodecyl heptaoxyethylene ether. Peptide concentrations: (A) 38 μM; (B) 20 μM; (C) 33 μM.

(Figure 4B). The small positive band at 229 nm is probably due to the tyrosine chromophore.

The undecapeptide T-3 is highly hydrophobic (it has only one charged side group). It is moderately soluble at alkaline pH values and sparingly soluble at acidic pH values, but the peptide solution remains clear upon lowering the pH below 7 if the peptide concentration is less than 0.5 mg/mL. In 25 mM $NaDodSO_4$ the CD spectrum of fragment T-3 shows a distinctive helical conformation (Figure 4C, curve 3), which remains unchanged from pH 2 to 12.5. The CD magnitude indicates a 45–50% helix for a helical segment of five to six residues. The percent helix of fragment T-3 is about the same in $DodNH_3Cl$ (curve 2), $NaDecSO_4$, and $Dod(CH_3)_3NCl$ solutions (not shown) but much less in nonionic $C_{12}E_7$ solution (curve 4).

Potentiometric Titration. In Figure 5 the degree of binding, x, in terms of moles of $NaDecSO_4$ per mole of peptide and the corresponding $[\Theta]_{222}$ are plotted against the total concentration of the surfactant. The binding occurs at C_t = 0.08 mM. Surprisingly, the number of $NaDecSO_4$ molecules bound levels off at x = 4 (C_t = 0.6 mM) instead of 5 (glucagon has five positive charges including the α-NH_3^+ at the N terminal). The peptide–surfactant complex begins to precipitate at x =

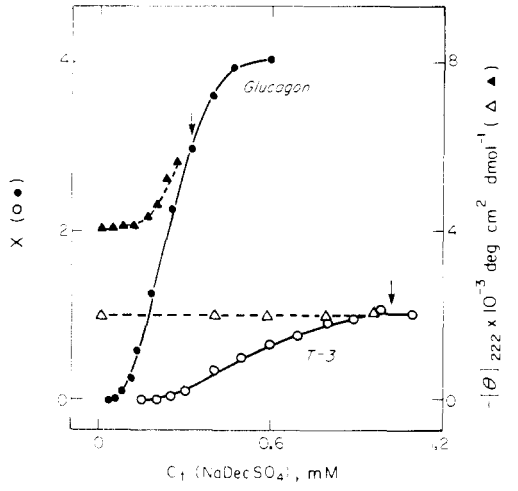

FIGURE 5: Binding isotherm of glucagon by $NaDecSO_4$ and the corresponding changes in CD. x is the moles of surfactant bound per mole of peptide. Peptide concentration: 49 μM in NaCl–glycine buffer (pH 2; ionic strength 0.1) at 25 °C. Arrows indicate precipitation (on the right side).

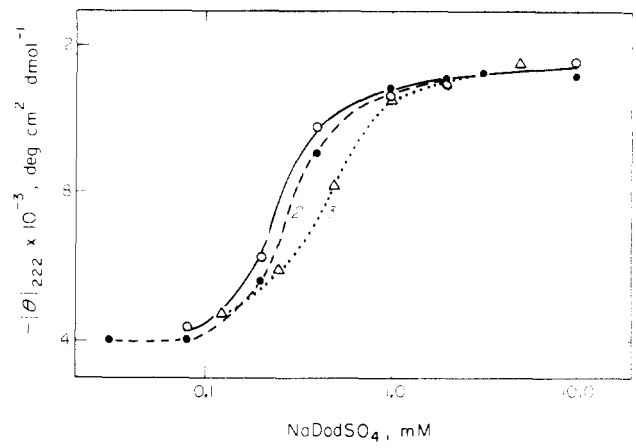

FIGURE 6: Effect of surfactant concentration on the CD of glucagon at constant $NaDodSO_4$/glucagon ratios. Glucagon was dissolved in 10 mM $NaDodSO_4$ without buffer and diluted with water. Molar ratios: (1) 4.4; (2) 7.8; (3) 29.

3; the corresponding change in $[\theta]_{222}$ is relatively small. The precipitate redissolves when C_t reaches ~10 mM where the $-[\theta]_{222}$ increases to 14000 deg cm^2 dmol^{-1}.

The binding of $NaDecSO_4$ to fragment T-3 starts at C_t = 0.3 mM and levels off at 1 mM as $x \rightarrow 1$. This corresponds well with a single positive charge at the N terminal of fragment T-3. This binding is not accompanied by any conformational change ($[\theta]_{222}$ remains constant). The complex at $x = 1$ becomes insoluble and does not redissolve until C_t reaches ~5 mM. The $-[\theta]_{222}$ increases to 7500 deg cm^2 and gradually to a maximum of 12000 dmol^{-1} at 10 mM $NaDecSO_4$. Both fragments T-1 and T-2 did not bind $NaDecSO_4$ within the range of surfactant concentrations studied (up to 3 mM).

Judged from the change in $[\theta]_{222}$, the binding of $NaDodSO_4$ by glucagon and its fragments is similar to that of $NaDecSO_4$. The only difference is that the binding starts at a lower surfactant concentration and that the insoluble peptide–surfactant complexes redissolve at 1 mM $NaDodSO_4$ in a buffer of 0.1 ionic strength as compared with 5–10 mM $NaDecSO_4$.

Figure 6 shows the dependence of $[\theta]_{222}$ on the $NaDodSO_4$ concentration when the molar $NaDodSO_4$/glucagon ratio is kept constant. Curves 1, 2, and 3 represent molar ratios of 4.4, 7.8, and 29, respectively. Above 1 mM $NaDodSO_4$ the ellipticity is identical in the three solutions. Below 1 mM $NaDodSO_4$ the CD magnitude falls off rapidly and more so at higher molar ratios. In this region, say at 0.4 mM surfactant, the ellipticity increases with the peptide concentration and levels off at ~0.3 mg/mL glucagon. This is in contrast to the results shown in Figure 1, where the CD magnitude is independent of the peptide concentration.

Discussion

Globular proteins are generally believed to have the same secondary structures in crystals and in solution. This may not be true for polypeptides without disulfide bonds. For instance, β-endorphin (31 residues) and β-lipotropin (91 residues) show little secondary structure in water, but about one-half of their molecules adopts a helical conformation in $NaDodSO_4$ solution (Yang et al., 1977). Although X-ray studies of these two polypeptides are not yet possible, the sequence-predictive method of Chou & Fasman (1974) does support the presence of the helical conformation. Glucagon is another such example. Despite its high helicity in crystals (Sasaki et al., 1975), this hormone is essentially unordered in water but becomes partially helical in an amphiphile solution. In the initial phase, $NaDodSO_4$ or $NaDecSO_4$ binds with the positively charged side groups of the polypeptide (at pH 2 the negatively charged Glu and Asp residues are protonated). Neutralization of the charges and exposure of the amphiphile tails to water cause the glucagon–surfactant complex to precipitate when three or more positive charge are neutralized (Figure 5). Further additions of the surfactants enable them to cluster around the polypeptide chain probably in a double-layer fashion with the polar heads of the amphiphiles exposed toward the aqueous medium. Thus, the glucagon–surfactant complexes can redissolve in excess surfactant solution.

In acidic solution the positively charged side groups of the polypeptide chain will repel the amphiphile heads having charges of the same sign. Unlike $NaDodSO_4$, $DodNH_3Cl$ cannot form ionic bonds with glucagon at pH 2. Because glucagon residues 19–29 are quite hydrophobic, the amphiphile tails may approach the polypeptide chain. Thus, the induced helical conformation of glucagon at pH 2 is the same in $DodNH_3Cl$ and $C_{12}E_7$, which are somewhat less effective than $NaDodSO_4$ (Figure 1, curves 2 and 3).

Glucagon fragment T-3 can have a partial helical conformation in nonionic $C_{12}E_7$ as well as both ionic surfactants (Figure 4). In this case, $NaDodSO_4$ and $DodNH_3Cl$ appear to be more effective than $C_{12}E_7$ for reasons that are still not clear (the binding affinity of the surfactant toward the polypeptide chain may be an important factor). The pentapeptide T-2 is probably too short to form one helical turn in any surfactant solution, even though it is part of the helix in the intact glucagon molecule. For glucagon fragment T-1, residues 10, 11, and 12 are located at the C terminal and therefore are unlikely to twist into a helical turn, whereas residues 5–9 preceding them may not have a helix-forming potential that is strong enough for a regular helix. Accordingly, fragment T-1 is also unordered in all three surfactant solutions. In $NaDodSO_4$ solution the helical segment of intact glucagon may extend from fragment T-3 into fragment T-2. Another short helix could be localized around Lys-12, as judged from the loss of some helicity at high pH (Figure 3). The results in Figures 1 and 4 indicate that the number of helical residues in intact glucagon is substantially higher than the number found in fragment T-3 when surfactant is present. However, the surfactant-induced helicity does not reach the level found in the crystalline state.

The concentration dependence of the CD of glucagon in aqueous solution has been attributed to the association of the

peptide molecules (Blanchard & King, 1966; Gratzer & Beaven, 1969; Srere & Brooks, 1969). On standing for several days, the helical conformation formed by association is transformed to β structure, as evidenced from infrared (Gratzer et al., 1967) and CD (Moran et al., 1977) spectra. This aggregation disappears in $NaDodSO_4$ solution (Figure 2A); the surfactant provides a hydrophobic environment that stabilizes the helical conformation without association. The salt effect (Figure 2B) on native glucagon almost linearly enhances the CD (glucagon in water precipitates at 2 M NaCl) and therefore cannot be attributed to electrostatic interaction alone. Rather, it must arise from the aggregation of the peptide molecules prior to its salting out. Here again the salt effect is small in the presence of $NaDodSO_4$. The small increase in the CD magnitude before precipitation may also be due to aggregation. Raising the temperature of the peptide solution "unfolds" the native glucagon, but addition of $NaDodSO_4$ stabilizes the helical conformation of glucagon against high temperature (Figure 2C).

Like globular proteins, glucagon "denatures" at extreme pH values (Figure 3, top curve). The situation is different in $NaDodSO_4$ solution, where carboxylate ions can destabilize the induced helical conformation. Accordingly, protonation of Glu and Asp residues enhances the ordered structure. The acidic transition at pH 5.2 compares well with the apparent pK_a of 5.9 for Leu-15 human gastrin in $NaDodSO_4$ solution (Wu & Yang, 1978). On the alkaline side, Lys and His residues will deprotonate above pH 12 and at the same time the phenyl group of Tyr residues will ionize. The combined reduction in positive charges and the increase in negative charges will disrupt the helical conformation that is induced by $NaDodSO_4$. At pH 13 glucagon still retains some helicity as evidenced by the CD magnitude. Very likely this helical segment is located in the fragment T-3 segment at the C terminal whose CD is pH independent.

Potentiometric titration of fragment T-3 with an anionic surfactant at pH 2 (Figure 5) indicates an initial binding of 1 mol of $NaDecSO_4$ per mol of fragment T-3 before the onset of precipitation of the complex. It suggests that the positive charge at the N terminal is neutralized. For intact glucagon four out of five positive charges are titrated by $NaDecSO_4$. Whether precipitation of the glucagon–surfactant complex with three or more $NaDecSO_4$ molecules bound obscures the titration of the fifth charge is difficult to answer at present. The binding of $NaDecSO_4$ to glucagon (Figure 4) cannot be interpreted in terms of multiple equilibria with identical and independent sites. In fact, the apparent K_a was found to increase rather than decrease with the number of $NaDecSO_4$ molecules bound. More surprising is that even fragment T-3 with one binding site lacks a constant K_a. One plausible explanation is that the aggregated T-3 fragments behave as if they were a single molecule containing several positive charges and the binding of $NaDecSO_4$ to such aggregates is cooperative.

The results in Figure 6 demonstrate that the critical micelle concentration of the surfactant is not required for inducing a maximum change in conformation. $NaDodSO_4$ has a critical micelle concentration of 8.3 mM in water, which is reduced to $\sim$1.3 mM in a solution of ionic strength 0.1 (Reynolds & Tanford, 1970). The CD magnitude reaches its maximum in the plateau region which covers both above and below the critical micelle concentration. Steinhardt et al. (1974) have shown that (dimethylamino)azobenzene is solubilized by the serum albumin–$NaDodSO_4$ complex well below the critical micelle concentration of $NaDodSO_4$. This is attributed to the formation of micelle-like structure on the protein–surfactant complexes. This is the same as our postulate that surfactant molecules can cluster around the polypeptide chain below their critical micelle concentration. However, when the $NaDodSO_4$/peptide ratio is small (Figure 6, curve 1), the number of surfactant molecules bound is not large enough to form micelles, but the peptide–surfactant complex can still adopt a partial helical conformation. In this case the hydrocarbon tails of the bound surfactant molecules may possibly be juxtapositioned to the hydrophobic side of the induced helical segment in the same or another glucagon molecule. The drop in CD magnitude below 1 mM $NaDodSO_4$ in Figure 6 is not due to the dissociation of the peptide–surfactant complexes since $NaDodSO_4$ is known to have a strong binding affinity toward proteins (Steinhardt et al., 1974). It is likely to be related to the population of peptide–surfactant complexes (without micelles) which can aggregate to form molecular species of higher helical content.

We were not able to detect the β form in glucagon or its fragments at low $NaDodSO_4$ concentration in spite of the high β-forming potential of residues 5–10 and 19–27 (Chou & Fasman, 1975). This could be due to the lack of cationic residues on or near the β-forming segments. Presumably, these residues bind $NaDodSO_4$ monomers which in turn associate with another bound surfactant molecule to form interchain aggregates. Our previous observation of β structure in angiotensin II (Wu & Yang, 1978), renin substrate, and insulin B chain residues 23–29 (Yang & Wu, 1978) supports this contention.

Acknowledgments

We thank Dr. Keishiro Shirahama for $NaDodSO_4$ and $NaDecSO_4$ samples and Yu-Hua Chang for her valuable assistance in the amino acid analysis.

References

Bedarker, S., Blundell, T. L., Dockerill, S., Tickle, I. J., & Wood, S. P. (1978) *Ciba Found. Symp. 60*, 105–121.

Blanchard, M. H., & King, M. V. (1966) *Biochem. Biophys. Res. Commun. 25*, 298–303.

Bornet, H., & Edelhoch, H. (1971) *J. Biol. Chem. 246*, 1785–1792.

Bromer, W. W., Sinn, L. G., & Behrens, O. K. (1957a) *J. Am. Chem. Soc. 79*, 2807–2810.

Bromer, W. W., Staub, A., Sinn, L. G., & Behrens, O. K. (1957b) *J. Am. Chem. Soc. 79*, 2801–2805.

Chen, Y. H., Yang, J. T., & Chau, K. H. (1974) *Biochemistry 13*, 4120–4131.

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

Chou, P. Y., & Fasman, G. D. (1975) *Biochemistry 14*, 2536–2541.

Contaxis, C. C., & Epand, R. M. (1974) *Can. J. Biochem. 52*, 456–468.

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

Epand, R. M. (1972) *J. Biol. Chem. 247*, 2132–2138.

Epand, R. M., Jones, A. J. S., & Schnider, S. (1977) *Biochim. Biophys. Acta 491*, 296–304.

Gratzer, W. B., & Beaven, G. H. (1969) *J. Biol. Chem. 244*, 6675–6679.

Gratzer, W. B., Bailey, E., & Beaven, G. H. (1967) *Biochem. Biophys. Res. Commun. 28*, 914–919.

Jirgensons, B. (1973) in *Optical Activity of Proteins and Other Macromolecules*, 2nd ed., p 121, Springer-Verlag, West Berlin, Heidelberg, and New York.

Mattice, W. L., Riser, J. M., & Clerk, D. S. (1976) *Biochemistry 15*, 4264–4272.
Moran, E. C., Chou, P. Y., & Fasman, G. D. (1977) *Biochem. Biophys. Res. Commun. 77*, 1300–1306.
Reynolds, J. A., & Tanford, C. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 1002–1007.
Sasaki, K., Dockerill, S., Adamiak, D. A., Tickle, I. J., & Blundell, T. (1975) *Nature (London) 257*, 751–757.
Satake, I., & Yang, J. T. (1976) *Biopolymers 14*, 1841–1846.
Schneider, A. B., & Edelhoch, H. (1972) *J. Biol. Chem. 247*, 4992–4995.
Srere, P. A., & Brooks, G. C. (1969) *Arch. Biochem. Biophys. 129*, 708–710.
Steinhardt, J., Stocker, N., Carroll, D., & Birdi, K. S. (1974) *Biochemistry 13*, 4461–4468.
Thomsen, J., Kristiansen, K., Brunfeldt, K., & Sundby, F. (1972) *FEBS Lett. 21*, 315–319.
Visser, L., & Blout, E. R. (1971) *Biochemistry 10*, 743–752.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.
Wu, C.-S. C., & Yang, J. T. (1978) *Biochem. Biophys. Res. Commun. 78*, 914–919.
Yang, J. T., & Wu, C.-S. C. (1978) in *Versatility of Proteins* (Li, C. H., Ed.) pp 99–118, Academic Press, New York.
Yang, J. T., Bewley, T. A., Chen, G. C., & Li, C. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3235–3238.

Proton Nuclear Magnetic Resonance Studies on Dideoxyribonucleoside Methylphosphonates†

Lou S. Kan,* Doris M. Cheng, Paul S. Miller, Junichi Yano, and Paul O. P. Ts'o

ABSTRACT: A series of dideoxyribonucleoside methylphosphonates, d-A*p*A, d-A*p*T, d-T*p*A, and T*p*T, were synthesized chemically and the diastereoisomers of each dimer were separated [Miller, P. S., Yano, J., Yano, E., Carroll, C., Jayaraman, K., & Ts'o, P. O. P. (1979) *Biochemistry 18*, 5134]. The ^{1}H NMR spectra of these compounds are similar to those of their parent diester compounds. Specifically, the assignments of the ^{1}H resonances of the two diastereoisomers of d-A*p*A (designated as 1 and 2) were reaffirmed by comparing with the unmodified, parent d-ApA. The absolute configuration of the phosphonate methyl group of the two isomers (d-A*p*A)$_1$ and (d-A*p*A)$_2$ was determined by the NOE technique. The ^{1}H NMR spectra of the diastereoisomers of d-A*p*A, as well as the corresponding monomer components d*A*p and CH_3*p*dA, and T*p*T were analyzed by spectrum simulation techniques. Thus, all the coupling constants and chemical shifts of the proton resonances of the deoxyribofuranose ring and the phosphonate methyl group could be precisely determined. These data provide the information for an analysis of the sugar puckering and backbone conformations of these novel nonionic nucleic acid analogues. It was found that the conformations of the sugar–phosphate backbones of each isomer are similar to each other and are similar to the conformations of the parent dinucleoside monophosphates. The average adenine stacking conformations of (d-A*p*A)$_1$ and (d-A*p*A)$_2$ were described in numerical coordinates derived from a computer analysis which included both ring-current magnetic anisotropy and atomic diamagnetic anisotropy effects. The two computer-derived conformational models are similar to those derived from the graphic approximation based only on the ring-current effects. For each pair of dimer analogues, the base stacking mode of isomer 1 is similar to that of its parent diester while the extent of base overlap in isomer 2 is less than that in isomer 1. The results of the conformational analysis based on NMR data are consistent with the results obtained from ultraviolet and circular dichroism measurements on these dimers.

Recently we described the synthesis of a series of deoxyribonucleoside methylphosphonates (Miller et al., 1979b). These nonionic nucleic acid analogues contain a 3′–5′ methylphosphonyl internucleoside linkage in place of the naturally occurring 3′–5′ phosphodiester linkage. These analogues not only serve as useful models for studying the conformational properties of nucleic acids (Miller et al., 1979b) but they can also serve as probes of nucleic structure and function within living cells (Miller et al., 1979a). This is because these nonionic analogues are very resistant to nucleases and enter the living mammalian cells with ease. The conformational properties of the individual diastereoisomers of each dimer, which differ only in the configuration of the phosphonate methyl group, were examined by UV and CD spectroscopy. The results of these studies suggested that each diastereoisomer has a distinct conformation in solution. This conclusion was further supported by the finding that the diastereoisomers of the dideoxyadenosine methylphosphonates each form complexes with poly(uridylic acid) having unique melting temperatures.

The detailed conformation of these dimers in solution has been studied by ^{1}H NMR[1] spectroscopy, including spin simulation, sequential-selective decoupling, nuclear Overhauser effects, and conformational model analyses by computer programs. In this paper, the ^{1}H NMR data are used to derive the conformation of each diastereoisomer of each dideoxyribonucleoside methylphosphonate, and the results are com-

† From the Division of Biophysics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205. *Received September 19, 1979.* This work was supported by a grant from the National Institutes of Health (GM016066-11) and a grant from the National Science Foundation (PCM77-25226). This paper was presented in part at the 1978 Biophysics Annual Meeting, Washington, DC.

[1] Abbreviations used: d-N*p*N, a deoxyribonucleotide dimer analogue containing a 3′–5′ internucleoside methylphosphonate linkage; (d-N*p*N)$_{1+2}$, a mixture of two stereoisomers of d-N*p*N; (d-N*p*N)$_{1and2}$, two individual compounds, i.e., (d-N*p*N)$_1$ and (d-N*p*N)$_2$; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; d-ApA, deoxyadenylyl(3′–5′)deoxyadenosine; poly(U), poly(uridylic acid).

0006-2960/80/0419-2122$01.00/0