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Lipid-Induced Ordered Conformation of Some Peptide Hormones and Bioactive Oligopeptides: Predominance of Helix over β Form†

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ABSTRACT: The conformation of several naturally occurring peptide hormones and bioactive oligopeptides in phospholipid solutions was studied by circular dichroism. Phosphatidylcholine induced a partial helix in human gastrin I at neutral pH, but phosphatidylserine did not unless the five consecutive glutamic acid residues in gastrin were protonated. Reduced somatostatin with two lysines and substance P with one arginine and one lysine were partially helical in phosphatidylserine, but not phosphatidylcholine, solution. Both lipids induced a helical conformation in glucagon and its COOH-terminal fragment (19–29) probably because the helical segment is primarily located at the uncharged COOH terminus. Thus, polypeptides with a helix-forming potential can have the helical conformation only when the peptides carry no charge or charges opposite to those on the polar head of the lipid. Renin substrate, which has potentials for the β form and β turn, seemed to form a mixture of the two conformations in phosphatidylserine solution. Angiotensin I with a strong probability for the β form adopted the β form in phosphatidylserine solution and sleep peptide with no structure-forming potential remained unordered in lipid solutions. The helix usually predominated over the β form in lipid solutions if the peptide has potentials for both conformations. This could account for the preponderance of helices in bacteriorhodopsin of the purple membrane, which according to its amino acid sequence would have favored the β form.

Previously, we reported that surfactants in solution, which provide a hydrophobic environment, may stabilize an ordered conformation in polypeptides and proteins that would have otherwise been disrupted by the peptide backbone–water interactions (Wu & Yang, 1978). We have hypothesized that the induced conformation of a polypeptide is related to the structure-forming potential of the peptide segments as dictated by the amino acid sequence of the peptides. Oligopeptides and short polypeptides in surfactant solutions can be classified into four types according to their induced conformation: (1) helix forming, (2) β forming, (3) either helix forming or β forming, and (4) unordered (Wu & Yang, 1978). Charged side groups of a peptide having the same sign as the surfactant and located

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in a helix or β segment tend to destabilize the ordered conformation (Wu et al., 1981). Peptide segments rich in hydrophobic residues can even adopt an ordered conformation in nonionic surfactant solutions (Wu & Yang, 1980). The presence of disulfide bonds may constrain the formation of an ordered conformation unless these linkages are broken by reduction (Wu & Yang, 1981a). Our hypothesis was supported by studies of many naturally occurring oligopeptides and short polypeptides in both ionic and nonionic surfactant solutions (Wu & Yang, 1978, 1980, 1981a; Yang & Wu, 1978).

We have since extended our studies to β-endorphin in cerebroside sulfate solution (Wu et al., 1979). To avoid the artifacts in optical measurements due to the light scattering of lipid vesicles we solubilized the lipid in a nonionic surfactant solution (Shirahama & Yang, 1979). We report herein the induced conformation of several peptide hormones and bioactive oligopeptides in solutions of two phospholipids (PhSer and PhCh).[1] On the basis of their CD spectra, the induced conformation of polypeptides in lipid solutions usually agreed with the surfactant-induced conformation. One notable exception was the type 3 peptides for which the helical conformation usually predominates over the β form, even though the average conformational parameters (Chou & Fasman, 1974, 1978) favor the β form. Because these parameters are derived from the secondary structure of crystalline proteins, their numerical values might have to be modified in a lipid environment. Our contention was supported by comparison of the predicted and the observed secondary structures of bacteriorhodopsin from the purple membrane of *Halobacterium halobium*.

Experimental Procedures

Materials. Human gastrin I, ovine somatostatin, renin substrate, angiotensin I, substance P, and sleep peptide were purchased from Peninsula Laboratories. Glucagon was from Elanco, and its COOH-terminal peptide (19–29) was prepared by trypsin digestion of the parent hormone (Bromer et al., 1957a,b). Bovine PhSer and PhCh (Supelco) and the nonionic surfactant $C_{12}E_7$ (Nikko Chemical, Japan) were used without further purification, whereas $C_{16}E_{13.5}$ (Kao Soap Co., Tokyo) was purified from 1-butanol (Shirahama & Yang, 1979).

The peptide concentration was determined spectrophotometrically and expressed in molar (mean residue) concentration. The molar absorption coefficients at 280 nm were assumed to be 1200 M^{-1} L^{-1} for tyrosine and 5500 M^{-1} L^{-1} for tryptophan. For substance P with no Tyr and Trp residues, the sample weight (0.5 mg) provided by the manufacturer was accepted without correction.

Circular Dichroism. The CD spectra were measured with a JASCO J-500A spectropolarimeter under constant nitrogen flush. It had been standardized with *d*-10-camphorsulfonic acid (Chen & Yang, 1977). The cell holder was a specially designed aluminum block with a water-circulating jacket; its temperature was maintained at 25 °C with a Haake constant-temperature regulator. Fused silica cells of various path lengths were used to cover the wavelength range of 190–240 nm. The cells were calibrated with a sucrose (National Bureau of Standards grade) solution of known optical rotation on a Cary 60 spectropolarimeter.

[1] Abbreviations: PhSer, phosphatidylserine; PhCh, phosphatidylcholine; $C_{12}E_7$, dodecyl heptakis(oxyethylene) ether; $C_{16}E_{13.5}$, hexadecyl poly(oxyethylene) ether (average condensation number 13.5); NaDodSO$_4$, sodium dodecyl sulfate; CD, circular dichroism.

Sample solutions were prepared by mixing a stock solution of the peptide in water and the lipid solution that had been solubilized in 3–4× molar concentration of either $C_{12}E_7$ or $C_{16}E_{13.5}$ at room temperature. All CD spectra of freshly prepared solutions were measured within 2 h. The CD data were expressed in mean residue ellipticities, $[\theta]$, in degrees centimeters squared per decimole (Yang et al., 1976). Because the lipids are optically active and contribute a considerable noise below 210 nm to the CD measurements, the experimental errors in the far-UV region may be larger than that in water alone.

Prediction of Secondary Structure of Peptides. The ordered conformation of the peptides was predicted from their amino acid sequences (Chou & Fasman, 1974, 1978). This method is based on a statistical analysis of 29 proteins of known atomic coordinates from X-ray diffraction studies. Three conformational parameters, P_α, P_β, and P_t, for the helix, β form, and β turn are assigned to each of the 20 amino acid residues from their frequency of occurrence in a particular conformation. For a segment of n residues, the average parameter of an ith conformation is simply $\langle P_i \rangle = \sum P_i/n$. The segment is considered to have the potential of forming a helix when $\langle P_\alpha \rangle \geq 1.03$ or a β-form when $\langle P_\beta \rangle \geq 1.05$. The choice of either of the two forms becomes equivocal if overlapping segments have the potentials for both conformations unless $\langle P_\alpha \rangle$ is much greater than $\langle P_\beta \rangle$ or vice versa. A tetrapeptide segment has the β-turn potential when $\langle P_t \rangle > 1$ and $\langle P_\alpha \rangle < \langle P_t \rangle > \langle P_\beta \rangle$. In addition, the product of the four bend frequencies in a β turn, p_t, must be greater than 0.75×10^{-4}.

Results

Human Gastrin I. This peptide has 17 residues:

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

According to the Chou–Fasman method (Chou & Fasman, 1974, 1978) residues 3–11 (underlined) have a $\langle P_\alpha \rangle = 1.31$, which indicates a high probability of adopting a helical structure in a suitable solvent. This agrees with the CD spectra of gastrin I, whose conformation is solvent dependent. In water at neutral pH the electrostatic repulsion among the five consecutive Glu's in the middle of the peptide chain is so great that it will disrupt any ordered conformation. Thus, the CD spectrum showed a strong negative band at 200 nm, which is typical of an unordered conformation (Figure 1, curve 1). The addition of either PhSer or PhCh at a molar lipid/peptide ratio, R, of 1 did not affect the peptide conformation. However, raising R to more than 3 for PhCh altered the CD spectrum; the appearance of a double minimum at 208 and 222 nm is characteristic of the presence of a helical conformation, and the magnitude of the minimum indicates a partial helix (curve 3). On the other hand, PhSer did not promote a helical conformation under similar conditions (curve 2). Both lipids became effective helix inducers when the Glu residues were protonated (curves 4 and 5). At $R = 4.6$ the mean residue ellipticities at 222 nm changed from 0 to −12 200 deg cm^2 dmol^{-1} in PhSer solution (curves 2 and 4) and from −2600 to −16 800 deg cm^2 dmol^{-1} in PhCh solution (curves 3 and 5) when the pH was lowered from above pH 6 to below pH 3. The $[\theta]_{222}$ of gastrin I in acidic PhCh solution was identical with that in acidic NaDodSO$_4$ solution (Wu & Yang, 1978; Yang & Wu, 1978), but the magnitude of the corresponding ellipticity in PhSer solution was smaller. The nonionic surfactant, $C_{16}E_{13.5}$ or $C_{12}E_7$, alone at a concentration that solubilized the lipid also induced a partial helical conformation;

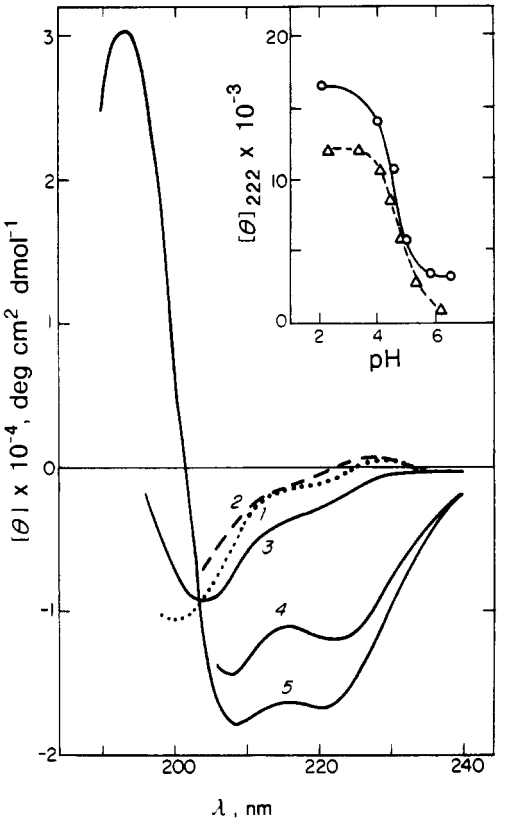
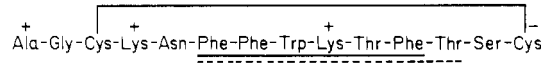


FIGURE 1: CD of human gastrin I: (curve 1) 0.55 mM in water at pH 5.7, (curve 2) 0.22 mM in 1.02 mM phosphatidylserine at pH 6.4, (curve 3) 0.22 mM in 1.02 mM phosphatidylcholine at pH 6.1, (curve 4) same as curve 2 but at pH 2.4, and (curve 5) same as curve 3 but at pH 2.1. (Inset) pH dependence of mean residue ellipticity at 222 nm.

$[\theta]_{222} = -10\,800$ deg cm^2 dmol^{-1} at pH 4, which was smaller (in magnitude) than that in the mixed micelles of the lipid and the nonionic surfactant. The change in $[\theta]_{222}$ of gastrin I with pH in either of the two lipids showed a midpoint at pH 4.4–4.8 (Figure 1, inset), which may represent the apparent ionization constant of the Glu's in the lipid solutions.

Somatostatin. This tetradecapeptide



has two Lys's separated by four residues and Cys-3 and Cys-14 linked by a disulfide bond. The peptide has the potentials for both the helix (solid underline) and the β form (broken underline). With $\langle P_\alpha \rangle = 1.08$ and $\langle P_\beta \rangle = 1.23$, the β form should be favored, but our CD results indicated otherwise.

The CD spectrum of somatostatin was again unordered in water (Figure 2, curve 1). It was unaltered when the single disulfide bond was reduced with dithioerythritol. However, Hollady & Puett (1976) have suggested an ordered conformation of the unreduced somatostatin in water. In PhSer solution ($R = 1.8$) the CD minimum at 200 nm was red shifted to 207 nm for the unreduced somatostatin (curve 2). Once the disulfide bond was broken, the CD spectrum resembled that of a partial helical conformation with a minimum at 208 nm, a shoulder at 222 nm, and a strong positive band near 193 nm (curve 3). The mean residue ellipticity at 222 nm was about −3000 deg cm^2 dmol^{-1}, which was about half of that in 25 mM $NaDodSO_4$ (Wu & Yang, 1981a; Wu et al., 1981). Lowering the PhSer concentration did not induce a β form, as 2 mM $NaDodSO_4$ did (Wu et al., 1981). The addition of PhCh did not promote a helical conformation in both native and reduced somatostatin probably because of the charge repulsion between the lipid and the peptide.

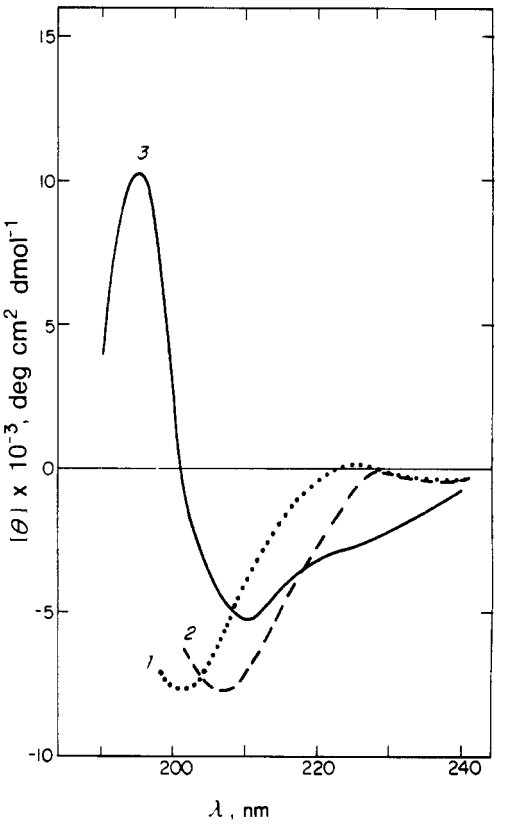


FIGURE 2: CD of somatostatin: (curve 1) 0.74 mM in 1.3 mM dithioerythritol at pH 6.6, (curve 2) 0.077 mM in 0.13 mM phosphatidylserine at pH 7.1, and (curve 3) 0.30 mM in 0.51 mM phosphatidylserine plus 0.5 mM dithioerythritol at pH 7.3.

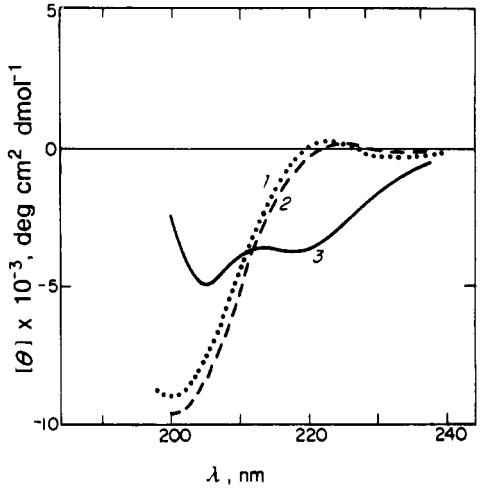
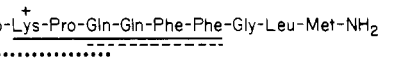


FIGURE 3: CD of substance P: (curve 1) 1.94 mM in water at pH 6.0, (curve 2) 0.23 mM in 0.51 mM phosphatidylcholine at pH 5.8, and (curve 3) 0.28 mM in 0.47 mM phosphatidylserine at pH 6.3.

Substance P. This undecapeptide



has a weak helical potential for residues 3–8 (solid underline) with $\langle P_\alpha \rangle = 1.04$, a β-form potential for residues 5–8 (broken underline) with $\langle P_\beta \rangle = 1.24$, and probably a β turn at residues 2–5 (dotted underline).

The CD spectrum of substance P indicated an unordered conformation in water (Figure 3, curve 1). The addition of

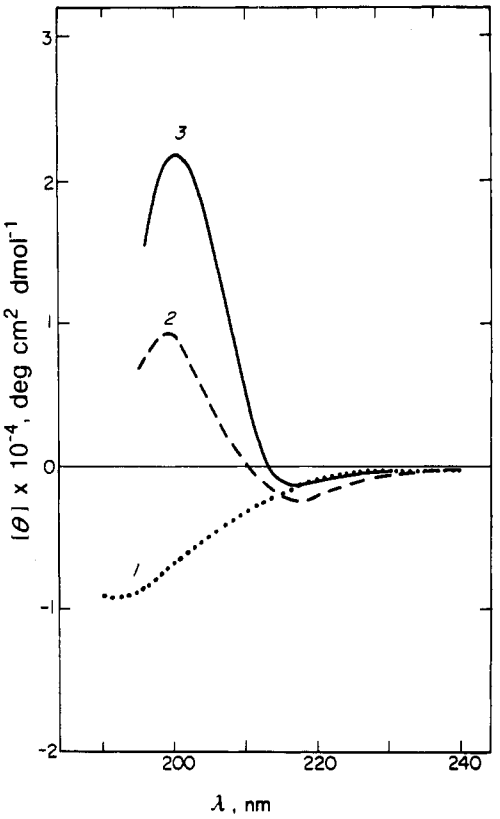
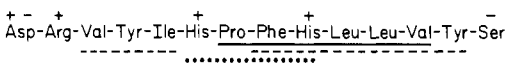


FIGURE 4: CD of renin substrate. The peptide concentration was 0.31 mM. The following solvents were used: (1) water at pH 6.5, (2) 0.31 mM phosphatidylserine at pH 6.5, and (3) same as (2) but at pH 4.0.

PhCh, which carries a positive charge on its polar head, could not induce an ordered structure in this peptide (curve 2). In PhSer solution at $R = 1.6$ the CD spectrum showed a double minimum with $[\theta]_{222} = -3700$ deg cm^2 dmol^{-1} and $[\theta]_{205} = -4900$ deg cm^2 dmol^{-1}, suggesting the formation of a partial helix. The magnitude of these ellipticities was similar to that obtained in $NaDodSO_4$ solution (Wu & Yang, 1978). Previously (Wu et al., 1982), we are unable to detect an ordered conformation of substance P in the lipid solutions probably because the peptide concentration used was too low and the noise of the instrument too high.

Renin Substrate. This tetradecapeptide



has a marginal helix potential between residues 7 and 12 (solid underline) with $\langle P_\alpha \rangle = 1.03$, strong β-form potentials at residues 3–5 ($\langle P_\beta \rangle = 1.59$) and 8–13 ($\langle P_\beta \rangle = 1.34$) (broken underlines), and also a β-turn potential at residues 6–9 in between the β forms (dotted underline).

The CD spectrum of renin substrate in water indicated an unordered conformation (Figure 4, curve 1). In 0.3 mM PhSer, the spectrum showed a small minimum at 217 nm and a strong maximum at 200 nm (curve 2). This profile suggested the presence of a β form, although the magnitude of the negative band was too small for a typical β form. The mean residue ellipticity at 217 nm was about half as large as that observed in 1 mM $NaDodSO_4$ (Yang & Wu, 1978). Lowering the pH of the peptide solution from pH 6.5 to pH 4.0 reduced the ellipticity at 217 nm to one half but more than doubled that at 200 nm (curve 3). This spectrum in acidic solution resembles that of the β turn formed by a model tetrapeptide, Cbz-Gly-L-Ser(*O*-Bu^t)-L-Ser-Gly-*O*-stearyl ester, in cyclohexane (Kawai & Fasman, 1978). However, the minimum was slightly blue shifted, and the magnitude of the ellipticities was smaller for renin substrate than for the model compound. Thus, the conformation of renin substrate in acidic phosphatidylserine solution was probably a mixture of the β form and β turn. Presumably, the protonation of Asp-1 and the COOH terminus facilitated the formation of the β turn.

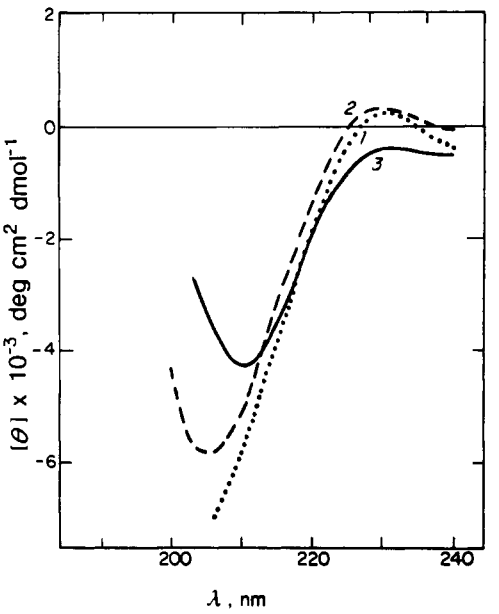


FIGURE 5: CD of angiotensin I: (curve 1) 2.3 mM in water at pH 4.4, (curve 2) 0.46 mM in 0.51 mM phosphatidylcholine at pH 4.8, and (curve 3) 0.46 mM in 0.51 mM phosphatidylserine at pH 5.9.

Angtiotensin I. This decapeptide is simply the fragment (1–10) of renin substrate without the four COOH-terminal residues. Removal of these residues further destabilized the potential helical segment and one of the two β-form segments, but residues 3–5 still retains a strong β-form potential.

The CD spectra of angiotensin I in Figure 5 indicated that the minimum around 200 nm (curve 1) for the unordered conformation in water was red shifted to 205 nm (curve 2) in PhCH solution and 210–212 nm in PhSer solution (curve 3). The latter suggested a β form. Comparison of the sequences of angiotensin I and renin substrate seemed to further suggest that the removal of four residues from renin substrate at the COOH terminus also destabilized the formation of a β turn. On the other hand, angiotensin II, which has two less residues than angiotensin I at the COOH terminus, formed a β structure in $NaDodSO_4$ solution (1.7 or 25 mM) (Wu & Yang, 1978) but did not have a β form in PhSer or PhCh solution (data not shown).

Glucagon and Glucagon (19–29). Glucagon is a polypeptide with 29 residues and no disulfide bonds. Its X-ray diffraction studies 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 helices (Bedarker et al., 1978). Yet in dilute aqueous solution glucagon is essentially in an unordered form (Wu & Yang, 1980). Dimyristoylglycerophosphocholine was reported to complex with glucagon and induce a helical structure in the peptide below the transition temperature of the lipid (Epand et al., 1977a,b). On the basis of the CD spectra of glucagon in lipid solutions (not shown), both PhCH and PhSer that had been solubilized in a nonionic surfactant could promote a partial helical conformation in glucagon at 25 °C. The CD spectra at pH 4 had a characteristic double minimum for the helix. At an equimolar mixture of the lipid and the peptide (residue) $[\theta]_{222}$ was −9800 and −8400 deg cm^2

Table I: Comparison of Induced Conformation of Short Polypeptides by Phosphatidylserine and $NaDodSO_4$[a]

peptides	concn (mM) PhSer	concn (mM) $NaDodSO_4$	conformation	$[\theta]_{222}$ (helix) PhSer	$[\theta]_{222}$ (helix) $NaDodSO_4$
gastrin I	0.31	25[b]	H	−12 200	−16 400
somatostatin	0.51	25[c]	H	−2 900	−6 100
		2	β		
substance P	0.47	25[b]	H	−3 700	−3 800
		1	β		
glucagon	0.31	25[d]	H	−9 800	−13 800
glucagon (19–29) fragment	0.51	25[d]	H	−6 100	−11 500
renin substrate	0.31	1[b]	β[g]		
		25	H		−5 000
angiotensin I	0.51	1[b]	β		
		25	β		
β-endorphin	0.18[e]	25[f]	H	−8 200	−13 000

[a] 25 mM $NaDodSO_4$ was used to fully develop the ordered conformation; actually, in many cases as low as 3 mM $NaDodSO_4$ would suffice. [b] Taken from Wu & Yang (1978). [c] Taken from Wu et al. (1981). [d] Taken from Wu & Yang (1980). [e] Taken from Wu et al. (1979). [f] Taken from Yang et al. (1977). [g] Probably a mixture of β form and β turn.

$dmol^{-1}$ in PhSer and PhCh, respectively. These values were slightly lower than the mean residue ellipticity reported by Epand et al. (1977a,b) probably because of the use of different lipids under different experimental conditions. Even the nonionic surfactant $C_{12}E_7$ (20 mM) alone could promote an appreciable amount of the helical conformation in glucagon (Wu & Yang, 1980). However, in 1.2 mM $C_{16}E_{13.5}$, which was enough to solublilze the amount of lipids used, the $[\theta]_{222}$ of glucagon was only −3000 deg cm^2 $dmol^{-1}$, which represented a low helical content. Thus, in the mixed micelles of the lipid and nonionic surfactant the lipid was mostly responsible for the observed helical conformation of glucagon.

On the basis of the CD spectra (not shown), the COOH-terminal fragment (19–29) of glucagon also assumed a partial helical conformation in the phospholipid solutions when the molar lipid/peptide (residue) ratio was kept at 1–3. But $[\theta]_{222}$ of −6100 to −6900 deg cm^2 $dmol^{-1}$ was smaller than that found in $NaDodSO_2$ or $DodNH_3Cl$ solution (Wu & Yang, 1980).

Sleep Peptide. This nonapeptide

$$\overset{+}{\text{Trp}}\text{-Ala-Gly-Gly-Asp-Ala-Ser-Gly-}\overset{-}{\text{Glu}}$$

has no helix or β-form potential by the criteria of Chou & Fasman (1974, 1978). It was previously classified as type 4 (unordered) peptide in $NaDodSO_4$ solution (Yang & Wu, 1978; Wu & Yang, 1978). This was also true in both PhSer and PhCh solutions.

The nonionic surfactant, either $C_{12}E_7$ or $C_{16}E_{13.5}$ alone, did not affect the conformation of native and reduced somatostatins, substance P, renin substrate, angiotensins I and II, and sleep peptide at a surfactant concentration used to solubilize the lipids. But the same nonionic surfactant could induce a partial helical conformation in protonated gastrin I, glucagon, and glucagon (19–29) even in the absence of lipids (see Discussion).

Discussion

The first step of the complex formation between a peptide and a lipid seems to be electrostatic, unless the peptide has no charged side groups. PhSer carrying a positive and negative charge on its polar head behaves like $NaDodSO_4$, whereas PhCh with only a positive charge on its polar head resembles dodecylammonium chloride in the complex formation. Apparently, the negative charge on the phosphate group or the positive charge on the serine moiety of PhSer does not participate in the binding of the lipids to the peptides. The second stage of the binding involves the clustering of the micellar amphiphiles around the peptide chain. This in turn provides a hydrophobic environment that includes an ordered conformation that would have otherwise been disrupted by the peptide backbone–water interactions.

The concentration of the amphiphile or the molar amphiphile/peptide ratio necessary for the induction of an ordered conformation in a polypeptide is always lower for the lipids than for the surfactants (Table I). For instance, 0.5 mM PhSer or less could induce a helical conformation in gastrin I, reduced somatostatin, substance P, glucagon, and glucagon (19–29), but $NaDodSO_4$ at such a low concentration could not (a β form was observed for reduced somatostatin and substance P instead when the $NaDodSO_4$ concentration was slightly above 1 mM). Likewise, renin substrate assumed an ordered structure in 0.3 mM PhSer as compared with 1 mM $NaDodSO_4$.

In general, the magnitude of the mean residue ellipticity for the induced conformation of the peptides was smaller in lipid solutions than in $NaDodSO_4$ solution (Table I). The $[\theta]_{222}$ of glucagon in acidic solutions was −9800, −8300, and −13 800 deg cm^2 $dmol^{-1}$ in PhSer, PhCh, and $NaDodSO_4$ solutions, respectively. Previously, we reported that the $[\theta]_{222}$ magnitude of β-endorphin was also smaller in PhSer solution than in $NaDodSO_4$ solution (Wu et al., 1979). The helicity of these peptides did not seem to increase with increasing lipid concentration, which, however, was limited to less than 1 mM because of excessive noise due to absorbance of the lipid. Thus, the possibility that the helicity might be enhanced beyond 1 mM lipid cannot be completely ruled out.

Like the lipids and $NaDodSO_4$, nonionic surfactants also provide a hydrophobic environment that could stabilize an ordered conformation of the peptides. However, nonionic surfactants usually fail to exert any effect whenever the peptides carry charged side groups. Such is the case for somatostatin, substance P, renin substrate, and angiotensins I and II (sleep peptide has no structure-forming potential and therefore does not have any ordered conformation in either lipid or ionic and nonionic surfactant solutions). Even when the nonionic surfactants can induce an ordered conformation in glucagon, glucagon (19–29), and protonated gastrin I, the amount of helicity produced was less than that found in the presence of lipids. Furthermore, the type of induced conformation in nonionic surfactant solution alone was the same as that in lipid solutions. Thus, the conformation of the peptides as seen in the mixed micelles of a lipid and a nonionic surfactant may represent the peptide conformation in the lipid solution alone.

The induced conformations in phospholipid solutions were qualitatively similar to those found in surfactant solutions (cf. Wu & Yang (1981b)]. Hence, gastrin I and glucagon, which are rich in helix potential (type 1), became partially helical in both lipid and surfactant solutions. Angiotensin I, which has the β-form potential, adopted a β form in PhSer and $NaDodSO_4$ solutions. Peptides having both helix and β-form potentials (type 3), reduced somatostatin and substance P, can only have the helical conformation in lipid solutions regardless of the concentration of the lipid used, whereas the same peptides adopted a β-form in less than 2 mM $NaDodSO_4$ and were converted to the helix in more than 5 mM $NaDodSO_4$ (Wu & Yang, 1978; Yang & Wu, 1978). On the other hand, renin substrate behaved like reduced stomatostatin and substance P in $NaDodSO_4$ solutions, but it appeared to form a mixture of the β form and β turn in PhSer solution.

The most striking difference in the binding of a peptide with a lipid and a surfactant such as $NaDodSO_4$ is the induced conformation of the type 3 peptides. In general it is the helical conformation that predominates over the β form in a lipid solution, even though the β-forming potential might be stronger than the helix-forming potential according to the Chou–Fasman method (1978). In this study renin substrate is the only exception that appeared to form a mixture of the β form and β turn in PhSer solution. Since the Chou–Fasman method is originally based on the three-dimensional structure of soluble proteins, there is reason for caution against its literal application to proteins in a lipid environment. Quite possibly the conformational parameters of the 20 amino acids must be modified under these conditions. This in turn will affect the average conformational parameters, $\langle P_\alpha \rangle$ and $\langle P_B \rangle$, of peptide segments and alter the relative potentials for the helix and the β form. It may be relevant to mention that poly(L-lysine) adopts a β conformation in $NaDodSO_4$ but has a helical conformation in PhSer solution (Shirahama & Yang, 1979). To support our contention that lipids usually favor the helical conformation over the β form for type 3 peptids, we will compare the conformation of bacteriorhodopsin obtained from X-ray diffraction and CD studies with the predicted secondary structure from its primary structure.

Bacteriorhodopsin from *H. halobium* is a transmembrane protein. The electron-scattering density map of this purple membrane shows that each bacteriorhodopsin molecule consists of seven rods oriented perpendicular to the membrane surface (Henderson & Unwin, 1975). They are believed to be made up of α-helical segments. The complete amino acid sequence of this protein has been determined by two laboratories with minor differences in nine places. Ovchinnikov et al. (1979) found a tryptophan at position 138, whereas Khorana et al. (1979) reported one extra tryptophan at positions 138 and 139. Thus, the total number of amino acid residues is 247 or 248 according to the two laboratories. Engelman et al. (1980) have attempted to fit the 247 amino acids to the three-dimensional density map of bacteriorhodopsin and suggested a most probable model for the arrangement of the polypeptide chain across the membrane. The seven helices are located at residues 8–31, 41–65, 77–101, 107–130, 133–156, 166–190, and 197–223. The length of the helical segments varies from 24 to 27 residues, and the six interrod links vary from 2 to 11. We used the Chou–Fasman method for predicting the secondary structure of the 248 amino acid sequence of bacteriorhodopsin (Table II); the minor discrepancy in the sequences reported by Ovchinnikov et al. (1979) and Khorana et al. (1979) will not affect the predicted results. Clearly, many segments of this protein possess both the helix and β-form potentials. If the choice of the two conformations were based on the published conformational parameters of the Chou–Fasman method (1978), the predicted structures would have favored the β form (about 60%) over the helix (about 20%) (the β turn was about 10%). [If we accept the model of Engelman et al. (1980), the seven rodlike segments would have a $\langle P_\alpha \rangle$ varying from 1.00 to 1.06 and a $\langle P_\beta \rangle$ varying from 1.12 to 1.17]. On the other hand, the CD spectra of the unsonicated purple membrane (Beeher & Cassim, 1976; Long et al., 1977) and the membrane solubilized in Triton X-100 solution (Reynolds & Stoeckenius, 1977) suggested a high helicity of 70–80% (the Beeher and Cassim's value was only about 45% probably because the distorted spectrum was not corrected for the light scattering effect). To reconcile this discrepancy between the CD results and the predicted structure, we suggest that the correct $\langle P_\alpha \rangle$ might be greater than the correct $\langle P_\beta \rangle$ for bacteriorhodopsin when the protein is buried inside a lipid environment. The predicted secondary structure of bacteriorhodopsin is not necessarily inconsistent with the suggested model of Engelman et al. (1980). The absence of conformational parameters of 20 amino acids for proteins in a lipid environment makes it difficult to quantitatively predict the secondary structure of membrane proteins.

The Chou–Fasman method also predicts six potential β turns between the seven ordered segments, just as the electron-scattering density map shows six interrod links. However, the locations of these β turns are not all identical with the positions of the six interrod links. Three β turns at residues 34–39, 104–107, and 192–195 are found in the links of the model of Engelman et al. (1980). This is as good as can be expected.

To summarize, for peptide segments having both the helix and β-form potentials the helix usually predominates over the β form in lipid solutions unless the average conformational parameters based on current literature values show a much larger $\langle P_\beta \rangle$ than $\langle P_\alpha \rangle$. For renin substrate residues 3–5 and 8–13 have $\langle P_\beta \rangle$ of 1.59 and 1.34 and residues 7–12 a $\langle P_\alpha \rangle$ of 1.03; thus, this peptide adopted a β form and possibly a β turn too in lipid solution. On the other hand, for substance P residues 5–8 have a $\langle P_\beta \rangle$ of 1.24 and residues 3–8 a $\langle P_\alpha \rangle$ of 1.04, and this peptide was partially helical in lipid solution.

Table II: Prediction of Secondary Structure of Bacteriorhodopsin[a]

residues	$\langle P_\alpha \rangle$	$\langle P_\beta \rangle$	$\langle P_t \rangle$	$p_t \times 10^4$
8–21	1.08	1.09		
22–29	1.00	1.24		
34–37			1.23	1.05
36–39			1.28	4.59
42–49	1.01	1.24		
50–69	1.03	1.14		
75–90	0.99	1.12		
91–103	1.16	1.06		
104–107			1.24	2.16
105–112	1.01	1.18		
113–116			1.32	2.18
115–119	0.96	1.15		
120–123			1.19	1.47
121–140	1.00	1.19		
143–157	0.97	1.25		
158–164	1.11	0.81		
167–191	1.00	1.23		
192–195			1.32	1.66
196–224	1.05	1.16		
225–235	1.13	0.82		
238–241			1.29	0.75
240–243			1.31	1.39

[a] Based on the 248 amino acid sequence (Khorana et al., 1979) and estimated by the Chou–Fasman method (Chou & Fasman, 1978).

Because the sequence-predictive method is empirical, no demarcation lines can be drawn for $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$, above which the peptide segment would adopt the helical and β conformation in a lipid environment. Cautiously interpreted, the Chou–Fasman method can still provide useful information about the secondary structure of membrane proteins.

Since most of the peptides studied in this work are known to exert their activities on the membrane, the conformations of these peptides observed in lipid solutions in vitro may better represent their conformation in vivo than those found in surfactant solutions. It is tempting to suggest that renin substrate may actually adopt a mixture of the β form and β turn rather than a helix in vivo. Likewise, glucagon may have a lower estimated helicity (about 30%) at the receptor site than that estimated in $NaDodSO_4$ solution (about 50%).

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