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CONFORMATION OF NATURALLY-OCCURRING PEPTIDES IN SURFACTANT SOLUTION: ITS RELATION TO THE STRUCTURE-FORMING POTENTIAL OF AMINO ACID SEQUENCE[1]

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SUMMARY: Short polypeptides are unordered in aqueous solution. Surfactants provide a proteinaceous environment in which ordered conformation of the peptides can be induced if they have a structure-forming potential. Ten peptides studied are classified into four types: (I) helix-forming, (II) β-forming, (III) either helix- or β-forming, and (IV) neither structure-forming. At low molar ratio, R, of sodium dodecyl sulfate to peptide (residue) ($R < 10$), type II peptides appear to be aggregated, but they dissociate at $R \cong 100$ or more.

Surfactants such as $NaDodSO_4$[2] can alter the conformation of proteins in solution. Unlike guanidine hydrochloride (5-6 M) or urea (8-10 M) which unfolds most protein molecules, $NaDodSO_4$ as low as 1 mM can enhance the helicity of certain proteins (1 and earlier references therein). No systematic studies of protein denaturation by surfactants have been available. Nor is there a general consensus about the shape of the protein-surfactant complexes; the proposed models range from rigid rod (2), to flexible necklace (3), deformable prolate ellpsoid (4) and non-draining coil (1). We report here the effect of $NaDodSO_4$ and $DodNH_3Cl$ on the conformation of several oligo- and polypeptides. They can be classified into four types according to their structure-forming potential: (I) helix, (II) β-form, (III) either structure (equivocal), and (IV) neither structure.

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[2]Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; $DodNH_3Cl$, dodecylammonium chloride; CD, circular dichroism.

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Each of the 20 amino acids in a polypeptide chain has a structure-forming potential for the helix, β-form, β-turn or aperiodic form. A sequential oligopeptide possessing these potentials can adopt a particular conformation under suitable conditions. Our working hypothesis is that surfactants, be they ionic or nonionic, can provide a proteinaceous environment which could overcome the hydrophilic interactions between peptide backbone and water in aqueous solution and any coulombic repulsion among charged side groups of the same sign. We have overlooked long-range interactions among residues far apart in a sequence, although these interactions are important in stabilizing the protein structure.

In the absence of x-ray diffraction results, several sequence-predictive methods have recently been proposed to predict the secondary structure of a protein molecule from its primary structure (5). These methods are empirical and applicable to compact, rigid proteins. However, we will show that the conformation of short polypeptides in surfactant solution can be related to their amino acid sequence. We use the Chou-Fasman method for its simplicity (6, 7), although other methods can equally well be employed.

Experimental

Angiotensin I and II (Lot #C0311 and C1231), Leu^{15} human gastrin I (Lot #D9656), substance P (Lot #D0814) and its fragment 4-11 (Lot #D0820) and Lys-bradkinin (Lot #D0430) were purchased from Beckman. ACTH fragment 4-11 (Lot #801031) and renin substrate (Lot #800623) were from Pennisula Lab and sleep peptide (Lot #700702) was from Calbiochem. Insulin B chain fragment 23-29 was prepared by tryptic digestion of insulin (8). Because of limited supplies (0.5-1 mg, except insulin), the sample weights provided by the manufacturers were accepted without correction. For those peptides containing aromatic residues their concentrations were further checked spectrophotometrically with molar extinction coefficients of 1,200 for Tyr and 5,500 for Trp at 280 nm. $NaDodSO_4$ and $DodNH_3Cl$ were gifts of Dr. K. Shirahama.

CD was measured with a JASCO SS-10 spectropolarimeter at 25°C after 1 hr mixing of the peptide and surfactant solutions. No change in spectra was observed after storing three to six days in excess surfactant solution (see Results and Discussion).

Results and Discussion

Judged from the CD spectra, all 10 peptides studied have little or no ordered structure in water. In surfactant solution the peptides can be classified into four types. Leu^{15} human gastrin I (17 residues) belongs

to type I (Fig. 1A). In water the CD spectrum shows a typical unordered conformation (curve 1). This peptide has an unusual sequence with five Glu's at residues 6-10, one Glu and Asp each at positions 1 and 16, and no positively charged groups (the α-amino group of Glu 1 is linked to the γ-carboxyl group). In 20 mM $DodNH_3Cl$ the CD shows a double minimum near 220 and 210 nm and a maximum at 193 nm, which is typical of a helical form (curve 2). $NaDodSO_4$ at neutral pH has no effect on the peptide conformation but induces partial helix at pH 5.5 or lower (curve 3). The titration of the Glu's in $NaDodSO_4$ solution indicates an apparent pK of 5.9 (Fig. 1A, inset). It suggests that the surfactant anions cluster around the unionized glutamic acid rediues. Probably the coulombic repulsion among the negatively charged Glu's and between them and the surfactant anions in neutral solution is too strong to stabilize the induced helical conformation. At low pH's $NaDodSO_4$ promotes the helical formation more effectively than $DodNH_3Cl$, noting that the critical micelle concentration of $NaDodSO_4$ and $DodNH_3Cl$ in water is about 8 and 14 mM, respectively. Thus, the hydrophobic interaction among the surfactant molecules seems to be a primary factor and the coulombic attraction may be of secondary importance. The partial helical formation can take place well below the critical micelle concentration, for instance, in 3 mM $NaDodSO_4$. This strongly suggests the clustering of surfactant molecules around the helical segment (3).

Type II includes insulin B23-29 and angiotensin I and II. At low molar ratios, R, of $NaDodSO_4$ to peptide (residue) the peptide solution is slightly turbid. For instance, the CD of angiotensin II at $R \cong 7$ shows a strong negative band at 219 nm and a positive one at 202 nm (Fig. 1B, curve 2). The profile resembles that of the β-form of poly(L-valine) in water (9). In excess $NaDodSO_4$ solution ($R \cong 100$) the solution becomes clear, but the CD magnitude is greatly reduced (curve 3). Similar results were obtained for angiotensin I, which has two more residues (His-Leu) added to angiotensin II at the C-terminal. According to the x-ray diffraction study,

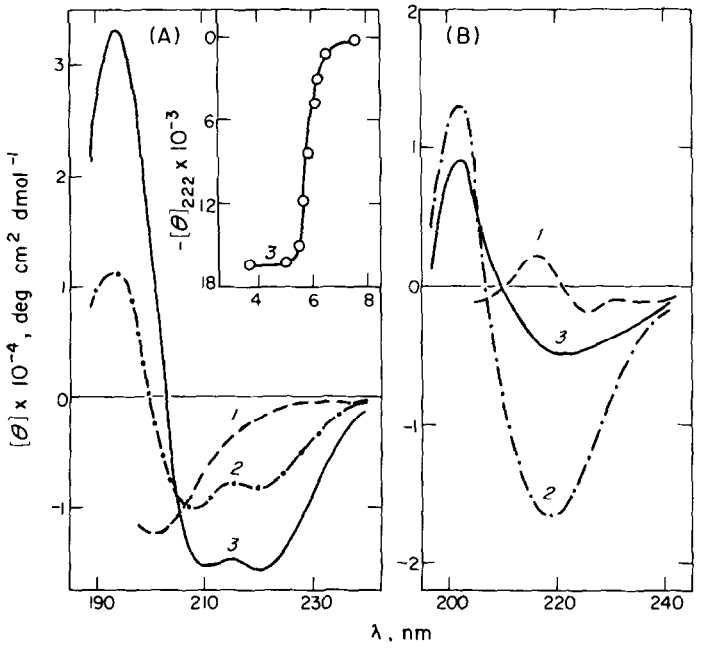

Figure 1(A). CD spectra of Leu^{15} human gastrin I at 25°C. Solvents: 1, water; 2, 20 mM $DodNH_3Cl$; and 3, 25 mM $NaDodSO_4$. Peptide: 0.31 mM (residue) at pH 5.5. Inset: pH dependence of the ellipticities in $NaDodSO_4$.
Figure 1(B). CD spectra of angiotensin II at 25°C. Solvents: 1, water; 2, 1.66 mM $NaDodSO_4$; and 3, 25 mM $NaDodSO_4$. Peptide: 0.24 mM (residue) at pH 5.2.

insulin dimerizes through the formation of an antiparallel β-form between two monomers at B23-29 (10). Our CD result (not shown) is consistent with this conclusion. However, in 25 mM $NaDodSO_4$ the CD of insulin B23-29 reverses to that in water. Mattice and Harrison have suggested that inter-chain β-form is promoted in dilute $NaDodSO_4$ solution, but the aggregates dissociate in concentrated $NaDodSO_4$ solution (11). Twenty mM $DodNH_3Cl$ has no effect on the conformation of the three peptides.

Substance P (11 residues) and renin substrate (14 residues) are classified as type III. At low R's the peptide solution again is slightly turbid, indicating some aggregation. The CD of substance P at R ≅ 3 shows a 210 nm minimum with a shoulder around 228 nm (Fig. 2A, curve 2). In most cases helices having strong light scattering show a skewed CD minimum near 208-210 nm (12, 13) instead. In 25 mM $NaDodSO_4$ the substance P solution is

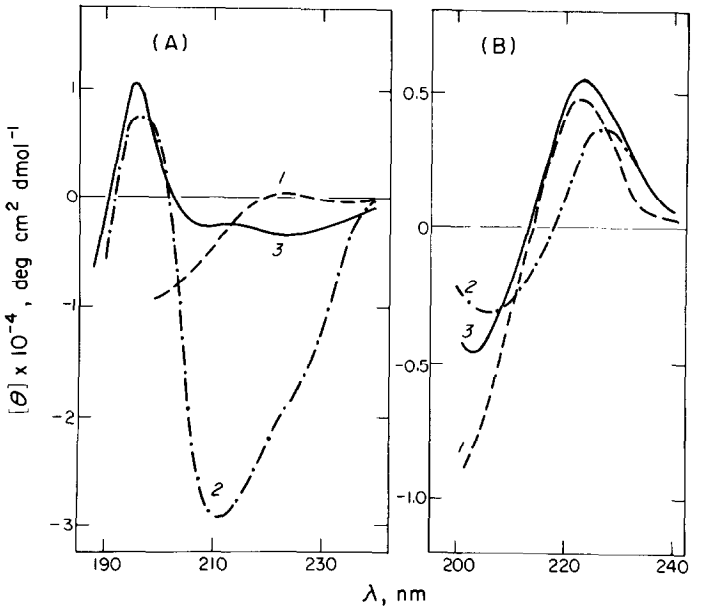

Figure 2(A). CD spectra of substance P at 25°C. Solvents: 1, water; 2, 1.25 mM $NaDodSO_4$; and 3, 25 mM $NaDodSO_4$. Peptide: 0.41 mM (residue) at pH 7.0.
Figure 2(B). CD spectra of ACTH fragment 4-11 at 25°C. Solvents: 1, water; 2, 1.66 mM $NaDodSO_4$; and 3, 25 mM $NaDodSO_4$. Peptide: 0.29 mM (residue) at pH 5.4.

clear and its CD differs markedly from that at low R value (curve 3). Renin substrate, which adds four more residues (Leu-Val-Tyr-Ser) to the C-terminal of angiotensin I, seems to adopt a β-form at low R's; its CD at R ≅ 3 has a single minimum near 215 nm, but it converts to a double minimum at high R with extremely small magnitude (to be published). Substance P fragment 4-11 has no ordered structure regardless of the surfactant concentration. The type III conformation is quite uncertain; an satisfactory explanation is still lacking.

Type IV includes ACTH fragment 4-11, sleep peptide (9 residues), and Lys-bradkinin (10 residues). Based on the CD spectra, neither anionic nor cationic surfactant promotes any helix or β-form in these peptides. Figure 2B shows the results with ACTH fragment 4-11. Lys-bradkinin is rich in Pro (residues 3, 4 and 8) and also contains one Gly (residue 5); its CD resembles

that of poly(L-proline).

We are as yet unable to observe the β-turn in surfactant solution, although some peptides studied may have such a structure-forming potential. The experimental and theoretical CD of the β-turn is also not completely resolved (Chang, Wu and Yang, to be published).

According to the Chou-Fasman method (6, 7), the four peptides in the figures should have the following structure-forming potential for residues inside the paratheses:

Leu^{15} human gastrin I:

	pGlu	Gly	Pro	Trp	Leu	Glu	Glu	Glu	Glu	Glu	Ala	Tyr	Gly	Trp	Leu	Asp	Phe-NH_2
						–	–	–	–	–						–	
Helix	H	B	B	(h	H	H	H	H	H	H)	b	B	h	H	I	h	
β-Form	B	b	b	h	h	B	B	B	B	B	i	H	b	h	h	b	h

Angiotensin II:

	Asp	Arg	Val	Tyr	Ile	His	Pro	Phe
	–	+				+		
Helix	I	i	h	b	h	I	B	h
β-Form	b	i	(H	H	H)	i	b	h

Substance P:

	Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met-NH_2
	+		+								
Helix	i	B	(h	B	h	h	h	h)	B	H	H
β-Form	i	b	b	b	(h	h	h	h)	b	h	h

ACTH Fragment 4-11:

	Met	Glu	His	Phe	Arg	Trp	Gly	Lys
		–	+		+			+
Helix	H	H	I	h	i	h	B	h
β-Form	h	B	i	h	i	h	b	b

The predicted structures for Leu^{15} human gastrin I, angiotensin II and ACTH fragment 4-11 are consistent with our types I, II and IV based on the CD spectra. Based on the sequence-predictive method, ACTH fragment 4-11 could have a helix-forming potential between residues 1 and 6, but the end effects near the N-terminal might have destabilized the helical formation. The structure for substance P is equivocal, so does the CD for type III. One

uncertainty is that the prediction parameters of the charged residues such as Lys^+ and Glu^- might be modified after binding to the surfactant ions.

We have only presented qualitative results of the four types. The magnitudes of the CD spectra for various conformations in surfactant solutions are still uncertain. The end effects of short peptides can also alter the spectra, so do the presence of any non-peptide chromophores that are optically active. The finding that the β-form dissociates in concentrated $NaDodSO_4$ solution might be related to the effect of surfactants on certain proteins. For instance, elastase has some helical conformation in excess $NaDodSO_4$ but assumes the β-structure after the bulk of $NaDodSO_4$ is removed through dialysis (14); likewise, the CD of concanavalin A that is rich in the β-form actually shows a double minimum typical of a helix in concentrated $NaDodSO_4$ solution (1). Any empirical method, be it CD or sequence-predictive model, should be used with caution.

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