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The conformation of fusogenic B18 peptide in surfactant solutions‡

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Abstract: The interaction of B18 peptide with surfactants has been studied by circular dichroism spectroscopy and fluorescence measurements. B18 is the fusogenic motif of the fertilization sea urchin protein. The peptide forms an α-helix structure when interacting with positively or negatively charged surfactants below and above the critical micellar concentration (CMC). The α-helix formation is due to binding of surfactant monomers rather than the formation of surfactant micelles on the peptide. Fluorescence measurements show that the CMC of the negatively charged surfactant increases in the presence of B18, supporting the fact that there is a strong interaction between the peptide and monomers. Nonionic surfactant monomers have no effect on the peptide structure, whereas the micelles induce an α-helical conformation. In this case the helix stabilization results from the formation of surfactant micelles on the peptide. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: conformational analysis; B18 peptide; charged and nonionic surfactants; circular dichroism; fluorescence measurements

INTRODUCTION

In biological cells, fusion of membranes is induced by proteins [1] that contain short hydrophobic sequences of 20–25 amino acid residues, known as fusion peptides, which interact directly with the cell membrane [2]. Fusion peptides exhibit a polymorphic structural behaviour, which seems to be crucial for the fusion process. Evidences suggest that the fusion-promoting state is the obliquely inserted α-helix [3]. The proposed mechanism is that the peptides are initially in a random coil conformation and adopt an α-helix structure when inserted in the membrane. Although β-sheet structure has been described as the fusion-promoting state for certain peptides, these results appeared to be a consequence of high peptide concentration [3]. Sample conditions, as well as peptide concentration, seem to determine the structures of these peptides in lipid bilayers. Nevertheless, fusion peptides have a tendency to self-associate into β-sheets at membrane surfaces, although its functional relevance is not known [3].

B18 is the amino acid sequence 103 to 120 of the protein bindin, which is found in the membrane of sperm cells of *strongylocentrotus purpuratus* (purple sea urchin) [4]. Bindin plays a key role in the fertilization process, and the sequence B18 (LGLLLRHLRHHSNLLANI) is recognized as the minimal membrane binding and fusogenic motif [4,5]. These 18 amino acids are perfectly conserved among all known sea urchin species.

B18 exhibits a high conformational flexibility: at slightly acidic pH, the peptide shows a random coil conformation, whereas at neutral and alkaline pH, the sequence has a tendency to self-assemble [6,7]. In trifluoroethanol and pH 7.5, B18 undergoes a transition from coil to helix structure, but at high peptide concentrations, an intermediate state constituted by oligomeric species appears to exist [6]. Zinc ions bind to B18, inducing an α-helix conformation and leading to the formation of oligomeric metallo-peptide complexes [8].

Given that B18 retains the fusogenic properties of the parent protein, the peptide has to interact strongly with the membrane and to be immersed into the bilayer. B18 binds to neutral lipid vesicles and induces their fusion, which is strongly enhanced by zinc ions [7]. An oblique penetration of the peptide into the bilayer had been observed by ATR IR spectroscopy and solid state NMR [8,9]. At low peptide-to-lipid ratio, B18 adopts an α-helix structure at zwitterionic membranes. At high ratio, however, solid state NMR spectroscopy and electron microscopy studies revealed an oligomeric β-sheet structure upon binding to membranes [7,10]. There is some controversy about the fusion promoting state of B18 peptide as both α-helix and β-sheet are claimed to be the fusogenic conformation.

This work focuses on conformational changes of B18 peptide in surfactant solutions. By using surfactants the influence of both monomers and micelles on the peptide structure can be studied. In addition, since micelles are fluid and dynamic systems, B18 can easily

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access to the hydrophobic core, allowing the structural characterization of the immersed peptide. The effect of (i) nonionic, (ii) cationic and (iii) anionic – hydrogenated and perfluorinated – surfactants on B18 was characterized by circular dichroism spectroscopy. Furthermore, fluorescence measurements of anionic surfactant were performed in presence of B18 to analyse the effect of the peptide on the critical micellar concentration (CMC).

MATERIALS AND METHODS

Materials

B18 peptide (Mw = 2090 g/mol) was purchased from Genscript (95%). Peptide stock solutions were prepared by dissolving B18 in 10 mM Hepes buffer (99.5%, Sigma-Aldrich) to obtain a pH of 7.4. Sodium dodecyl sulphate (SDS), pentadecafluorooctanoic acid (PFOA), cetyltrimethylammonium chloride (CTAC), and 1-*O*-*n*-octyl-β-D-glucopyranoside (OG) were from Fluka. Probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was from Molecular Probes. All chemicals were used as supplied.

Circular Dichroism (CD) Spectropolarimetry

Circular dichroism (CD) spectra were recorded at room temperature (25 °C) using a Jasco J-715 spectropolarimeter (Tokyo, Japan) equipped with a temperature-regulated sample chamber. A 0.05 cm optical path length quartz cell was used to record spectra of B18 in the far ultraviolet region (190–260 nm) at a peptide concentration of 96 μM. All CD spectra were acquired at a scan speed of 20 nm/min, 0.2 nm bandwidth, and a response time of 1 s. Spectra were signal-averaged over eight scans. All spectra were corrected by subtracting the buffer or surfactant solution baseline. The spectra were smoothed using the noise reduction routines provided with the J-715 spectropolarimeter, for secondary structure estimation. The content of structural motifs was calculated using the method CONTIN/LL [11]. Normalized root mean standard deviation (NRMSD) was used to determine the quality of the fit of the calculated structure to the data, and was between 0.022 and 0.069.

Fluorescence Measurements

Fluorescence measurements were performed on a microplate reader (H.T. Synergy, BIO-TEK) with excitation and emission wavelengths of 358 and 430 nm, respectively.

The CMC of SDS was determined by a fluorimetric method based on the use of a fluorescent probe DPH. This fluorescence probe has a high quantum yield and low fluorescence in water, [12] and thus, can be employed to determine the CMC according to a previously described procedure [13].

The samples were prepared in Hepes buffer as follows: in a final volume of 200 μl, 2 μl of DPH dissolved in tetrahydrofuran was added to increasing concentrations (2 mM–50 mM) of SDS solution (SDS in buffer) to achieve a final concentration of the probe of 1 μM. The mixture was incubated in the dark at room temperature (25 °C) for 30 min with gentle mixing to allow complete incorporation of the probe. After incubation, B18 peptide was added to reach a final concentration of 50 μM. In other set of samples considered as blanks, the peptide solution was absent.

RESULTS

The effect of surfactants on the secondary structure of B18 peptide was investigated at pH 7.4, with a final peptide concentration of 96 μM. The properties of the surfactants used in this study are shown in Table 1 [14–17]. B18 peptide exhibits, in the absence of surfactants, a CD spectrum characteristic of random coil conformation (minimum at 198 nm). The content of structure motifs was estimated to be 3% α-helix, 19% β-sheet, 10% β-turn and 68% random coil. Figure 1 shows the circular dichroism spectra of B18 peptide in SDS solutions at concentrations below (1.7 mM) and above (50.3 mM) the CMC. At low concentration of SDS, B18 peptide exhibits a spectrum with two negative bands, at 222 and 208 nm, and a positive band at 190 nm, which is an indication of the presence of α-helix structure (Figure 1, curve 2). Quantitative analysis of the fraction of secondary structures showed an α-helix content of 36% at 1.7 mM SDS. It can be seen in the Table inserted in Figure 1, that the increase in α-helix upon addition of SDS surfactant (from 3 to 36%) is more due to a reduction of the random coil content (from 68 to 25%) than to the reduction of β-sheet or β-turn structures. At higher SDS concentrations (50.3 mM), the spectrum shows as well characteristic bands of α-helix, but the bands are less pronounced when compared with the spectrum at 1.7 mM SDS (Figure 1, curve 3).Comparing the two CD line shapes (Figure 1, curve 2 and 3), it can be observed that they are proportional to one another. The presence of small peptidic aggregates is generally known to reduce the signal intensity across the full spectral width due to light scattering and a shadowing effect [18]. Therefore, the decrease in the signal intensity of the CD spectrum of B18 at high SDS concentrations is likely due to the formation of peptidic aggregates. Hence, the self-associated peptide has probably a secondary structure comparable to that of soluble state at SDS concentrations below the CMC, although the estimation of structural motifs indicates lower helical content.

The effect of PFOA on B18 peptide is shown in Figure 2. The CD spectrum of B18 peptide in 1.8 mM PFOA, which is below the CMC, shows characteristics of α-helix structure and is more pronounced at micellar PFOA concentrations. The content of helicity of B18 was estimated to be in the range of 34–40% (Table of Figure 2). The random coil fraction decreased from 68%, in absence of PFOA, to 32% upon addition of the anionic perfluorinated surfactant. The β-sheet and β-turn structure content changed only slightly.

Interaction of B18 with CTAC showed a change of the peptide spectrum towards α-helix even at concentrations low as 0.3 mM of CTAC (Figure 3,

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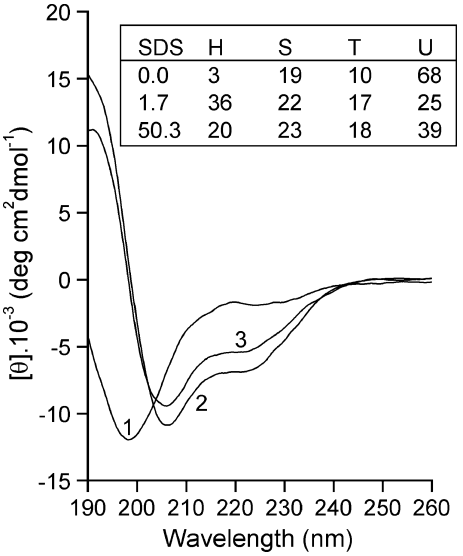

Figure 1 Circular dichroism spectra of B18 peptide in aqueous solution containing SDS at concentrations of 0.0 mM (curve 1), 1.7 mM (curve 2), and 50.3 mM (curve 3). The inserted table shows the percentage of structural motifs of B18 (H-helix, S-strand, T-turn, U-unordered, ±3%).

Table 1 Properties of the surfactants used in the study of interaction effects with B18. CMC is the critical micellar concentration. For OG, *R* is the radius of the cylinder, and *L* is its length

Surfactant	CMC (mM)	Shape of micelle	Size of micelle (nm)
SDS[a]	8.0	Spherical	4.8
PFOA[b]	9.1	Disk-like	—
CTAC[c]	1.4	Spherical	4.8
OG[d]	25	Cylinder	$R = 1.3$; $L = 9.6$

[a,c] [14,15].
[b] [16].
[d] [17].

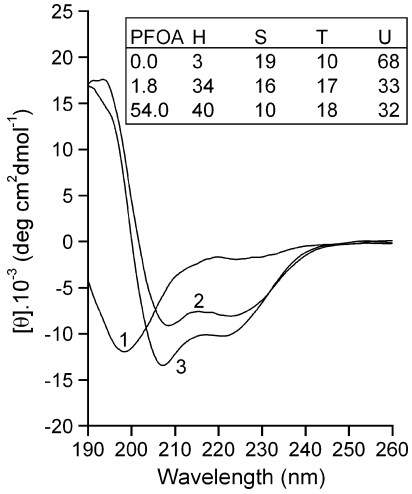

Figure 2 Circular dichroism spectra of B18 peptide in aqueous solution containing PFOA at concentrations of 0.0 mM (curve 1), 1.8 mM (curve 2), and 54.0 mM (curve 3). The inserted table shows the percentage of structural motifs of B18 (H-helix, S-strand, T-turn, U-unordered, ±3%).

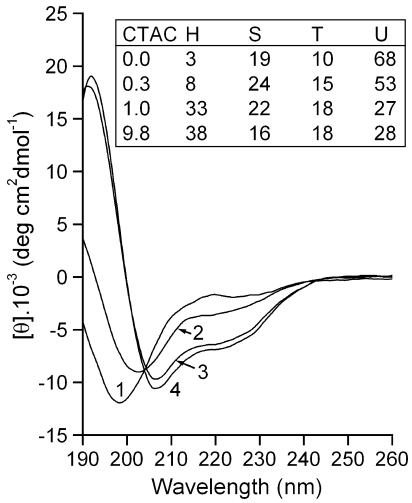

Figure 3 Circular dichroism spectra of B18 peptide in aqueous solution containing CTAC at concentrations of 0.0 mM (curve 1), 0.3 mM (curve 2), 1.0 mM (curve 3) and 9.8 mM (curve 4). The inserted table shows the percentage of structural motifs of B18 (H-helix, S-strand, T-turn, U-unordered, ±3%).

curve 2). The content of α-helix increased to 33% at a concentration of surfactant just below the CMC (1.0 mM) and was 38% at concentration above the CMC (Table of Figure 3). The content of random coil decreased from 68 to 28%, whereas the β-sheet and β-turn did not vary significantly. This is supported by an isosbestic point observed at 203 nm indicating that the transition occurs mainly between two states, random coil and α-helix.

To discern the role of surfactant charges on the peptide structure, samples of B18 in nonionic OG solutions were prepared. CD measurements showed no significant influence of the nonionic surfactant on B18 structure at concentrations below the CMC (Figure 4, curve 2). However, at micellar concentrations, the CD spectrum of B18 changed compared with the spectrum of the peptide in absence of the surfactant (Figure 4, curve 3). A positive band at 190 nm and small negative bands at 208 and 222 nm were observed for B18 in the presence of nonionic micelles. Secondary structure estimation indicates that the content of α-helix increased from 3 to 21% upon addition of OG micelles, whereas the random coil content decreased from 68 to 41%. The β-sheet and β-turn content were 20 and 18%, respectively.

Figure 5 shows the concentration dependence of DPH fluorescence in both pure SDS and SDS in presence of B18 and yields to two intersecting straight lines.

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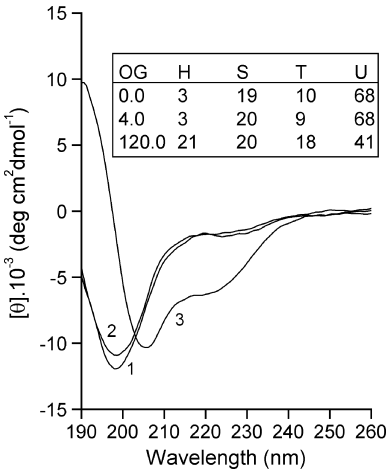

Figure 4 Circular dichroism spectra of B18 peptide in aqueous solution containing OG at concentrations of 0.0 mM (curve 1), 4.0 mM (curve 2), and 120.0 mM (curve 3). The inserted table shows the percentage of structural motifs of B18 (H-helix, S-strand, T-turn, U-unordered, ±3%).

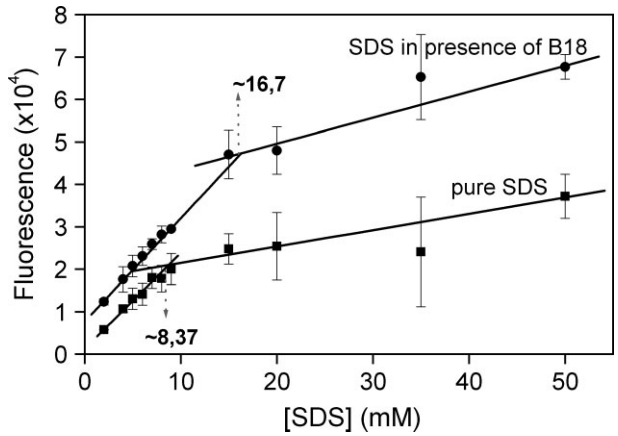

Figure 5 Fluorescence intensity of DPH at 430 nm as a function of SDS concentration in absence and presence of B18 peptide (50 μM).

The first line corresponds to surfactant concentration below the CMC. The increase in DPH fluorescence is most probably due to the formation of premicellar aggregates of SDS [19] which consist of a small number of surfactant species aggregated around a DPH molecule. Upon mixing detergent solutions with the neutral fluorescent molecule DPH, a large increase in fluorescence is observed if detergent exceeds the CMC. This property was used to determine the CMC of the anionic detergent SDS. Indeed, as the SDS concentration was increased and typical spherical micelles were formed, the micellar interior allowed the penetration of the probe which has low fluorescence in water. Therefore, a second linear line shows the enhancement of fluorescence intensity of DPH. The point of intersection of the two straight lines must thus correspond to the concentration at which the free SDS molecules start to aggregate, i.e. the CMC. The CMC values obtained with this approach were very similar to the reported values for SDS micelles (~8 mM) [13]. The addition of B18 increases the CMC of the SDS micelles to ~17 mM. This is in agreement with the CD measurements, because B18 interacts with free SDS molecules, forming mixed micelles with a major SDS content.

DISCUSSION

Anionic (alkylated and fluorinated) and cationic surfactants were found to induce α-helix structure on B18 peptide at concentrations below and above the CMC, as determined by CD spectroscopy. Nonionic surfactant has no influence on the B18 structure at concentrations below the CMC, whereas at micellar concentrations an α-helix is formed. Fluorescence measurements showed that B18 alters the CMC of negatively charged surfactant SDS to higher values.

Given that B18 peptide is positively charged at pH 7.4, these observations strongly suggest that the main interaction between B18 and charged surfactants is due to bound surfactant monomers, whereas the interaction with nonionic surfactants results from the formation of surfactant micelles on the peptide.

At high SDS concentrations, B18 forms peptidic aggregates, as it has been observed in trifluoroethanol at high peptide concentrations and pH 7.5 [6]. Under these conditions, an intermediate state constituted by oligomeric species appeared to exist. These species differ from the aggregates formed by β-sheet structures, since self-associated peptide has a secondary structure comparable to that of the TFE-induced soluble state, that is α-helix. Zinc ions, which promote the membrane fusion, bind to B18, leading as well to the formation of oligomers. These oligomeric species must be related to the biologically activate state of fusogenic B18 [7].

The increasing of the CMC value of SDS by B18 is in agreement with the observation that structural changes occur in the peptide molecule already at surfactant concentrations below the CMC. This means that B18 interacts strongly with the charged surfactant monomers, which gain in terms of screening their hydrocarbon-water contact through interaction with hydrophobic peptide residues, due to the unfavorable contact between water and the hydrocarbon chains. The interactions between monomers are, in their turn, weakened due to their interaction with the peptide. Therefore, a higher surfactant concentration is needed to form micelles.

Fluorinated surfactant gives the largest increase in α-helix content, which is explained by the higher hydrophobicity of the fluorinated carbons when compared to their hydrogenated counterparts.

Since the OG has no influence on the B18 structure at concentrations below the CMC it is assumed

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that the size of the polar head plays a role in the interaction of B18 with uncharged surfactants. This result agrees with the previous study in which the size of the head group was observed to influence the efficiency of nonionic surfactants as α-helix stabilizer [20]. Increasing the head group size of surfactants leads to a decreasing of the α-helix content, which was attributed to steric effect. By increasing the surfactant concentration to values above the CMC, B18 undergoes α-helix formation. The formation of an α-helix turn in random coil is entropically costly. However, the entropy of a random coil peptide chain is lower when bound to a surfactant micelle as compared to a free peptide chain [20]. The cause of α-helix formation upon interaction with nonionic micelles can, therefore, be understood from the fact that the entropy loss suffered upon coil-helix transition can be assumed to be considerably lower than in the case for a free peptide molecule.

The results showed that B18 helical content is lower in nonionic micelle solution when compared to charged micelle solutions. Once more, the reason is the bulky head group that sterically hinders the hydrophobic residues on the peptide from interacting with the micelle core. It has previously been shown that nonionic surfactants interact with polymers, only if the polymer is sufficiently hydrophobic [20–22]. This suggests that hydrophobic interactions play an important role in the helix formation and that an appreciable number of the hydrophobic residues is immersed into the micelle core region. This is consistent with the finding that B18 inserts in zwitterionic membranes [8,9]. Micelles are simple models which have the ability to establish chemical interactions with either hydrophilic or lipophilic character molecules. The peptide–micelle interactions provide additional and significant information to the current level of understanding of the fusion process once it stresses the need of α-helix structures in the peptide–membrane interactions. The data suggest that the affinity of B18 for a membrane is driven predominantly by hydrophobic interaction.

CONCLUSIONS

B18 peptide was observed to adopt α-helix structure in presence of charged surfactants independently from the fact they form micelles or not. At pH 7.4, B18 has a net positive charge, indicating that the helical state results from hydrophobic interactions. In this case the coil–helix transition is a result of the binding of surfactant monomers to B18. The strong interaction of B18 with monomers is supported by the increase of SDS CMC, meaning that the binding of the surfactant to the peptide has a large effect on the surfactant monomer concentration.

The nonionic surfactant monomers had no significant influence on the peptide structure mainly due to the steric effect of the surfactant head group. In contrast, the nonionic micelles induced a partial α-helix structure. This is, at least partly, explained by the fact that the entropy loss suffered upon coil–helix transition is considerably lower when the peptide is bounded to a surfactant micelle than is the case for a free peptide chain.

B18 peptide is known to induce fusion of zwitterionic lipid vesicles and considering the results presented here, the active fusion-promoting state is the helical structure.

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