Reversed micellar fibres in organic media as a new model of the parallel-chain β-sheet structure of peptides

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In aggregates of tripeptide-containing amphiphiles, the tripeptide groups possess a parallel-chain β-sheet structure not only in water but also in CCl_4, in which the amphiphiles form a reversed micellar fibre structure.

Although recent research reports examples of self-organization of molecules in organic media,[1] structural aspects of these aggregates remain to be elucidated. We describe here the details of molecular arrangements within the self-assemblage in organic media. The molecules **1–3** used in the study are amphiphilic, and contain a tripeptide group.† These amphiphiles can form fibrous aggregates not only in water but also in some non-polar organic solvents,[2,3] which makes it possible to compare the aggregate structures within different media using the same molecule. The amide modes of the interpeptide hydrogen bonding will help to resolve the structures, because secondary structures in peptides have been extensively studied by FTIR spectroscopy.[4–7] The major problem is that the IR modes of the parallel-chain β-sheet structure have not yet been identified.[4] However, throughout the study, we will show that the parallel-chain β-sheet structure is probably formed in our aggregate irrespective of the medium and hence the aggregates in organic solvents possess a reversed micellar fibre structure.

The amphiphiles used in this work formed fibrous aggregates both in water and in CCl_4, while no aggregates were formed in $CHCl_3$.[1] Fig. 1 shows FTIR spectra of amphiphile **1** in three different solvents.‡ Remarkable band shifts accompany aggregate formation, clearly showing the presence of strong hydrogen bonding within the aggregates both in water and in CCl_4. Emphasis is placed on the identity of the spectra of the aqueous and the CCl_4 solutions in the region 1500–1800 cm^{-1}. In these solvents, the maximal wavenumbers of the amide I and II bands are located at 1637–1638 and 1538–1542 cm^{-1}, respectively. The coincidence of the spectra at this region suggests that the modes of the hydrogen bonding are the same irrespective of whether the solvent is water or CCl_4. Similar spectra were obtained at the same region, for amphiphiles **2** and **3** (Table 1). Therefore, the mode of the interpeptide hydrogen bonding within the aggregates of the three amphiphiles is obviously independent of the amino acid structures. The same result has been reported by Tonioro and Palumbo.[5]

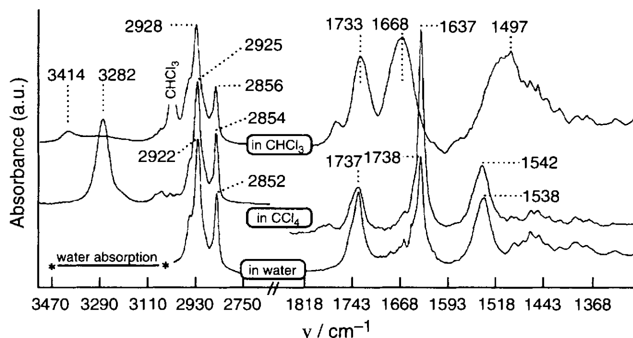
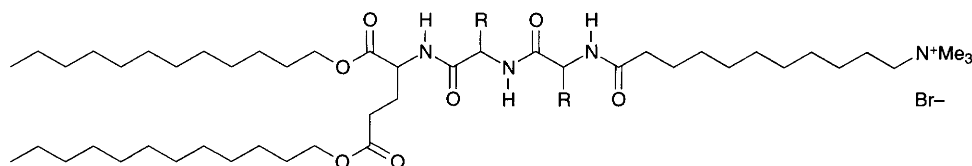


Fig. 1 FTIR spectra of amphiphile **1** in different solvents

Amphiphiles **1–3** form extended micellar fibres in water. Because every component is arranged in the same direction as its adjacent neighbours, the adjacent neighbouring molecules have parallel alignments and never an antiparallel counterpart. Thus, all the peptide groups should have parallel alignments, and hence a parallel-chain β-sheet is the only plausible structure in the aqueous bilayer membrane. Formation of a reversed-turn structure is undoubtedly impossible in this case. An α-helix structure is, of course, excluded because it requires at least four amino acid residues. So far there are few reports of amide modes of a parallel-chain β-sheet structure because of the lack of a good model.[4] However, Toniolo and Palumbo have reported that the heptamers of valine, isoleucine and phenylalanine gave the same amide I bands at 1635–1639 cm^{-1}.[5] Susi and Byler reported that the amide I band corresponding to the parallel β-sheet motif in two proteins absorbed at 1626–1639 cm^{-1} using a deconvolution of the amide I band.[6] Bandekar and Krimm calculated the wavenumber of the amide I band in the parallel β-sheet structure as 1640 cm^{-1}.[7] These values are quite similar to our results.§ Therefore, the spectral evidence above implies that the tripeptide groups should form a parallel β-sheet structure even in CCl_4. This structure is possible only in the reversed micellar fibre illustrated in Fig. 2. The strong interpeptide hydrogen bond should be formed along the long axis of the aggregate [Fig. 2(c)].

Table 1 Wavenumbers of the major bands of aggregates in CCl_4 solution

	Maximal wavenumber/ cm^{-1}		
	1	**2**	**3**
ν(N–H)	3282	3280	3281
$\nu_{as}(CH_2)$	2925	2927	2926
$\nu_s(CH_2)$	2854	2853	2854
ν(C=O) (Ester)	1737	1734	1736
ν(C=O) (Amide I)	1637	1632	1632
δ(N–H) (Amide II)	1542	1545	1545



R = $PhCH_2$ **1**, Me_2CH **2**, $MeCH_2CHMe$ **3**

The FTIR spectra provide other important information, *e.g.* the structure of the ammonium-head region. Despite its strong hydrophilicity, no boundary water exists in this region, since the O–H stretching band was absent in the FTIR spectra of the CCl_4 solutions (Fig. 1). Another aspect is the fluidity of the alkyl tail region. The antisymmetric and symmetric CH_2 stretching bands are known to shift to higher wavenumbers upon an increase in the fluidity of the polymethylene chain.[8] For example, the crystal to liquid crystal phase transition, a fundamental property of lipid and synthetic bilayer membranes, is accompanied by a shift in the antisymmetric CH_2 stretching band from 2914–2918 to 2923–2926 cm^{-1}.[9] On the other hand, the maximal frequency of the antisymmetric CH_2 stretching band of the amphiphile **1** was 2922 (in water), 2925 (in CCl_4) and 2928 cm^{-1} (in $CHCl_3$). This result shows that the alkyl chains in CCl_4 solution are more ordered than those in $CHCl_3$ solution, since the antisymmetric CH_2 stretching band of the disordered alkyl chains absorbed at higher wavenumber than that of the ordered alkyl chains.

In conclusion, we have proposed a reversed micellar fibre structure based on IR spectral evidence. On the other hand, there is controversy about the amide modes due to the parallel-chain β-sheet structure which is an important secondary structure of peptides.[4] However, the reversed micellar fibre structure could be an adequate model for the parallel chain β-sheet structure of peptides, since no strong molecular interaction except for the hydrogen bonding is present in the aggregate in non-polar organic solvents.

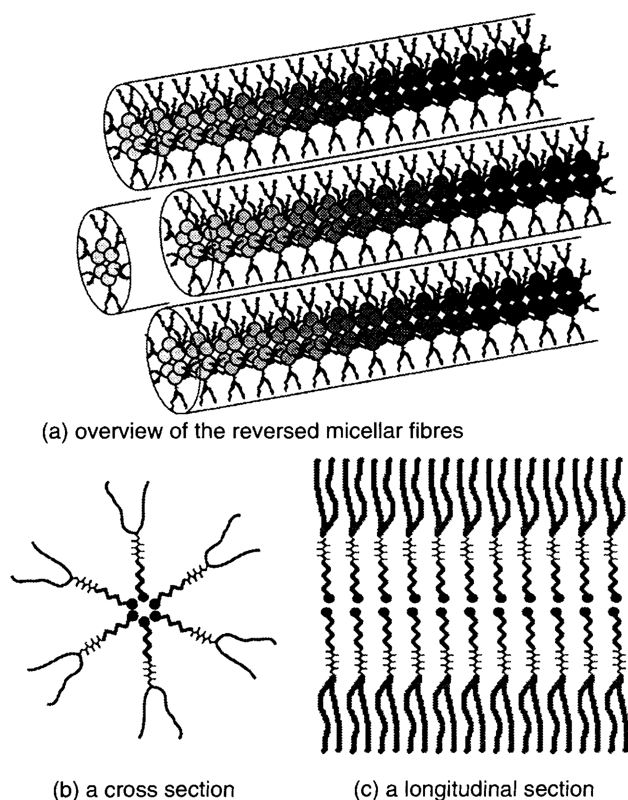


Fig. 2 Schematic illustration of the reversed micellar fibre formed from the tripeptide-containing amphiphiles in non-polar organic solvents

Footnotes

† All amphiphiles were prepared in the same manner as described elsewhere.[2,3] They gave the expected IR, NMR and microanalytical data. These amphiphiles formed fibrous aggregates not only in water but also in organic solvents with relative permittivities in the range 2.0–2.3. ^1H NMR spectroscopy gave direct evidence of interpeptide hydrogen bonding in the aggregate. On the other hand, polar organic solvents, such as $CHCl_3$, ethanol and methanol, gave transparent solutions with the amphiphiles molecularly dispersed, and no broadening of NMR peaks was observed. Details have been described in ref. 2.

‡ All the sample solutions were sandwiched in CaF_2 windows with a spacer, which were mounted on a temperature-controlled through-flow cell (TFC-19; Harrick Co.). The concentration of the sample solutions and thickness of the spacer were 1 mmol dm^{-3}, 50 μm for CCl_4 and $CHCl_3$ solutions, and 10 mmol dm^{-3}, 19 μm for aqueous solutions. The FTIR spectra were recorded using a Nicolet 740 spectrophotometer equipped with TGS detector at 25 °C. Two hundred interferograms were coadded and Fourier transformed, giving FTIR spectra with 4 cm^{-1} optical resolution.

§ For the α-helix and antiparallel β-sheet structure of poly-L-alanine, the amide I mode absorptions were at 1658 and 1632 cm^{-1}, respectively.[4] The amide I mode of a β-or γ-turn can give two or more strong bands in this region.[4] These wavenumbers are entirely different from our results.

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