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SHORT COMMUNICATION

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Micellar structure of an oligopeptide surfactant "trimeric *N*-dodecanoyl-L-proline potassium salt" in aqueous solution – small-angle neutron scattering study

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Abstract Structures of the micelles which are formed by the chiral oligopeptide surfactant *N*-dodecanoyl-L-proline tripeptide anions have been examined using small-angle neutron scattering spectral analysis. Results show that the chiral *N*-dodecanoyl-L-proline trimeric anions may form a spherical micelle with an aggregation number of 36 and that the oligopeptide portions with a poly-L-proline I-type helical structure are saturated with water.

Key words Micelles · Chiral oligopeptide surfactant · SANS

Introduction

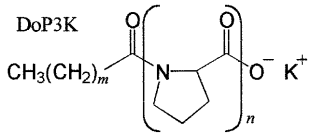
In previous work [1], the small-angle neutron scattering (SANS) spectra for micellar solutions of *N*-decanoylglycine and *N*-decanoyl-L-alanine oligomeric K-salts were measured and analyzed in order to elucidate the correlation between the secondary structure of the oligomeric portion and the observed SANS spectra. For micelles formed by the trimeric salts, a helical structural model provided the best fit to the observed SANS intensity, while for micelles of the monomeric and dimeric salts, a β-sheet model provided the best fit to the observed data. In particular, it was emphasized that for the trimeric micelles, the aggregation number depends upon the species of amino acid residue.

In this present study, for an aqueous micellar solution of an oligopeptide surfactant, *N*-dodecanoyl-L-proline trimeric anions with a helical structure, the structure of the micelles, obtained using SANS spectral analysis, is discussed.

Experimental

Materials

Chiral *N*-dodecanoyl-L-prolyl-L-prolyl-L-proline was synthesized by the stepwise procedure previously described [2]. The acid-type oligomer was identified by ^{1}H NMR. The chiral oligomeric acid-type was then dissolved in methanol–water and the pH of the solution was adjusted to 7.0 by slow addition of dilute KOH–H_2O at 273 K. The potassium salt of this chiral trimer (DoP3K) was collected by lyophilization and dried under high vacuum at room temperature over P_2O_5.



$m = 10, n = 3$

Critical micelle concentration measurements

The critical micelle concentration (cmc) of DoP3K was determined by the refractive index method using an Abbé refractometer (Atago Optical Works) at room temperature (around 298 K). The cmc of DoP3K thus obtained is 3.8×10^{-2} mol/l.

Neutron scattering measurements

SANS measurements were carried out using the SANS-U installed at the JRR-3M reactor at the neutron scattering laboratory in the Institute for Solid State Physics of the University of Tokyo, Tokai, Japan. The sample solutions were placed in a quartz cell of 4-mm path length at 298 K. The scattering length density (ρ) of each component was calculated using the following equation,

$$\rho = \sum b_i / V \ , \tag{1}$$

where b_i is the scattering length of atom i and V is the molecular volume. The Σb_i values quoted from Ref. [3] and the V values calculated from the partial molar volume data [4–6] are listed in Table 1. The magnitude of the momentum transfer (Q) is given by Eq. (2),

$$Q = \frac{4\pi}{\lambda} \sin\left(\frac{\theta}{2}\right) \ , \tag{2}$$

where λ is the incident wavelength (5 Å for SANS-U). The intensity of the scattered neutrons was recorded on a position-sensitive 2D detector. Normalization of the data to an absolute intensity scale was made by using the transmission of a 1-mm water sample. Corrections for the attenuation were also made.

Results and discussion

The dependence of the neutron scattering intensity on the magnitude of the scattering vector, Q, depends upon both the particle structure factor, $P(Q)$, and the interparticle structure factor, $S'(Q)$. $S'(Q)$ is a function of the diameter, δ, the charge, Z, and the number density of a particle and of the dielectric constant of the solvent.

Table 1 Partial molar volumes (V) and scattering length (Σb_{coh})

Species	V (Å^3)	Σb_{coh}(Å)
CH_3	42.6[a]	-4.57×10^{-5}
CH_2	28.2[a]	-8.32×10^{-6}
$-N(CH_2CH_2CH_2)CH-CO-$	112.3[b]	2.23×10^{-4}
COO^-	25.7[b]	1.83×10^{-4}
K^+	9.85[c]	3.71×10^{-5}

[a] Ref. [4]
[b] Ref. [5]
[c] Ref. [6]

For a monodispersed system of charged hard particles, the scattering intensity can be expressed as the product of $S'(Q)$ and $P(Q)$ in the following form,

$$\frac{d\Sigma(Q)}{d\Omega} = n_p 10^{-16}\left[(\rho_p - \rho_c)\right]V_c + (\rho_s - \rho_p)V_m]^2 P(Q)S'(Q) \ , \tag{3}$$

$$n_p = \frac{(c - \mathrm{cmc})N_A}{1000n}\left(\mathrm{cm}^{-1}\right) \ , \tag{4}$$

where n_p denotes the number density of the particles and n the average aggregation number of a micelle, and

$$P(Q) = \int_0^1 |F(Q,\mu)|^2 d\mu \ , \tag{5}$$

$$F(Q,\mu) = x\left(\frac{3[\sin(QR_1) - QR_1\cos(QR_1)]}{(QR_1)^3}\right) + (1-x)\left(\frac{3[\sin(QR_2) - QR_2\cos(QR_2)]}{(QR_2)^3}\right) \ , \tag{6}$$

$$x = \frac{(\rho_p - \rho_c)V_c}{(\rho_p - \rho_c)V_c + (\rho_s - \rho_p)V_m} \ , \tag{7}$$

where $V_c(\text{Å}^3)$ and $V_m(\text{Å}^3)$ are the volumes of the micellar core and the overall micelle, respectively. $\rho_p(\text{Å}^{-2})$, $\rho_c(\text{Å}^{-2})$ and $\rho_s(\text{Å}^{-2})$ are the average neutron scattering length densities of the polar shell, the hydrophobic core and the solvent, respectively.

When the micellar shape is spherical, R_1 and R_2 are given by

$$R_1 = b \ , \tag{8}$$

$$R_2 = b + t \ , \tag{9}$$

where b is the diameter of the micellar axes and t the diameter of the polar group.

$S'(Q)$ can be calculated approximately by use of the following equation

$$S'(Q) = 1 + \beta(Q,\mu)[S(Q) - 1] \ , \tag{10}$$

where

$$\beta(Q,\mu) = \frac{\left|\langle F(Q,\mu)\rangle^2\right|}{\left\langle |F(Q,\mu)|^2 \right\rangle} \tag{11}$$

and

$$S(Q) = \frac{1}{[1 - 24\eta a(Q)]} \ , \tag{12}$$

Table 2 Parameters extracted from small-angle neutron scattering analysis of the DoP3K–D_2O (0.10 $moll^{-1}$) system. n: the average aggregation number; n_{cw}: the number of hydrated methylenes of an n-dodecanoyl chain; n_{po}: the number of hydrophobic peptide residues in a peptide chain; α: the degree of ionization; b: the minor axis of a spherical micelle; t: the thickness of the hydrophilic layer; N_s: the number of water molecules associated with an oligopeptide anion; $1/\kappa$: the inverse Debye–Hückel screening length; σ: the macroion diameter; Δ: the average deviation per datum point

Model	n	n_{cw}	n_{po}	α	b/Å	t/Å	N_s	$(1/\kappa)$/Å	σ/Å	Δ/%
I	36	0.2	–	0.90	13.9	8.9	22.9	11.7	45.6	4.0
II	39	–	0.2	0.75	14.8	12.0	45.6	12.1	53.6	12.7

where η is the volume fraction of the macroion and $a(Q)$ is as given by Hayter and Penford [7]. In this model, the micelle is assumed to be a rigid charged sphere of diameter σ [8], interacting through a dimensionless screened Coulomb potential. The dimensionless screened Coulomb potential is calculated by using the inverse screening length of the Debye–Hückel theory, defined by the ionic strength, I, of the solution.

When the concentration of the surfactant is very low and the intermicellar interaction is neglected, the intensity spectrum is dominated by $P(Q)$, and $S(Q)$ is unity throughout the observed Q range; however, when the interaction cannot be neglected as the concentration increases, $S(Q)$ deviates from unity and the interaction peak appears in the intensity spectrum.

The conformation of poly-L-proline has already been studied by several investigators. It was found that poly-L-proline exists in two conformations (forms I and II). The structures of forms I and II have been determined, using the X-ray diffraction method, by Traub and Shmueli [9], Cowan and McGavin [10] and Sasisekharan [11]. The results showed that form I has a right-handed helical structure and has each peptide bond in a cis configuration, while the structure of form II is a left-handed helix with the peptide bonds in a trans configuration (Fig. 1).

We examined the conformations of the oligopeptide moieties for the salts of the *N*-acyl-L-proline oligomers, with exact residue numbers of 3 and 6, by use of IR spectra [12]. The results indicate that these oligopeptide moieties take up a conformation in the solid state and in aqueous solutions similar to that of the poly-L-proline I form. Accordingly, in a micellar model, it is assumed that the peptide portion of a DoP3K anion takes up a helical structure similar to that of poly-L-proline I and that this portion belongs to the hydrophilic layer.

It is possible to predict the existence of the micelles formed by the DoP3K anions using the spherical, prolate and oblate spheroid models by assuming monodispersity; however, it has been found that only a spherical model provides a reasonable result. The structural model of the spectral DoP3K micelle which was used in the calculation of the single particle form factor $P(Q)$ is shown in Fig. 2.

The b and t values are given by the following equations, which are similar to the equation presented by Tanford [13], and are used to determine the border between the hydrophobic and hydrophilic moieties in the hydrocarbon chain,

$$b = 1.50 + 1.27(11 - n_{cw}) \ , \tag{13}$$

$$t = 1.27n_{cw} + l_{pep}n_{pep} + 3.30 \ , \tag{14}$$

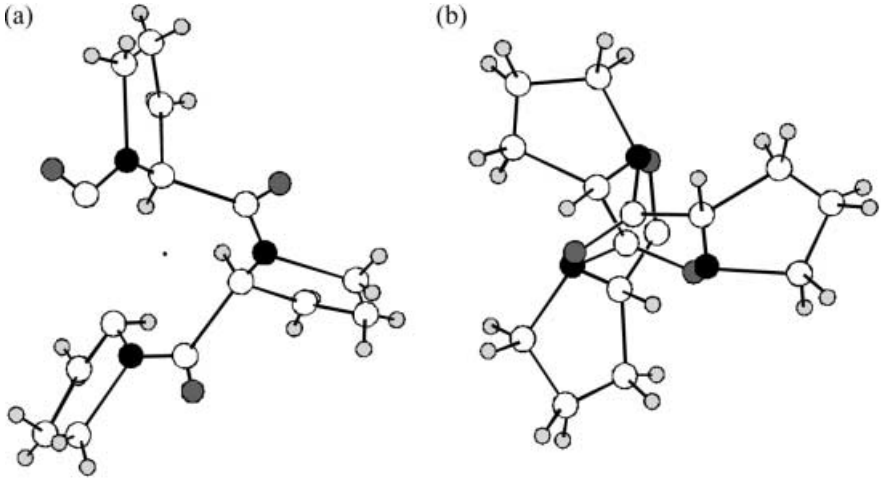


Fig. 1 Structural models of poly-L-proline **a** I form and **b** II form

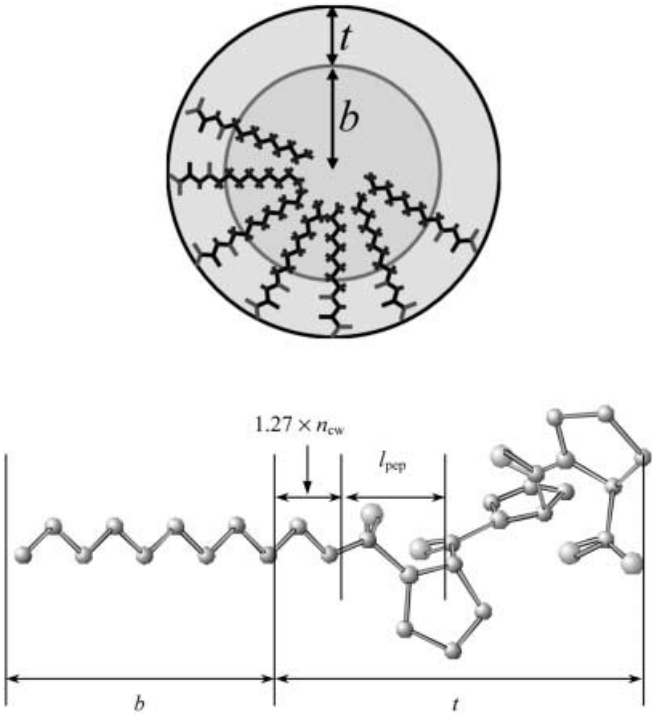


Fig. 2 Schematic micellar model used in the calculation of $P(Q)$. t is the thickness of the hydrophilic (Stern) layer, $(b + t)$ is the diameter of a spherical micelle

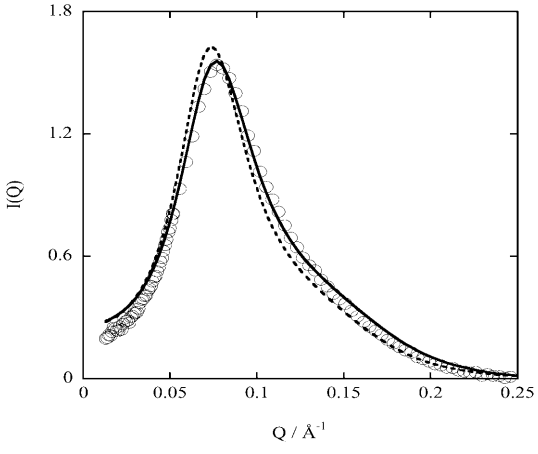


Fig. 3 Observed scattering intensity spectrum (*open circles*) for the DoP3K–D_2O (0.10 $moll^{-1}$) system at 298 K and fitted scattered intensity profiles (*solid line*: poly-L-proline I-type helical model; *broken line*: poly-L-proline II-type helical model)

while the values which are used to determine the border between the hydrophobic and hydrophilic portions in the peptide chain are

$$b = 1.50 + 1.27 \times 10 + l_{pep} n_{po} \ , \tag{15}$$

$$t = l_{pep} \times (n_{pep} - n_{po}) + 3.30 \ , \tag{16}$$

where n_{cw} is the number of hydrated methylenes of an n-dodecanoyl chain, n_{po} is the number of hydrophobic peptide residues in a peptide chain, l_{pep} is the length of an oligopeptide chain, and n_{pep} is the number of peptide residues. The l_{pep} value for one helical turn is 1.77 Å for I form and 3.10 Å for II form, a value obtained from geometrical dimensions of the helical structures for polypeptides as determined by X-ray diffraction analysis. The sum of the number of methyl and methylene carbon atoms in a dodecanoyl chain is 11. The length of a terminal COO^- group calculated using both geometrical dimensions of a COO^- group and the van der Waals radius of oxygen atoms [14] is 3.30 Å.

Since the diameter of a spherical micelle is b, the aggregation number (n) is expressed by

$$n = (4/3)\pi b^3 / V_t \ , \tag{17}$$

where b is the length of a hydrophobic chain and V_t is the volume of the hydrophobic portion of one molecule.

Figure 3 shows the neutron scattering intensity spectrum of the DoP3K–D_2O sample, in which the scattered intensity was corrected for the detector background and incoherent scattering and the intensity spectrum of the sample solution measured at concentrations below the cmc was subtracted from the observed intensity of the micellar solution. A very broad peak is seen in the SANS spectrum, indicating the presence of intermicellar interactions in this surfactant system.

The observed scattering intensity spectrum was analyzed with the aggregation number (n), the number of hydrated methylenes of an n-dodecanoyl chain (n_{cw}) or the number of hydrophobic peptide residues in a peptide chain n_{po}) and the degree of ionization of a micelle (α) as fitting parameters. The extracted parameters are listed in Table 1. The best-fit scattering intensity profile for the DoP3K (0.1 $moll^{-1}$)–D_2O sample is also shown in Fig. 3. The closeness of the fit between the observed and the calculated data is excellent. The average percentage of deviation per datum point was about 4%.

Thus, as a consequence of the SANS spectral analysis, we may assume that the N-dodecanoyl-L-proline trimeric anions form spherical micelles with an aggregation number of 36 and that the oligomeric portions are saturated with water.

We also analyzed the SANS spectrum of a DoP3K–D_2O sample, using a micellar model in which the oligomeric portion takes up a helical structure similar to that of poly-L-proline II. In this calculation, the thickness of the hydrophobic layer (t) was varied by changing the conformation of the L-proline oligopeptide moiety from form I to form II, thus altering the SANS intensity profile. The best-fit profile is also shown in Fig. 3. However, in this model, the average percentage deviation per datum point was about 13%, implying

that the I-type helical model of the peptide moiety provides a better fit to the SANS intensity data than does the II-type helix.

We may compare the steric structural of poly-L-proline I [9] with that of form II [10, 11], in order to enhance our understanding as to why the L-proline oligomeric portion favors the form I-type sterical structure in the micelles.

Figure 1 shows the relative configurations of the pyrrolidine rings on the projection plane perpendicular to the helical axis for the I and II forms. In form I, the helical structure is extended in the direction of the long molecular axis compared with that of form II and the pyrrolidine rings orient themselves in a direction approximately parallel to that of the helix axis [9], while for form II the pyrrolidine rings are oriented in a direction approximately vertical to the helical axis [10]. Therefore, the DoP3K anion with the extended I-type structure may easily form a self-assembly system through hydrophobic interactions.

In previous work [2, 15], we demonstrated that the carboxylate anion for *N*-acylglycine oligomers contributes markedly to the stabilization of the polyglycine II-type structure [15] and that for the *N*-acylglycine oligomers, the long acyl chains induce a further polyglycine II-type structure in the hydrogen-bonding system and peptide skeleton.

Thus, the existence of the singly charged groups (CO_2^-) [16], in addition to the long acyl chain effect, probably plays a significant role in preferential stabilization of the poly-L-proline I-type structure in micelles formed by DoP3K anions.

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