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Interfacial behaviour and micelle formation of novel amphiphilic sequential lipid–lysine oligomers☆

Shigeo Yanai, Thiagarajan Sakthivel, Alexander T. Florence *

Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK

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Abstract

As part of work on the design and synthesis of new supramolecular carrier systems for drugs, a series of novel linear oligomers of alternating α-amino tetradecanoic acid and lysine having positively charged groups and lipid chains was synthesised. The smallest member of the series ($n = 2$) is insoluble in water and diluted acid solutions, but the larger members are soluble in acid conditions and poorly soluble in alkaline conditions. Hence, in one series, one can conduct experiments both on the determination of micelle formation and spread monolayer behaviour. The surface pressure–area isotherms revealed limiting surface areas at the air/water interface ranged from 0.04 to 0.9 nm^2 according to the oligomer size, and a linear correlation between the observed area per molecule and that projected by computer-generated molecular models was demonstrated. The surface tension of the soluble members in dilute acid solution fell as the concentration of the oligomers was increased, indicating that all of these polymers were surface active with quite clearly defined critical micelle concentrations. The fluorescence intensity ratio of third to first band in the emission spectra of pyrene as a function of the polymer concentrations demonstrated that, even after normalising the data for the amount of lipid chains in the system, the ($n = 3$) oligomer had fewer accessible hydrophobic sites for pyrene, and the forces of the repulsion between the charged head groups was crucial on the formation of micelles, especially in the case of the $n = 3$ oligomer. Supramolecular fibre-like structures were observed in aqueous solution only when $n = 3$ by transmission electron microscopy (TEM). Cryogenic TEM observation of the ($n = 3$) solution also revealed that the micelles might elongate to form long cylindrical or fibrous structures. The diameter of these structures was estimated to be 6.0–13 nm, although their length varied.

Keywords: Interfacial behaviour; Micelle formation; Sequential lipid–lysine polymers

1. Introduction

In order to manipulate molecular devices on a nanoscopic scale that could be useful in various fields, understanding of assembly mechanisms of several specific molecules has been challenged

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* Corresponding author. Tel.: +44-20-7753-5819; fax: +44-20-7837-5092.

E-mail address: a.t.florence@ulsop.ac.uk (A.T. Florence).

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(Alivisatos et al., 1996; Sakthivel et al., 1998; Jenekhe and Chen, 1999). Soluble amphiphilic molecules usually self-assemble into micelles in aqueous solutions above their critical micelle concentration, but insoluble or poorly soluble amphiphilic molecules form vesicles (In et al., 1999). As part of work in our laboratories on the design and synthesis of new supramolecular carrier systems for drugs, a series of novel linear polymers of alternating α-amino tetradecanoic acid and lysine having positively charged groups and lipid chains was synthesised (Fig. 1) (Sakthivel et al., 2000). In this paper, the surface activity, micelle formation and further self-assembly into fibrous structures of the soluble member of the oligomer series are described.

2. Materials and methods

2.1. Surface pressure measurement

The oligomer solution in chloroform was spread on purified water in a Langmuir film balance system (Nima 601S; Nima Technology) at 25°C. The solvent was allowed to evaporate for 5 min and the monolayer of the polymer was then compressed at a rate of 100 cm^2/min. The surface pressure was measured by the Wilhelmy plate method and the compression isotherms were recorded.

2.2. Surface tension measurement

The oligomers were dissolved in 0.1 mM HCl (pH 4) in the concentration range from 0.01 μM to 0.1 mM and the surface tension at the air/liquid interface at 25°C was measured by the Wilhelmy plate method (DCA-312; CAHN Instruments). Data were plotted as a function of the logarithm of the oligomer concentration.

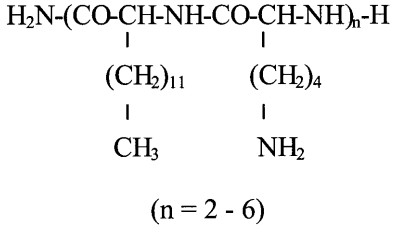

Fig. 1. Chemical structures of the amphiphilic lipid–lysine oligomers.

2.3. Observation of supramolecular structures

The oligomers were dissolved in 0.1 mM HCl (0.1 mM or 1 mg/ml) and the solutions were filtered through a cellulose acetate membrane (pore size, 0.22 μm). The samples were examined by transmission electron microscopy (TEM). The samples were also examined by TEM at cryogenic temperature (cryo-TEM). A small droplet of the solution was applied to a 700 mesh hexagonal grid and blotted, with the thickness of the liquid film about 0–200 nm, forming menisci in the holes. The grid was then immediately plunged in liquid nitrogen at its freezing temperature. This ultrafast cooling allows complete vitrification of the specimen.

2.4. Fluorescence measurement

Fluorescence spectra were recorded using a spectrofluorometer (LS50B, Perkin Elmer). Pyrene was used as a hydrophobic fluorescent probe. Pyrene solution in ethanol (0.4 mM, 6 μl) was added to the polymer solutions in 0.1 mM HCl (1.6 μM to 0.1 mM, 4 ml) in glass vials. These samples containing 0.6 μM pyrene were kept for 24 h at 20°C before measurements were made. Excitation was carried out at 340 nm and emission spectra were recorded ranging from 350 to 600 nm. The excitation and emission band widths were 10 and 3 nm, respectively. The intensity (peak height) ratios of the third band (385 nm) to the first band (374 nm) (I_3/I_1) in the emission spectra were determined as a function of concentration of the oligomers.

3. Results and discussion

The lowest member of the series ($n = 2$) is insoluble in water and diluted acid solutions, but the higher members are soluble in acid conditions and poorly soluble in alkaline conditions. Hence, in

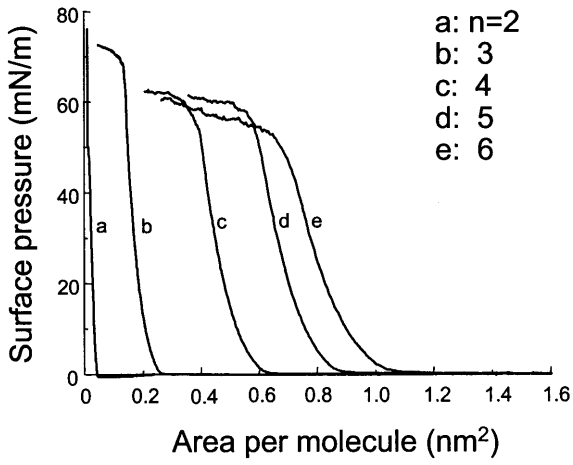

Fig. 2. Surface pressure isotherms of the oligomers at the air/water interface.

one series, one can conduct experiments both on the determination of micelle formation and spread monolayer behaviour.

The surface pressure–area isotherms revealed that the limiting surface areas at the air/water interface ranged from 0.04 to 0.9 nm^2 according to the polymer size (Fig. 2), and a linear correlation between the observed area per molecule and that projected by computer-simulated molecular models was recognised, although the observed values for $n = 2$ and $n = 3$ polymers were smaller than expected, suggesting that they possibly aggregate or form multilayers at the air–water interface in this experimental system (Maget-Dana et al., 1997).

Table 1
CMCs and area per molecule calculated from the Gibbs' equation

n	CMC (mM)	Area per molecule (nm^2)
3	0.018	0.65
4	0.025	0.44
5	0.046	0.58
6	0.056	0.65

The surface tension of the soluble members in 0.1 mM HCl fell as the concentration of the compounds was increased, indicating that these

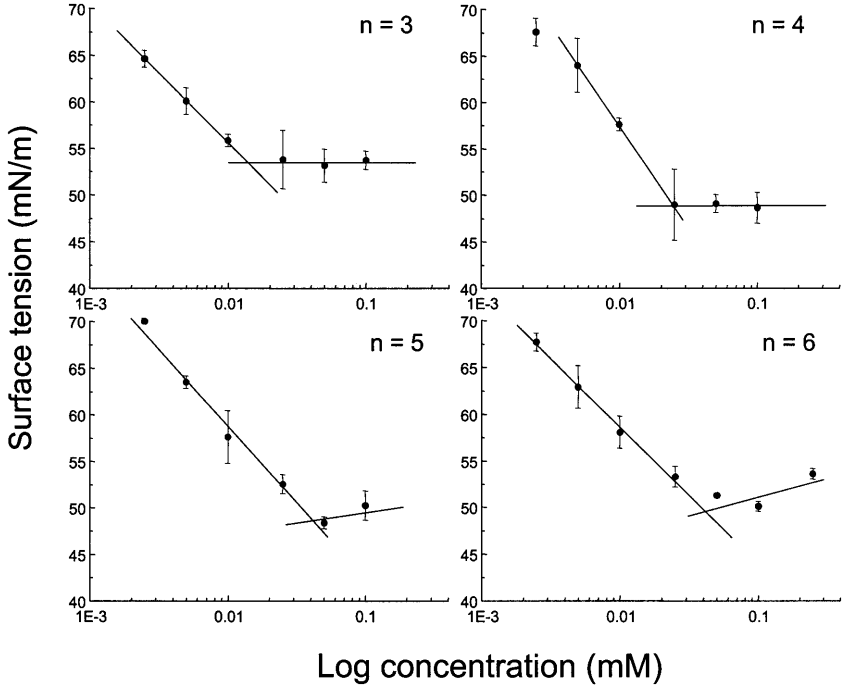

Fig. 3. Surface tension against log concentration curves for the oligomers in 0.1 mM HCl.

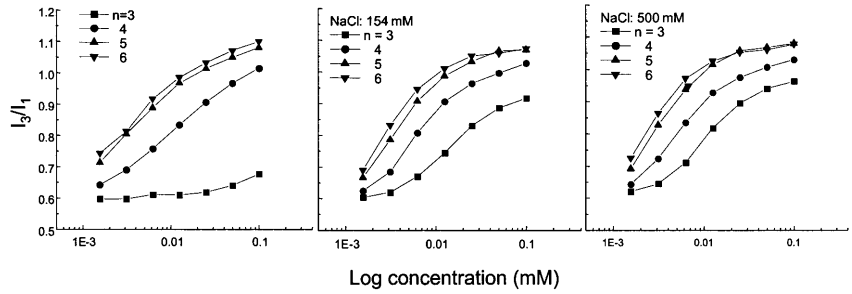

Fig. 4. Fluorescence intensity ratio of pyrene in the oligomer solutions on 0.1 mM HCl in the presence and absence of the salt.

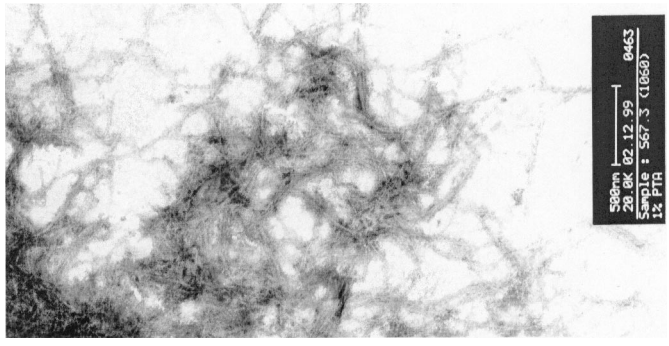

Fig. 5. Supramolecular structures of the ($n = 3$) oligomer (1 mg/ml) in 0.1 mM HCl observed by TEM.

oligomers were surface active. Limiting surface tension values were 53 and 48–50 mN/m in the cases when $n = 3$ and $n = 4$–6, respectively (Fig. 3). The discontinuity in the surface tension data was identified with the critical micelle concentration (CMC), and the values for each compound determined from the plots are listed in Table 1, which showed that the CMC was closely related to n. As the polymer molecules were closely packed in the surface over the CMC, the surface area occupied per molecule (A) was determined from the equation, $A = 1/N_A\Gamma$, where Γ is the concentration of the polymer in the surface phase calculated from the Gibbs' equation, and N_A is the Avogadro constant. The calculated values of A are listed in Table 1, showing that the area in the case of $n = 3$ was larger than that expected from the relationship between n and the area per molecule for the other members of the series.

The I_3/I_1 value of pyrene as a function of the oligomer concentrations in the presence or absence of NaCl are shown in Fig. 4. It was demonstrated that, even though the amount of the lipid chain in the system was normalised where $n = 3$, there were fewer accessible hydrophobic sites for pyrene in the absence of the salt. By adding the salt, these curves shifted to the left, particularly

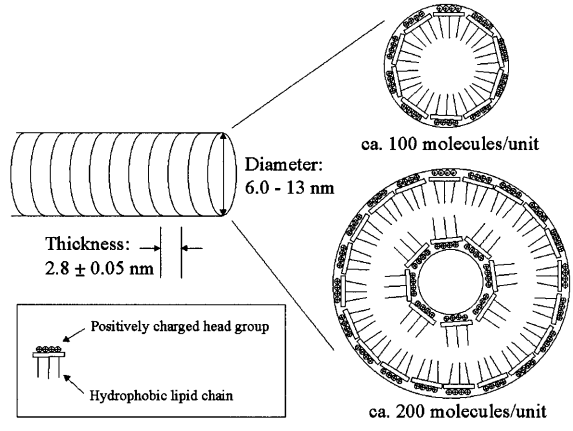

Fig. 6. Possible models of supramolecular structures of the ($n = 3$) oligomer in aqueous solution.

remarkable in the case of the $n = 3$ polymer, indicating that the forces of the repulsion between the charged head groups is crucial on the formation of micelles.

Supramolecular fibre-like structures in aqueous solutions were observed by TEM only in the case of the $n = 3$ oligomer. The smaller or larger members did not form such structures in any of the experiments conducted under the same conditions. The diameter of these fibres was about 10 nm, their length varying considerably (Fig. 5). Cryo-TEM observation of the ($n = 3$) oligomer solution also revealed that the micelles might elongate to form long cylindrical or fibrous structures. Highly magnified TEM images of the ($n = 3$) oliogomer solution revealed that the fibrous structures were composed of disk-like units stacking in a file. The thickness of the disk unit was analysed to be 2.8 nm with little variance, although the diameter varied from 6.0 to 13 nm. Fig. 6 speculates on the manner in which the polymer forms fibre-like structures in aqueous systems. It is possible that due to the apparently flat shape of the molecule, they form the disk-like units that associate to form fibres. Further investigation is necessary to understand the nature of the structural or physical specificity of the molecule where $n = 3$ that determines aggregation into fibrous structures.

In conclusion, the sequential lipid–lysine polymers were demonstrated to be surface active. Their CMCs were related to polymer length, although when $n = 3$ there are abnormalities in behaviours not only in fluorescence studies but in the fact that it formed fibre-like supramolecular structures in aqueous solution. Work will continue to further understand the association behaviour.

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References

Alivisatos, A.P., Johonsson, K.P., Peng, X., Wilson, T.E., Loweth, C.J., Bruchez, M.P., Jr, Schultz, P.G., 1996. Organization of 'nanocrystal molecules' using DNA. Nature 382, 609–611.

In, M., Aguerre-Chariol, O., Zana, R., 1999. Closed-looped micelles in surfactant tetramer solutions. J. Phys. Chem. B 103, 7747–7750.

Jenekhe, S.A., Chen, X.L., 1999. Self-assembly of ordered microporous materials from rod-coil block copolymers. Science 283, 372–375.

Maget-Dana, R., Brack, A., Lelievre, D., 1997. Amphiphilic peptides as models for protein-membrane interactions: interfacial behaviour of sequential lys- and leu-based peptides and their penetration into lipid monolayers. Supramol. Sci. 4, 365–368.

Sakthivel, T., Toth, I., Florence, A.T., 1998. Preparation and physicochemical properties of lipidic peptide dendrimers. Pharm. Res. 15, 776–782.

Sakthivel, T., Yanai, S., Toth, I., Florence. A.T., 2000. Synthesis and characterization of linear amphiphilic lysine-lipid conjugates. Proceedings of the Millennial World Congress of Pharmaceutical Sciences, abstract, p. 124.