THE JOURNAL OF PHYSICAL CHEMISTRY B

 VOLUME 107, NUMBER 51, DECEMBER 25, 2003

LETTERS

Vesicle Formation in Amylose−Surfactant Mixtures

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Received: April 9, 2003; In Final Form: October 31, 2003

Images by cryo-transmission electron microscopy show two types of structure in dilute aqueous mixtures of amylose (AM) and sodium dodecyl sulfate (SDS): Loose fibrous networks and unilamellar vesicles. Neither of the structures was observed in reference solutions of AM or SDS alone. It is proposed that both types of structure consist of inclusion complexes where SDS molecules are enclosed in V-type single helices of amylose. In the vesicles, the complexes are packed, by folding of the AM helices, into lamellae.

Introduction

Amylose (AM) is an essentially linear polymer composed of glucose residues linked by (1→4)-α-D-glucosidic bonds. AM forms single-helical inclusion complexes together with various hydrophobic ligands such as iodine,[1] alcohols,[2−4] fatty acids,[4−6] and surfactants.[4,7,8] The normal procedure to produce the inclusion complexes is to cool a hot aqueous mixture of AM and the ligand. Two types of complexes have been observed, distinguished by their melting temperatures and their crystallinity.[4,5,9−12] Crystalline complexes have high melting temperatures, whereas the noncrystalline complexes have lower melting temperatures. Either type of complex may be produced for uncharged ligands, such as alcohols or fatty acids, depending on the thermal history of the sample. By contrast, only noncrystalline complexes have been found for charged ligands, such as alkyl sulfates and soaps.[4]

The crystalline form, referred to as amylose-V, is well studied and is based on a hexagonal close-packing of single-stranded AM helices, where the hydrophobic ligand molecules are included in the central cavities of the single helices.[13−15] For ligands containing unbranched alkyl chains, each helix turn contains six glucose units. Several groups have found evidence of a lamellar structure of amylose-V.[2,9,10,13,16−18] Thus, Godet et al.[13] studied the complexation between AM and fatty acids by transmission electron microscopy and found crystalline areas composed of lamellar layers. It is believed that the lamellar structure is due to alternating crystalline and amorphous layers.[13,17,18] According to this model, a crystalline layer contains the hexagonally close-packed AM helices with their central axes perpendicular to the layer. The thickness of the crystalline layer corresponds to the length of two fatty acid molecules, packed head to tail inside a helical section, and the headgroups of the fatty acid molecules protrude into the amorphous layers on either side of a crystalline layer.

By contrast, little is known about the structure of the noncrystalline inclusion complex. It is generally believed to contain V-type single helices, but the helices are not packed in crystals with long-range order. Gunning et al. have recently used atomic force microscopy (AFM) to image mixed inclusion complexes, formed in the presence of iodine and a nonionic surfactant.[19] The images showed single chains with dimensions corresponding to the V-type single helix. The authors proposed that the observed structures represented single-helical complexes containing both iodine and surfactant, with the surfactant headgroups protruding from the helix ends.

Here we have used cryo-transmission electron microscopy (cryo-TEM) in an attempt to visualize the structures formed in

10.1021/jp034946h CCC: $25.00
Published on Web 11/25/2003

aqueous mixtures of AM and surfactant. We have found two types of structure: Loose fibrous networks and unilamellar vesicles. As far as we are aware, the latter type of structure has not previously been observed or predicted for AM−surfactant mixtures.

Experimental Section

Materials. Potato amylose with a molecular weight of about 800 000 (high-molecular-weight AM) was obtained from Sigma Chemical Co. Prior to use, the high-molecular-weight AM was placed in an oven at 80 °C for 1 h to minimize solvent impurities such as butanol. Low-molecular-weight AM samples with molecular weights of 2800, 15 000, and 160 000 were obtained from CarboMer, Inc. and used as received. Sodium dodecyl sulfate (SDS) with a critical micelle concentration (cmc) of 8.1 mM[20] was obtained from BDH and used without further purification. Degassed Millipore water was used in all samples.

Sample Preparation. Aqueous solutions of 0.50% w/w AM were prepared by heating mixtures to approximately 155 °C for 30 min followed by cooling to 90 °C. To this solution was added an SDS solution, yielding final concentrations of 8 mM SDS and 0.25% w/w AM. Reference binary samples containing either 8 mM SDS or 0.25% w/w AM were also prepared.

Cryo-TEM. The technique, which has been described in detail by Bellare et al.,[21] leads to vitrified specimens, where component rearrangement and water crystallization are prevented. The original microstructure is thus preserved. A sample was placed in a controlled environment vitrification chamber close to room temperature, where the relative humidity was kept close to saturation to prevent water evaporation. A 5 μL drop of the solution was put on a lacey carbon film grid supported by a TEM copper grid. The lacey carbon film grid had been glow discharged prior to its use. To create a thin liquid film over the grid, the drop was gently blotted with filter paper. Quickly after the blotting, the sample was rapidly plunged into liquid ethane close to its freezing point (−177 °C), to obtain a vitrified film less than 200 nm thick. The vitrified specimen was transferred under a liquid nitrogen environment by use of a cold stage unit (Oxford CT3500) into the electron microscope (Philips CM120 BioTWIN). The working temperature was kept below −183 °C. The images were digitally recorded with a CCD camera (Gatan MSC 791) on Kodak 50−163 film.

Results

High-Molecular-Weight AM. Most experiments were performed on high-molecular-weight AM. All the prepared solutions were clear and colorless and had a low viscosity. Other studies on the same AM sample in our laboratory, using the same preparation protocol, indicate that soluble AM-SDS complexes, showing no sign of precipitation, are formed with 0.25% w/w AM, if the SDS concentration is kept within the range 1−50 mM.[22]

Approximately 20 h after mixing, the various samples were plunged into liquid ethane to be vitrified for experiments with cryo-TEM. The ref 8 mM SDS solution gave featureless images, as expected, because few aggregates should be present at the cmc. By contrast, the images from the 0.25% w/w AM sample (Figure 1) showed branched structures similar to those previously reported by Putaux et al.[23] No other features were found for this sample.

Cryo-TEM images of AM-SDS samples showed two types of structure. Parts of the images contained finite loose networks, reminiscent of balls of yarn (Figure 2), whereas other parts showed unilamellar vesicles, with a wall thickness of a few nanometers, quite similar to the unilamellar vesicles observed by the same technique for lipids and catanionic surfactant mixtures.[24] During the allowed mixing time, only a few complete vesicles were formed (Figure 3a), whereas most of the vesicles were incomplete (Figure 3b,c). Repeated cryo-TEM imaging was performed on the same solution after 12 days. The images from the aged solution showed only closed vesicles with diameters in the range 200−600 nm.

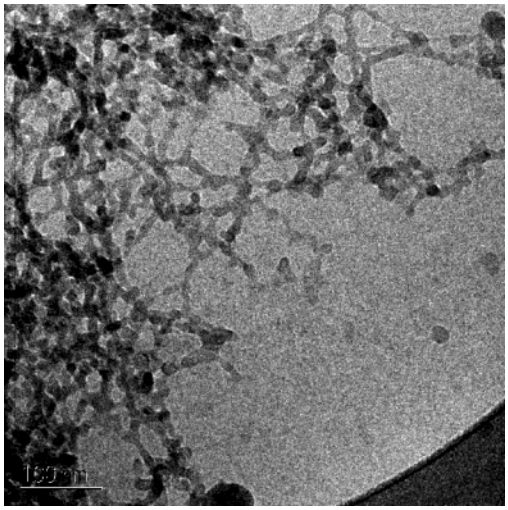

Figure 1. Cryo-TEM image of a sample of 0.25 wt % high-molecular-weight AM.

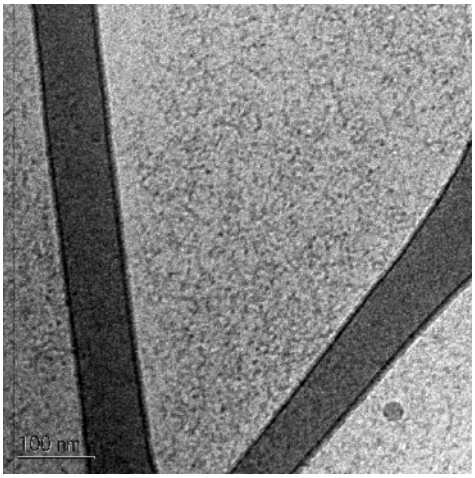

Figure 2. Cryo-TEM image showing the loose network structure in a sample of 0.25 wt % high-molecular-weight AM and 8 mM SDS.

Low-Molecular-Weight AM. For the low-molecular-weight AM samples, mixed solutions with SDS yielded only network structures of the type shown in Figure 2, except for the sample with the molecular weight of 15 000, where a few vesicles were found. The number of vesicles found in this sample was, however, significantly lower than that found for high-molecular AM.

Discussion

Two types of structure were observed in the AM−SDS mixtures: Loose, finely stranded networks and vesicles. Both structures differed from the coarse networks observed for surfactant-free AM. A network structure is not surprising in a system of linear polymers. The resolution of the cryo-TEM technique does not allow detailed conclusions regarding the fibrous strands making up the networks in the AM−surfactant mixtures, but it is quite possible that they could correspond to

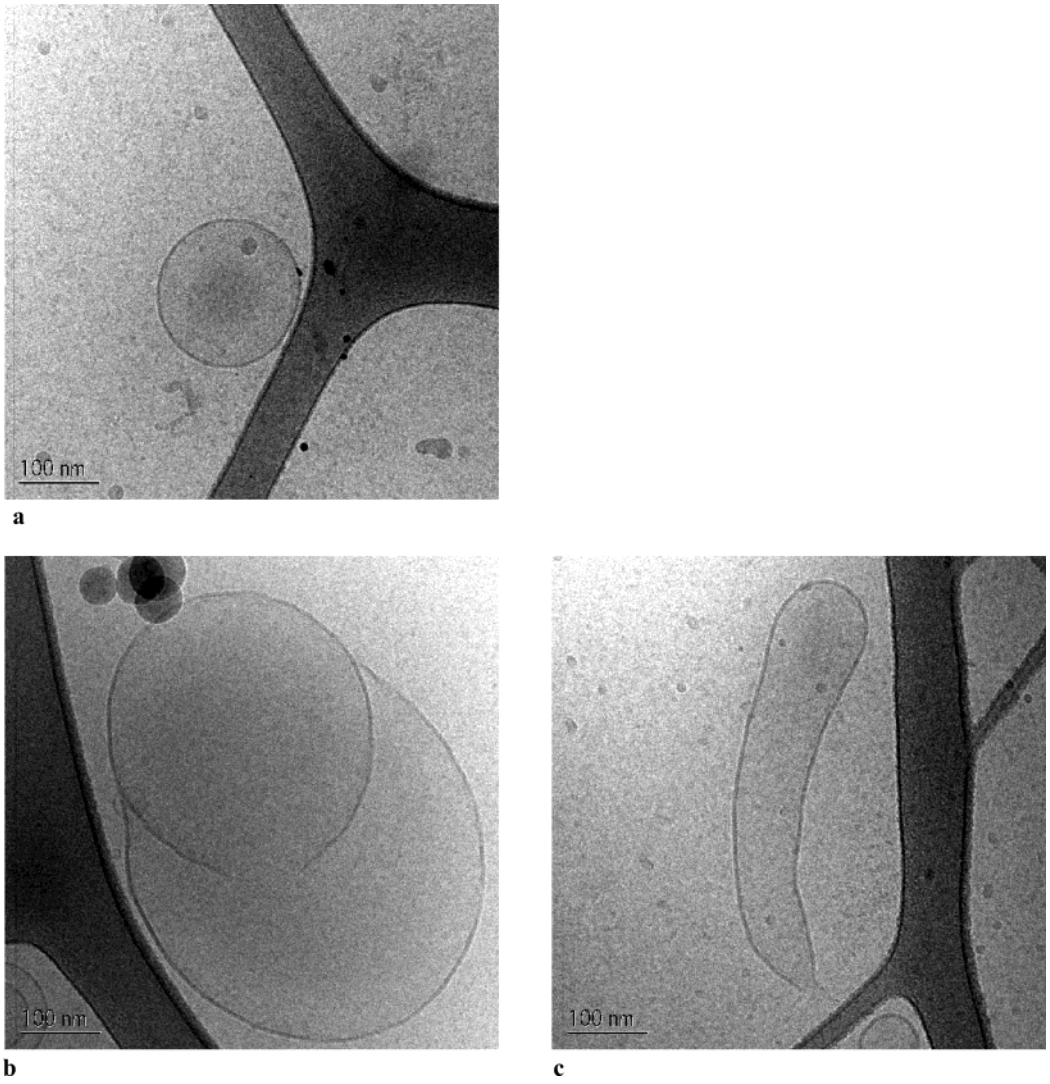

Figure 3. Cryo-TEM image showing closed (a) and open (b, c) vesicles in a sample of 0.25 wt % high-molecular-weight AM and 8 mM SDS.

V-type helices, as was proposed for the strands found for AM−iodine−surfactant complexes in the AFM studies by Gunning et al.[19] In contrast to the latter study, however, we always found networks of strands, rather than individual strands in our systems. This may reflect the different compositions of the two systems; moreover, the solutions studied by Gunning et al. were much more dilute (3 mg of AM per liter).

The occurrence of vesicles in AM−surfactant mixtures is much less obvious. Vesicles were not found in all mixtures, and only a few vesicles were found for the AM samples that did produce vesicles. Nevertheless, every time a sample of the latter type was prepared, similar vesicles were seen, whereas no vesicles could be detected for either of the binary reference solutions, containing 8 mM SDS, or 0.25% w/w of AM. It is thus clear that the formation of the vesicles requires the presence of both components. Still, the possibility must be seriously considered that the vesicles could be due to some trace impurity of lipid in the AM sample. In principle, added SDS could form vesicles together with such a lipid impurity. We regard this possibility as highly unlikely, however, for the following reasons.

1. The high-molecular-weight AM sample is not expected to contain any lipid impurities. The source, potato starch, is generally regarded as being essentially lipid-free.[25] Moreover, our previous NMR analysis of the same type of high-molecular-weight AM revealed the expected presence of small amounts of butanol, but no other impurity.[26]

2. At 8 mM SDS, which corresponds to the cmc, trace impurities of amphiphilic molecules should simply be dissolved in SDS micelles. This holds true even if we allow for the consumption of a small fraction of SDS through inclusion complexation with AM. The maximum amount of SDS that can be included in 0.25% AM is 0.6 mM, according to published binding isotherms,[27] and unpublished surface tension measurements performed in our laboratory.[22]

We are left with the conclusion that the vesicles seen in Figure 2 consist of AM−SDS complexes. The question remains as to the probable nature of such vesicles. Because lamellar structures have been observed for crystalline inclusion complexes (see the Introduction), a candidate structure is the one depicted in Figure 4, where the single lamella surrounding the vesicle has a structure similar to one layer of AM helices in the amylose-V crystal. This would require chain folding, such that each AM helix makes several folds along the lamella. Such a structure, with antiparallel AM helices, has indeed been proposed on the basis of X-ray diffraction studies on amylose-V crystals.[15] The unilamellar structure of the vesicle, as opposed to the multilayered structure formed in the crystal, would then have its origin in electrostatic repulsions between surfactant-loaded lamellae. We note that the thickness of the vesicle membrane in Figure

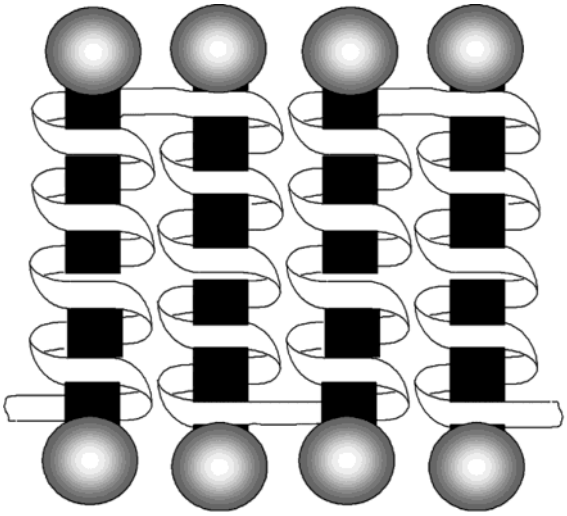

Figure 4. Schematic picture showing the proposed structure of a segment from a vesiclecreated by AM−surfactant inclusion complexes.

3 is in the same range as the lamellar thicknesses observed for crystalline AM−fatty acid complexes.[13]

One may ask whether the repulsion between charged SDS molecules would not be too strong to prevent the folding of a chain into a lamellar structure. A conclusive answer to this question would obviously require quantitative information on the strength of the attraction between the amylose helices in a lamella. To our knowledge, this information is not available. However, we can note that the packing of SDS molecules in the proposed lamellar structure is not a priori unreasonable, because the distance between charged headgroups is significantly larger than that of conventional SDS aggregates. The headgroup area in a lamella should correspond to the cross-sectional area of the V-type single helix, i.e., 144 Å^2, using the reported value 13.5 Å for the outer diameter of a single helix with 6 glucose units per turn.[28] This may be compared with the much smaller headgroup area of 62 Å^2 for SDS in a spherical micelle.[29]

A more difficult question is why the observed samples contained only few or sometimes no vesicles, coexisting with network structures. However, we should recall that the occurrence of two types of structure, one amorphous and one featuring regular lamellae, is well established for amylose inclusion complexes with neutral ligands.[4,5,9−12] For the latter type of complex, the thermal history during sample preparation has been shown to have a strong influence on the relative proportions of the two structures. In particular, annealing at a high temperature is typically required to obtain a large fraction of the regular (lamellar) structure. Moreover, an increased chain length of the amylose also favors the lamellar structure.[13] The latter trend agrees with the general trend found in our observations, although the sample with a molecular weight of 160 000, where no vesicles were found, deviates from this trend.

Much more extensive studies, involving variations in sample composition and in the sample preparation procedure, are obviously needed to clarify the conditions required for vesicle formation, and the optimum conditions for obtaining a high yield of vesicles. Such studies are in progress in our laboratory.

Acknowledgment. We thank Gunnel Karlsson for expert cryo-TEM microscopy, Maria Karlberg for help with sample preparations, and Ann-Charlotte Eliasson, Ali Khan, and Håkan Wennerström for valuable discussions. This work was financed by the Centre for Amphiphilic Polymers from Renewable Resources (CAP) at Lund University (M.E.) and by the Swedish Research Council (L.P.).

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