Biochimica et Biophysica Acta, 1029 (1990) 67–74
Elsevier

BBAMEM 75008

Solubility properties of the alkylmethylglucamide surfactants

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(Received 20 April 1990)

Key words: Nonionic surfactant; Alkylmethylglucamide; *N*-D-Gluco-*N*-methylalkanamide

The critical micelle concentration (CMC) and the ability to solubilize and form vesicles from phospholipids are important criteria for the selection of a surfactant for reconstitution protocols. The CMC and its temperature dependence were determined for an homologous series of alkylmethylglucamides (MEGA-8, MEGA-9, MEGA-10). Each detergent was added continuously from a concentrated solution to a saline buffer with the environment-sensitive fluorescent probe ANS, held in a thermojacketed cuvette; ANS fluorescence increases at the CMC. The CMCs at 25 °C were 51.3, 16.0 and 4.8 mM for MEGA-8, MEGA-9 and MEGA-10. The free energy change for transfer to a micellar environment per -CH_2- was −740 cal/mol, similar to other alkyl series. The CMCs decreased slightly with increasing temperature (T = 5–40 °C) for MEGA-9 and MEGA-10 while that of MEGA-8 was virtually insensitive to temperature in this range. MEGA-9 solubilization of egg PC in aqueous solutions was determined as a function of [PC] and temperature. The lamellar-micellar phase boundaries were determined by simultaneous 90° light scattering and the resonance energy transfer using the headgroup labeled lipid probes NBD-PE and Rho-PE. The [MEGA-9] at solubilization was linear with [PC]; the MEGA-9 to egg PC ratio in the structures at optical clarity was 2.3 while the monomeric [MEGA-9] was 14.3 mM or slightly lower than the CMC at 25 °C. Solubilization of egg PC by MEGA-9 was somewhat more temperature-dependent than the CMC of this detergent. Vesicles formed from MEGA-9 tended to be multilamellar. MEGA-9 is clearly different from octyl glucoside, despite its chemical similarity, in terms of its temperature sensitivity and vesicle forming characteristics.

Introduction

Determining the physicochemical properties of surfactants and phospholipids in aqueous solution is a prerequisite for their effective use in biochemical applications such as protein isolation and reconstitution. The critical micelle concentration of the surfactant alone and its temperature dependence will help determine if that particular surfactant is reasonable to consider for a particular use. Then, knowledge of the specific behavior of the mixed surfactant phospholipid system, e.g., the aqueous-micellar concentration distribution of surfactant at solubilization, can be applied to the design of an effective protocol. Similarly, the selection of a particular surfactant may depend on the characteristics of the vesicles formed from surfactant-phospholipid mixtures.

Members of the *n*-alkyl-MEGA series of surfactants have been used to solubilize membranes and appear to maintain protein function (e.g., Refs. 1, 2). MEGA-8 and MEGA-9 proved similarly effective as octyl glucoside at selectively solubilizing a subunit from the protein chloroplast coupling factor 1 [3], but neither MEGA-9 nor MEGA-12 proved suitable for solubilization of dopamine-D_2 receptors from synaptosomal membranes [4]. Thus, from these few experiments it seems the MEGA series might prove to be a set of useful, quite mild, nonionic surfactants for biochemical application. However, the solubility properties of these have not been fully characterized for lipid or lipid-protein mixtures.

In this study, we characterize three members of the methylalkylglucamide surfactants known as MEGA-8, MEGA-9, and MEGA-10 and the interactions of MEGA-9 with phospholipid. These are nonionic

Abbreviations: CMC, critical micelle concentration; OG, octyl glucoside; PC, phosphatidylcholine; NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine; Rho-PE, *N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine; ANS, anilinosulfonic acid; SUV, sonicated unilamellar vesicles; DUV, unilamellar vesicles prepared by dialysis; cryo-TEM, cryotransmission electron microscopy.

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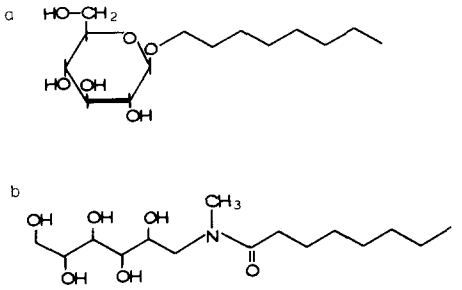

Fig. 1. Chemical structure of (a) octyl glucoside and (b) octylmethylglucamide (MEGA-8), also called octanoyl-*N*-methylglucamide and *n*-octanoyl-*N*-methylglucamine; systematic name: *N*-D-gluco-*N*-methyloctanamide.

surfactants with a methylglucamide polar region and a single *n*-alkyl chain 8, 9, or 10 carbons in length (Fig. 1). It was though that due to their chemical similarity to octyl glucoside (OG), their solubilization properties also would be similar. Here we determine the temperature dependence of the CMCs of MEGA-8, MEGA-9 and MEGA-10 and subsequently characterize the MEGA-9 egg phosphatidylcholine (PC) interactions using a combination of fluorescence and light scattering techniques [5]. When the data are compared to our previous results on OG-PC mixed systems [5,6], it is observed that although the CMCs of all three MEGA surfactants have relatively shallow temperature dependencies, the MEGA-9-PC mixed system is closest to that of OG-PC. Moreover, the mole fraction of MEGA-9 in the mixed surfactant-lipid structures at solubilization is similar to that for the OG-PC system. In contrast, however, vesicles prepared from OG-PC mixed micelles by dialysis or OG-removal by a hydrophobic resin are generally unilamellar and about 100 nm in diameter whereas those from MEGA-9-PC mixtures tended to be multilamellar regardless of initial conditions or the method of the surfactant removal.

Materials and methods

Materials. Egg phosphatidylcholine (egg PC) (> 99%) and the fluorescent lipids *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine (NBD-PE) and *N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine (Rho-PE) were purchased from Avanti Polar Lipids (Birmingham, AL). Octanoyl, nonanoyl and decanoyl methylglucamides were from CalBiochem (La Jolla, CA) and used without further purification. Anilinosulfonic acid (ANS) was from Sigma (St. Louis, MO).

Vesicle formation. Both sonicated unilamellar vesicles (SUV) and vesicles prepared from octyl glucoside by dialysis (DUVs) were used. For either preparation, the lipids were added in a chloroform solution to a small clean glass vial. The chloroform was evaporated under a stream of nitrogen leaving a thin lipid film. This was held under vacuum for about 30 min to remove any remaining traces of organic solvent. After evaporation, buffer was added and allowed to equilibrate with the lipid, on ice, with intermittent vortex mixing for at least 30 min. This preparation was either sonicated (Branson probe tip sonicator model 200, Danbury, CT) until the solution appeared characteristically clear (no longer than 6 min), or prepared for detergent dialysis by addition of concentrated octyl glucoside to an effective mole ratio of 5 or greater. Mixed micelles were dialyzed against at least four changes of 500-fold excess buffer at 5 °C. When NBD-PE and/or Rho-PE were used, these were incorporated at 1% by weight. The aqueous buffer was 150 mM NaCl, 10 mM Na-Hepes, 0.1 mM EDTA, and 0.02% sodium azide at pH 7.2. No differences in the stages of solubilization were observed between the two vesicle types so all data are combined. Final lipid concentrations were determined from hydrolyzable phosphate [7].

Critical micelle concentration (CMC) determinations. The CMC determinations were based on the property that ANS has a significantly greater fluorescent yield in a hydrophobic low dielectric environment than in aqueous solution: thus, micelle formation, providing microscopic hydrophobic regions, is marked by a sudden increase in solution fluorescence (e.g., Refs. 6, 8). Stock solutions at concentrations above the CMC were prepared for each of the *n*-alkyl-MEGAs. These were added with the aid of a syringe pump (Harvard Apparatus, South Natick, MA) to a continuously stirred cuvette containing a known quantity of buffer with 18 μM ANS, and held in a thermojacketed cuvette holder. Fluorescence was monitored simultaneously ($\lambda_{ex} = 410$ nm; $\lambda_{em} = 490$ nm) (SLM-8000 spectrofluorometer SLM Instruments, Urbana, IL). The MEGA-8 stock solution was prepared between 400 mM and 1M, and, the MEGA-9 stock solution was 200 to 400 mM. MEGA-10 appeared to be soluble only up to 33 mM at room temperature and generally a 20 mM stock was used.

Vesicle solubilization. The procedure used to monitor vesicle solubilization was essentially that outlined in Ollivon et al. [5]. In brief, a concentrated stock solution of MEGA-9 was added slowly and continuously to a defined amount of egg PC vesicles in a well-stirred thermojacketed cuvette as described above for the CMC measurements. NBD-PE and Rho-PE are an energy transfer pair that will monitor the average distance between lipids [9] and NBD-PE is sensitive to the local dielectric environment. NBD-PE fluorescence (F_{NBD}) was monitored ($\lambda_{ex} = 475$ nm, $\lambda_{em} = 535$ nm with an added 515 nm long pass filter). F_{NBD} increases as the two fluorophores are increasingly separated, and then catastrophically increases as small mixed micelles are formed. The size of the mixed surfactant-phospholipid structures was monitored indirectly by optical density, or, more usually by 90 ° scattering, simultaneously with

the fluorescence determinations. The fluorometer was in T-format and a 470 band pass (±5 nm) filter used to monitor scattering. Distinct breakpoints are observed by both methods. Three of these were chosen to analyze.

Vesicle formation from MEGA-9-egg PC mixed micelles. The procedure for forming vesicles from MEGA-9 solutions of egg PC was essentially identical to the DUV formation from octyl glucoside described above. The protocol was varied to see if it would alter the final outcome. Slow dialysis, which means the solution was allowed to equilibrate at the point of solubilization prior to equilibration against a large excess of buffer, was tried. We attempted to form vesicles by detergent removal using SM-2 Bio-Beads (Bio-Rad, Richmond, CA) following the method suggested by Vainstein et al. [10].

Cryotransmission electron microscopy (cryo-TEM). To determine the disposition of dialysed egg-PC MEGA-9 preparations, these were visualized using cryo-TEM, an electron microscopy technique that requires no staining or other perturbation of the sample; thus it is ideal for lipid systems. The methods followed were essentially those of Vinson et al. [11]. In brief, a 3 μl drop of vesicle solution was placed on a formvar-treated copper grid that was held in a temperature and humidity-controlled environment. The drop was wicked across the grid with a piece of filter paper and the sample rapidly plunged into liquid ethane at its boiling point to solidify the sample. The grid was subsequently transferred to liquid nitrogen and viewed on a liquid nitrogen-cooled stage (ca. 100 K) (model 626, Gatan Inc., Warrendale, PA) of an analytical electrion microscope (JEOL model 120 CX or 100 CX electronic Optics Division, Peabody, MA). Images were formed at a nominal underfocus of 3.9 μm on Kodak S0-163 film.

Results

The CMCs of the alkylmethylglucamides

The critical micelle concentration of an amphipathic solute is defined as the monomer concentration that is in equilibrium with self-aggregates or micellar structures in which the hydrocarbon chains are excluded from the aqueous solvent and the hydrophilic regions of the molecule are in contact with water. Thus, one way to identify the CMC is by determining the initial formation of microhydrophobic regions in aqueous solution during continuous addition of the surfactant. The large increase in fluorescence associated with ANS partitioning into these regions provides a sensitive means of detecting the CMC as seen in Fig. 2a where fluorescence was monitored during continuous addition of surfactant as described in Materials and Methods.

The CMCs at 25°C for the three homologous surfactants are 51.5 ± 5.6, 16.0 ± 1.0 and 4.8 ± 0.2 mM (±S.D., $n = 7$). As expected, the CMCs were inversely

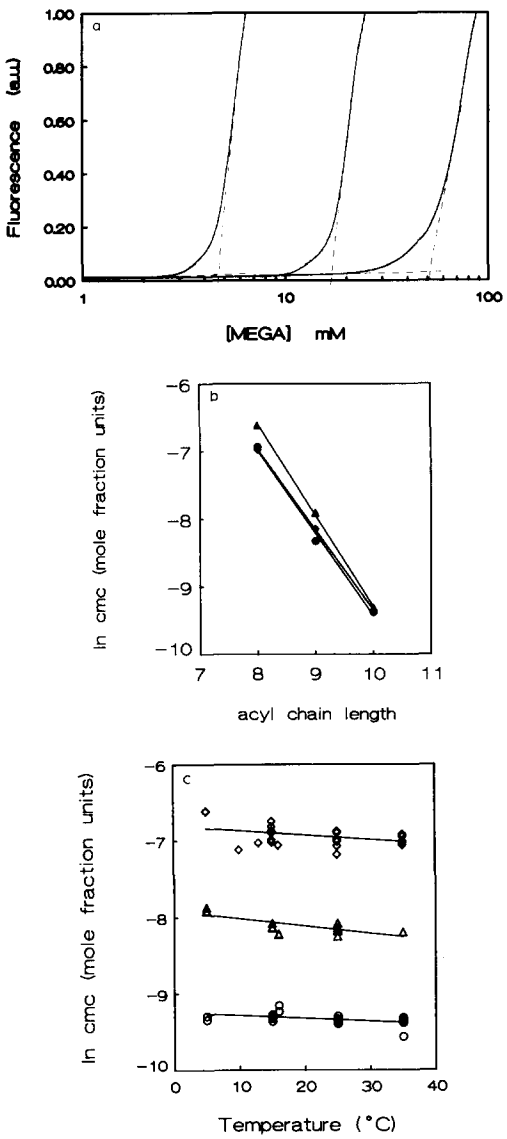

Fig. 2. Critical micelle concentration (CMC) determination for MEGA-8, MEGA-9 and MEGA-10. (a) The CMC determined by the increase in ANS (18 μM) fluorescence during continuous addition of concentrated surfactant at 25°C. Please note that the concentration of each MEGA is depicted on a log-scale. From left to right the traces are examples of addition of (i) MEGA-10 (20 μl/min of 33 mM stock solution); (ii) MEGA-9 (10 μl/min of a 400 mM stock solution); and, (iii) MEGA-8 (10 μl/min of a 1 M stock solution). The dashed lines indicate the breakpoints used to identify the respective CMCs. Excitation and emission wavelengths were 410 and 490 nm, respectively. (b) The CMCs of MEGA-8 (□), MEGA-9 (△) and MEGA-10 (●) as a function of their alkyl chain lengths. The data are plotted as −ln CMC in mole fraction units at 5, 25 and 35°C. (c) The CMCs of MEGA-8 (□) MEGA-9 (△) and MEGA-10 (●) as a function of temperature from 5 to 35°C.

proportional to alkyl chain length. For a homologous series varying only in the number of methylene groups, the solubility or conversely, the CMC is expected to vary by a difference in ΔG, standard of free energy, of 600 to 800 cal/mol [12]. The relationship that allows calculation of the free energy change associated with a

monomer transferring from the aqueous to micellar environment is, $\Delta G^{a \rightarrow m} = -RT$ ln CMC. A plot of $-\ln$ CMC vs. alkyl chain length shows that the alkylmethylglucamide CMCs change linearly with alkyl chain length at 5, 25 and 35 °C (Fig. 2b). The average ΔG per $-CH_2-$ is -740 cal/mol.

The temperature dependence of the CMCs for each of the three methylglucamide surfactants was determined in the range of biological interest, 5–35 °C. In all cases, the CMC decreased very slightly with increasing temperature over this range (Fig. 2c). For example, MEGA-9's CMC changed from 20.5 to 13.5 mM or by 7 mM in a 30 °C change in temperature. The ln CMC (mol fraction units) appeared to be a linear function of temperature, suggesting that the micellar composition was constant over this range; however, the variability in the data and the very shallow slope both contribute to the uncertainty of this conclusion.

Solubilization of egg PC vesicles by MEGA-9

Since the 9-carbon alkylmethylglucamide's CMC was, at 16 mM, the closest to that of octyl glucoside (20.5 mM [5]), this surfactant was chosen for studies to characterize its interaction with egg phosphatidylcholine vesicles. As with the CMC determinations, concentrated MEGA-9 (usually 400 mM) was added slowly and continuously to a suspension of egg PC vesicles in a well-stirred thermojacketed cuvette in a spectrofluorometer. Two parameters were monitored (see Materials and Methods): the relative interaction of two fluorescent lipid probes that form an energy transfer pair (NBD-PE and Rho-PE) and the 90° light scattering. Two different parameters are monitored by these two measurements; the fluorescent lipids indicate the relative proximity of the lipids and the 90° scattering is indicative of the size and number of macromolecular structures. Together these provide a sensitive means of observing the changes in lipidic particle structure during vesicular or lamellar phase lipid solubilization by the surfactant (e.g. Refs. 5, 11).

TABLE I

Free and lipid-associated MEGA-9 at the break points (T = 25 °C)

Breakpoint	[MEGA-9]aq (mM)	MEGA-9 : PC (mol : mol)
B	13.22	2.34
C	14.10	3.15

Three points were identified during the lamellar-micellar transition as indicated in Fig. 3a: one of RET breakpoints was clearly identical to one of the turbidity breakpoints so both of these were designated as 'C'. The total amount of MEGA-9 needed to reach each stage was a linear function of the egg PC concentration as seen in Fig. 3b. The slope of each line gives the average ratio of MEGA-9 to egg PC in the structures of lipid phase and the intercept is equal to the aqueous monomeric MEGA-9 concentration. If we *assume*, by analogy to the OG egg PC system, that these three points indicate vesicle opening or the initial formation of cylindrical mixed micelles, and the two boundaries of a mixed region containing both cylindrical and spherical micelles [11], then we can assign the compositions listed in Table I to (a) cylindrical micelles, and (b) lipid-saturated spherical micelles. Caution *must* be taken with these assignments, however, since the structures themselves have not been examined directly.

The aqueous concentration of MEGA-9 at each point identified is lower than the CMC of MEGA-9 alone in aqueous solution. For example, at solubilization, when the mixed vesicle-MEGA-9 concentration becomes clear presumably due to spheroidal micelle formation, the aqueous [MEGA-9] is only 14.1 mM compared to the CMC of 16 mM at 25 °C. This is somewhat higher than the value expected at this point if the two were mixing

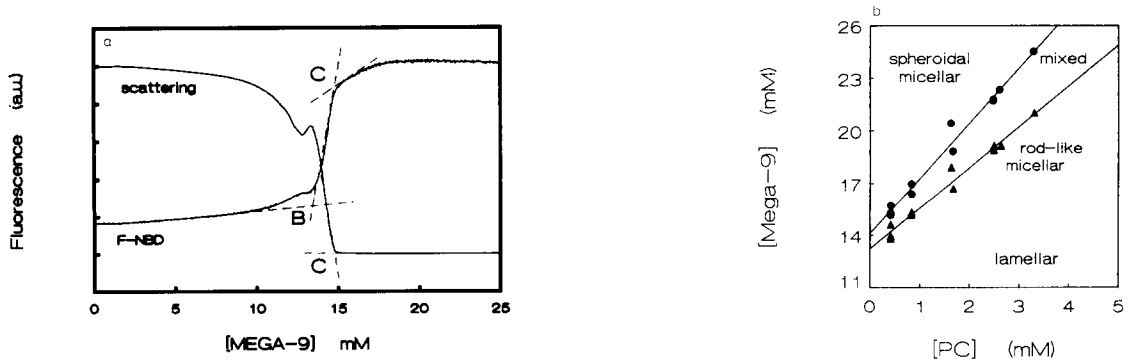

Fig. 3. The dissolution of egg PC vesicles by MEGA-9 monitored by changes in energy transfer efficiency between the fluorescent probes NBD-PE and Rho-PE and by light scattering. (a) Data were collected by continuous addition of MEGA-9 from a concentrated stock solution and simultaneous observation of NBD-PE fluorescence (λ_{ex} 475 nm, λ_{em} 535 nm) and light scattering ($\lambda_{em} = 470$ nm bandpass filter). Three breakpoints were identified as indicated. (b) The concentration of MEGA-9 at each of the three break points shown in (a) are plotted as a function of total lipid concentration.

ideally according to the relationship: CMMC = $X \cdot$ CMC (Eqn. 1) where CMMC is the critical mixed micellar concentration and X is the mole fraction of surfactant in the mixed structures [12].

To determine if the solubilization process would have the same temperature dependence as the MEGA-9 CMC, the two fluorescent breakpoints were determined at one egg PC concentration (0.46 mM) at five different temperatures between 5 and 35 °C. The total amount of MEGA-9 at each point is plotted as a function of temperature in Fig. 4. About 9 mM more MEGA-9 was needed to form spheroidal mixed micelles at 5 than at 35 °C, which is more than twice the difference in the MEGA-9 CMC for this temperature range.

Vesicles formed from MEGA-9-egg PC mixed micelles

Vesicles were prepared from MEGA-9-egg PC solutions using several approaches. The surfactant : PC ratio was varied from about 8.3 to 16 and the total lipid concentration ranged from 1.0 to 10 mg/ml. The means of surfactant removal was varied by changing the rate of dialysis and by using hydrophobic SM-2 Bio-Beads in batch. All preparations appeared extremely turbid for the amount of lipid. The hydrodynamic diameter estimated by laser light scattering (Coulter N4) was about 270 nm for vesicles prepared by dialysis from MEGA-9. Cryo-TEM images from several types of preparations revealed that the vesicles were multilamellar with many closely packed lamellae or were extremely large (Fig. 5). One preparation, made by dialysis from a dilute lipid suspension, did contain some unilamellar but non-spherical vesicles, but was extremely turbid.

Variations in the concentration of lipid, MEGA-9 or the means of surfactant removal did not alter the vesicle structure significantly, although lower total lipid concentration may have reduced the number of MLVs. It is highly unlikely that the appearance of multilamellar structures was induced by preparing the sample for cryo-TEM since the sample is completely unperturbed by chemicals or dehydration and only rapidly frozen to prepare it for viewing. This technique has been applied to numerous other vesicular preparations and revealed unilamellar vesicles [11,13].

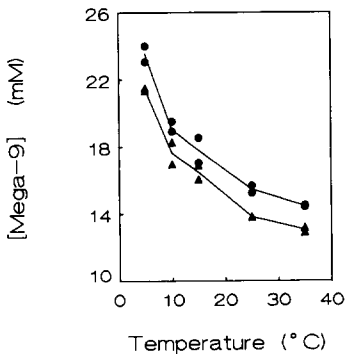

Fig. 4. Temperature dependence of the MEGA-9 dissolution of egg PC vesicles (0.46 mM). The total amount of MEGA-9 at each of the two break points considered to be the boundaries of the cylindrical and spherical micelle mixed region.

Discussion

The methylalkylglucamides were developed and presented as an alternative to the alkyl glucosides and other nonionic relatively mild surfactants designed for biochemical applications [1]. Although these three surfactants, MEGA-8, MEGA-9 and MEGA-10 are similar in some respects to octyl glucoside, it is clear that they are not necessarily a direct substitute as will be discussed below.

The critical micelle concentrations determined in this study using ANS for MEGA-8, MEGA-9 and MEGA-10 are similar but somewhat lower than those reported previously at 25 °C either by ANS [3] or by changes in transmittance of an iodine solution [2] or at 30 °C using surface tension or light scattering techniques [14]. Reported CMC values range from 54.5 to 73 mM (MEGA-8), 17 to 25 mM (MEGA-9), and 5 to 7 mM (MEGA-10) compared to the present determinations of 51.5, 16 and 4.8 mM, respectively. This variability among laboratories may be indicative of variability among the preparations of these surfactants. For example, some of the material examined in the present study contained a lower CMC component *. The CMCs of the three MEGA surfactants decrease exponentially with increasing alkyl chain length as expected for a homologous series. The average standard free energy change per methylene group for incorporation into a micelle determined at three temperatures is 740 cal per mol, in the range expected [12]. This value compares remarkably well to 744 cal/mol determined from the CMCs at 30–40 °C and to 687 cal/mol methylene groups calculated from the partition coefficient of these surfactants into dipalmitoylphosphatidylcholine above the sol-gel transition temperature (calculated from data in Ref. 15). The correspondence of these results would be expected only from relatively pure samples.

One remarkable feature of the three alkylmethylglucamide surfactants examined is the relative insensitivity of the CMCs to temperature. This result is suggested by previous studies, since the published values do not vary systematically from room temperature to 40 °C

* A small 'bump' was present in some MEGA-8 and MEGA-9 preparations and its position relative to the CMC is temperature dependent in a way that might by consistent with it being due to the formation of mixed MEGA-8 and octanoic acid or mixed MEGA-9 and nonanoic acid structures. Nonanoic acid is used in the synthesis of MEGA-9 and is extremely hard to remove from the final product. The 'bump' could be removed by equilibrating a pH-solution with an anion exchange resin.

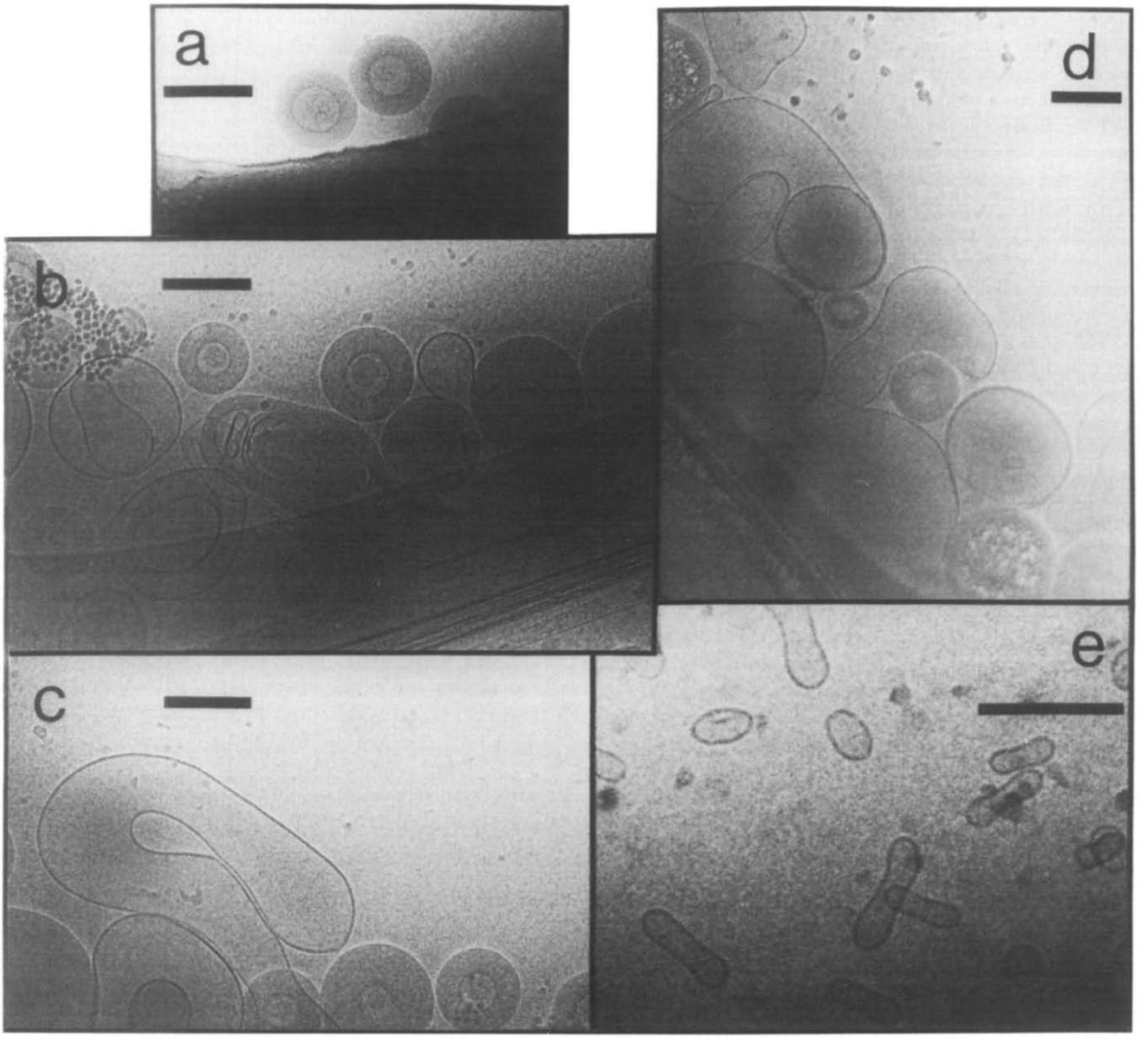

Fig. 5. Cryo-TEM images of egg PC vesicles prepared from mixed egg PC-MEGA-9 micelles. All scale bars represent 200 nm. (a) Vesicles prepared by slow dialysis from about 5 mM PC. (b)–(d) Examples of images observed from vesicle preparations prepared by a normal dialysis protocol. (e) An example of a dialysis preparation using 1 mM PC. This image is unlikely to represent the range or majority of structures existing in the sample since the sample appeared extremely turbid.

determinations [2,3,14,15]. Nonionic surfactant CMCs are generally considered to be inversely related to temperature since solubility is entropically determined in the temperature range 5–40 °C [16]. Eventually, the enthalpic term will dominate at higher temperature and all surfactants will become increasingly soluble with a rise in temperature. It appears that the alkyl methyl glucamides are at the transition where both entropic and enthalpic terms contribute significantly to their overall solubility in this temperature regime.

The insensitivity to temperature of the three MEGAs is in direct contrast with the octyl glucoside critical micelle concentration which decreases from 31 to 16 mM from 5 to 40 °C [6], whereas MEGA-8 CMCs change just a few mM. The difference presumably is in the nature of the headgroup interaction since the acyl chains alone would predict a rather steep negative temperature dependence.

Differences between the methylglucamide and glucoside moiety must account for the somewhat greater solubility of the MEGA series of surfactants compared to the alkyl glucosides. For example, the CMC of octyl glucoside with an 8-carbon alkyl chain is at 21 mM ($T = 25$ °C, Ref. 5) closer to the CMC of MEGA-9 (16

mM at $T = 25\,^{\circ}C$) than to the CMC of MEGA-8 (52 mM at $T = 25\,^{\circ}C$). The linear headgroup on the methylglucamides and amino substitution (see Fig. 1) may both contribute to differences in steric packing constraints or hydrogen bonding possibilities. Other differences between the alkylmethylglucamides and octyl glucoside behavior in aqueous solution are suggested by the apparent relative proximity of the cloud point to the CMC. For example, MEGA-10 will precipitate at about 30 mM when held at room temperature, as determined by general observation while preparing and holding stock solutions. Similarly, Hanatani et al. [2] report precipitation of 40 mM MEGA-9 and 15 mM MEGA-10 at temperatures between 0 and 15 °C. Unfortunately, this property is clearly awkward for protein reconstitution when solutions are often held on ice to minimize proteolysis or irreversible denaturation.

Solubilization of sonicated or large unilamellar PC egg vesicles by MEGA-9 does appear to be similar qualitatively and quantitatively to solubilization by octyl glucoside (and other nonionic surfactants, e.g., Triton X-100) [5,17]. On the basis of monitoring changes in lipid–lipid interactions, the dielectric environment of the lipids (fluorescence) and the morphology of the mixed MEGA-9-egg PC structures (indirectly by turbidity or light scattering), the points considered to be the boundary of a putative cylindrical-spheroidal micelle transition region and final solubilization into small micelles could be clearly identified. As for the case of octyl glucoside, the total amount of MEGA-9 needed at each point increased linearly with total PC concentration indicating a defined average composition of the mixed structure(s) at each of these points. Moreover, the values of the average surfactant to egg PC ratios at each point, i.e., 1 : 1, 2.3 : 1 and 3.15 : 1 are nearly identical to those for octyl glucoside and egg PC at the equivalent stage of solubilization [5,6,17,18].

It was expected, based on the temperature-insensitive nature of the CMC, that MEGA-9 dissolution of egg PC vesicles would be relatively independent of temperature. However, the total concentration of MEGA-9 needed to solubilize egg PC vesicles decreased from 24 mM to 14 mM as temperature increased from 5 to 35 °C, making the mixture about twice as temperature-sensitive as the CMC. Moreover, at solubilization, the mixture did not behave as ideally predicted for perfectly mixed amphiphiles. Although addition of egg PC modified the temperature sensitivity somewhat in the octyl glucoside-PC mixed system, the changes were slight and at solubilization (Spheroidal micelles) the mixture behaved ideally by the criterion in Eqn. 1 [6]. This is not the case for MEGA-9 and egg PC. At sub-solubilizing concentrations, MEGA-8, MEGA-9 and MEGA-10 are reported to have no effect on the width of the DPPC sol-gel transition temperature although the value is depressed in proportion to the amount of surfactant in the membrane [15]. This result might predict ideal mixing as it implies little or no interaction between the methylglucamide and choline headgroups, at least at temperatures of 30 °C and greater. However, as noted anecdotely above by us and others [2], the cloud point is not far from the CMC and these surfactants may become increasingly non-ideal at lower temperatures.

Vesicle formation from MEGA-9 and egg PC mixed micelles by surfactant removal results in multilamellar structures with a large number of closely spaced lamellae. Similar vesicles were formed regardless of the temperature (room or 4 °C), the rate of MEGA-9 removal, the total amount of egg PC, or the initial MEGA-9 : egg PC ratio (5 : 1 or 10 : 1 in the mixed micelles). In contrast, quite uniform unilamellar vesicles are formed from octyl glucoside/egg PC mixtures under all of these equivalent conditions. Clearly there is something about the MEGA-9 interactions with egg PC that promote the multilamellar structures. These large multilamellar bilayers may be useful for some specialized purposes such as preparations of protein for structural studies where high concentrations of properly reconstituted protein are needed (suggested by B.A. Wallace). However, in general, this type of vesicle is not desirable. MEGA-9 may prove useful in mixtures of surfactants; for example, MEGA-9-cholate mixtures have resulted in unilamellar vesicles (Mary Blackwell, personal communication).

In conclusion, the alkyl methylglucamide surfactants are interesting for the relatively flat temperature dependence of their critical micelle concentrations, their low cloud points and the provocative nature of their interactions with phosphatidylcholines. There is clearly some critical aspect of the headgroup structure, possibly the methylamine moiety or the open conformation of the glucamide. Regardless of these interesting questions, it appears that MEGA-8, MEGA-9 and MEGA-10 each have characteristics to make them unsuitable as the sole surfactant in a protein reconstitution procedure.

Acknowledgements

The authors are exceedingly grateful to Dr. Mary Blackwell for providing a sample of MEGA-9 that she had prepared. We thank Debbie Clark for providing excellent technical assistance, and Ofer Eidelman for advice during manuscript preparation. The work was supported in part by the American Heart Association, Miami Valley Chapter.

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