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Amphiphiles modify the properties of detergent solutions used in crystallization of membrane proteins

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The effect of the amphiphile heptanetriol on the properties of solutions containing several detergents commonly used for crystallization of membrane proteins was characterized. The critical micelle concentration was found to be relatively unchanged by the presence of the amphiphile. In contrast, the addition of heptanetriol to solutions containing both detergent and polyethylene glycol exhibited significant shifts in the clouding behavior, with the largest shifts being for lauryl dimethylamine oxide. These results suggest that conditions favorable for crystallization of integral membrane proteins can be inferred from the properties of the detergents and amphiphiles.

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1. Introduction

The determination of three-dimensional structures of integral membrane proteins remains a challenging effort owing to the difficulties in obtaining crystals suitable for X-ray diffraction experiments (Sowadski, 1996; Bowie, 2000). Although the development of effective crystallization screens coupled with many technical improvements in X-ray diffraction measurements and analysis has led to thousands of structures of water-soluble proteins being solved each year, preferred crystallization conditions have not been established for membrane proteins. The purification of proteins from the membrane requires the presence of detergents and crystallization is often favored when small amphiphiles are present (Michel, 1983). Thus, crystallization trials for membrane proteins involve sparse-matrix sampling of many different detergent and amphiphile combinations (Jancarik & Kim, 1991; Garavito *et al.*, 1996; Song & Gouaux, 1997), resulting in investigation of tens of thousands of conditions (Chang *et al.*, 1998). Understanding the effects of detergents on the properties of crystallization solutions should provide a basis for developing more efficient screens. In this paper, the impact of amphiphiles on the critical micelle concentration (CMC) and the cloud-point temperature is described for several of the most commonly used detergents for crystallization: lauryl dimethylamine oxide, octyl glucoside, dodecyl maltoside, decanoyl glucamide and octyl tetraoxyethylene.

2. Experimental

2.1. Effect of heptanetriol on the CMC

One of the key properties describing any detergent is the critical micelle concentration or CMC. Below the CMC, the detergents are in solution as monomers. Once the detergent concentration is at or above the CMC, the detergent molecules will form micelles, which are detergent aggregates with the polar groups exposed to the water and the hydrophobic groups buried. The CMC for each detergent was determined through measurements of the surface tension using capillary action, in which the height that a solution travels up a uniform glass capillary is directly proportional to the surface tension. The surface tension was observed to linearly decrease owing to the presence of detergent monomers until the concentration of detergent reached the CMC (Fig. 1). Above the CMC, increasing the detergent concentration does not change the surface tension owing to the added detergent molecules being incorporated into micelles. The CMC measured for a variety of detergents (Table 1) was found to agree with published values (Michel, 1991). The CMC was also determined in the presence of 3% heptanetriol. For lauryl dimethylamine oxide, the data indicate that the CMC may have increased owing to the presence of the heptanetriol, although the change was within the error of the measurement. For the other detergents, the CMC either did not change or slightly increased owing to the heptanetriol. Heptanetriol alone does not exhibit a CMC owing to its small size, with the surface tension simply decreasing with increasing heptanetriol concentration.

2.2. Effect of heptanetriol and polyethylene glycol on the cloud-point temperature

Detergents can exhibit complex phase behavior, with one striking example being clouding phenomena. The distinct turbidity seen at the cloud point of a detergent solution is generally a consequence of the separation of the detergent from the aqueous solution (Laughlin, 1994). Systems with a lower consolute boundary will separate into an aqueous and detergent region upon heating, whereas phase separation occurs on cooling in systems that possess an upper consolute boundary. The temperature at which the phase boundary occurs is the cloud-point temperature. Of the detergents measured, only octyl tetraoxyethylene exhibited a clouding behavior in water, while the other detergents remained clear throughout the entire measured range 277–363 K (Table 2). Octyl tetraoxyethylene had a lower consolute boundary and was clear below 323 K in the presence of heptanetriol, compared with a cloud-point temperature of 313 K measured for the detergent alone. The addition of heptanetriol did not affect the behavior of the other detergent solutions in water.

Conversely, all of the detergents underwent a clouding process when polyethylene glycol was present, as has been previously noted for octyl glucoside and octyl tetraoxyethylene (Zulauf, 1991). In all cases, the addition of heptanetriol changed the cloud-point temperature. Several of the detergents, including octyl glucoside, showed an upper consolute boundary, with the solution being cloudy from 277 K until the cloud-point temperature was reached. For these solutions, the addition of heptanetriol decreased the cloud-point temperature. In contrast, octyl tetraoxyethylene had a lower consolute boundary, with the solution being cloudy above the cloud-point temperature, which was increased in the presence of heptanetriol. The largest changes were seen for lauryl dimethylamine oxide, for which the cloud-point temperature decreased from 305 to 281 K upon the addition of heptanetriol. The value of the cloud-point temperature of the lower consolute boundary was dependent on the concentration of lauryl dimethylamine oxide, with values ranging from 293 to 281 K as the concentration was extended from 7.5 to 10% lauryl dimethylamine oxide with both 10% polyethylene glycol 4000 and 3% heptanetriol present in the solutions.

3. Discussion

The initial choice of detergent concentration for solutions containing integral membrane proteins is usually the CMC of the detergent. This value was not affected by the presence of heptanetriol, indicating that alteration of the detergent concentration in crystallization solutions containing amphiphiles such as heptanetriol is not necessary. Other observations have also indicated that the formation of micelles does not change owing to the presence of heptanetriol. For example, neutron-scattering measurements reveal only a slight change in the size of micelles of lauryl dimethylamine oxide upon addition of 3% heptanetriol (Timmins *et al.*, 1991; Thiyagarajan & Tiede, 1994), although octyl glucoside micelles have a different sensitivity to heptanetriol than micelles of lauryl dimethylamine oxide. Thus, both the CMC and neutron-scattering measurements are consistent with the conclusion that the composition of the micelle is not significantly altered by the addition of heptanetriol.

In contrast to the CMC, the cloud-point temperature changed significantly when heptanetriol was present. The difference between the effects of the amphiphile on the CMC and the cloud point of the detergents reflects the differences in the level of organization of the detergent at these points. The CMC is primarily a measure of the interaction between detergent monomers, with the formation of micelles driven by detergent interactions with water. It can be inferred that the amphiphile does not affect interactions at the level of the detergent monomers. The cloud point represents conditions under which detergent micelles aggregate owing to attractive interactions among micelles. The effect of the amphiphile on the cloud-point temperature suggests that the interaction of the surface of the micelle with water changes as a result of the amphiphile addition.

Integral membrane proteins have a large amount of bound detergent and interactions involving both amino-acid residues and the bound detergent will contribute to the crystallization. The cloud point is a parameter that is directly related to interactions between detergent micelles and presumably between protein–detergent complexes and it is these interactions that appear to be altered by the addition of amphiphiles. Although measurements of clouding can only be made at much higher detergent concentrations than are used in crystallization solutions, conditions that approach the consolute boundary are favorable for crystallization (Garavito *et al.*, 1996). The correlation is physically relevant because the interactions measured by the bulk properties at high concentration are related to the local interactions in the small fraction of the protein–detergent complexes that drive nucleation of crystals.

Ideally, the crystallization solution should be poised so that it is near the cloud point at room temperature without undergoing phase separation. Both the direction and the extent of change of the cloud-point temperature owing to the amphiphile will affect its usefulness in attaining these conditions. For example, phase separation is often a problem for crystallization solutions containing octyl glucoside and polyethylene glycol. The addition of heptanetriol lowers the cloud-point temperature so that phase separation is no longer observed at room temperature, although the solution remains near the consolute boundary. Similarly, crystallization may be favored by the significant decrease of the boundary for

Table 1
CMC (m*M*) for various detergents.

Detergent	Without heptanetriol	With 3% heptanetriol
Dodecyl maltoside	0.3 ± 0.2	0.4 ± 0.2
Decanoyl glucamide	7 ± 1	8 ± 1
Octyl glucoside	20 ± 10	20 ± 10
Lauryl dimethylamine oxide	1.5 ± 0.5	2.0 ± 0.5
Octyl tetraoxyethylene	7 ± 1	8 ± 1

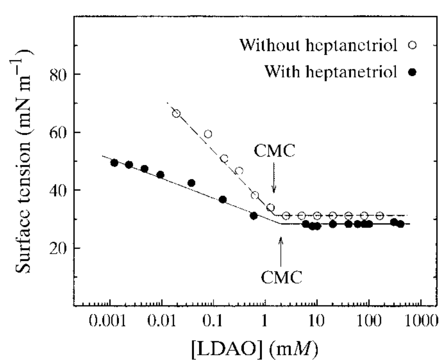

Figure 1
The surface tension of a solution as a function of the concentration of the detergent lauryl dimethylamine oxide, LDAO. At each detergent concentration, the surface tension was determined by measuring the height that the solution rose up a vertical microcapillary tube of inner radius 0.3 mm. Without the amphiphile heptanetriol (open circles), the surface tension steadily decreased from 72 mN m^{-1}, the surface tension for pure water, until a detergent concentration of 1.5 ± 0.5 m*M* was reached, which represents the CMC value. When heptanetriol was present (closed circles) a similar pattern was observed, with the surface tension having a lower value of 50 mN m^{-1} at low detergent concentration owing to the presence of the amphiphile.

Table 2
Cloud-point temperature (K) for various detergents.

The cloud-point temperature is the temperature at which the solutions become turbid. Measurements were made at temperatures from 277 to 363 K and the range for which the solution was cloudy is indicated. The cloud-point temperature was measured as described in Laughlin (1994). A continuously stirred detergent solution was immersed in a water bath that was either cooled or heated. The temperature of the bath was adjusted at ~1 K min^{-1} to ensure thermal equilibrium between the detergent solution and bath. When clouding was visually noted, the temperature was repeatedly increased and decreased slowly around this temperature to accurately determine the cloud-point temperature. The nature of the detergent phase was not determined in these measurements. Detergent concentration was 10% except for decanoyl glucamide (3%), although altering the detergent concentration did not change the trends observed. Estimated error is ±2 K. The values obtained for octyl glucoside and octyl tetraoxyethylene agree with previous measurements (Zulauf, 1991).

	Without polyethylene glycol		With 10% polyethylene glycol	
Detergent	Without heptanetriol	With 3% heptanetriol	Without heptanetriol	With 3% heptanetriol
Dodecyl maltoside	None	None	≤341	≤327
Decanoyl glucamide	None	None	≤313	≤290
Octyl glucoside	None	None	≤295	≤285
Lauryl dimethylamine oxide	None	None	305–323	281–333
Octyl tetraoxyethylene	≥313	≥323	≥292	≥306

lauryl dimethylamine oxide in polyethylene glycol from a relatively high cloud-point temperature owing to the addition of heptanetriol. The effects of heptanetriol on decreasing the cloud-point temperature of dodecyl maltoside and decanoyl glucamide suggest that this amphiphile would have a limited benefit in preventing phase separation for a crystallization solution containing these detergents at room temperature. In contrast, the addition of heptanetriol to octyl tetraoxyethylene would move the cloud-point temperature of the solution further away from the temperature used for crystallization. Although the cloud-point temperature is influenced by many different parameters and the actual value of the cloud-point temperature will depend on the concentrations used in the crystallization solutions, these results are consistent with heptanetriol being generally useful in crystallization in combination with the detergents octyl glucoside and lauryl dimethylamine oxide.

The interactions that drive the clouding probably contribute to the formation of crystals, but the crystallization process of membrane proteins is complex and dependent upon many aspects. For example, heptanetriol has a tremendous impact on the solubility of the protein (Rosenow *et al.*, 2001). Detergent properties are also critical and since the properties in polyethylene glycol were unique for each detergent, the use of the same set of precipitation conditions for every detergent is not efficient. Furthermore, the addition of amphiphiles such as heptanetriol will alter the most favored conditions to different extents for each detergent. While amphiphiles are not necessarily required for crystallization, they can improve the range of conditions that may be successful for crystallization.

These results indicate that one of the reasons amphiphiles are useful in crystallization is that they influence the physical properties of the detergent micelles, in particular the cloud-point temperature. The ability of amphiphiles to modulate the consolute boundary provides a mechanism for how amphiphiles such as heptanetriol influence the crystallization of certain membrane proteins. In general, amphiphiles that can poise attractive interactions between micelle complexes should be favored for crystallization. The effect of amphiphiles on the cloud-point temperature of detergent solutions with polyethylene glycol thus serves as an indicator of their role in crystallization solutions.

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