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The influence of detergents and amphiphiles on the solubility of the light-harvesting I complex

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The effect of detergents and amphiphiles on protein solubility and their use in crystallization solutions was examined for an integral membrane protein, the light-harvesting I complex from *Rhodospirillum centenum*. Measurement by a centrifugation assay of the solubility of the protein in different detergents and amphiphiles showed high protein-solubility values when either octyl glucoside or lauryldimethylamine-*N*-oxide was present with heptanetriol or when deoxycholate was present with spermine. The detergent/amphiphile combinations that resulted in high protein solubility were shown to be successful for crystallization of the protein, suggesting that crystallization is favored for detergents and amphiphiles that optimize the solubility of integral membrane proteins. The amphiphiles effective for crystallization were found using laser mass spectrometry to displace the lauryldimethylamine-*N*-oxide bound to the protein. These results suggest that mass spectrometry can be used for screening of favorable crystallization conditions.

1. Introduction

Protein solubility is a key parameter in the crystallization process. To achieve crystals, the concentration of a protein is initially poised near the solubility limit. The solubility of the protein is gradually decreased by inclusion of salts or polyethylene glycol which are concentrated by a technique such as vapor diffusion (McPherson, 1999). The decrease in solubility causes the protein to either precipitate as disordered aggregates or to form crystalline arrays. For membrane proteins, solubilization requires the use of detergents that replace the lipid molecules surrounding the protein in the cell membrane. The presence of detergents adds to the complexity of the crystallization solutions and increases the number of conditions that must be investigated. In addition, detergents are often supplemented by the inclusion of small amphiphiles that were originally introduced for the crystallization of the photosynthetic reaction center from *Rhodopseudomonas viridis* (later renamed *Blastochloris viridis*) (Michel, 1983). The mechanism by which amphiphiles act has not been experimentally established. It has been proposed that amphiphiles associate with detergents on the surface of a membrane protein to form a complex that allows the protein to pack better in a crystalline array (Michel, 1983). The addition of the amphiphile heptanetriol has been found to reduce the radius of the micelles and to decrease the amount of the detergent lauryldimethylamine-*N*-oxide (LDAO) bound to the reaction center (Timmins *et al.*, 1991; Thiyagarajan & Tiede, 1994; Gast *et al.*, 1994, 1996).

In photosynthetic bacteria, the light-harvesting I complex is an integral membrane protein that captures light and transfers the energy to the bacterial reaction center where the primary photochemistry occurs (Blankenship *et al.*, 1995). The functional properties of the complex have been extensively characterized by optical spectroscopy, but the structure has not yet been determined. The three-dimensional structure of a related complex, the light-harvesting II complex, shows a large open ring formed by eight or nine pairs of two subunits, α and β, that are related by a central rotation axis (McDermott *et al.*, 1995; Koepke *et al.*, 1996). The structure of the light-harvesting I complex has been modeled as a larger ring composed of 16 pairs of the two α- and β-subunits, but projections of the structure in the membrane plane using electron microscopy reveal that the complex forms an irregular ring that does not have the predicted rotational symmetry (Karrasch *et al.*, 1995; Cogdell *et al.*, 1999).

In this report, we describe a systematic study of the effects of detergents and amphiphiles on the solubility of the light-harvesting I complex from *Rhodospirillum centenum*. This organism was chosen because the light-harvesting complex I is abundantly expressed and the purification is simplified by the lack of a light-harvesting II complex in the organism (Yildiz *et al.*, 1991, 1992). Both ionic and non-ionic detergents were investigated in combination with three different amphiphiles: heptanetriol, benzamidine and spermine. The solubility was determined using a centrifugation assay that had been previously developed using the bacterial reaction center (Rosenow *et al.*, 2002). For the bacterial reaction center, it had been found that the detergent/amphiphile combinations that yielded the highest protein solubility were also those combinations that resulted in large protein crystals. To determine if the protein solubility was also correlated with favorable crystallization conditions for the light-harvesting I complex, crystallization trials were performed. In order to better understand the mechanism by which amphiphiles alter the crystallization process, the binding of the different amphiphiles to the light-harvesting I complex was characterized using laser mass spectrometry.

2. Materials and methods

2.1. Sample preparation

Light-harvesting I complex was isolated from *R. centenum* grown anaerobically under illuminated conditions in sealed 4 l glass containers at 310 K for 5 d. The growth medium, a vitamin B_{12} modified CENMED (Favinger *et al.*, 1989), contained 20 m*M* phosphate buffer pH 6.8, 0.1% EDTA and 0.04 mg vitamin B_{12}, 5 g yeast extract, 6 g casamino acids, 5 ml concentrated base and 7.5 ml vitamin solution per litre. The concentrated base contained 7.1 g tetrasodium EDTA, 1.0 g $ZnSO_4{\cdot}7H_2O$, 0.04 g $CuSO_4{\cdot}5H_2O$, 0.02 g $NaB_4O_7{\cdot}10H_2O$, 0.03 g $CoCl_2{\cdot}6H_2O$, 2.5 g nitrilotriacetic acid, 0.02 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 6.9 g $Ca(NO_3)_2{\cdot}4H_2O$, 40 g $MgSO_4{\cdot}7H_2O$ and 0.75 g $FeSO_4{\cdot}7H_2O$ adjusted to pH 7.0 per litre. The vitamin solution contained 100 mg nicotinic acid, 50 mg thiamine·HCl and 2 mg biotin per litre.

Harvested cells were resuspended in 10 m*M* Tris–HCl pH 8.0 and lysed using a French Press. The broken cell solution was supplemented with 0.1 m*M* $CaNO_3$, 0.1 m*M* $MgNO_3$, 0.1 *M* NaCl, 1 m*M* EDTA and 0.4% LDAO and centrifuged at 3500*g* for 10 min at 277 K to remove cell debris and any unlysed cells. Membranes in the supernatant contained the light-harvesting I complex and were separated from the water-soluble portion by centrifugation at 138 000*g* for 90 min at 277 K. The pellet was resuspended in 20 m*M* Tris–HCl pH 8.0, 1.0 m*M* EDTA (TE buffer) containing 0.4% LDAO and centrifuged at 138 000*g* for 90 min at 277 K. The light-harvesting I complex was solubilized from the membrane by resuspending the pellet in TE buffer with 1.0% LDAO for 1 h at 277 K and centrifuging at 138 000*g* for 2 h at 277 K. The supernatant was diluted to a LDAO concentration of 0.3% and purified using ion-exchange (DEAE) chromatography. The light-harvesting I complex bound to the column was washed with 20 m*M* Tris–HCl pH 8.0, 1.0 m*M* EDTA and 0.025% LDAO (TLE buffer) with 20 m*M* NaCl and eluted using TLE buffer with 200 m*M* NaCl. The purest fractions of the light-harvesting I complex, determined by an A_{280}/A_{873} ratio of 0.3–0.4, were collected and dialyzed against TLE buffer to remove the salt. The protein concentration was determined from the optical spectrum using an extinction coefficient of ε_{875} = 125 mM^{-1} cm^{-1} (Cogdell, 1986). To exchange the detergent, the protein was bound to a DEAE column, washed in buffer containing the new detergent and then eluted with salt and dialyzed. The most pure grades of detergents and amphiphiles were purchased from the following sources: LDAO (Fluka), octyl glucoside (Calbiochem), dodecyl maltoside (Calbiochem), Triton X-100 (Fluka), deoxycholate (Calbiochem), 1,2,3-heptanetriol (Fluka), benzamidine (Fluka) and spermine (Sigma).

2.2. Determination of solubility

To determine the protein solubility, a high-speed centrifugation assay was used as previously described (Rosenow *et al.*, 2002). For each condition, solutions were prepared with protein concentrations ranging from very low to saturating values. The solutions were centrifuged in a micro-airfuge (Beckman) at 100 000*g* for 60 min and the concentration of the protein remaining in solution after centrifugation was measured. At low protein concentrations, the protein concentration of the supernatant was essentially equal to the initial protein concentration. As the protein concentration increased, the protein in the supernatant approached the solubility limit of the protein. The protein solubility could be determined in the range 0.1–80 mg ml^{-1}. The accuracy was approximately ±5%, except for solutions in which the solubility was low (0.1–2 mg ml^{-1}), where the accuracy was approximately 20%.

Table 1
Solubility of the light-harvesting I complex in different detergents and amphiphiles.

	Solubility† (mg ml^{-1})			
Detergent	No amphiphile	Heptanetriol	Benzamidine	Spermine
Octyl glucoside	27.0	45.0‡	32.0	31.0
LDAO	<0.1	36.0‡	4.8	1.5
Deoxycholate	5.0	11.0	10.0	15.0‡
Dodecyl maltoside	<0.1	<0.1	<0.1	<0.1
Triton X-100	0.2	2.0	<0.1	<0.1

† Solubility values determined at an ionic strength of 4.5 in ammonium sulfate with detergent concentrations of 0.8% octyl glucoside, 0.025% LDAO, 0.3% deoxycholate, 0.01% dodecyl maltoside and 0.05% Triton X-100 and amphiphile concentrations of 3% 1,2,3-heptanetriol, 3% benzamidine and 50 m*M* spermine. ‡ Conditions successful in the crystallization of the light-harvesting I complex as shown in Fig. 1.

Table 2
Solubility of the light-harvesting I complex in LDAO at different ionic strengths.

	Solubility† (mg ml^{-1})			
Ionic strength	No amphiphile	Heptanetriol	Benzamidine	Spermine
0.3	7.5	12.3	3.4	7.5
0.75	9.6	14.2	5.3	8.2
1.5	11.0	19.3	6.3	10.2
2.25	10.0	20.1	7.3	9.2
3.0	8.0	16.8	5.8	4.7

† Solubility values determined in potassium phosphate with a detergent concentration of 0.025% LDAO and amphiphile concentrations of 3% 1,2,3-heptanetriol, 3% benzamidine and 50 m*M* spermine.

2.3. Measurement of amphiphile binding using mass spectrometry

Matrix-assisted laser desorption ionization (MALDI) spectrometry was performed using a Voyager-DE STR Biospectrometry Workstation (Applied Biosystems, Foster City, CA, USA). This instrument incorporates a nitrogen laser producing pulses of 3 ns duration with a wavelength of 337 nm. Mass spectra were acquired in the positive-ion mode using delayed extraction and the reflectron for improved ion focusing. Detergent and amphiphile samples were prepared by mixing 0.025% LDAO, 3.0% heptanetriol, 0.02% benzamidine or 50 m*M* spermine in a 1:10 ratio with a matrix solution consisting of 4-hydroxybenzylidenemalononitrile as a saturated solution in 50% methanol. Protein samples were prepared by adjusting the protein concentration to 1.5 mg ml^{-1} and adding as needed 3.0% heptanetriol, 0.02% benzamidine or 50 m*M* spermine. Each solution was dialyzed against 4 l of water overnight at 277 K. The protein was not soluble after dialysis but could be suspended in water. The protein solution was centrifuged at 18 000*g* for 5 min at 277 K and the pellet was resuspended in 1 ml of water. The centrifugation and resuspension was repeated twice. The protein suspended in water was then mixed with the matrix solution. For all samples, 1 µl aliquots were dried on a stainless-steel sample plate for measurements.

2.4. Crystallization of light-harvesting I complex

For any chosen combination of detergents and amphiphiles, the light-harvesting complex was tested for crystallization using the precipitation and buffer conditions of Crystal Screens 1 and 2 from Hampton Research at 277 K. Sitting drops with volumes of 5 µl were diluted with an equal volume of the reservoir. The detergent concentrations were fixed at either 0.025% LDAO, 1.0% octyl glucoside or 0.3% deoxycholate. The amphiphiles heptanetriol and benzamidine, when present in the protein solution, were poised at concentrations of 3% and 50 m*M*, respectively. Those conditions that yielded small crystals were adjusted to optimize the size and quality of the crystals.

3. Results

3.1. Light-harvesting I complex solubility in different detergents and amphiphiles

The solubility of the light-harvesting I complex in solutions containing five types of detergents and three amphiphiles was measured at an ionic strength of 4.5 in ammonium sulfate using the centrifugation assay (Table 1). The protein solubility in different solutions was found to have a wide range of values from less than 0.1 to 45 mg ml^{-1}. The highest protein solubility was found for the detergents octyl glucoside, LDAO and deoxycholate. For the detergents dodecyl maltoside and Triton X-100, the highest protein solubility measured was 2.0 mg ml^{-1}. For octyl glucoside, addition of the amphiphile heptanetriol increased the solubility almost twofold, while addition of benzamidine and spermine resulted in only minor increases. For LDAO, addition of heptanetriol significantly increased the solubility to 36 mg ml^{-1}, compared with a value of less than 0.1 mg ml^{-1} for LDAO alone. The addition of benzamidine also resulted in a smaller but clear increase in solubility to 4.8 mg ml^{-1}. In deoxycholate alone the protein solubility was 5 mg ml^{-1}, but with the addition of spermine the solubility increased to 15 mg ml^{-1}, compared with only 10–11 mg ml^{-1} for the addition of heptanetriol or benzamidine. Thus, for each detergent the most soluble conditions were found for combinations of octyl glucoside and heptanetriol, LDAO and heptanetriol, and deoxycholate and spermine.

The protein solubility was also measured in potassium phosphate for the detergent LDAO at ionic strengths ranging from 0.30 to 3.0 (Table 2). Measurements above an ionic strength of 3.0 were not possible owing to the occurrence of phase separation. The measured solubilities were generally higher than those measured using ammonium sulfate; however, the influence of the amphiphiles on the solubility was similar. At every ionic strength, the highest solubility was measured for the combination of LDAO and heptanetriol, with the solubility being approximately twofold greater than the solubility without any amphiphile. The addition of benzamidine decreased the protein solubility and spermine resulted in only minor changes. The dependence was also measured for the detergents octyl glucoside and deoxycholate. For deoxycholate, the solubility was either too high to measure accurately, at values above 100 mg ml^{-1}, or the solutions

underwent phase separation. For octyl glucoside, the solubility exceeded 100 mg ml^{-1} for all ionic strengths less than 4.0. At higher ionic strengths the solubility decreased rapidly with increasing ionic strength, with the protein being most soluble with heptanetriol present. Solubilities above ionic strengths of 5 were difficult to accurately measure owing to the low solubility of the protein in this region. Preliminary measurements for other precipitants such as sodium chloride and polyethylene glycol were also consistent with the same relative dependence of the protein solubility for the different detergent and amphiphile combinations.

3.2. Crystallization of the light-harvesting I complex

Three sets of conditions were identified that optimized protein solubility: LDAO and heptanetriol, octyl glucoside and heptanetriol, and deoxycholate and spermine. Crystallization trials were performed for each of these detergent/amphiphile combinations using precipitant and buffer screens. Each of the three combinations was found to yield crystals of the light-harvesting I complex (Fig. 1). Crystals in the shape of a parallelepiped grew in 0.06% LDAO and 4.0% heptanetriol with a reservoir consisting of 4.0% heptanetriol, 6.0% polyethylene glycol 4000, 35 m*M* NaCl and 50 m*M* Tris–HCl pH 8.5. These crystals grew as thin plates, with the long axis being approximately 0.1 mm. Crystals in the shape of cubes grew using 0.3% deoxycholate and 50 m*M* spermine with a reservoir consisting of 15% polyethylene glycol 4000, 100 m*M* Tris–HCl pH 8.0. The largest crystals grew using 1.0% octyl glucoside and 2.0% heptanetriol with a reservoir consisting of 200 m*M* K_2HPO_4 and 50 m*M* CHES pH 9.8. These crystals grew to a maximal length of 0.2 mm, but the crystals were always aggregated together. In each case crystal formation was usually observed after 5–8 d. Crystallization trials were conducted at 277 K since it had been found that the light-harvesting I complex is unstable in LDAO and octyl glucoside at room temperature. No crystals were observed for crystallization trails performed with other combinations of the detergents octyl glucoside, deoxycholate and LDAO with the amphiphiles heptanetriol, benzamidine and spermine.

3.3. Determination of detergents and amphiphiles bound to the light-harvesting I complex

MALDI spectrometry was used to characterize the detergents and amphiphiles bound to the light-harvesting I complex (Fig. 2). This technique was used to determine the presence of molecules with molecular weights of less than 600 Da. All of the MALDI experiments were performed for the light-harvesting I complex in LDAO. Detergent solutions were made using pure commercial grade LDAO (Fluka); however, the spectra of the detergent alone in water indicate that the detergent is composed of two species: lauryl-dimethylamine-*N*-oxide (LDAO) and myristyl-dimethylamine-*N*-oxide (MDAO). Similar admixtures of different chain lengths were found for solutions of dimethylamine-*N*-oxides with other chain lengths (data not shown). These measurements were not performed for the light-harvesting I complex in non-ionic octyl glucoside or the negatively charged deoxycholate, as the peaks arising from sodium adducts of these two detergents were smaller than the matrix peaks under the experimental conditions.

The pronounced peaks at 230.1 and 258.1 Da arise from LDAO and MDAO monomers and the peaks at 459.2, 487.1 and 515.2 Da are from the three possible dimer forms of the detergents, namely LDAO, LDAO/MDAO and MDAO dimers, respectively. The presence of the dimers in the spectra probably reflects the experimental conditions and does not necessarily imply that the detergents bind to the protein as dimers. To confirm the effectiveness of the washing procedure, spectra were measured of the supernatant after the washing and no detergents were detected. The minor peaks seen in the spectra arise from the matrix.

The addition of heptanetriol to the light-harvesting I complex in LDAO resulted in alterations of the spectra compared with the spectra obtained for the protein with LDAO alone, with a reduction in the LDAO monomer peak

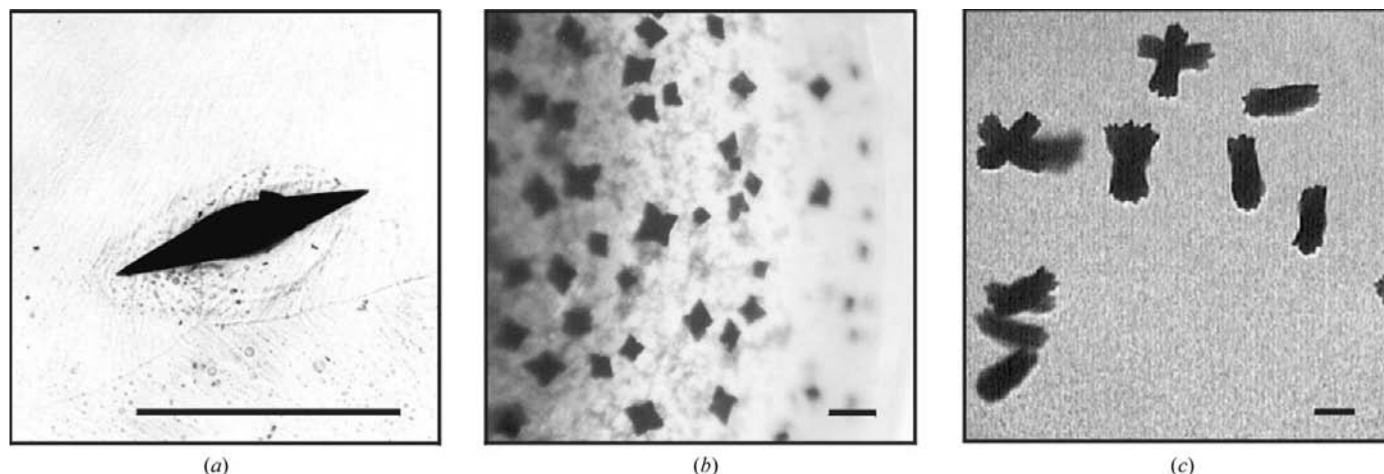

Figure 1
Crystals of the light-harvesting I complex grown using vapor diffusion under different conditions. The crystals were grown in the presence of (*a*) LDAO and heptanetriol, (*b*) deoxycholate and spermine and (*c*) octyl glucoside and heptanetriol. The scale in each panel is 0.1 mm.

amplitude compared with the MDAO monomer peak. A reduction in peak amplitude is also seen for the peaks corresponding to LDAO and LDAO/MDAO dimers. Heptanetriol is a small neutral molecule and did not contribute to the spectra under these conditions. For the spectrum of the protein with benzamidine, a reduction in the peaks corresponding to the LDAO monomer, LDAO dimer, LDAO/MDAO dimer and MDAO dimer are seen relative to the spectra of the protein with LDAO alone. The spectrum obtained after addition of spermine was the same as the spectrum obtained for the protein and LDAO alone within experimental error (data not shown).

For quantitative analysis of the relative amounts of detergents, three samples were prepared for each detergent/amphiphile combination in water and each sample was measured between three and six times. For each spectrum, the peak area was integrated for the LDAO monomer at 230.1 Da and for the MDAO monomer at 258.1 Da and the ratio of these areas was calculated (Table 3). Saturation of individual laser shots was minimized by maintaining the MDAO signal to within the upper 50–80% of the detector range. For spectra of the detergent and amphiphiles in water, the relative areas were found to be always in the range 1.3–1.4. Since the relative area of the LDAO peak was unchanged in all cases within the experimental error of ± 0.1, the results demonstrate that the presence of the amphiphile did not alter the detection of the detergent molecules by changes in the ionization or desorption of the detergent molecules.

Table 3
Relative integrated peak areas of mass spectra.

	Relative peak area† (mg ml^{-1})	
Detergent and amphiphile	In water	Bound to LHI
LDAO	1.3 ± 0.1	0.29 ± 0.09
LDAO and heptanetriol	1.3 ± 0.1	0.06 ± 0.02
LDAO and benzamidine	1.4 ± 0.1	0.03 ± 0.02
LDAO and spermine	1.4 ± 0.1	0.22 ± 0.08

† The values are the ratio of the integrated areas for the LDAO peak at 230.1 Da compared with the MDAO peak at 258.1 Da. The errors are statistical deviations based upon the results from three samples that were each measured three to six times.

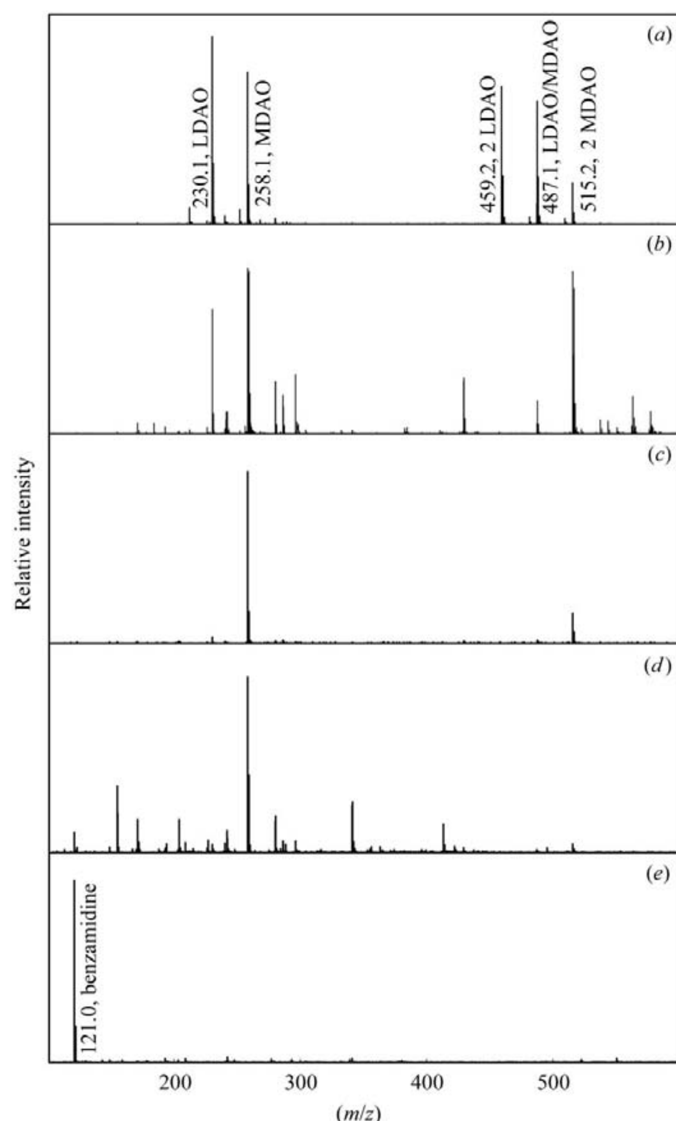

Figure 2
MALDI spectra of (*a*) LDAO, (*b*) the light-harvesting I complex (LHI) with LDAO, (*c*) LHI with LDAO and heptanetriol, (*d*) LHI with LDAO and benzamidine and (*e*) benzamidine. The spectra shown are normalized to the largest peak of the spectrum. The peak for benzamidine arises from its protonated form that has a mass of 121.0 Da.

These measurements were also performed for the protein samples in LDAO with different amphiphiles. The amount of LDAO relative to MDAO was found to be strongly dependent upon the presence of amphiphiles. Without any amphiphile present, the ratio is only 0.29, showing that the relative amount of LDAO bound to the protein is less than expected based upon the amount in solution. The addition of heptanetriol or benzamidine also resulted in much lower ratios of 0.06 and 0.03, respectively, compared with the value of 0.29 for LDAO alone, suggesting that the amphiphiles displace LDAO molecules bound to the protein. For the spectrum of the protein with benzamidine, a peak at 121.0 Da is evident arising from benzamidine, indicating that this amphiphile is bound to the protein. The spectrum obtained after the addition of spermine shows a ratio equal, within experimental error, to the ratio for the protein and LDAO alone, indicating that the addition of spermine does not alter the relative proportion of LDAO to MDAO (spectra not shown).

4. Discussion

The influence of amphiphiles on the physical properties of membrane proteins was investigated. The solubility of an integral membrane protein, the light-harvesting I complex, was measured to range from over 100 mg ml^{-1} to less than 0.1 mg ml^{-1} depending upon the choice of amphiphile, detergent and ionic strength. The displacement of bound detergent molecules was characterized by MALDI experiments. To relate the changes in solubility and detergent binding to crystal formation, crystallization trials were

performed using different detergent/amphiphile combinations. The relationships among the results of these measurements are discussed below.

4.1. Dependence of protein solubility on detergent and amphiphile

The solubility of the light-harvesting I complex was measured for different combinations of detergents and amphiphiles. One of the most useful amphiphiles for crystallization is heptanetriol. The solubility of the protein was enhanced to values of 36.0 and 45.0 mg ml^{-1} owing to the addition of heptanetriol for the detergents LDAO and octyl glucoside, respectively. The other amphiphiles tested, benzamidine and spermine, did not improve the solubility significantly for these detergents. For dodecyl maltoside and Triton X-100 the solubility remained low in the presence of heptanetriol and for deoxycholate the use of the amphiphile spermine rather than heptanetriol resulted in a significant improvement in solubility.

Previously, we have measured the solubility of the bacterial reaction center in the same combinations of detergents and amphiphiles (Rosenow *et al.*, 2002). In that case, the highest solubilities were also found for heptanetriol with octyl glucoside and LDAO. The use of spermine with deoxycholate, but not heptanetriol, resulted in a significant increase in the reaction-center solubility in that detergent. Thus, the same detergent/amphiphile combinations were found to be effective in improving protein solubility. This agreement for the two proteins suggests that heptanetriol is most effective with the detergents octyl glucoside and LDAO and that spermine would be the choice for deoxycholate or cholate.

For LDAO, the addition of heptanetriol and benzamidine, but not spermine, resulted in measurable changes in the amount of the detergent bound as determined by mass spectrometry. MALDI has become an important analytical tool in quantitative analysis of low-mass analytes as well as biomolecules (see, for example, Gobom *et al.*, 2000; Tubbs *et al.*, 2001; Bucknall *et al.*, 2002; Griffin *et al.*, 2003). Known problems associated with this technique included sample heterogeneity, which gives rise to low shot-to-shot reproducibility, signal suppression and nonlinear detector response. By performing many independent measurements of each sample, a reliable estimate of the reproducibility was obtained. The use of internal standards similar in composition to the sample analyte can also be used to compensate for these problems. In a sense, the MDAO can be considered as an internal standard against which the concentration of LDAO was determined. However, because the absolute concentration of MDAO was unknown in these experiments, the absolute concentration of LDAO likewise could not be determined. Making the assumption that the amphiphiles increase the MDAO:LDAO ratio by preferentially displacing bound LDAO, rather than by increasing MDAO binding, one can conclude that the total amount of bound detergent, both LDAO and MDAO, is substantially decreased by the amphiphiles.

The solubility and MALDI results are consistent with a model that amphiphiles are effective when they bind to the protein. In this model, the binding of amphiphiles causes a reduction in the amount of bound detergent and consequently changes the properties of the protein–detergent complex. The fundamental role of detergents in determining the biochemical properties of integral membrane proteins has long been recognized (Helenius & Simons, 1975; Tanford & Reynolds, 1976). The results reported here suggest that one of the primary effects of amphiphiles in crystallization solutions is to improve the solubility of the protein. Crystallization may also depend upon other factors that are dependent upon amphiphiles, such as facilitating specific interactions between proteins. While bridging molecules are often ions, such as the gold compound found at the contact sites in crystals of the MscL channels (Chang *et al.*, 1998), heptanetriol can also serve this role as found in crystals of the light-harvesting II complex from *Rhodospirillum molischianum* (Koepke *et al.*, 1996).

4.2. Impact of detergents and amphiphiles on protein crystallization

A critical role for amphiphiles is probably in improving the protein concentration to above 10 mg ml^{-1}, which is generally associated with the conditions used to crystallize membrane proteins. For the bacterial reaction center it was found that the detergents and detergent/amphiphile combinations that resulted in a high protein solubility were the same combinations that have been successful in growing the three crystal forms of the reaction center from *Rhodobacter sphaeroides* (Allen *et al.*, 1987; Chang *et al.*, 1991; Ermler *et al.*, 1994; Stowell *et al.*, 1997). The light-harvesting I complex has not yet been crystallized in a form suitable for X-ray diffraction. The initial conditions identified in the solubility assays provided the basis for the crystallization trials that yielded three crystal forms. Crystals grown in deoxycholate grew to a maximal length of 0.3 mm and diffracted to a resolution limit of 8 Å. Thin plates were grown in LDAO, but these diffracted X-rays very poorly. The largest crystals were grown in octyl glucoside but formed as clusters of crystals (Fig. 1).

Thus, for two different integral membrane proteins, amphiphiles that enhanced protein solubility were also useful for crystallization. These results indicate that solubility tests can be a highly effective means of identifying conditions that are likely to produce crystals. For the pigment–protein complex under study, the protein concentration could accurately and easily be measured from the optical absorption of the pigments. In general, protein concentrations can also be measured for proteins without pigments by measuring the absorption at 280 nm, assuming that the 280 nm extinction is known and that interfering substances are not absorbing in that region. Alternatively, the concentrations can also be determined by a variety of other methods, such as the Bradford method, Peterson method or the bicinchoninic acid assay.

5. Conclusion

Although many factors have an impact on the crystallization of membrane proteins and crystals can grow from solutions containing low concentrations of protein, the results indicate that crystallization is favored if the protein solubility can be improved by the use of an amphiphile. Thus, solubility tests can be a highly effective means of identifying conditions that favor crystallization. In addition, the usefulness of an amphiphile can be predicted based upon MALDI experiments for amphiphiles that can become charged, typically by protonation or by binding a metal cation. Adjustment of the experimental conditions, such as altering the matrix to produce a charged adduct, can enable measurement of neutral amphiphiles. The use of internal standards, such as isotopically labeled detergents, could help overcome the variable effects of the desorption/ionization process. These results suggest that amphiphile and detergent combinations can be rapidly screened based upon the outcomes of the solubility and binding assays.

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