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Static light scattering studies of OmpF porin: Implications for integral membrane protein crystallization

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Abstract

Integral membrane proteins carry out some of the most important functions of living cells, yet relatively few details are known about their structures. This is due, in large part, to the difficulties associated with preparing membrane protein crystals suitable for X-ray diffraction analysis. Mechanistic studies of membrane protein crystallization may provide insights that will aid in determining future membrane protein structures. Accordingly, the solution behavior of the bacterial outer membrane protein OmpF porin was studied by static light scattering under conditions favorable for crystal growth. The second osmotic virial coefficient (B_{22}) was found to be a predictor of the crystallization behavior of porin, as has previously been found for soluble proteins. Both tetragonal and trigonal porin crystals were found to form only within a narrow window of B_{22} values located at approximately -0.5 to -2×10^{-4} mol mL g^{-2}, which is similar to the "crystallization slot" observed for soluble proteins. The B_{22} behavior of protein-free detergent micelles proved very similar to that of porin-detergent complexes, suggesting that the detergent's contribution dominates the behavior of protein-detergent complexes under crystallizing conditions. This observation implies that, for any given detergent, it may be possible to construct membrane protein crystallization screens of general utility by manipulating the solution properties so as to drive detergent B_{22} values into the crystallization slot. Such screens would limit the screening effort to the detergent systems most likely to yield crystals, thereby minimizing protein requirements and improving productivity.

Keywords: cloud point; membrane protein crystallization; porin; protein-detergent complex; second osmotic virial coefficient; static light scattering

Knowledge of a protein's three-dimensional (3D) structure is critical for any thorough understanding of its function. Consequently, much effort has been devoted to the problem of structure determination, and the number of proteins of known structure has grown explosively in the past several decades. Now that complete genomic sequences are becoming available for many organisms, structural biologists are redoubling their efforts and are crafting structural proteomics initiatives aimed at keeping pace with the flood of sequence information (Terwilliger et al., 1998). However, the explosion in our knowledge of protein structure has not extended to integral membrane proteins. Even though 20–30% of the open reading frames found in a genome are likely to encode membrane-bound proteins (Wallin & von Heijne, 1998), far fewer than 1% of the structures found in the Protein Data Bank represent membrane proteins. This disparity persists despite tremendous interest in membrane protein structure, fueled by the critical biological functions of these molecules and by their importance as drug targets.

The relative lack of information about membrane protein structure can be traced directly to the technical difficulties associated with working with these molecules. Detergents are required to isolate and maintain membrane-bound molecules in stable, water-soluble form; the detergent molecules adsorb onto the hydrophobic faces of the protein, forming micelle-like structures and producing a protein-detergent complex (PDC). A PDC can easily contain as much as 50% detergent by weight. Thus, the PDC formed by any given membrane protein will be substantially larger than a soluble protein of comparable molecular weight. This leads to large tumbling times and complicates the use of NMR for structure determination. X-ray crystallography does not suffer from this size limitation, but has the absolute prerequisite of a large single crystal of the protein under study. The production of protein crystals is extremely complex, and, in the absence of a clear picture of the mechanisms underlying protein crystal growth, investigators are forced to employ arduous, expensive, and risky trial-and-error methods to produce suitable crystals. In the case of integral membrane

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proteins, the presence of detergents greatly increases the complexity of the system and exacerbates these difficulties. As a consequence, the success rate for crystallization of integral membrane proteins is very much lower than that for soluble proteins.

Many mechanistic studies have examined the crystallization of soluble proteins in the hopes of developing a more rational approach to crystallogenesis (McPherson, 1999). Such studies are gradually yielding fundamental information about the factors controlling crystal nucleation and growth. Particularly interesting results have come from examining the behavior of the second osmotic virial coefficient (B_{22}) under crystallization conditions. B_{22} is a measure of the intermolecular forces between two particles in dilute solution (Stigter & Hill, 1959); these, of course, are the same forces which control crystal nucleation and growth. The value of B_{22} for a protein solution has been demonstrated to be related to protein solubility (Guo et al., 1999; Haas et al., 1999). Positive B_{22} values indicate that protein-solvent forces are favored over protein–protein forces, leading to a highly soluble protein. Negative B_{22} values signal that attractive protein–protein interactions are favored over protein–solvent interactions and are indicative of reduced solubility and crystal formation and growth.

B_{22} can be obtained from static light scattering (SLS) measurements conducted on subsaturated solutions. Wilson and colleagues have demonstrated that B_{22} measurements are predictive of the crystallization behavior of a diverse set of soluble proteins (George & Wilson, 1994; George et al., 1997). They have shown that when conditions favor crystal growth, B_{22} values invariably lie within a narrow range of slightly negative values, the so-called "crystallization slot." This slot lies between -0.8 and -8.0×10^{-4} mol mL g^{-2}; such B_{22} values correspond to small attractive interactions between protein molecules in solution. Solutions with positive or large negative values do not yield crystals, instead remaining as stable solutions ($B_{22} > 0$) or forming amorphous precipitates ($B_{22} \ll 0$).

We have extended the work of Wilson et al. to integral membrane proteins to test whether a crystallization slot exists for PDCs. Specifically, using the bacterial outer membrane protein OmpF porin as a model system, we have used static light scattering to quantify the B_{22} values of protein-free detergent micelles and PDCs under crystallizing and noncrystallizing conditions. Our results suggest that a crystallization slot may indeed exist for PDCs and that it is approximately the same as that described by Wilson and colleagues. Hence, the use of B_{22} as a predictor of crystallization may be of general utility, valid for integral membrane proteins as well as for soluble proteins. In addition, our results suggest that the detergent moiety contributes significantly to the net forces between PDCs. We anticipate that quantitative information about the molecular interactions between detergent micelles and PDCs will be useful in designing more successful crystallization experiments for integral membrane proteins.

Results

Crystal growth

To delineate the precise conditions where crystals form, and as a control to ensure that the buffers and protein preparations used were of appropriate quality, crystallization experiments were carried out in parallel with the light scattering studies. Large single crystals were observed in both tetragonal and trigonal crystallization conditions, in a range of PEG concentrations that in each case was in good agreement with published values (Garavito & Rosenbusch, 1986; Pauptit et al., 1991). Tetragonal crystals were found to form at PEG concentrations of 12% and higher, while trigonal crystals formed at PEG concentrations of 9% and above. Some typical crystals are shown in Figure 1.

Light scattering: Protein-free micelles

Since the molecular size of the particles under study (micelles and PDCs) does not exceed $\lambda/20$, the excess scattered intensity is expected to be independent of scattering angle. The lack of angular dependence allows for the use of the Debye analysis of the SLS results (Kratochvil, 1987):

$$\frac{K_c}{R_{90}} = \frac{1}{Mw} + 2B_{22}c \tag{1}$$

where

$$K = \frac{4\pi^2 n_0^2 (dn/dc)^2}{N_A \lambda^4}; \tag{2}$$

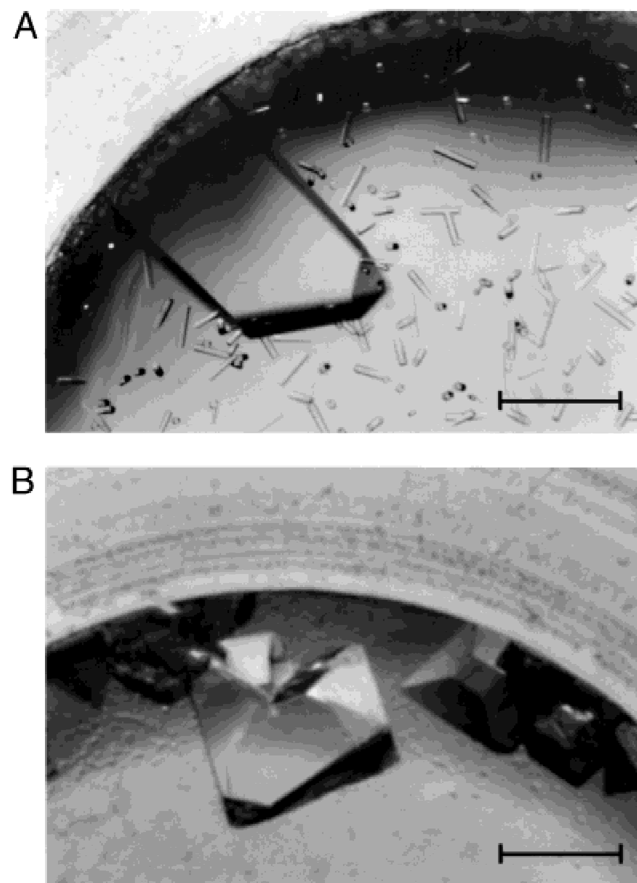

Fig. 1. Crystals of OmpF porin grown in this study. (**A**) Trigonal crystals; (**B**) tetragonal crystals. The curved surfaces in both panels are the walls of the microdialysis chamber. Scale bars correspond to ~0.25 mm.

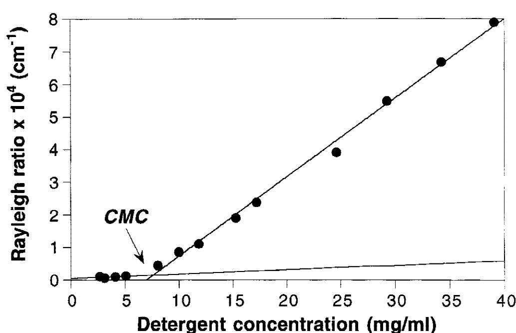

Fig. 2. Light scattering observed from a solution containing only PEG and detergent. The Rayleigh ratio is shown as a function of detergent concentration in a buffer containing 2% PEG. The detergent used is a mixture of n-octyl-2-hydroxyethylsulfoxide and octyl POE, corresponding to the trigonal crystallization condition for OmpF. Note the sharp break at roughly 7.5 mg/mL of detergent indicative of the critical micelle concentration (CMC). Error bars are typically smaller than symbol size.

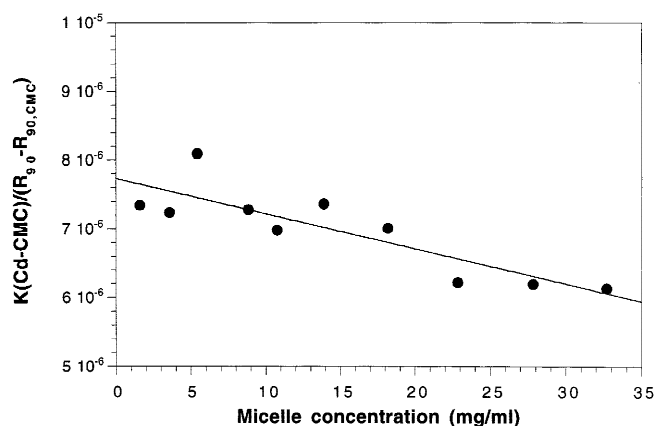

Fig. 3. Representative Debye plot for protein-free detergent micelles in trigonal buffer containing 2 (w/w)% PEG. Micelle concentration is obtained by subtracting the CMC from the total detergent concentration. B_{22} and the micelle molecular weight are obtained from the slope of the line and the y intercept, respectively (see Equation 3).

c is the concentration of the of the scattering species (g/mL) in solution; R_{90} is the excess Rayleigh ratio at 90°; dn/dc is the specific refractive index; N_A is Avogadro's number; λ is the wavelength of the laser light source; n_0 is the refractive index of the solvent; Mw is the weight average molecular weight of the scattering species; and B_{22} is the second osmotic virial coefficient of the scattering species.

The concentration of the scattering species was determined differently for protein-free detergent micelles vs. PDCs. For protein-free detergent micelles, the exact concentration of detergent was determined gravimetrically. The concentration of micelles (scattering species) was then assumed to be the detergent concentration in excess of the critical micelle concentration (CMC). The CMC was measured by light scattering in a manner similar that described by Kameyama and Takagi (1990); typical results are shown in Figure 2. The characteristic sharp break in the plot of Rayleigh ratio vs. detergent concentration is indicative of the formation of detergent micelles, and thus identifies the CMC. Since micelles are the scattering object of interest in these studies, only concentration in excess of the CMC is utilized to calculate the light scattering properties for micelles. Thus, Equation 1 becomes modified to the following form:

$$\frac{K(Cd-\mathrm{CMC})}{(\mathrm{R}_{90}-\mathrm{R}_{90,\mathrm{CMC}})} = \frac{1}{Mw} + 2B_{22}(Cd-\mathrm{CMC}) \quad (3)$$

where Cd is the total concentration of the detergent and $R_{90,\mathrm{CMC}}$ is the Rayleigh ratio of the solution at the CMC. Once an estimate of the CMC is available, light scattering is measured at various concentrations of the scattering species. A typical Debye plot of the micellar data is shown in Figure 3. Equation 3 then yields an estimate of the micellar molecular weight and the second virial coefficient (B_{22}).

These parameters were measured as a function of precipitant concentration, and the results are shown in Table 1 (tetragonal crystallization buffer) and Table 2 (trigonal crystallization buffer) along with corresponding values for the specific refractive index (dn/dc). For these protein-free detergent solutions, sufficient sample was available to measure dn/dc at all PEG concentrations.

The general trend in the tetragonal data (Table 1) suggests that the CMC increases slightly and micelle molecular weight decreases as the PEG concentration increases in the system. The

Table 1. *Effect of PEG concentration on protein-free detergent micelles in tetragonal crystallization buffer*

PEG (w/w%)	dn/dc (g/mL)$^{-1}$	CMC (mg/mL)	Mw (Da)	B_{22} (mL mol/g^2)
0	0.144 ± 0.002	5.9 ± 1.3	41,800 ± 700	−5.9E−05 ± 1.1E−05
2	0.126 ± 0.002	6.4 ± 1.3	52,200 ± 3,700	−3.2E−05 ± 3.4E−05
4	0.132 ± 0.003	7.4 ± 1.3	41,300 ± 2,200	−4.1E−05 ± 3.1E−05
6	0.112 ± 0.002	6.1 ± 1.3	58,900 ± 900	−8.8E−05 ± 6.6E−06
8	0.129 ± 0.002	8.7 ± 1.3	40,300 ± 1,600	−1.3E−04 ± 2.6E−05
10	0.119 ± 0.002	6.9 ± 1.3	44,200 ± 1,600	−1.5E−04 ± 2.3E−05
12	0.123 ± 0.003	7.9 ± 1.3	29,100 ± 1,400	−2.2E−04 ± 3.6E−05
14	0.113 ± 0.002	9.1 ± 1.3	32,800 ± 800	−2.7E−04 ± 2.3E−05

Table 2. *Effect of PEG concentration on protein-free detergent micelles in trigonal crystallization buffer*

PEG (w/w%)	dn/dc (g/mL)$^{-1}$	CMC (mg/mL)	Mw (Da)	B_{22} (mL mol/g^2)
0	0.139 ± 0.002	7.5 ± 0.9	131,000 ± 1,300	1.4E−05 ± 1.7E−06
2	0.134 ± 0.003	6.4 ± 0.9	129,000 ± 3,300	−2.6E−05 ± 5.4E−06
4	0.125 ± 0.003	6.3 ± 0.9	106,000 ± 6,500	−4.8E−05 ± 1.5E−05
6	0.135 ± 0.002	7.9 ± 0.9	98,000 ± 5,000	−4.3E−05 ± 1.2E−05
8	0.132 ± 0.004	5.7 ± 0.9	85,000 ± 5,100	−1.0E−04 ± 1.7E−05
10	0.125 ± 0.002	7.5 ± 0.9	87,000 ± 3,900	−9.6E−05 ± 1.5E−05

zig-zag trend seen for the micelle molecular weight suggests that errors may be underestimated for these samples; error estimates are derived from the slope estimates of a given Debye plot and do not account for the minor (but additive) errors introduced for each new solution condition (e.g., slight variations in buffer constituent concentrations). Overall, however, these results are consistent with observations made by Thiyagarajan and Tiede (1994). They found, as is seen here, that the buffer solutions without PEG produced aggregated or extended micelle structures of relatively high molecular weight. As the PEG was added to the system, the detergent micelles dissociated, producing small noninteracting micelles conducive for the crystallization of membrane proteins. The molecular weights obtained at the relatively high PEG loadings are consistent with reports that the molecular weight of β-octyl glucoside (BOG) micelles in water ranges from 22–28 kDa depending on temperature (Kameyama & Takagi, 1990). The second virial coefficient shows an essentially noninteracting system ($B_{22} \sim 0$) at low PEG concentrations, with interactions becoming more attractive as PEG is added to the system. George et al. (1997) have shown that the ideal interaction range lies between -0.8 and -8×10^{-4} mol mL g^{-2} for soluble proteins. It is noteworthy that only in the presence of PEG do the B_{22} values for protein-free detergent micelles enter this range.

The data for the trigonal buffer system (Table 2) shows similar trends, although the absolute values for micelle molecular weight are substantially larger, reflecting the different properties of the detergent system and a possibly anisotropic shape. The second virial coefficient again reflects a change from an essentially noninteracting system to slightly attractive system as the concentration of PEG is increased. The virial coefficients of protein-free micelles in both the tetragonal and trigonal systems are in the range of -0.5 to -2×10^{-4} mL mol g^{-2} under crystallization conditions, which lies within the crystallization slot observed by George and Wilson (1994).

Light scattering: Protein-detergent complexes

The concentration of PDCs was monitored spectroscopically. However, this measurement provides the concentration of the protein only, not that of the combined protein-detergent complex. Defining the complexed detergent loading (δ) as the weight of detergent per weight of protein, the concentration of the complex can be calculated from the spectroscopically determined protein concentration:

$$c = (1 + \delta)c_p \qquad (4)$$

where c is the concentration of the scattering species (PDC) and c_p is the protein concentration as measured by ultraviolet (UV) spectroscopy. The value for δ can be estimated from the specific refractive index of the neat micellar solution and the complex as outlined by Hayashi et al. (1989) based on the specific refractive index for the protein as well as the protein-free detergent micelles.

Unlike the experiments on protein-free detergent solutions, the PDC light scattering experiments require a constant level of detergent and varying protein concentration. To assure that the background solvent structure was truly matched for all solutions, the samples were brought to dialysis equilibrium with respect to all components (including PEG) except protein. The use of Equations 1 and 4 yields the Debye equation for this system in terms of the protein concentration:

$$\frac{K'c_p}{R_{90}} = \frac{1}{Mw} + 2B_{22}(1+\delta)^2 c_p \qquad (5)$$

where

$$K' = \frac{4\pi^2 n_0^2 (dn/dc_p)^2}{N_A \lambda^4} \qquad (6)$$

which are the same equations utilized by Takagi et al. (1980). Mw in this case is the molecular weight of the porin alone; this provides a good check on the results since the molecular weight of the porin trimer is known to be 111 kDa (Inokuchi et al., 1982). A Debye plot (Equation 5) for porin utilizing the trigonal buffer at varying PEG concentrations is shown in Figure 4. The slopes of these lines provide estimates of B_{22} and the intercepts provide estimates of the molecular weight via Equation 5.

The dn/dc values for solutions containing the protein–detergent complex were measured in the same manner as for the detergent-only systems. These measurements require several hundred microliters of sample which is unrecoverable. To conserve precious protein sample, for some experiments dn/dc was not measured but rather adjusted until the proper molecular weight was obtained for the porin. In cases where dn/dc values were both measured experimentally and calculated from Equation 5, the agreement was good (see Table 3).

The B_{22} data for PDCs in the tetragonal and trigonal crystallization buffers are shown in Figures 5 and 6, respectively. While these two crystallization conditions are different in most respects, the B_{22} values show the same trend in both cases: Initial B_{22} values

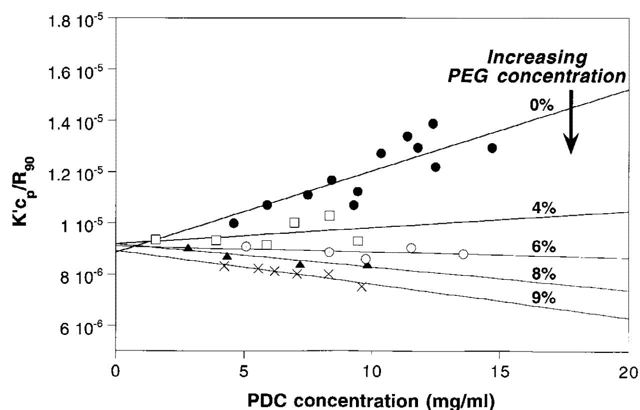

Fig. 4. Debye plot for porin-detergent complexes in trigonal buffer conditions at varying PEG concentrations (0, 4, 6, 8, 9 (w/w)%). The general trend of decreasing B_{22} with increasing PEG concentration is evident.

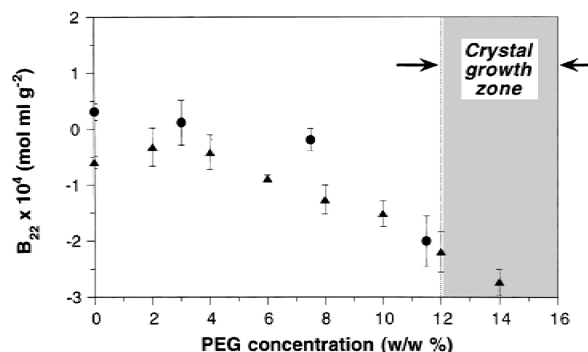

Fig. 5. B_{22} values for both porin-detergent complexes (circles) and protein-free detergent micelles (triangles) at varying PEG concentrations in the tetragonal crystallization buffer. The range of PEG concentrations in which crystals can be grown is shaded.

are positive, and addition of precipitating agent causes them to drop to increasingly negative values. At the highest PEG concentrations studied (which correspond to concentrations that give rise to crystals), B_{22} values for both buffer conditions lie in the range -0.5 to -2.0×10^{-4} mol mL g^{-2}.

Discussion

While PEG is commonly thought of as a precipitating agent for macromolecules such as proteins and nucleic acids, it exerts a similar effect on detergent micelles and protein–detergent complexes. This has been noted previously (Zulauf, 1991), and is clearly demonstrated by the results presented in this paper. The current study makes use of the second virial coefficient, as measured by static light scattering, to quantitate the effects of PEG addition on the interparticle attractive potentials for PDCs and micelles near the crystallization boundary.

It is interesting to consider the results of this study in light of the observation that PDCs can be crystallized near the cloud point of the detergent used (Garavito & Picot, 1990; Rosenbusch, 1990; Zulauf, 1991). The cloud point of a detergent solution (also known as the consolute boundary) represents the phase boundary between a single-phase micellar solution and a two-phase micellar system.The two-phase system (which is turbid, hence the name "cloud point") is comprised of one phase containing almost all detergent micelles, and a second containing detergent at a concentration slightly greater than the CMC. Micelle structure is not thought to change significantly during this transition; the cloud point is therefore essentially a condensation or coalescing of micelles, mediated by attractive micelle–micelle interactions (Zulauf & Rosenbusch, 1983). It has been suggested that as the cloud point is approached, attractive forces between the micellar regions of PDCs become significant well before actual phase separation can be observed, and that these attractive forces are responsible for bringing PDCs into close contact and enabling crystal formation. This is confirmed by the current study that shows that, for both pure detergent micelles and PDCs, the interparticle attractive forces become significantly attractive well before the actual cloud point is reached. For both the tetragonal and trigonal crystal forms of OmpF, no clouding can be observed unless the PEG concentration is raised to levels substantially higher than those that yield large single crystals. This implies that B_{22} values are likely to be more useful for predicting crystallization conditions for PDCs than simple cloud point observations.

Table 3. *Comparison of measured and calculated dn/dc values for protein–detergent complexes in the tetragonal crystallization buffer system*

PEG (w/w%)	dn/dc measured $(g/mL)^{-1}$	dn/dc calculated $(g/mL)^{-1}$
0	0.252	0.252
3	—	0.270
7.5	—	0.242
8.2	0.221	—
9	0.221	—
11	0.209	0.192

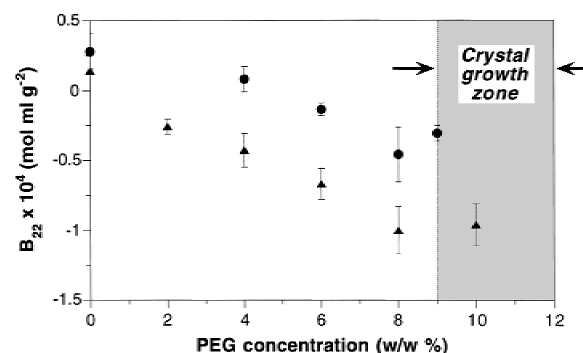

Fig. 6. B_{22} values for both porin-detergent complexes (circles) and protein-free detergent micelles (triangles) at varying PEG concentrations in the trigonal crystallization buffer. The range of PEG concentrations in which crystals can be grown is shaded.

We interpret this observation as meaning that the detergent portions of the PDCs are playing a constructive role in the crystallization process, by making the PDCs slightly "sticky" and thereby allowing them to come together and sample possible lattice packing arrangements. The actual lattice contacts are expected to be formed by protein–protein interactions, since the micellar portions of the PDCs are too unstructured to support well-ordered packing; but the micellar groups play a significant (or even dominant) role in bringing the PDCs together in the first place. Furthermore, analysis of detergent structure in several different PDC crystal structures indicates that micellar groups are brought into close apposition or even fused in the process of crystal formation (Roth et al., 1991; Pebay-Peyroula et al., 1995; Garavito et al., 1996). It stands to reason that micelle–micelle forces must be neutral or attractive to allow this close packing to occur.

Perhaps the most significant result to emerge from this study derives from a comparison of the relative effects of PEG on B_{22} values for PDCs and protein-free detergent micelles (Figs. 5, 6). For tetragonal OmpF crystals, the B_{22} behavior of PDCs near the crystallization point is essentially mimicked by the behavior of the protein-free micelles, suggesting that the dominant forces controlling the attractive potential between PDCs near the crystallization point are micelle–micelle forces. In the case of the trigonal crystals, B_{22} values for PDCs parallel those for the protein-free micelles, but are less negative, suggesting that favorable micelle–micelle attractions may be compensating for unfavorable forces derived from the protein moieties. Hence, the detergent is likely to be a major player in the control of crystal formation for both OmpF crystal forms studied, despite differences in crystal packing, pH, buffer composition, and detergent.

It is instructive to compare the porin results with those obtained by Wilson's group for soluble proteins. They have shown that under crystallizing conditions the B_{22} values for a diverse group of proteins cluster in the so-called crystallization slot (George et al., 1997). In the appropriate crystallization conditions, B_{22} values for both porin-detergent complexes and the corresponding protein-free detergent micelles also fall close to this range. This suggests that the notion of a crystallization slot is not limited to soluble proteins, but is applicable to PDCs as well. Both B_{22} values measured for porin fall at the low end of the crystallization slot (where "low" refers to the absolute value of B_{22}); because of the low sample number, it is not yet clear whether this represents a significant difference between soluble proteins and PDCs.

The observation of slightly negative B_{22} values under crystallizing conditions implies weak, but significant, intermolecular interactions. This may at first appear to be contrary to results reported for the reaction center from *Rhodobacter sphaeroides*, which indicate that the reaction center exists as noninteracting monomers during crystallization (Marone et al., 1998), and indeed that conditions favoring crystallization actually prevent micelle aggregation (Thiyagarajan & Tiede, 1994). However, the reaction center studies were carried out using small-angle neutron scattering, which is not as sensitive as static light scattering to the small interactions of the type we have measured for porin-detergent complexes. In fact, preliminary SLS data for reaction center-detergent complexes suggests that their B_{22} values behave similarly to those for porin (J. Wiencek, unpubl. results).

The results presented in this paper have important implications for the construction of crystallization screens for PDCs. First, we observe that B_{22} values for porin-detergent complexes must fall within the crystallization slot in order for crystals to grow; this is true for two different crystal forms that grow under distinctly different crystallization conditions. If this observation holds true for other membrane proteins, it would argue strongly for the generality of the crystal slot requirement for crystallization and imply that efforts to crystallize novel membrane proteins should focus on placing PDCs into the slot. Second, we have demonstrated that, at least in the porin model system, the detergent moiety contributes significantly to the B_{22} behavior of the PDC. For this system, measurements of B_{22} values from detergent micelles alone would be sufficient to predict appropriate PEG concentrations for crystallization of entire PDCs. If this holds true for other membrane proteins, it may be possible to construct screens that are detergent-specific, but of general utility for different membrane proteins that will minimize the amount of protein required to produce a useful crystal.

Materials and methods

OmpF purification

OmpF porin was purified from the *ompC*$^{-}$ *Escherichia coli* strain MH225, containing the *ompF* overexpressing multicopy plasmid pPR272 (Misra & Reeves, 1987). Cells were grown overnight at 37 °C in 40 L batches of LB containing 50 μg/mL kanamycin, typically yielding 320 g of wet cells. Membrane materials were prepared, ompF porin was extracted, and size exclusion and anion exchange chromatography were performed essentially as described (Kim, 1995). Pooled fractions from the anion exchange steps were concentrated and exchanged into 25 mM methylpiperazine·Cl, pH 5.7, plus 0.5% (v/v) *n*-octyl-polyoxyethylene (octyl-POE; Bachem, King of Prussia, Pennsylvania), using a Filtron Technology OMEGA 50 ultrafiltration cell. The concentrated sample was loaded onto a 4 mL Pharmacia MonoP column at room temperature. Chromatofocusing was carried out using 35 mL of 10% (v/v) polybuffer 74, pH 4.0, 0.5% (v/v) octyl-POE. Porin was found to elute at pH 4.5. This purified material was subjected to a final round of anion exchange chromatography, performed as before. Tetragonal and trigonal porin crystals were prepared as described (Garavito & Rosenbusch, 1986; Pauptit et al., 1991). The porin concentration in solution was determined by UV absorbance using an extinction coefficient of 1.41 (276 nm, 0.1%) (Rosenbusch, 1974). Protein composition and purity were analyzed by SDS-PAGE. Typical yields of highly purified porin were one to two milligrams per liter of cell culture.

Preparation of crystallization buffers

Porin is known to crystallize in several different crystal forms. In this paper, conditions used to prepare the tetragonal (spacegroup $P4_2$) crystals are referred to as the tetragonal buffer (Garavito & Rosenbusch, 1986), and those used to prepare the trigonal crystals (spacegroup $P321$) are referred to as the trigonal buffer (Pauptit et al., 1991). The tetragonal buffer consists of 0.5 M NaCl, 0.1 M sodium phosphate, 1 mM sodium azide, 0.9% (w/w) n-octyl-beta-D-glucoside (BOG; Anatrace, Maumee, Ohio) and 0.09% (w/w) octyl-POE at pH 6.5. Two solutions (0.5 M NaCl, 0.1 M sodium phosphate monobasic and 0.5 M NaCl, 0.1 M sodium phosphate dibasic) were titrated to yield a pH 6.5 stock. After the titration, sodium azide, BOG, octyl-POE were all added at their specified concentrations. Varying amounts (0–13% w/w) of PEG 2000 (Fluka,

Buchs, Switzerland) were added to this buffer. The trigonal buffer system consisted of 0.05 M Tris base, pH 9.8, containing 0.6% (w/w) n-octyl-2-hydroxyethylsulfoxide (Bachem) and 0.1% w/w octyl-POE, as well as 0.7 M $MgCl_2 \cdot 6H_2O$. Varying amounts (0–10% w/w) of PEG 2000 were added to this buffer. In the case of protein-free detergent micelle solutions, when preparing solutions of varying detergent concentration the weight ratio of the two surfactants was held constant while varying the total surfactant loading from 0 to 40 mg/mL.

Crystal growth

All crystals were grown using microdialysis at room temperature (~22 °C), using 10 μL dialysis buttons (Hampton Research, Laguna Niguel, California) and Spectra/Por 12–14 kDa cutoff RC dialysis tubing. Tetragonal crystals were prepared by concentrating purified porin to a concentration of ~15 mg/mL. The concentrated sample was dialyzed overnight against two changes of tetragonal crystal buffer. This was diluted to 10 mg/mL with dialysate and concentrated PEG 2000 (60% w/v in water) was added to a final concentration of 7.5% (w/v). Microdialysis was carried out against a series of 3 mL reservoirs of tetragonal crystal buffer containing 11–15.5% PEG 2000 (w/w). Crystals formed within one to two weeks at PEG concentrations ≥12%. Trigonal crystals were prepared by exchanging purified porin into the trigonal crystal buffer by repeated dilution and ultrafiltration. The sample was then concentrated and dialyzed overnight against two changes of trigonal crystal buffer containing 6% (w/w) PEG 2000. The concentration was adjusted to 10 mg/mL by addition of dialysate, and microdialysis was carried out using reservoirs of trigonal crystal buffer with 7–11.5% PEG 2000 (w/w). Crystals formed within three weeks at PEG concentrations ≥9%.

Equilibrium dialysis of protein solutions

Static light scattering methodology requires the analysis of a series of protein samples that have different concentrations, but are in osmotic equilibrium with one another. This was achieved via dialysis. The purified porin samples were divided into 500 μL aliquots of varying protein concentration. Each sample was spiked with polyethylene glycol (PEG 2000) to yield a final (w/w%) concentration ranging from 0–14% PEG; these samples were then dialyzed against a buffer with the desired PEG concentration. The buffer was exchanged daily. It was found that dialysis in the presence of detergent required surprisingly long times to reach equilibrium. For example, 1–2 weeks were required when using a 50k MWCO Spectra/Por (RC) membrane. However, the dialysis time was decreased to 2–3 days by dialyzing with 100k MWCO Spectra/Por (CE) membrane. Times required to reach equilibrium were determined by monitoring the specific refractive index.

Specific refractive index

The change in refractive index as a function of concentration (*dn/dc*, or specific refractive index) was measured using a Bellingham + Stanley 60/ED Abbe Refractometer (if sufficient volume was available) or the ALV Differential Refractometer (ALV-DR1). Both instruments give identical results to ± 0.01 $(g/mL)^{-1}$. All measurements were conducted at 22 °C.

Static light scattering

All static light scattering experiments were conducted using an ALV Model 5000 Compact DLS/SLS goniometer at 22 °C. The laser light source was a 35 mW He-Ne Uniphase laser operating at 632.8 nm. All measurements were collected at a scattering angle of 90°.

To minimize the corruption of the data by small amounts of dust, samples were filtered using a 0.22 μm centrifugal filter (Millipore Ultra-Free-MC) in an Eppendorf centrifuge at 3,000 RPM for 1 min. Data collection followed the methodology outlined by Farnum (1997).

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