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Characterization of Protein Detergent Complexes by NMR, Light Scattering, and Analytical Ultracentrifugation

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Abstract

Bottlenecks in expression, solubilization, purification and crystallization hamper the structural study of integral membrane proteins (IMPs). Successful crystallization is critically dependent on the purity, stability and oligomeric homogeneity of an IMP sample. These characteristics are in turn strongly influenced by the type and concentration of the detergents used in IMP preparation. By utilizing the techniques and analytical tools we earlier developed for the characterization of protein-detergent complexes (PDCs) (Maslennikov et al., 2007), we demonstrate that for successful protein extraction from *E. coli* membrane fractions, the solubilizing detergent associates preferentially to IMPs rather than to membrane lipids. Notably, this result is contrary to the generally accepted mechanism of detergent-mediated IMP solubilization. We find that for one particular member of the family of proteins studied (*E. coli* receptor kinases, which is purified in mixed multimeric states and oligomerizes through its transmembrane region), the protein oligomeric composition is largely unaffected by a 10-fold increase in protein concentration, by alteration of micelle properties through addition of other detergents to the PDC sample, or by a 20-fold variation in the detergent concentration used for solubilization of the IMP from the membrane. We observed that the conditions used for expression of the IMP, which impact protein density in the membrane, has the greatest influence on the IMP oligomeric structure. Finally, we argue that for concentrating PDCs smaller than 30 kDa, stirred concentration cells are less prone to over-concentration of detergent and are therefore more effective than centrifugal ultrafiltration devices.

Keywords

Membrane protein; Protein detergent complex; NMR; Light scattering; Analytical Ultracentrifugation

Introduction

Structural studies of integral membrane proteins (IMPs) are hampered by inherent difficulties in heterologous protein expression and in purification of solubilized protein-detergent complexes (PDCs). The type and concentration of detergents used in IMP preparation are critical determinants of protein homogeneity and stability, and are thus important variables in IMP crystallization experiments. In a previous paper (Maslennikov et al. 2007), we presented simple techniques for NMR-based monitoring of the presence and concentration of detergents during all stages of IMP sample preparation, and for characterization of PDCs by analytical

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ultracentrifugation. The ability of NMR to accurately measure the concentrations of buffer and detergent components in IMP preparations allowed us to trace detergent during protein extraction, to assess the completeness of detergent exchange, and to quantify the actual detergent concentration during ultrafiltration.

In this paper, we examine in more detail practical and mechanistic aspects of detergent-mediated protein extraction from *E.coli* membrane fractions. Membrane solubilization has been studied extensively, and the mechanistic view of this process is based on a three stage model. In the first stage, the detergent is incorporated into the membranous phase; in the second stage, fragmented membrane coexists with mixed lipid-detergent micelles; and in the final stage, membrane components are fully solubilized and incorporated into mixed lipid-detergent micelles (Lichtenberg 1985; Banerjee et al. 1995; Kragh-Hansen et al. 1993; de Foresta et al. 1996; Kragh-Hansen et al. 1998; le Maire et al. 2000; Lichtenberg et al. 2000; Almgren 2000; Seelig 2004). Most of the detailed experimental studies of membrane solubilization were performed using phospholipidic liposomes or relatively homogeneous membrane vesicles, for example those from sarcoplasmic reticulum (SR), which contain mostly phoshatidylcholine and Ca2+ATPase (Meissner and Fleischer 1971). In contrast, native *E.coli* membrane is complex: its phospholipid composition (70 to 80% phosphatidylethanolamine, 15-25% phosphatidylglycerol, and 5-10% cardiolipin) is different from that of SR, and about half of its weight is contributed by hundreds of natively encoded proteins embedded in the membrane (Kadner 1996). It is therefore not surprising that the properties of *E. coli* membranes can differ substantially from those of model membrane systems. The partial and selective solubilization of membrane components from a complex membrane environment (like the *E.coli* membrane) remains poorly characterized (Lichtenberg et al. 2000). Detailed studies with SR vesicles from rabbit skeletal muscle have indicated that during the first stage of membrane solubilization, the membrane lipid serves as the major detergent binding component (Kragh-Hansen et al. 1993). Although it has been shown that there is no abrupt transition from membranous to the detergent-solubilized state, a general viewpoint is that as a consequence of detergent partitioning into the membrane, the membrane lamellar structure is disturbed and solubilized species that are composed of protein, detergent and lipid are released to the non-sedimentable fraction. In this view, however, the protein does not have an active role in the solubilization process. Yet, abundant data suggest that detergents are selective for IMPs, which means that treatment with a specific detergent may fail to solubilize certain proteins and instead results in incomplete solubilization of membrane. Three distinct types of membrane domains, lipid rafts, detergent-resistant membranes and liquid-ordered lipid phases, have been introduced by researchers to explain such resistance to solubilization by detergents (for review see Lichtenberg 2005). But again, in these models, the IMP components play a rather passive role. We feel that often overlooked in the membrane-solubilization process is the competition between detergent and lipid for interaction with the IMP. In contrast, our current studies of the solubilization of *E.coli* membrane fractions with and without an overexpressed IMP suggest the importance of detergent-IMP interactions as we find that during solubilization, detergent incorporates into the membrane most probably by association with membrane-embedded protein and that this association is a determining factor in successful protein solubilization.

In order to check the homogeneity of the sample and to characterize the oligomeric state of the PDC, we analyzed the data from the velocity experiment by analytical ultracentrifugation (AU) and used physical constraints on the Tanford equation (Tanford et al. 1974) to roughly estimate the composition of the PDC (Maslennikov et al. 2007). This method was more accurate for monomers and dimers of IMP and less accurate for higher-order multimers. Searching for a more robust method we found that static light scattering (LS) measurements are perfectly suited to our needs. The basic idea behind static LS analysis is that the amount of light scattered by a particle is proportional to particle mass and particle concentration, so by measuring the amount of light scattered by a particle and the concentration of the particle we can derive its

averaged molar mass (Wyatt 1993). Coupling LS with size exclusion chromatography (SEC) is therefore a logical step, because particles can be fractionated according to size and their concentration can be measured using UV absorbance or refractive index (RI) (Folta-Stogniew and Williams 1999). To apply this methodology to IMPs, decoupling protein and detergent from the PDC is necessary. This is done by measuring the concentration of PDC particles both by UV absorbance and RI. Since the molar concentration detected by both devices is the same, comparing the signals from UV and RI gives the basis for the estimation of the amount of both the protein and the detergent in the PDC (Hayashi et al. 1989; Wen et al. 1996).

Comparing the results of the two methods, we observed that the determination of the oligomeric composition of the truncated form of the receptor histidine kinase ArcB(1-83) by AU is in good agreement with that by LS. Additionally, we found that at a high concentration (OD_{280} = 10) the oligomeric composition of ArcB(1-83) determined by AU is the same as it is at a low concentration. Following this observation, we examined a large number of *E. coli* receptor kinases for possible changes in their oligomeric state when purified in a particular detergent after the PDC is perturbed by addition of another detergent, a procedure commonly used in crystallization of IMPs (Newstead et al. 2008). Our main focus was on those receptor kinases which were purified as a mixture of oligomers. In most cases we observed no change in the oligomeric composition of the investigated protein. This observation allowed us to conclude that for *E. coli* receptor kinases altering protein concentration and adding a different detergent to the existing PDC is unlikely to change the oligomeric structure of the IMP once it is stabilized in the primary detergent. However, we observed that the conditions used for expression of the IMP, which impact protein density in the membrane, has the greatest influence on the IMP oligomeric structure.

We have previously reported that during ultrafiltration by centrifugation of some detergents the concentration factor depends on the initial concentration of these detergents (Maslennikov et al. 2007). We excluded the possibility that micelle size increases with concentration and we found that concentration polarization during ultrafiltration, which not only limits the tangential flow and decreases the permeability of the membrane but also effectively decreases membrane molecular weight cutoff, is solely responsible for this phenomenon (Bowen and Jenner 1995; Bhattacharjee et al. 1999).

Material and methods

DHPC was purchased from Avanti Polar Lipids; all other detergents were purchased from Anatrace and all chemicals from Sigma. NuPAGE gels are from Invitrogen. The Vivaspin ultrafiltration device is from Vivascience.

Membrane fractions preparation

The genes of *E. coli* receptor kinases were cloned to pMIS vector (Roosild et al. 2005; Kefala et al. 2007) and expressed in *E.coli* BL21 DE3 cells. The clone of GFP-fused-YcjF was obtained from the von Heijne group (Drew et al. 2001) and also expressed in *E. coli* BL21 DE3 cells. To obtain membranes without overexpressed protein we grew untransformed *E. coli* BL21 DE3 cells. The truncated gene of *E. coli* receptor kinases ArcB(1-83) was cloned to pHIS8 vector and expressed in *E.coli* BL21 DE3 cells in two different ways: first both samples were expressed at 37°C until OD_{600} reached 0.6 and then, after IPTG induction, one sample was expressed at 18°C and the other at 37°C. The membranes from these cells were prepared by a standard protocol as described in Kefala et al. (2007).

Protein extraction from cell membrane

Aliquots of EnvZ, YcjF and ArcB(1-83) membrane fractions were mixed 1:1 with extraction buffer (20 mM Tris-HCl pH 7.6, 200 mM NaCl, 1 mM $MgCl_2$, 1 mM BME) containing extraction detergent at twice the desired concentration and incubated with stirring overnight at 6°C. The detergent-solubilized fraction was separated by centrifugation at 100,000 g for 1 hour and extraction efficiency was estimated from SDS-PAGE by comparing fractions before and after the 100000 g spin.

Solubilization studies for E. coli membranes without and with overexpressed proteins (NarX and YcjF)

The concentration of the membranous phase in the membrane fraction used in this study was adjusted to 25 mg/ml. The primary concentration was measured by weighing the pellet of the membrane fraction after the 100000 g centrifugation during membrane preparation. Aliquots of membrane fractions were mixed 1:1 with extraction buffer containing detergent at twice the desired concentration. The fractions were incubated with stirring overnight at 6°C, the detergent-solubilized fraction was separated by centrifugation at 100000 g for 1 hour, and the detergent concentration in this fraction was measured by NMR (Maslennikov et al. 2007).

Using detergents as additives

The stocks of detergents listed in Table 1 were prepared at concentration set to 10x the destination concentration, also listed in Table 1. The concentrated protein was mixed with each detergent stock in the 10:1 ratio and incubation time varied between 2 to 72 hours before the sample was analyzed by LS.

Homogeneity and oligomeric state of IMP analyzed by light scattering

20 ul of each of the FPLC-purified *E. coli* kinase receptors concentrated to ~10 mg/ml was injected into the Waters HPLC system equipped with Wyatt miniDawn (LS) and Optilab (RI) devices in line, which were pre-equilibrated with 20 mM Tris 7.4, 200 mM NaCl, 2 mM FC12. Data recorded by the UV spectrometer and the RI and LS devices were analyzed using the Protein Conjugate module of Wyatt's ASTRA V software. The extinction coefficient for protein was calculated from the amino acid composition (Gasteiger et al. 2005; Gill at al.1989). dn/dc = 0.185 was used for proteins (Folta-Stogniev et al. 1999). To measure dn/dc of FC12, 5ml of the detergent at 8 different concentrations (0.5 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mM) were injected directly to the Optilab RI detector and the obtained RI values were used to calculate dn/dc = 0.131 (Strop and Brunger 2005).

Analytical Ultracentrifugation measurements

Two samples of ArcB(1-83) were prepared at OD_{280} = 0.8 and OD280 = 10 in a 20 mM Tris pH 7.4, 200 mM NaCl, and 2 mM FC12 buffer. Velocity measurements were completed at 40,000 rpm and 20°C in a AN60 rotor by collecting 200 absorbance and 200 interference scans for the OD_{280} = 0.8 and OD280 = 10 samples, respectively. The scans were first analyzed by the van Holde-Weischet method (van Holde and Weischet 1978) and then by the C(s) analysis using ultrascan software (Demeler 2005). Detergent contribution to the PDC buoyant mass was negligible because buffer density was very close to the density of FC12 (Maslennikov et al. 2007), so the detergent buoyant mass was close to zero.

Detergent ultrafiltration by centrifugation

Four samples of 10 ml of 20 mM Tris pH 8.0 buffer containing 10 mM of LDAO were concentrated independently in four 30kD cutoff Vivaspin concentrators down to about 1 ml using the Beckman Allegra 25R centrifuge at 3000 rpm and 20°C. The spin was stopped every

2 min and two of the four samples were mixed by gentle pipetting. The concentration factor (*cf*) was calculated as follows: *cf*=(*fc*-*ic*)/(*n***ic*-*ic*), where *fc* is the final concentration, *ic* - the initial concentration, and *n* - fold concentration = *iv*/*fv*, where *iv* is the initial and *fv* is the final volume.

Results and Discussion

Protein extraction from the E. coli membrane by detergents

In our previous study (Maslennikov et al. 2007), we hypothesized that during the detergent-mediated solubilization of membrane proteins overexpressed in *E. coli*, the detergent preferentially binds to the protein, and that the protein is extracted from membrane lipids only after the detergent exceeds a critical threshold concentration, below which no protein and almost no detergent exists in the supernatant after 100000g centrifugation (referred to as a hard spin). The specific affinity of a detergent for a protein is consistent with the observation that certain proteins can be solubilized only by particular detergents. For example, solubilization profiles of EnvZ and GFP-fused-YcjF with 20 mM of commonly used detergents show that EnvZ can only be extracted by FC14, FC12, FC10, Zw-3.14, DPh, and SDS (as illustrated in Figure 1A), whereas GFP-fused-YcjF is extracted by most detergents except DHCP and Brij-30 (Figure 1B). Even a vast excess of an incompatible detergent will not effect the extraction of certain proteins, as illustrated in the last four lanes of Figure 1A, which show the inability of both 200mM DDM and DM to extract EnvZ.

To test our hypothesis that the solubilizing detergent binds preferentially to an overexpressed IMP, we compared the solubilization properties of several detergents with *E. coli* membrane fractions lacking overexpressed protein or containing the overexpressed proteins NarX or GFP-fused-YcjF (Figure 2). The same amount of membrane was used for each experiment, and therefore the membrane samples differed mainly in the amount of IMP. Our results clearly indicate that the appearance of the solubilization detergent in the pelleted membrane fraction is influenced by the amount of protein in the membrane and the affinity of the detergent to the protein. In other words, during the first stage of solubilization the detergent incorporates into membrane by association with membrane proteins. The detergent will not solubilize proteins for which it lacks affinity.

For the purpose of analyzing these results we introduce a detergent parameter, which we call the critical protein solubilization concentration (CPSC), below which only background (less than 10% of total) amount of protein is solubilized and most of the detergent is in the sedimentable fraction. It is worth noting that the amount of protein solubilized below CPSC hardly depends on the detergent concentration (Figure 2). When the membrane containing overexpressed protein is solubilized with a detergent capable of extracting this protein (for example, DDM and DM for YcjF - green lines in Figure 2A), about 80-90% of the detergent is pulled down during the hard spin when the detergent concentration is below CPSC (between 4 and 6 mM in this case). However, as the detergent concentration rises beyond this threshold, the protein becomes increasingly solubilized and the fraction of pulled-down detergent decreases. Conversely, when the same membrane sample is treated with a nonextracting detergent (for example DDM and DM for NarX - turquoise lines in Figure 2A), the fraction of detergent pulled down is similar in behavior to that observed for the detergent treatment of membrane with no overexpressed protein (NOP) (red lines in Figure 2A). There is a non-negligible amount of various endogenous *E. coli* proteins in membranes with NOP, but the total amount of protein is considerably smaller than in the case of membranes with overexpressed protein. That is why the fraction of pulled-down detergent solubilizing a NOP membrane decreases much faster when detergent concentration increases. Also, different detergents will have different affinities to the endogenous *E. coli*. proteins, which explains for example why about 70% of DDM and only 40% of FC14 at 1mM are pulled down during the

hard spin. It is then clear that the pull-down of the solubilization detergent during hard spin depends on the affinity of the detergent to the solubilized protein. The CPSC also depends on the amount of protein in the membrane. When the amount of solubilized membrane containing YcjF was increased four times, the CPSC also increased about four times from 3-4 mM to 10-20 mM (Figure 2B).

Homogeneity and oligomeric state of IMP analyzed by light scattering

We confirmed the principle of decoupling protein and detergent in PDC by light scattering experiments on numerous *E. coli* receptor kinases. For example, Figure 3 shows a typical light scattering experiment for the *E.coli* tyrosine kinase receptor Etk. The analysis yielded that in FC12 Etk is a monomer with an estimated average molecular mass of 100.3 kDa and an estimated protein-to-detergent weight ratio of 1.2. The molecular weight calculated from the sequence of this protein is 99.8 kDa, so experimental error is less than 1% in this case.

In order to check if the LS results are in agreement with the AU results, we used a truncated version of the receptor histidine kinase ArcB(1-83) purified in FC12 and estimated its average molar mass from LS as well as AU experiments. We found that both methods rendered similar results (Figure 4A and 4B). The protein is a mixture of monomers, dimers and tetramers. The monomers are eluted under the dimer peak shoulder, as illustrated in the chromatogram by light scattering at 90deg (Figure 4A), and are not easily identified, but it is evident from the figure that most of the protein is dimeric. Similar results can be concluded from the C(s) type of analysis of the absorbance scans from the AU velocity experiment (Figure 4B). Protein concentration used in LS experiments usually cannot exceed OD_{280} = 1 in order to allow for a reliable UV measurement. Since we knew from previous experiments that ArcB(1-83) can form mixtures of various oligomers, we measured its oligomeric distribution by AU at OD_{280} = 10 (Figure 4C) and found that at higher concentrations ArcB(1-83) does not change its oligomeric composition.

Following this observation, we decided to check whether the oligomeric state of the protein could be changed by, for example, addition of different detergents. We have been using detergents as additives (Table 1) for crystallization for a long time. The main idea is to change micelle properties by forming mixed micelles of the main and the additive detergents. Changing the size of the micelle could influence protein stability and accessibility of the soluble domains and allow protein-protein contacts, which might positively affect crystallization. We used LS to follow changes in the oligomeric state of several different receptor kinases upon addition of the detergents listed in Table 1 and different incubation times, which varied from 2 to 72 hours. A receptor kinase purified in FC12 was mixed with the additive detergent in such a way as to achieve the final concentration of the additive detergent specified in Table 1. We focused mainly on those proteins which are a mixture of different oligomers after purification, because previous experiences indicated that the oligomeric state of such proteins depends highly on the preparation process. This raises the question of whether the addition of a second detergent would also affect the oligomeric composition. Using ArcB(1-83) purified in FC12 as illustrated in Figure 5 as an example, we can see that there are no changes in the oligomeric composition of IMP upon addition of a variety of additive detergents, however, our experiment showed that some proteins precipitated after addition of CHAPS, CHAPSO, OG or NG. Additionally, we did not observe any change related to incubation time. For the purpose of clarity Figure 5 shows the results for only two detergent families: the foscholine family with different hydrophobic chain lengths, which includes the main detergent FC12 (Figure 5A), and the maltoside family (Figure 5B).

Knowing that the oligomeric composition of ArcB(1-83) does not change with protein concentration or with the addition of another detergent, we were posed with the next question: at what stage of protein production does the mixture of different oligomers become “frozen”?

In order to see if detergent concentration during the solubilization stage may play a role we extracted ArcB(1-83) with 5 and 100mM of FC12 and, after parallel protein purification for both samples, we compared their oligomeric compositions. As evident from Figure 6A, these two extractions yielded similar compositions. However, when we repeated this experiment using protein from another preparation, its oligomeric composition was different (Figure 6B). The slight difference in oligomeric composition that could be observed between the 5 and 100mM detergent extractions both in panel 6A and 6B was that the 5mM extraction resulted in a slight shift toward higher oligomers. However, the difference in the oligomeric state between panels 6A and 6B had to be attributed to different expression or preparation. In order to separate expression of the protein from its preparation we expressed ArcB(1-83) overnight at 37°C and 18°C and conducted subsequent membrane preparation and purification for both samples in a parallel mode. From the difference between oligomeric states in the two samples presented in Figure 6C it is evident that the main factor governing the oligomeric composition of ArcB(1-83) is expression.

Detergent ultrafiltration by centrifugation

Ultrafiltration by centrifugation is the most commonly employed method of concentrating protein for crystallization. We previously provided some guidelines for using the concentrators (Maslennikov et al. 2007) and we recommended the use of a 50 kDa or higher cutoff Vivaspin device, since they are permeable for most commonly used detergents while the 30 kDa cutoff devices are not. We found in the previous study that the concentration factor for 30 kDa cutoff membranes increases upon raising the initial concentration of the detergent for both the regenerated cellulose (Amicon) and the polyethersulfone (PES) (Vivaspin) membrane. There are two possible explanations for this: (1) micelles increase in size; (2) there is a change in membrane permeation due to the concentration polarization effect. Since we determined by using LS that increasing detergent concentration does not change the average molar mass of micelles, we conclude that the latter takes place. To prove it, we compared the concentration factors for the 30kDa cutoff PES membrane from Vivaspin with partial mixing and without mixing during the concentration process, and we found that mixing decreases the concentration factor by 50% (Table 2). Therefore, we concluded that mixing partially overcomes the concentration polarization effect and results in increased membrane permeability. Since this problem appears mostly with the 30 kDa cutoff membrane, in order to concentrate PDCs smaller than 30 KDa, stirred cells instead of centrifugal ultrafiltration devices are suggested. However, it should be noted that even when using 50 kDa cutoff Vivaspin devices, examples of overconcentrating detergents may occasionally be found in PDC samples. We have not yet found such an example with the 100kDa devices.

Conclusions

After PDC has been formed, it is difficult to displace the detergent to allow protein-protein interactions of transmembrane protein domains to form protein oligomers. The results we present here confirm that for ArcB(1-83) increasing protein concentration 10-fold does not change the oligomeric composition of the protein. Moreover, changing micelle properties by adding other detergents to the PDC sample of an *E. coli* receptor kinase extracted and purified with the primary detergent typically does not result in a change of the oligomeric state of the protein, even for proteins extracted and purified as mixtures of oligomers. We have also found that modification of parameters of protein expression, such as temperature, changes the oligomeric composition of the protein more profoundly than modification of detergent concentration during protein solubilization. This finding is rather surprising because it has been shown before that the concentration of the solubilization detergent affects the homogeneity of the protein (le Maire et al. 2007). A possible explanation of our results is that, as the density of the overexpressed protein changes in the E.coli membrane from expression to expression,

different oligomers are formed in the membrane, which are subsequently extracted by the solubilization detergent. However, we would like to stress that our results can be only generalized to proteins which oligomerize through their transmembrane domains and that protein-protein interactions interplay with protein-lipid, protein-detergent, detergent-lipid, lipid-lipid, and detergent-detergent interactions and the final result of solubilization is the function of all these factors.

Our studies of protein extraction show that in the initial stages of solubilization of proteins overexpressed in *E. coli* membrane, the detergent partitions itself to the membrane and associates mostly with the protein present in the membrane. At concentrations below CPSC, most of the detergent is partitioned into the non-sedimentable fraction and as a consequence is pulled down by hard spin. Our data suggest that the protein is the main detergent-binding component. However, when the detergent concentration increases above CPSC, the interaction between detergent and the lipid molecules that surround IMP will likely destabilize the lipid structure around the protein, and, as a consequence, the protein, protected by the detergent or by mixed detergent-lipid micelles, is released from the membrane. One could argue that in accordance with the generally accepted view of the initial stages of solubilization, the lamellar structure of the membrane is compromised and then the protein is released to the non-sedimentable fraction but since the detergent concentration is too low (less than CPSC) to solubilize the IMP, the protein aggregates together with detergent and as a consequence is pulled down by hard spin, which would also explain our data. However, we feel that this argument is not substantiated because membrane proteins can stay soluble with detergents which cannot extract them from the membrane. As an example we showed that DDM cannot solubilize EnvZ even at the concentration of 200mM, however, it can effectively and completely replace FC12 and keep the protein soluble for days at the concentration as low as 3mM (Maslennikov et al. 2007).

It is known that membrane lipids can be efficiently solubilized by detergents (Kragh-Hansen et al. 1998). It is also known that some IMPs are sequestered within small compartments of the membrane for functional reasons (Persegian 1995). There is a possibility that overexpressed IMPs are also compartmentalized and depending on detergent-lipid and detergent-protein interactions, the individual compartments may not be soluble with particular detergents. This is especially true of detergents which have weaker affinity for protein than lipids and whose affinity for lipids is weaker than that of protein, which essentially means that neither the lipids enclosing IMPs nor the IMPs are soluble with such detergents. We agree therefore with Lichtenberg et al. (2000) that more research is required to clarify mechanistic aspects of detergent-mediated solubilization of membrane components in protein-rich highly heterogenous membranes.

Summary

In this paper we demonstrated that: (1) in successful IMP protein extraction, the solubilizing detergent interacts preferentially with IMPs rather than with membrane lipids; (2) the conditions used for the IMP expression have a greater influence on IMP oligomerization than the subsequent solubilization and purification steps; (3) for concentrating PDCs smaller than 30 kDa, , stirred cells perform better than centrifugal ultrafiltration devices, which can cause over-concentration of the detergent.

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Abbreviations

IMP, Integral membrane protein; PDC, Protein detergent complex; AU, Analytical ultracentrifugatino; LS, Light scattering; RI, Refractive Index; CPSC, Critical protein solubilization concentration.

Detergent abbreviations

Brij-35, polyethylene glycol(23)monododecyl ether
Brij-30, polyethylene glycol(4) dodecyl ether
C12E8, dodecyl octaethylene glycol ether
C10E8, decyl octaethylene glycol ether
C8E5, octyl pentaethylene glycol ether
TDM, n-tetradecyl-β-D-maltopyranoside
DDM, n-dodecyl-β-D-maltopyranoside
DM, n-decyl-β-D-maltopyranoside
β-NG, n-nonyl-β-D-glucopyranoside
β-OG, n-octyl-β-D-glucopyranoside
FC14, ntetradecylphosphocholine
FC12, n-dodecylphosphocholine
FC10, n-decylphosphocholine
FC9, n-nonylphosphocholine
FC8, n-octylphosphocholine
TX-100, α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxy-poly(oxy-1,2-ethanediyl)]
LDAO, lauryldimethylamine-Noxide
Zw-3.14, n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
Zw-3.12, ndodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
Zw-3.10, n-decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
Zw-3.8, n-octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate
SDS, sodium dodecyl sulfate
DPh, disodium-N-lauryl-β-iminodipropionate
DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine
Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
ChapsO, 3-([3-cholamidopropyl]dimethylammonio)-2-hydroxy-1-propanesulfonate
DOC, 3α,12α-dihydroxy-5β-cholanic acid
Mega-9, nonaoyl-N-methylglucamide
Mega-8, octanoyl-N-methylglucamide

References

Almgren M. Mixed micelles and other structures in the solubilization of bilayer lipid membranes by surfactants. Biochim Biophys Acta 2000;1508:146–163. [PubMed: 11090823]

Bhattacharjee S, Kim AS, Elimelech M. Concentration Polarization of Interacting Solute Particles in Cross-Flow Membrane Filtration. J Colloid Interface Sci 1999;212:81–99. [PubMed: 10072278]

Bowen WR, Jenner F. Theoretical descriptions of membrane filtration of colloids and fine particles: an assessment and review. Advances in Colloid and Interface Science 1995;56:141–200.

Banerjee P, Joo JB, Buse JT, Dawson G. Differential solubilization of lipids along with membrane proteins by different classes of detergents. Chem Phys Lipids 1995;77:65–78. [PubMed: 7586093]

Demeler, B. In Modern Analytical Ultracentrifugation: Techniques and Methods. Scott, DJ.; Harding, SE.; Rowe, AJ., editors. Royal Society of Chemistry; 2005. p. 210-229.

Drew DE, von Heijne G, Nordlund P, de Gier JW. Green fluorescent protein as an indicator to monitor membrane protein overexpression in Escherichia coli. FEBS Lett 2001;507:220–224. [PubMed: 11684102]

Folta-Stogniew E, Williams KR. Determination of molecular masses of proteins in solution: Implementation of an HPLC size exclusion chromatography and laser light scattering service in a core laboratory. J. Biomol. Tech 1999;10:51–63. [PubMed: 19499008]

de Foresta B, Legros N, Plusquellec D, le Maire M, Champeil P. Brominated detergents as tools to study protein-detergent interactions. Eur. J. Biochem 1996;241:343–354. [PubMed: 8917429]

Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, MR.; Appel, RD.; Bairoch, A. In The Proteomics Protocols Handbook. In: Walker, John M., editor. Humana Press; New York: 2005. p. 571-607.

Gill SC, von Hippel PH. Calculation of protein extinction coefficients from amino acid sequence data. Anal. Biochem 1989;182:319–326. [PubMed: 2610349]

Hayashi Y, Matsui H, Takagi T. Membrane protein molecular weight determined by low-angle laser light-scattering photometry coupled with high-performance gel chromatography. Methods Enzymol 1989;172:514–528. [PubMed: 2546016]

van Holde KE, Weischet WO. Boundary analysis of sedimentation velocity experiments with monodisperse and paucidisperse solutes. Biopolymers 1978;17:1387–1403.

Kadner, RJ. In Escherichia coli and Salmonella: cellular and molecular biology. In: Neidhardt, FC.; Curtiss, R., III; Ingraham, JL.; Lin, ECC.; Low, KB.; Magasanik, B.; Reznikoff, WS.; Riley, M.; Schaechter, M.; Umbarger, HE., editors. ASM Press; Washington DC: 1996. p. 58-87.

Kefala G, Kwiatkowski W, Esquivies L, Maslennikov I, Choe S. Application of Mistic to improving the expression and membrane integration of histidine kinase receptors from Escherichia coli. J Struct Funct Genomics 2007;8:167–172. [PubMed: 17985211]

Kragh-Hansen U, le Maire M, Noel JP, Gulik-Krzywicki T, Moller JV. Transitional steps in the solubilization of protein-containing membranes and liposomes by nonionic detergent. Biochemistry 1993;32:1648–1656. [PubMed: 8431445]

Kragh-Hansen U, le Maire M, Møller JV. The mechanism of detergent solubilization of liposomes and protein-containing membranes. Biophysical Journal 1998;75:2932–2946. [PubMed: 9826614]

le Maire M, Champeil P, Møller JV. Interaction of membrane proteins and lipids with solubilizing detergents. Biochim Biophys Acta 2000;1508:86–111. [PubMed: 11090820]

le Maire M, Møller JV, Menguy T, Velours J, Champeil P. Protein-protein contacts in solubilized membrane proteins, as detected by cross-linking. Anal Biochem 2007;362:168–171. [PubMed: 17250795]

Lichtenberg D. Characterization of the solubilization of lipid bilayers by surfactants. Biochim. Biophys. Acta 1985;821:470–478. [PubMed: 4074739]

Lichtenberg D, Opatowski E, Kozlov MM. Phase boundaries in mixtures of membrane-forming amphiphiles and micelle-forming amphiphiles. Biochim Biophys Acta 2000;1508:1–19. [PubMed: 11090815]

Lichtenberg D, Goni FM, Heerklotz H. Detergent-resistant membranes should net be identified with membrane rafts. Trends Biochem Sci 2005;30:430–436. [PubMed: 15996869]

Maslennikov I, Kefala G, Johnson C, Riek R, Choe S, Kwiatkowski W. NMR spectroscopic and analytical ultracentrifuge analysis of membrane protein detergent complexes. BMC Struct. Biol 2007;7:74. [PubMed: 17988403]DOI 10.1186/1472-6807-7-74

Meissner G, Fleischer S. Characterization of the Sarcoplasmic Reticulum from Skeletal Muscle. Biochim. Biophys. Acta 1971;241:356–378. [PubMed: 4258478]

Newstead S, Ferrandon S, Iwata S. Rationalizing alpha-helical membrane protein crystallization. Protein Sci 2008;17:466–472. [PubMed: 18218713]

Parsegian VA. The cows or the fence? Mol Membr Biol 1995;12:5–7. [PubMed: 7767382]

Roosild TP, Greenwald J, Vega M, Castronovo S, Riek R, Choe S. NMR structure of Mistic, a membrane-integrating protein for membrane protein expression. Science 2005;307:1317–1321. [PubMed: 15731457]

Seelig J. Thermodynamics of lipid-peptide interactions. Biochim. Biophys. Acta 2004;1666:40–50. [PubMed: 15519307]

Strop P, Brunger AT. Refractive index-based determination of detergent concentration and its application to the study of membrane proteins. Protein Sci 2005;14:2207–2211. [PubMed: 16046633]

Tanford C, Nozaki Y, Reynolds JA, Makino S. Molecular characterization of proteins in detergent solutions. Biochemistry 1974;13:2369–2376. [PubMed: 4364776]

Wen J, Arakawa T, Philo JS. Size-exclusion chromatography with on-line light-scattering, absorbance, and refractive index detectors for studying proteins and their interactions. Anal. Biochem 1996;240:155–166. [PubMed: 8811899]

Wyatt PJ. Light scattering and the absolute characterization of macromolecules. Analytica Chimica Acta 1993;272:1–40.

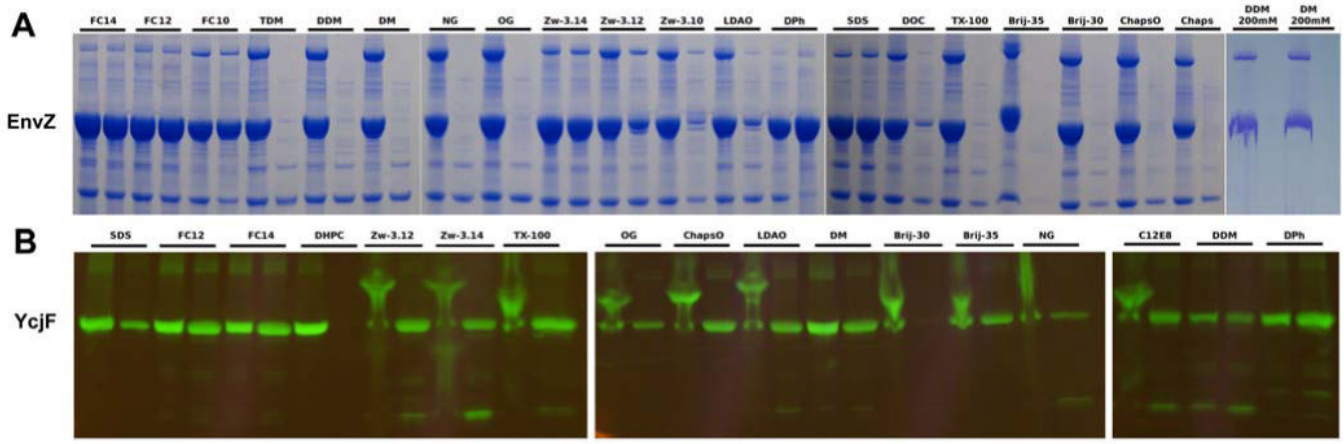

Figure 1.

Protein solubilization profile for commonly used detergents. (A) Coomassie-stained NuPAGE (4-12%) Bis-Tris SDS gel of the extraction profile for EnvZ. (B) GFP fluorescence NuPAGE (4-20%) Tris-Glycine SDS gel of the extraction profile for GFP-fused-YcgF. Each detergent in (A) and (B) is represented by 2 lanes on the gel. The first lane shows the total amount of protein in the membrane fraction and the second lane shows the amount of solubilized protein in the supernatant from an overnight extraction of the membrane fraction.

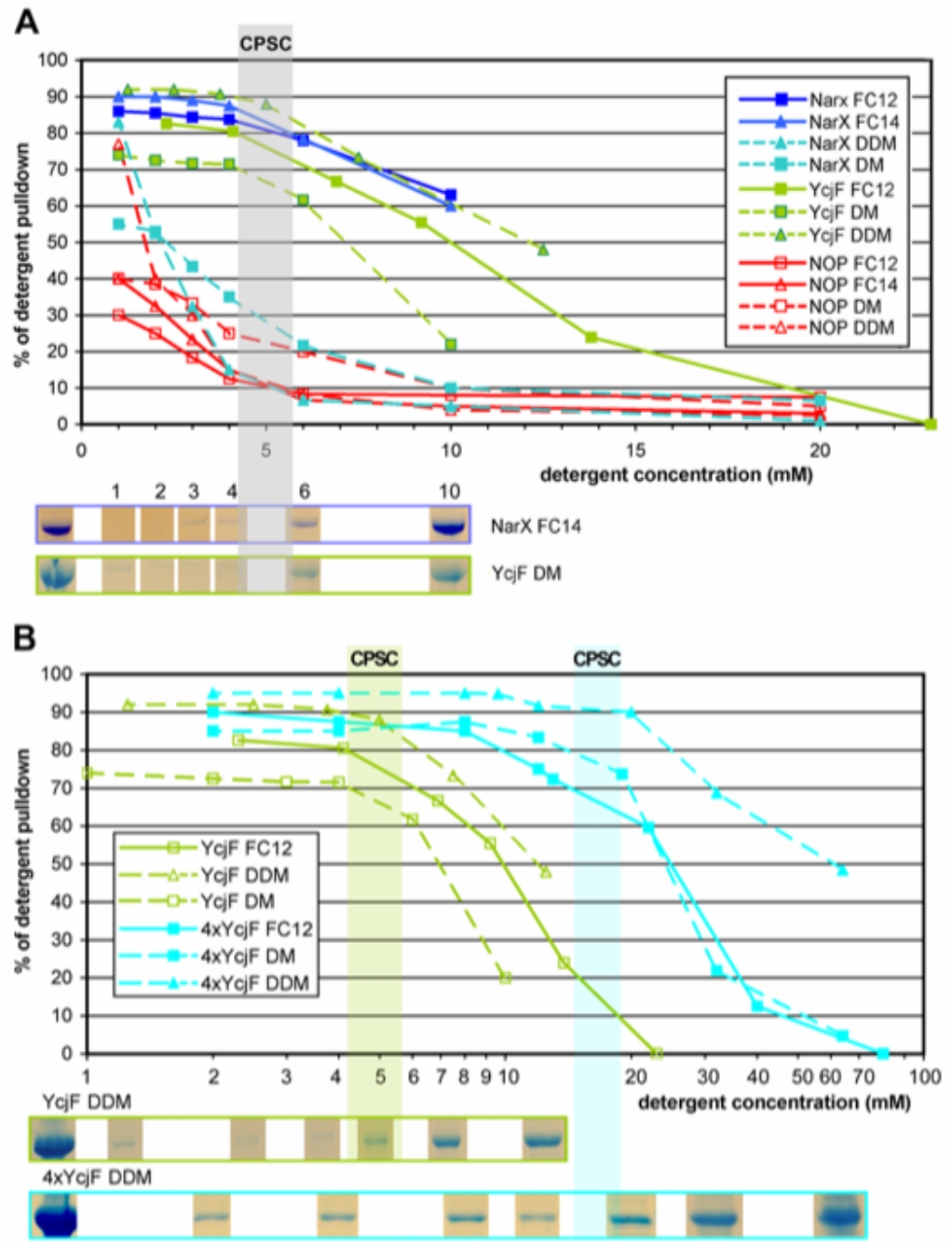

Figure 2.

Detergent extraction profiles for the *E. coli* membrane with and without overexpressed protein. (A) FC12, FC14, DM and DDM extraction profiles for the *E. coli* membrane without overexpressed protein (red lines) compared to the extraction profiles of the same detergents for the membrane with overexpressed NarX (blue lines) and GFP-fused-YcjF (green lines). (B) FC12, DM and DDM extraction profiles for the *E. coli* membrane with GFP-fused-YcjF (green lines) compared to the extraction profiles of the same detergents for 4x the amount of the same membrane (turquoise lines). The extraction profiles in (A) and (B) are represented by the percentage of detergent pull-down after the hard spin for a given detergent concentration used in extraction. The horizontal axis is represented in the logarithmic scale in (B). The amount

of protein solubilized by a given detergent concentration is illustrated below the profile plot on a coomassie-stained SDS PAGE gel for NarX in FC14 and YcjF in DM (A) and for YcjF in DDM and 4x YcjF in DDM (B). The position of the coomassie-stained bands corresponds to the concentration of the detergent on the horizontal axis of the plot. Critical protein solubilization concentration (CPSC) is marked on the plot.

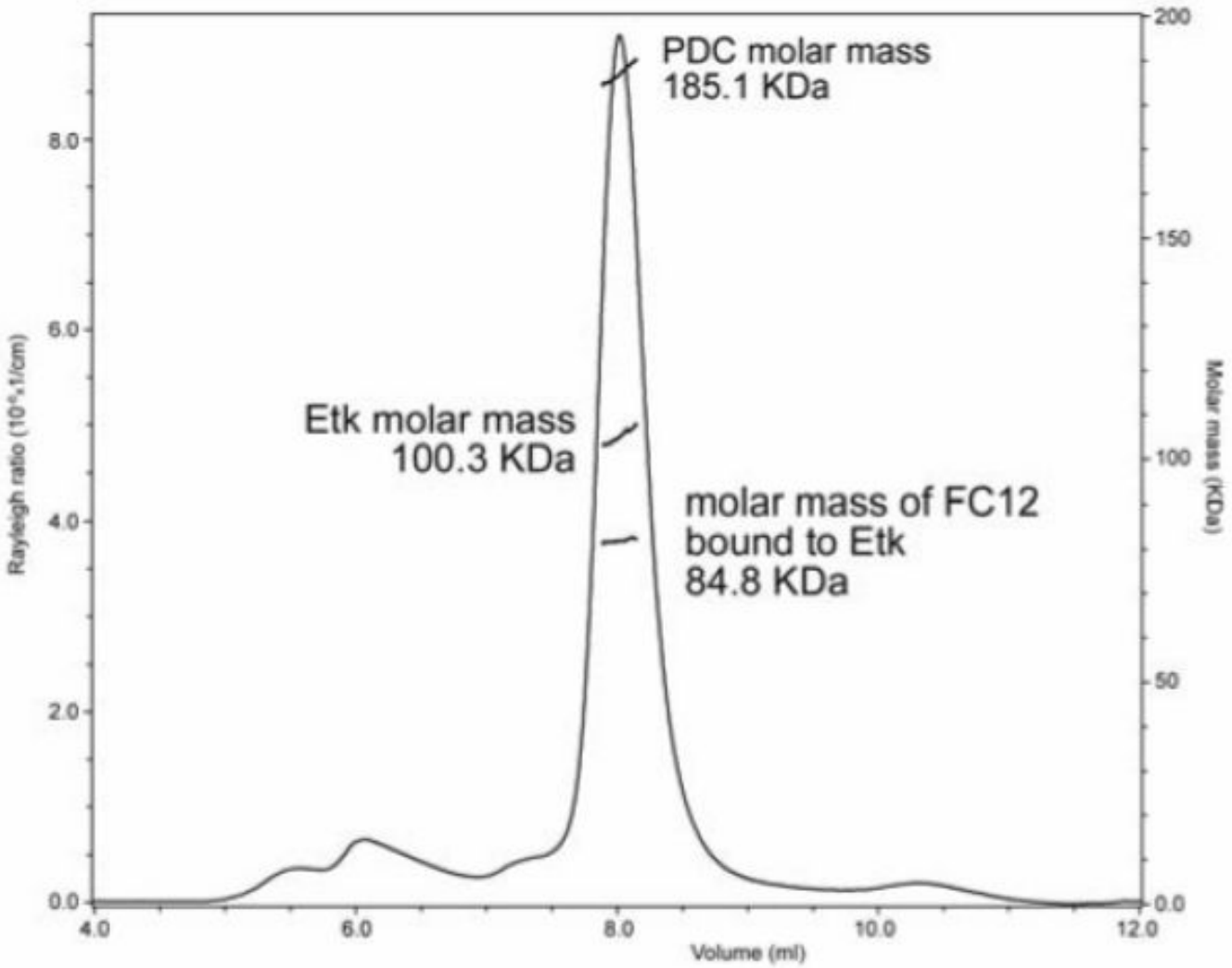

Figure 3.
Size exclusion chromatogram by LS for Etk in FC12. The LS profile at 90deg is shown together with the lines representing the distribution of the molar mass of PDC, Etk, and FC12 bound to Etk across the peak.

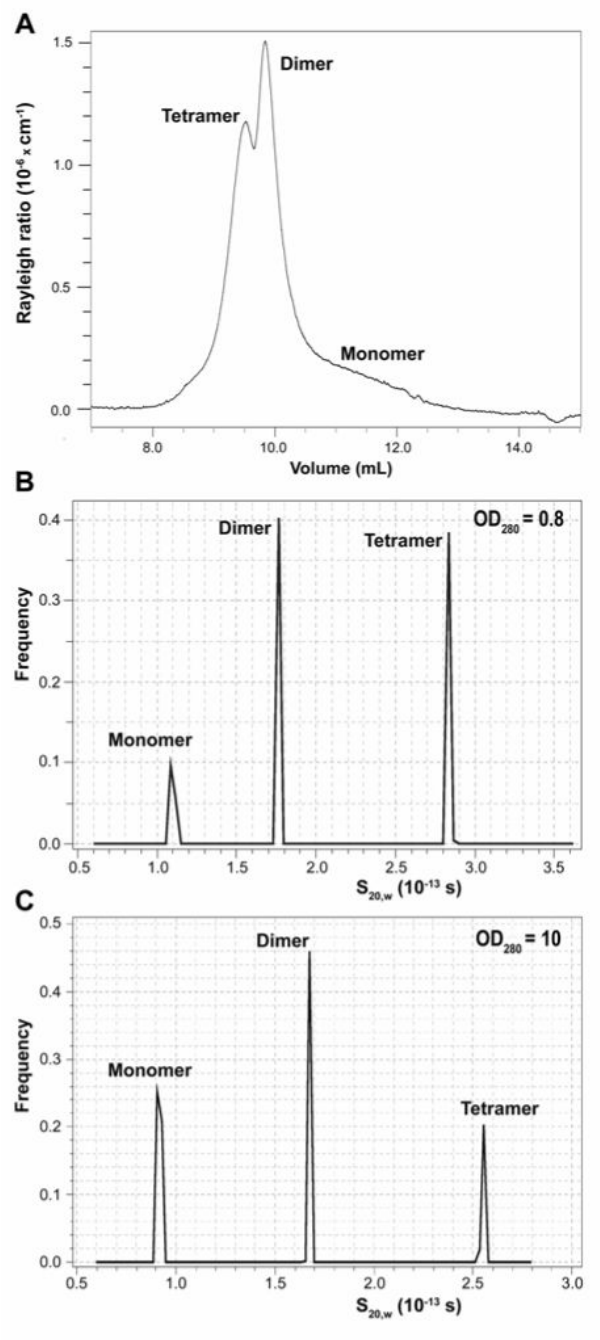

Figure 4.

Molecular weight distribution profile of ArcB(1-83) in FC12. (A) Size exclusion chromatogram by LS at 90deg. (B) C(s) analysis of the absorbance scans from the analytical ultracentrifugation velocity experiment at OD_{280} = 0.8. (C) C(s) analysis of the interference scans from the analytical ultracentrifugation velocity experiment at OD_{280} = 10.

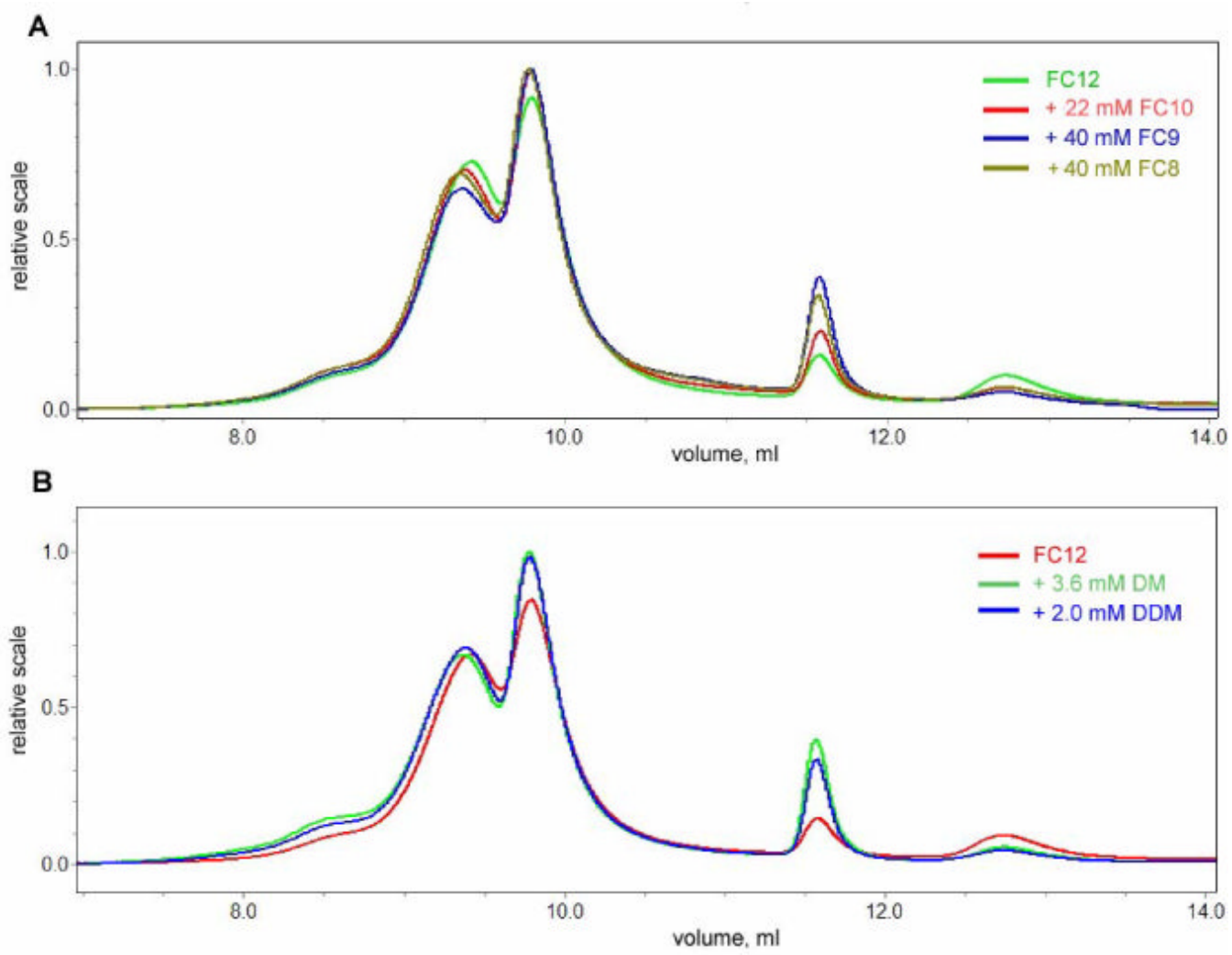

Figure 5.
Size exclusion chromatogram by LS at 90deg for ArcB(1-83) in FC12 (A) with the addition of FC8, FC9, and FC10 (B) with the addition of DM and DDM.

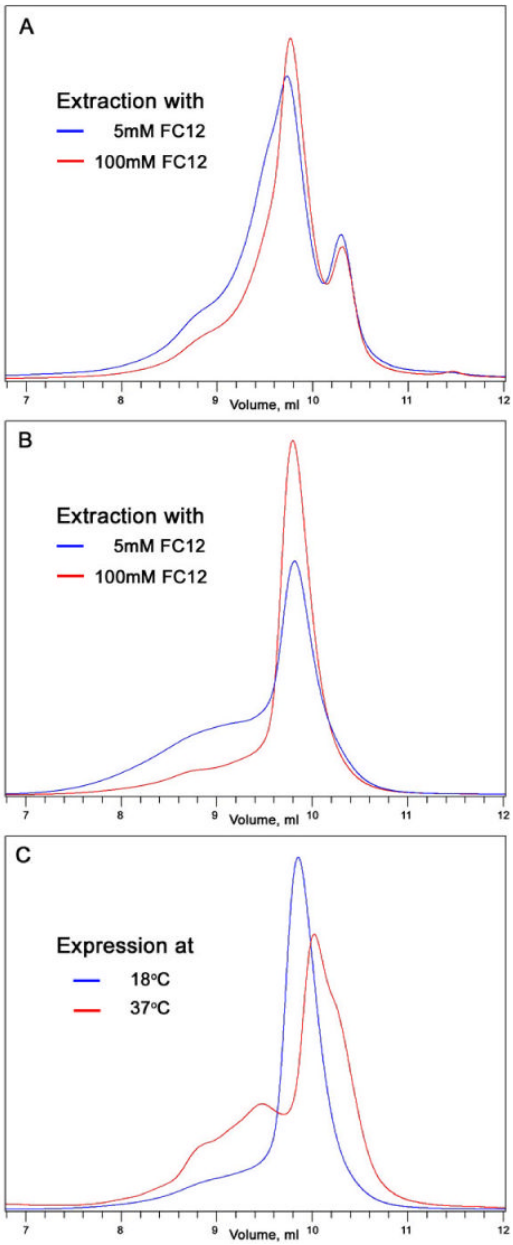

Figure 6.

Size exclusion chromatogram by UV for ArcB(1-83). (A) ArcB(1-83) extracted with 5 and 100mM FC12 (B). The second preparation of ArcB(1-83) extracted with 5 and 100mM FC12 (C) ArcB(1-83) expressed overnight at 18 and 37°C.

Table 1

List of detergents used as additives for crystallization screening and their corresponding concentrations

Detergent	cmc		destination concentration		
	mM	%	mM	%	x cmc
TX-100	0.23	0.015	2.0	0.129	8.70
OG	18.0	0.530	36.0	1.060	2.00
NG	6.50	0.200	13.0	0.400	2.00
DM	1.80	0.087	3.6	0.174	2.00
DDM	0.17	0.009	2.0	0.102	11.76
TDM	0.01	0.001	2.0	0.108	200.0
C12E8	0.09	0.005	2.0	0.108	22.22
C10E8	1.00	0.052	2.0	0.103	2.00
C8E5	7.10	0.250	14.2	0.500	2.00
Mega-8	79.0	2.500	40.0	1.286	0.51
Mega-9	25.0	0.840	40.0	1.342	1.60
Zw-3.8	390	10.90	40.0	1.118	0.10
Zw-3.10	39.0	1.200	40.0	1.230	1.03
Zw-3.12	2.80	0.094	5.6	0.188	2.00
Zw-3.14	0.20	0.007	2.0	0.073	10.00
DHPC	15.0	0.680	30.0	1.361	2.00
Chaps	8.00	0.492	16.0	0.984	2.00
ChapsO	8.00	0.505	16.0	1.009	2.00
FC8	114	3.368	40.0	1.182	0.35
FC9	39.5	1.222	40.0	1.238	1.01
FC10	11.0	0.356	22.0	0.711	2.00
FC12	1.50	0.053	3.0	0.105	2.00
LDAO	1.00	0.023	2.0	0.046	2.00

*Anatrace Catalog: Anatrace 2006 [http://www.anatrace.com/docs/catalog.pdf].

Table 2

Concentration factors for the Vivaspin 30 kDa ultrafiltration device used to concentrate the 10mM LDAO, with and without mixing during centrifugation

	ic, mM	*iv*, ml	*fc*, mM	*fV*, ml	Cf
mixed	8.7	10.0	22.9	0.69	0.12
	8.7	10.0	20.1	0.73	0.10
non-mixed	8.7	10.0	28.6	0.82	0.20
	8.7	10.0	29.3	0.70	0.18