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An evaluation of detergents for NMR structural studies of membrane proteins

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Abstract

Structural information on membrane proteins lags far behind that on soluble proteins, in large part due to difficulties producing homogeneous, stable, structurally relevant samples in a membrane-like environment. In this study 25 membrane mimetics were screened using 2D ^{1}H-^{15}N heteronuclear single quantum correlation NMR experiments to establish sample homogeneity and predict fitness for structure determination. A single detergent, 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)] (LPPG), yielded high quality NMR spectra with sample lifetimes greater than one month for the five proteins tested – *R. sphaeroides* LH1 α and β subunits, *E. coli* and *B. pseudofirmus* OF4 ATP synthase *c* subunits, and *S. aureus* small multidrug resistance transporter – with 1, 2, or 4 membrane spanning α-helices, respectively. Site-specific spin labeling established interhelical distances in the drug transporter and genetically fused dimers of *c* subunits in LPPG consistent with *in vivo* distances. Optical spectroscopy showed that LH1 β subunits form native-like complexes with bacteriochlorphyll *a* in LPPG. All the protein/micelle complexes were estimated to exceed 100 kDaltons by translational diffusion measurements. However, analysis of ^{15}N transverse, longitudinal and ^{15}N{^{1}H} nuclear Overhauser effect relaxation measurements yielded overall rotational correlation times of 8 to 12 nsec, similar to a 15–20 kDalton protein tumbling isotropically in solution, and consistent with the high quality NMR data observed.

Abbreviations: APPC – 1-palmitoyl-2-acetoyl-sn-glycero-3-phosphocholine; βOG – n-octyl-β-D-glucopyranoside; CFTR cystic fibrosis transmembrane conductance regulator; Chaps – 3-([3-cholamidopropyl] dimethylammonio)-1-propanesulfonate; Cholic acid – 3a,7a,12a-trihydroxy-5-β-cholanic acid; DHPC – 1,2-dicaproyl-1-sn-glycero-3-phosphocholine; DHPG – 1,2-dicaproyl-1-sn-glycero-3-[phospho-RAC-(1-glycerol)]; DLCP – dilysocardiolipin; DM – n-dodecyl-β-D-maltoside; DPC – dodecylphosphocholine; LDAO – N,N-dimethyldodecylamine N-oxide; LH1 – light-harvesting complex I; LMPC – 1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine; LMPE – 1-myristoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine; LMPG – 1-myristoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)]; LOPC – 1-oleoyl-2-hydroxy-sn-glycero-3- phosphocholine; LOPG – 1-oleoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)]; LPPC – 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine; LPPE – 1-palmitoyl -2-hydroxy-sn-glycero-3-phosphoethanolamine; LPPG – 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)]; MLPA – sn-(3-myristoyl-2-hydroxy)-Glycerol-1-phospho-sn-3′-(1′-myristoyl-2′-hydroxy)-glycerol; pMAL-B – [Poly (maleic anhydride-alt-1-tetradecene) substituted with 3-(Dimethylamino) propylamine]; 3-maleimido-PROXYL – 3-(maleimidomethyl)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy – free radical; RAC – racemic; SDS – Sodium-n-dodecylsulfate; Smr – small multi-drug resistance protein from *S. aureus*; TMH – transmembrane helix; TRITON – polyoxyethylene(10) isooctylphenyl ether; TSP – [3-trimethylsilyl)propionic acid]; Zwittergent 3-10 – Decyl-N-sulfobetaine; Zwittergent 3-12 – Dodecyl-N-sulfobetaine; Zwittergent 3-14 – Tetradecyl-N-sulfobetaine; Zwittergent 3-16 – Hexadecyl-N-sulfobetaine.

Introduction

Cells use membranes to establish biologically distinct compartments, and rely on membrane-embedded proteins to carry out transport, signaling, energy transduction and other cellular functions across these barriers. Roughly one-third of all structural genes code for membrane proteins (Fraser et al., 1995). However, less than one out of 500 high-resolution structures is from this class of proteins. The main high-resolution struc-

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tural techniques, X-ray crystallography and solution NMR, require removing the protein from the membrane. Many of the difficulties in obtaining suitable samples of membrane proteins for structural studies stem from the need to satisfy interactions typically provided by their multi-layered, quasi-planar environment. Structural studies require conditions where the protein is soluble at high concentrations, monodisperse, conformationally homogeneous, and stable for days to weeks.

From a solution NMR perspective, a protein associated with a membrane mimetic tumbles as part of a larger complex, which leads to signal broadening, poor sensitivity, and reduced spectral resolution. This is especially problematic for membrane proteins that often have low spectral dispersion due to the preponderance of similar amino acid types and helical secondary structures located in non-polar environments. However, recent advances, such as higher magnetic fields and TROSY methods (Pervushin et al., 1997), have extended the size of systems accessible to solution NMR towards 100 kDa (Wüthrich et al., 1998). And, despite difficulties in over-expression and purification, many membrane proteins are available in sufficient quantities for NMR studies. Still, few structures have actually been solved, in part due to the lack of optimal membrane mimetics.

Detergent micelles are the smallest membrane mimics that provide all of the interactions that integral membrane proteins require, prearranged in the appropriate order. Mixed organic solvents and amphipols likely organize in response to the characteristics of the protein. However, detergents spontaneously assemble into a hydrophobic core and an interfacial region providing the membrane-like ability to induce or accommodate structure (White et al., 2001). Bicelles are an appealing membrane mimic (Sanders et al., 1998), improving on micelles by providing a planar interface between the hydrophobic and hydrophilic domains. However, since bicelles are much larger than micelles, detergents are the most promising candidates for a general membrane mimetic for conventional NMR solution structure determination.

Here we used NMR spectroscopy to evaluate detergent micelle conditions that support concentrated, stable, structurally homogeneous membrane protein samples. The LH1 β subunit (5.5 kDa, 1 TMH) of the *R. sphaeroides* photosynthetic bacterial light harvesting 1 (LH1) complex had been tested previously with a subset of the detergents presented here, and Zwittergent 3-12 yielded NMR spectra of sufficient quality to determine the structure of the β subunit. (Sorgen et al., 2002). However, the zwittergents were poor mimetics for the LH1 α subunit (6.8 kDa, 1 TMH), subunit *c* monomers of the *E. coli* (8.3 kDa, 2 TMH) or *B. pseudofirmus* OF4 (7.0 kDa, 2 TMH) ATP synthase, and the small multidrug resistance (Smr) transporter from *S. aureus* (11.7 kDa, 4 TMH). In the work presented here, a number of detergents were screened for their ability to support high quality NMR spectra of *E. coli* subunit *c*, the β subunit, and Smr. A detergent derivative of a standard lipid, 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)] (LPPG), stood out as a superior membrane mimetic. Diffusion and ^{15}N relaxation measurements revealed proteins tumbling rapidly *within* the micelles, allowing higher spectral quality than expected for these large complexes. Two other membrane proteins, LH1 α subunit and OF4 subunit *c*, made poor NMR samples in other membrane mimetics but gave high quality 2D ^{1}H-^{15}N HSQC spectra in LPPG.

Materials and methods

Detergents

Lipid derivatives were purchased from Avanti Polar Lipids (Alabaster, AL). Poorer protein reconstitution was observed for about one-quarter of the LPPG lot numbers used, but no significant lot variations were observed for other detergents. pMAL-B was purchased from Anatrace (Maumee, OH), and other detergents from CalBiochem (San Diego, CA).

Subunit c of the E. coli *F_1 F_O ATP synthase*

Uniformly ^{15}N labeled wild type subunit *c* (*uncE* gene product) and subunit *c* mutants were over-expressed and purified as described previously (Girvin et al., 1998), but using *E. coli* strain C43(DE3) (Miroux and Walker, 1996) as the host. Typical yields from M63 minimal media were 4 mg/l. Subunit *c* and all other purified proteins were characterized by SDS-PAGE, NMR (2D ^{1}H-^{15}N HSQC) and MALDI-TOF mass spectrometry.

Dimers of E. coli *subunit* c

The expression vector for a fused subunit *c* dimer was made by directionally cloning (NdeI, XhoI) a

PCR product coding for *uncE* (G71W mutant) followed by the linker sequence ANGSLNDG plus a second *uncE* (G71C mutant) into the pET17b plasmid (Novagen). Subunit *c* dimer was over-expressed from this plasmid in *E. coli* C43(DE3) in M63 minimal media with $^{15}NH_4Cl$ as the sole nitrogen source. Two hours after IPTG induction (0.7 mM), cells were harvested by centrifugation, resuspended in 8 ml of 100 mM ammonium acetate per liter of culture, and stored frozen at −20 °C. Subunit *c* dimer was extracted with 10 volumes of 1:1 $CHCl_3/CH_3OH$ and the cell debris removed by centrifugation. Adjusting the extract to a ratio of 8:4:3 $CHCl_3/CH_3OH/H_2O$ caused phase separation. The organic phase was removed and concentrated to ∼4 ml, and the dimer was precipitated with 5 volumes of diethylether at −20 °C. The precipitate was air dried then dissolved in a minimal volume of 1:1 $CHCl_3/CH_3OH$ and purified on a 2.5 × 100 cm LH60 size exclusion column eluted isocratically with 1:1 $CHCl_3/CH_3OH$, 50 mM ammonium acetate. The LH60 fractions were evaporated under a stream of argon to less than 10 ml and the subunit *c* dimer was desalted and the solvent exchanged on a LH20 size exclusion column (2.5 cm × 30 cm) eluted with 1:1 $CHCl_3/CH_3OH$ and stored at 4 °C. Typical yields from minimal media were 3–5 mg/l.

Subunit c *from* B. pseudofirmus *OF4*

Uniformly ^{15}N labeled OF4 subunit *c* was over-expressed in *E. coli* C43(DE3) (Miroux and Walker, 1996), and purified as described for subunit *c* dimer. There was some overlap of the genomically exressed *E. coli* subunit *c* (8.3 kDa) and OF4 subunit *c* (7.0 kDa) peaks eluted from the LH60 size exclusion column. Calculated differences in optical absorbance (Pace et al., 1995) of OF4 subunit *c* (0 Trp, 0 Tyr, and 3 Phe, $\varepsilon_{280} \sim 0$, $\varepsilon_{257} = 564\ M^{-1}\ cm^{-1}$), and *E. coli* subunit *c* (0 Trp, 2 Tyr, and 4 Phe, $\varepsilon_{280} = 2530\ M^{-1}\ cm^{-1}$, $\varepsilon_{257} = 752\ M^{-1}\ cm^{-1}$) were used to identify unmixed fractions. *E. coli* subunit *c* was confirmed to be below the level of detection by 2D ^1H-^{15}N HSQC NMR experiments in mixed organic solvents before reconstituting the OF4 subunit *c*. Yields from M63 minimal media were variable, occasionally reaching 3–4 mg/l.

Small multidrug resistance protein (Smr) from S. aureus

The *S. aureus smr* gene (Grinius et al., 1992) was directionally cloned into pET17b. Over-expression and purification was as described for subunit *c* dimer except that the purified Smr was dried under a stream of argon and stored at −20 °C. Typical yields from M63 minimal media were 3–5 mg/l.

LH1 α *and* β *subunits from* R. sphaeroides

For uniform isotopic labeling, the α and β subunits were expressed in a genomic deletion strain of *R. sphaeroides* SK102 as described previously (Sorgen et al., 2002). Yields were typically 3–5 mg/l of each subunit from *R. sphaeroides* in minimal media. Bacteriochlorophyll *a* (BChl *a)* purification and binding assays were described previously (Sorgen et al., 2002).

Spin label-modified proteins

Single Cys and Trp substitutions were introduced using the Quick-Change mutagenesis protocol (Stratagene) or by PCR cassette mutagenesis. Unique Cys residues were spin labeled after LH60 chromatography. The protein containing fractions were concentrated to near dryness in a rotovap at 37 °C then diluted to approximately 0.1 mM in 4:4:1 chloroform/methanol/water, 20 mM TRIS pH 7.0 with a 10-fold molar excess of 3-maleimido-PROXYL, and incubated for 60 min at 30 °C. Excess reagent and buffer were removed, and the solvent exchanged on a LH20 size exclusion column (2.5 cm × 30 cm) eluted with 1:1 $CHCl_3/CH_3OH$.

Reconstitution in detergent micelles

Subunit *c*, subunit *c* dimer, OF4 subunit *c*, α subunit, and Smr were optimally reconstituted by mixing detergent with the protein dissolved in 3:1 $CHCl_3/CH_3OH$ and drying to a thin, clear film (approximately 1 cm^2 per mg of detergent present) under a stream of argon. $CHCl_3$ and/or CH_3OH were added a drop at a time as necessary during drying to prevent precipitation. The film was dried for 1–12 h more under argon to remove residual $CHCl_3$. Detergent/protein films were dissolved in 1–50 mM aqueous potassium phosphate buffer, pH 4.5–8 (as noted) containing 5% (v/v) D_2O and 1 mM TSP. When necessary, samples were heated to 42 °C for up to 12 h and/or bath sonified for up to 30 min to dissolve the film. Lyophilized β subunit readily dissolved in buffered aqueous detergent solutions.

NMR spectroscopy

NMR data were acquired at 600 MHz. The sensitivity enhanced version of the 2D ^{1}H-^{15}N HSQC (Kay et al., 1992; Palmer et al., 1991) was used. ^{1}H chemical shifts were referenced directly to TSP, and ^{15}N was referenced indirectly (Wishart et al., 1995). Translational diffusion coefficients were measured by a bipolar pulse pair longitudinal-eddy-current delay experiment (Wu et al., 1995). Protein/micelle sizes were estimated from comparisons to protein standards, normalized for differences in viscosity using the TSP standard. Spectra were processed and analyzed using NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevins, 1994).

^{15}N relaxation experiments

The pulse sequences used for measuring ^{15}N T_1 and T_2 relaxation times and ^{15}N{^{1}H} NOEs (Skelton et al., 1993) included water suppression (Piotto et al., 1992; Fushman et al., 1997) and for the NOE, water flip-back (Grzesiek and Bax, 1993). The T_1 and T_2 experiments were collected with 4 K and 192 complex points in F2 (^{1}H) and F1 (^{15}N) respectively, with 32 scans per t_1 point and a recycle delay of 1s. The NOE experiments were collected with 2 K and 64 complex points in F2 (^{1}H) and F1 (^{15}N) respectively, with 96 scans per t_1 point and a recycle delay of 6 s. All experiments used a ^{1}H sweep width of 7800 Hz and a ^{15}N sweep width of 2500 Hz with the ^{1}H and ^{15}N carriers set to 4.7 and 119 ppm, respectively. The T_2 relaxation delays were 8, 16 (×2), 24, 32, 40, 48, 64 (×2), 128, 160, 240 (×2) and 320 ms, with a 1 ms delay between ^{15}N pulses in the Carr-Purcell-Meiboom-Gill sequence. The T_1 longitudinal relaxation delays were 8, 20 (×2), 40, 100 (×2), 160, 200, 300 (×2), 500, 800 ms, 2 and 4 s. ^{15}N{^{1}H}steady-state NOE values were obtained from spectra with and without a 3 s GARP (Shaka et al., 1985) proton decoupling period applied in the middle of the amide spectral region during the recycle delay. Relaxation data were analyzed by the model free formalism (Lipari and Szabo, 1982a, b), using the program Dasha (Orekhov et al., 1995).

Results

Detergent screen

Detergents were chosen to represent those used successfully to extract and reconstitute membrane proteins, those that have been used previously for NMR and crystallographic studies, and those that are single chain analogs of the most common phospholipids. Three test proteins represented single TM helix (the *Rb. sphaeroides* LH1 β subunit), two TM helix (*E. coli* subunit *c*) and four TM helix (*S. aureus* Smr) classes. For screening, the proteins were initially dissolved at sub-millimolar concentrations (0.35 mM for β and 0.5 mM for *c* and Smr). Except where noted, the samples remained clear and free of precipitation for the duration of the studies. 2D ^{1}H-^{15}N HSQC spectra were used to evalute the sample properties in each detergent and representative spectra are shown in Figures 1–3. The area under the amide proton region in 1D ^{1}H spectra as a function of time was used as an indicator of sample stability for the relatively successful detergents. The percentage of expected cross peaks initially observed, and the sample half-lives are summarized in Table 1.

Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectra of the three test proteins in the best detergent identified, LPPG. Subunit *c* in a single conformation should give rise to 84 cross peaks in this spectrum. As seen in Figure 1A, 96% of the expected cross-peaks for subunit *c* were resolved in LPPG micelles, and the cross peaks were uniform in line width and intensity. All of the expected peaks were observed upon resolution into a third dimension in ^{13}C-separated experiments. As seen in Figure 1B, LPPG also yielded excellent spectra for the β subunit, where all 50 of the expected cross-peaks were observed. The Smr proton/drug antiporter from *S. aureus* was perhaps the best test of LPPG as a detergent for high-resolution NMR studies. Smr contains 107 residues and four transmembrane helices (Grinius and Goldberg, 1994; Paulsen et al., 1995), and was the largest monomer tested. 86% of the 113 cross-peaks expected in a 2D ^{1}H-^{15}N HSQC spectrum were observed for Smr in LPPG (Figure 1C). Several of the peaks had shapes and intensities consistent with two or more overlapping signals.

As summarized in Table 1, and depicted for representative detergents in Figure 2, many detergents yielded poorer quality NMR data for these proteins. The sparse cross peaks for subunit *c* and the β subunit in APPC (Figures 2A,D) displayed a wide range of

Table 1. 2D ^{1}H-^{15}N HSQC spectral quality and lifetime for protein in detergent micelles

Detergent	Subunit *c*		LH1 β subunit		Smr	
	Peaks (%)[a]	$T_{1/2}$(days)[b]	Peaks (%)	$T_{1/2}$(days)	Peaks (%)	$T_{1/2}$(days)
APPC	30	1	20	<1	n.a.[c]	
βOG	33	3	85	1–2	18	<7
Chaps	ins.[d]		6	<1	ins.	
Cholic acid	ins.		6	<1	ins.	
DM	25	9	6	<1	2	
DPC	99	9	93	<7	82	<2
DHPC	0		96	<1	8	<0.1
DHPG	8		n.a.		5	<0.1
DLCP	54	2	n.a.		n.a.	
LDAO	6		10	<1	8	
LMPC	81	<1	100	<14	50	<0.1
LPPC	77	2.8	76		28	<0.1
LOPC	46	4.6	20		18	<0.1
LMPG	92	>40	100	<7	29	<7
LPPG	96	>40	100	>30	86	>30
LOPG	89	>40	94	>14	86	>30
LPPE	ins.		n.a.		ins.	
LMPE	0		n.a.		n.a.	
MLPA	ins.		n.a.		n.a.	
PMAL-B	5		n.a.		n.a.	
SDS, 37 °C	143	2	126	<1	140	<2
SDS, 42 °C	93	>30	n.a.		88	>0
TRITON	0		2	<1	4	
Zwittergent 3–10	10	>7	44	<7	0	
Zwittergent 3–12	60	>7	100	7–14	18	<2
Zwittergent 3–14	24	>7	12	7–14	14	<2
Zwittergent 3–16	51	>7	34	<1	4	n.a

[a]Percentage of expected 2D ^{1}H-^{15}N HSQC cross-peaks actually observed. Subunit *c* and the β subunit were measured at 37 °C, unless otherwise noted. Smr was measured at 42 °C. Subunit *c* and Smr were tested at a minimum concentration of 0.5 mM and the β subunit at 0.3 mM. Detergent concentrations were 5% (w/v) for subunit *c*, 8% (w/v) for the β subunit and 10% (w/v) for Smr. For the most successful detergents, 5% (w/v) corresponds to 99 mM (LPPG), 94 mM (LOPG), 101 mM (LPPC), 142 mM (DPC) and 173 mM (SDS).
[b]Number of days at which average signal intensity decreases to half its initial value.
[c]Not attempted.
[d]Solubility below the level of detection in the NMR experiments (see text).

intensities and line widths that were typical of spectra where too few cross peaks were observed. Each protein had more than the expected number of cross peaks in SDS at 37 °C (Table 1, and Figures 2B,E). Although the intensities were high, and some line widths narrow, the large number of amides showing two or more cross peaks suggest multiple conformations for each protein in SDS at 37 °C. Increasing the temperature by 5 °C gave spectra with approximately the expected numbers of peaks for subunit *c* and Smr in SDS.

Some detergents gave protein spectra that had approximately the correct number of cross peaks, but had other undesirable properties. For all three proteins, the number of cross peaks in LPPG and DPC were similar. As seen in Figures 2C and F, the characteristics of subunit *c* and the β subunit were less than ideal in DPC (Smr was similar, data not shown). A wide range of intensities, somewhat broader lines, and significant cross peak overlap were observed. Additionally, DPC samples were not reproducible, especially for subunit *c*. Often fewer than half of the expected cross peaks were observed in contrast to the spectrum in Figure 2C. This unpredictable behavior persisted with

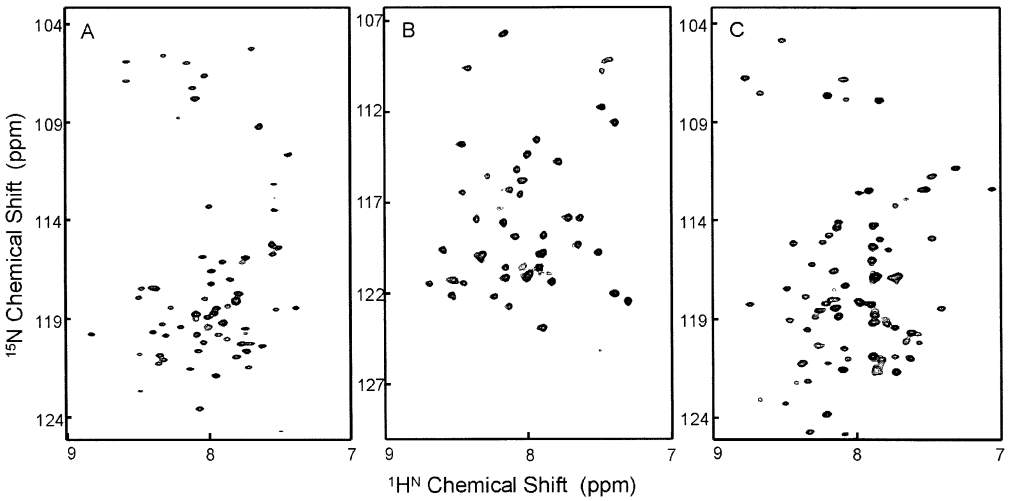

Figure 1. 2D ^{1}H-^{15}N HSQC NMR spectra of three membrane proteins in the most successful detergent discovered in the membrane mimetic screening, LPPG. Shown are (A) subunit *c* of the *E. coli* F_1F_O ATP synthase, 1.6 mM, 5% (w/v) LPPG, pH 6.8, 42 °C, (B) β subunit of the *R. sphaeroides* LH1 antenna 1.2 mM, 8% (w/v) LPPG, pH 6.5, 37 °C and (C) Smr from *S. aureus*, 1.3 mM, 10% (w/v) LPPG, pH 6.0, 42 °C.

different protein preparations, detergent sources, pH values (5 to 8), and temperatures (17 to 47 °C).

Detergent and protein comparisons

Several generalizations can be made from the data summarized in Table 1. Even in detergents where the spectral quality was relatively high, the number of peaks observed was lower than the expected total for the polytopic subunit *c* and Smr. Subsequent 3D experiments showed that for LPPG samples this was due to overlap (data not shown). In general the monotopic β subunit was the most soluble protein, and dissolved readily at 0.3 mM in all of the tested detergents. However, as seen in Table 1, subunit *c* and Smr did not dissolve at sufficient concentration for NMR studies in several detergents. The maximum stable protein concentrations in the lyso-phosphatidylglycerols were inversely proportional to size (1.3, 1.6 and 1.7 mM for Smr, subunit *c*, and the β subunit, respectively). Smr also required more detergent (10% w/v) to reach protein concentrations greater than 1 mM. Both subunit *c* and the β subunit could be dissolved at higher initial concentrations (2.5 to 6 mM), but NMR signal intensities declined over 4 to 24 h to a stable state with intensities and spectral quality similar to samples initially prepared at 1.6 mM.

Marked protein dependent differences in NMR spectral quality were observed for detergents that solubilized millimolar concentrations of protein. Notable examples were DHPC and βOG where nearly all of the expected cross-peaks were observed for the β subunit, but never more than one-third of the expected count for subunit *c* and Smr. The most successful Zwittergent, the 3–12 form, gave all of the expected cross peaks for the β subunit, but only slightly over one-half for subunit *c* and less than one-fifth for Smr. Short half-lives were observed for some detergents with otherwise good spectral properties. Samples of β subunit in Zwittergent 3–12 had excellent spectral properties but samples were only stable for about a week, forcing the use of several samples to solve the structure (Sorgen et al., 2002). The spectra of *c* in LPPC were initially quite favorable (see Figure 3A), but half of the signal intensity was lost in less than 3 days. A comparable example for the β subunit in DHPC is shown in Figure 3B, where 96% of the expected cross peaks were observed initially, but the samples degraded below useful concentrations in less than a day.

Spectral quality was sensitive to hydrocarbon tail length and varied in a similar manner for all three proteins. Hydrocarbon tail lengths from 10–16 for the zwittergent headgroup and 12–18 for the lysophophatidylcholines and glycerols were tested. Chemical shifts changed very little as a function of tail length, with maximal changes of ∼0.1 ppm for protons, and ∼0.4 ppm for nitrogens. However, as summarized in Table 1, signal intensity varied quite significantly. Quality ranged most widely in the zwittergents where subunit *c* and β subunit had maximal peak counts in Zwittergent 3–12 and quite low

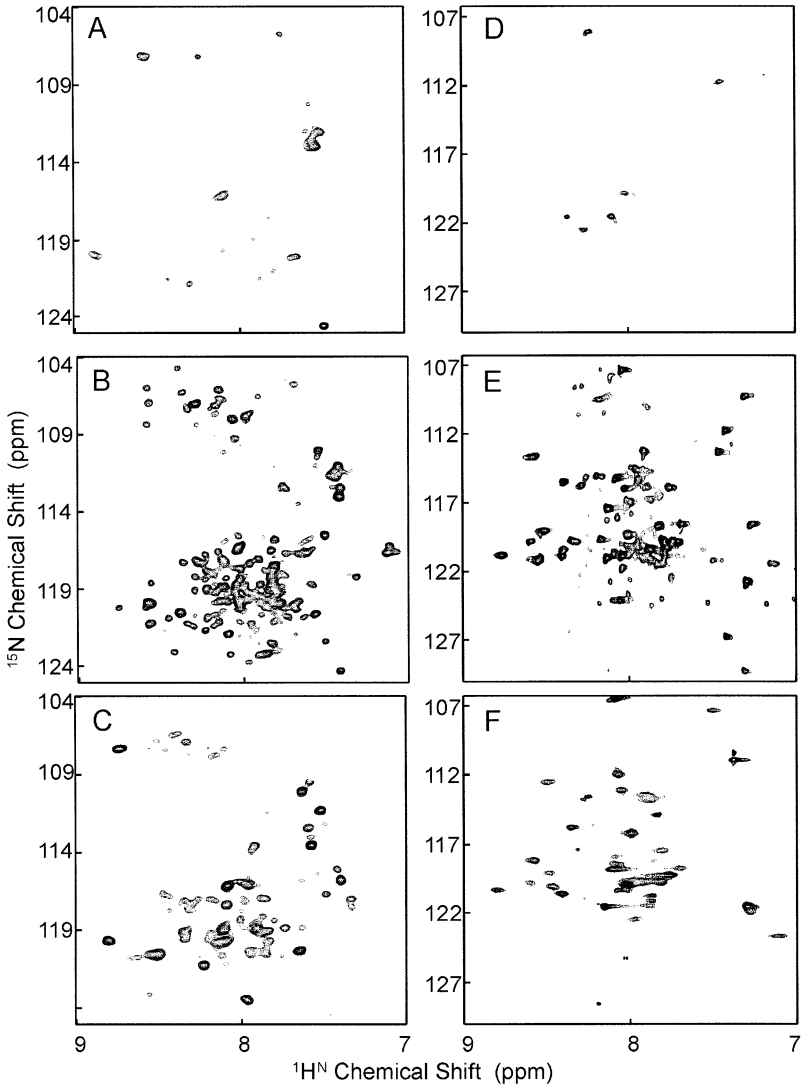

Figure 2. 2D ^{1}H-^{15}N HSQC NMR spectra illustrating the range of quality observed for two membrane proteins as a function of detergent type. Spectra were collected at 37 °C for (A–C) *E. coli* subunit *c* [1.0 mM protein and 5% (w/v) detergent] and (D–F) β subunit [0.8 mM protein and 8% (w/v) detergent] in three detergents: (A,D) APPC, (B,E) SDS and (C,F) DPC.

counts for the other chain lengths, and Smr had less than 20% or the expected number of peaks in the two chain lengths tested. All proteins showed an inverse relationship between peak counts and chain length in the lyso-phophatidylcholines. The lyso-phophatidylglycerol samples had high quality spectra with all chain lengths except for the largest protein (Smr) dissolved in the shortest hydrocarbon chain (LMPG, 14 carbons).

Optimization

Because the amounts of relatively poorly expressed membrane proteins were limited, the trials summarized in Table 1 varied only the membrane mimetic while holding other variables like protein and detergent concentration, temperature, pH and salt concentration constant. The most successful detergents from Table 1 were used in optimization trials.

Solution conditions for subunit *c* were optimized in the most promising detergents from Table 1: LOPG, LPPG, LPPC, DPC, and SDS. Subunit *c* was soluble in each of these detergents to at least 1.3 mM.

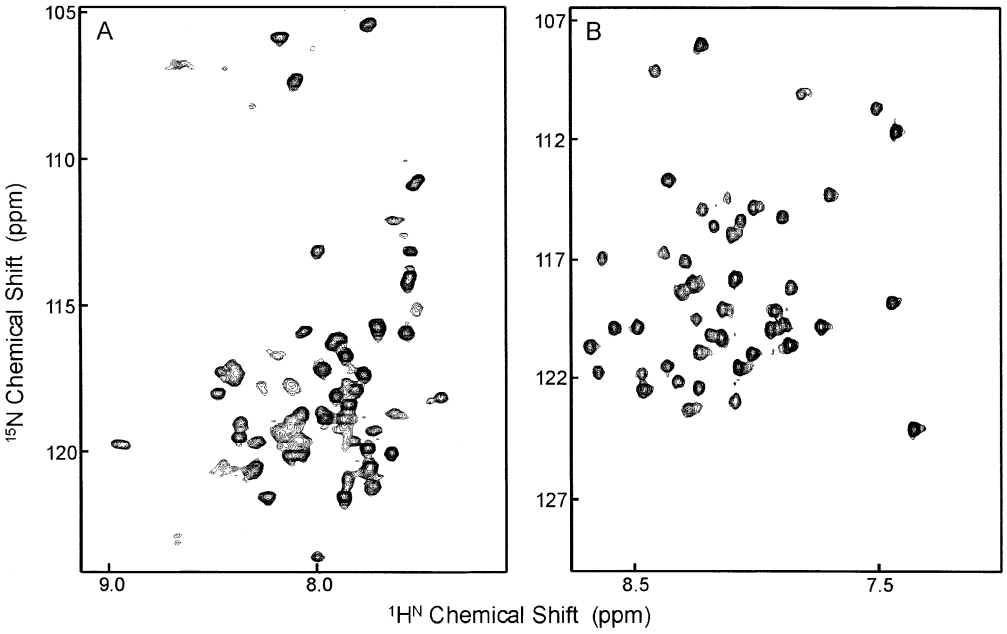

Figure 3. Two spectra typical of results observed when experiments were completed within an hour of dissolving short-lived samples. Initial signal intensities in 2D ^{1}H-^{15}N HSQC NMR spectra shown for (A) *E. coli* subunit *c*, 1.0 mM in LPPC 5% (w/v), 42 °C, pH 6.8 and (B) β subunit, 0.8 mM in DHPC 8% (w/v), 37 °C, pH 6.5, fell below useful levels in a few days.

The spectral quality and half-life as a function of temperature, pH, detergent concentration, and salt concentration were measured. Conditions were varied from the standard sample (1 mM protein, 50 mM potassium phosphate, pH 6.8, 5% (v/v) D_2O, and 5% (w/v) detergent in 300 μl) as described below, and 2D ^{1}H-^{15}N HSQC (eight scans per t_1 increment) and 1D proton (128 scans) NMR spectra at 42 °C (except where noted) were used for the comparisons.

Temperature had the most significant impact on spectral quality and sample lifetimes. For subunit *c* in LOPG and LPPG the number and overall pattern of peaks did not change dramatically over the temperature range from 22 to 48 °C, but the range of intensities and line widths varied. Spectral quality improved with increasing temperature up to 42 °C. At higher temperatures the signals began to broaden and merge. Subunit *c* had a long lifetime (>40 days) at temperatures up to 42 °C in LOPG and LPPG. Samples in LPPG often solidified at room temperature, but melted when the temperature was returned to 42 °C with some permanent loss of signal intensity. LOPG samples were stable at room temperature for as long as 4 months. Samples of subunit *c* in LPPC showed a similar temperature dependence, but with fewer cross peaks and shorter half-lives at all temperatures.

The quality and stability of subunit *c* samples reconstituted in DPC varied more significantly with temperature. Inconsistencies between DPC samples made it difficult to judge, but typically the number of cross peaks and the uniformity of intensity and line width improved at 42 °C relative to 37 °C. However, samples were stable for more than a week at 37 °C but only 1 day at 42 °C. Subunit *c* in SDS was even more sensitive to temperature; a 5-degree increase from 37 °C to 42 °C reduced the peak count from 143% to about the expected value, and *increased* the half-life from 2 days to several weeks. SDS samples typically solidified in a few minutes at room temperature, but melted and recovered their full NMR spectral quality immediately when they were reheated to 42 °C.

For subunit *c* it was especially important to find sample conditions that were stable over the range of pH values where the sidechain carboxylate of the critical Asp61 residue was either fully protonated or deprotonated, since changes in its protonation state are essential to function. The quality of the 2D ^{1}H-^{15}N HSQC spectra of subunit *c* in LPPG or LOPG did not vary widely over a pH range from 4.6 to 8.0. Several cross peaks shifted (the same ones and to approximately the same extent for each detergent), presumably as residues titrated and the C-terminal helix rotated exposing the residues to different chem-

ical environments (Rastogi and Girvin, 1999). Several cross peaks also varied in intensity, but the peak counts were comparable at all pHs. DPC, LPPC and SDS showed markedly different behavior; in these three detergents, several cross peaks began to disappear as the pH was changed from ~7.5 to ~6.5 and below pH 6 the sample half-lives were reduced to a few hours.

Varying the salt (KCl) concentration from 0 to 190 mM in the presence of 10 mM potassium phosphate affected the observed 2D ^{1}H-^{15}N HSQC intensities in a non-specific manner. Total salt concentrations of 50 mM or less were roughly equivalent; slight gains in some signals offset slight decreases in others. KCl at 190 mM concentration reduced the 2D ^{1}H-^{15}N HSQC cross-peak intensities by 8 to 80% compared to the intensities in the absence of KCl. These effects were additive with the salt-dependent decrease in NMR probe sensitivity, which was estimated to be <1% at 15, 3% at 40 and 13% at 190 mM added KCl based on the intensity of the internal TSP standard. Sample stability was insensitive to salt concentration. Salt concentrations below 50 mM had similarly minor effects on the spectral quality of subunit *c* in LPPC, SDS or DPC. Increasing the LPPG concentration from 5 to 15% (w/v) reduced some 2D ^{1}H-^{15}N HSQC signal intensities by as much as 10%, probably due to increased solution viscosity. The other detergents were not tested at concentrations higher than 5%.

Since two of the most successful mimetics (LPPG and LPPC) were derivatives of *E. coli* lipids (dipalmitoyl glyceorolphosphoglycerol and dipalmitoyl glycerolphosphocholine respectively), we attempted to further optimize subunit *c* samples using detergent mixtures that more closely resemble native membranes. Samples with 10, 30, 50 or 70% weight fraction of LPPC and the remainder LPPG (5% w/v total detergent) showed modest differences in their 2D ^{1}H-^{15}N HSQC spectra when compared to pure LPPG. As discussed later and shown in Figure 7, some cross peaks shifted by as much as a tenth of a ppm in proton and by a few tenths of a ppm in nitrogen. Some of the weakest resonances increased in intensity by 5–25% at 10% LPPC. However, at 30% weight fraction LPPC, these intensities fell by approximately the same amount. Further increasing the LPPC fraction reduced intensities across the spectrum. At 70% weight fraction LPPC, 15 peaks had merged or disappeared, thus matching the cross peak count observed for pure LPPC (see Table 1).

The major component of the *E. coli* inner membrane is dipalmitoyl glycerolphospho-ethanolamine. The detergent derivative (LPPE) was unsuccessful alone and in mixtures with LPPG and LPPC at producing suitable samples of subunit *c*. Samples with weight fractions of 50% each LPPG/LPPE or 33% each LPPG/LPPC/LPPE (5% w/v total detergent) did not dissolve completely when reconstituted in aqueous phosphate buffer. Starting with sufficient protein for a 1 mM sample, the final protein concentration (estimated by ^{1}H NMR) was ~ 0.5 mM in the binary detergent mixture, and ~ 0.7 mM for the ternary mixture. The 2D ^{1}H-^{15}N HSQC spectra of the LPPG/LPPE mix had 37%, and the LPPG/LPPC/LPPE mix 56% of the expected cross peaks. Sample lifetimes in mixed detergents were not determined.

Solution conditions were also optimized for Smr in LPPG. The spectral quality showed a broad temperature optimum at 42 °C, similar to subunit *c,* where 86% of the expected cross peaks were observed. The quality was relatively insensitive to changes in pH from 7 down to 4.6. As for subunit *c*, several of the cross peaks shifted, presumably as protonatable side chains titrated. In contrast with subunit *c*, spectral quality deteriorated rapidly above pH 7 with only about half of the peaks detected in a 1 h 2D ^{1}H-^{15}N HSQC at pH 7.4.

Sample conditions for the β subunit in Zwittergent 3–12 had been optimized previously (Sorgen et al., 2002). Spectral quality improved as the temperature was increased from 25 to 45 °C, presumably due to increased tumbling rates, but an irreversible loss of signal occurred above 50 °C. The useful pH range was defined by the need to keep His 38 deprotonated so it could coordinate BChl *a.* The β subunit in Zwittergent 3–12 gave good 2D ^{1}H-^{15}N HSQC spectra from pH 7.5 to 6.5, well within the range where we could demonstrate BChl *a* binding. Spectral quality was insensitive to ionic strength over a range of 0 to 100 mM NaCl.

General applicability of lyso-phosphatidylglycerols

To test the general applicability of LPPG, two other proteins that have proven difficult to reconstitute into detergents in the past were examined: *B. pseudofirmus* OF4 subunit *c* and *R. sphaeroides* LH1 α subunit. Subunit *c* from the ATP synthase of *B. pseudofirmus* OF4, an extreme alkaliphile, is 10 residues shorter than the protein from *E. coli*. Glu54 in the OF4 subunit replaces the critical Asp61 residue of the *E. coli* protein. Both subunits have two TMH and are responsible for coupling proton translocation to ring rotation in the enzyme

complex, but the OF4 subunit *c* must achieve this over a wide range of pH gradients. OF4 subunit *c* reconstitution required detergent concentrations of 10% for reproducible samples. As seen in Figure 4A, OF4 subunit *c* yielded excellent 2D ^{1}H-^{15}N HSQC spectra in LPPG. Over 90% of the expected cross peaks were observed at temperatures ranging from 34 to 47 °C. The half-life of OF4 subunit *c* was approximately 30 days at 42 °C, and approximately doubled at 34 °C. Preliminary trials with OF4 subunit *c* dissolved in DPC and Zwittergent 3–12 gave very poor spectra containing less than 20% of the expected cross peaks.

The LH1 α and β subunits from *R. sphaeroides* both have a single hydrophobic TM helix containing a histidine residue that coordinates BChl *a* in the native antenna. One α and one β subunit with their coordinated BChl *a* form the B820 sub-complex, and lowering the detergent concentration forms the B877 or LH1 complex. It might be expected that the two proteins would behave similarly, but this was not the case. Unlike the β subunit, aqueous detergent solutions (LPPG, LMPC, DPC, or Zwittergent 3–12) did not dissolve lyophilized α subunit. However, modifying the final step of the α subunit purification to desalting over an LH20 column (as in the purification of subunit *c* and Smr) rather than dialysis (as for the β subunit), and drying in the presence of LPPG gave the spectra shown in Figure 4B in which 95% of the expected cross peaks were detected. Other detergents (LMPC, DPC, and Zwittergent 3–12) were somewhat more successful at dissolving α subunit into an NMR sample with this final purification step and reconstitution scheme, but did not approach the quality of spectra observed in LPPG.

Native protein folding in LPPG

The most favorable solution conditions are of little use if the protein is not properly folded into a functional form. Subunit *c* functions by binding and translocating protons. Since proton translocation cannot be measured in the absence of the other F_O subunits and a sealed membrane, we used distance measurements within subunit *c* dimers in LPPG to test whether they were consistent with measurements made *in situ*. In the intact F_O complex, subunit *c* packs into decameric rings (Stock et al., 1999; Jones et al., 1998). A basic requirement for a subunit *c* dimer to represent the native quaternary structure is that the two linked monomers occupy a single micelle and pack against each other, N-terminal helix against N-terminal helix and C-terminal helix against C-terminal helix. A dimer of subunit *c* monomers genetically fused with an eight residue linker (17 kDa), which is fully functional in the F_O complex (Jones and Fillingame, 1998), was used to measure the packing of two subunits in LPPG micelles. Replacing Gly71 with a unique Cys in one subunit and a unique Trp residue in the other subunit of a fused dimer allowed us to estimate the distance between the two in the absence of full NMR assignments for the dimer. The Trp indole NH proton in the second subunit *c* is easily identified in an 2D ^{1}H-^{15}N HSQC or 1D proton spectrum by its downfield shift, and is broadened nearly to baseline by a 3-maleimido-PROXYL spin label linked to C71 (Figure 5). This limits the distance between the two positions to less than approximately 15 Å, and is evidence that the two subunits occupy a single micelle consistent with the positioning expected from inter-subunit cross-linking results in the intact complex (Jones et al., 1998).

As seen in Figure 6A wild type Smr contains a single native Cys residue at position 42 in helix 2, a single Trp reporter at position 62 in helix 3, and is suitable for spin label difference experiments to approximate the extent of helix packing. These two residues are predicted to lie at approximately the same depth in the membrane. Additionally, six of the eight Gly residues are distributed throughout the predicted transmembrane regions of all four helices. Characteristic ^{15}N chemical shifts make Gly residues easy to identify in an 2D ^{1}H-^{15}N HSQC spectrum, providing further indications of how many helices approach a 3-maleimido-PROXYL spin label attached to Cys42 within the 20 Å sphere of broadening. The signal from the side chain amide of Trp62 was reduced in intensity by 60% in the 1D proton NMR spectrum (not shown). The reduced minus oxidized 2D ^{1}H-^{15}N HSQC difference spectrum in Figure 6B showed that over 75% of the observed 2D ^{1}H-^{15}N HSQC peaks were affected by the spin label on Cys42. Significantly, five of the eight Gly peaks were affected. A soluble hydrophilic broadening agent, Chromium(maltolate)$_3$ (Burchfield et al., 1994), was used on another sample to identify the cross peaks for the solvent exposed Gly25 and Gly104, which were not affected by spin label at Cys42.

The β subunit functions as a scaffold that binds BChl *a* cofactors, and positions them to efficiently transfer energy within and between *R. sphaeroides* antennas and reaction center. While no energy transfer can occur in the absence of an intact antenna, BChl *a* binding can be demonstrated in LPPG micelles by observing the 'tuning' of the pigment. When

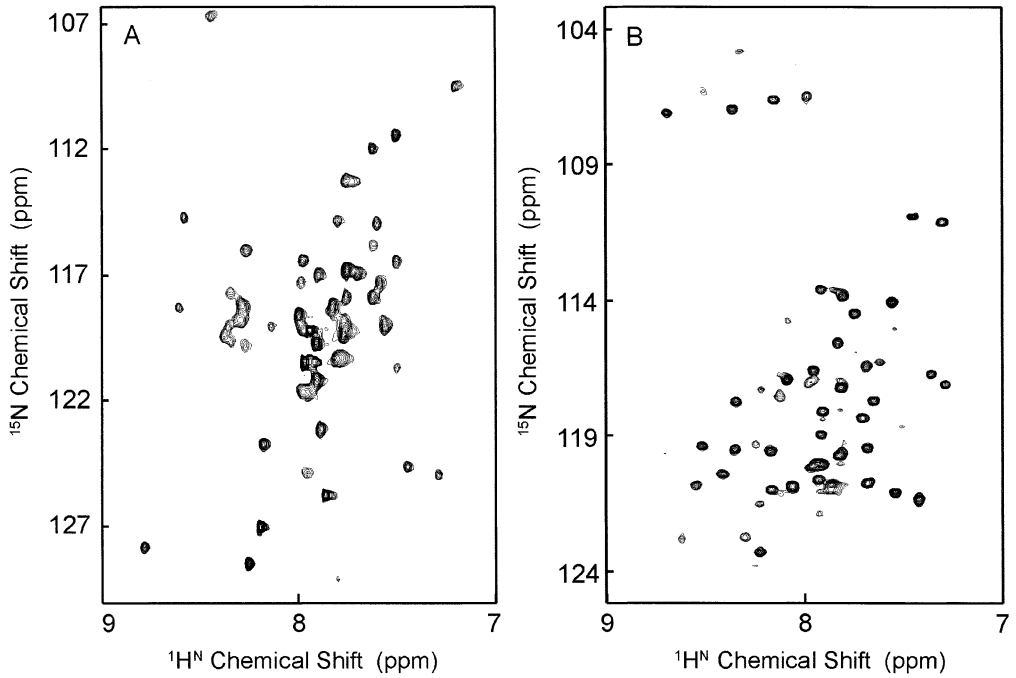

Figure 4. Two proteins that were not screened against a wide range of membrane mimetics were successfully dissolved and gave high quality 2D ^{1}H-^{15}N HSQC spectra on the first attempt in LPPG micelles. All of the expected cross peaks were observed for (A) subunit *c* of the *B. pseudofirmus* OF4 F_1F_O ATP synthase [1.3 mM, 5% (w/v) LPPG, 42 °C, pH 6.8] and (B) α subunit of the *R. sphaeroides* LH1 [0.8 mM 8% (w/v) LPPG, 37 °C, pH 6.5].

BChl *a* binds to the β subunit, its optical absorption maximum shifts from 774 nm to 777 nm. While a shift of this magnitude is difficult to detect, the absorption maximum changes significantly to 820 nm when two β subunit/BChls *a* associate. Optical spectroscopy was used to demonstrate that this dimeric species formed in β subunit/BChl *a*/LPPG mixtures in a detergent/protein concentration ratio dependent manner (data not shown) as had been shown for the β subunit in βOG (Parkes-Loach et al., 1998) and Zwittergent 3–12 (Sorgen et al., 2002). Since pigment binding is thought to involve many weak interactions with the protein, the ability to bind BChl *a* and form complexes provides strong evidence that the β subunit has a native-like fold in LPPG that can satisfy these interactions.

Table 2. Translational diffusion and rotational correlation times for protein-micelle complexes

Protein	Detergent	D_s(cm^2/s)[a]	τ_c (nsec)[a]
β subunit	Zwittergent 3–12	1.8×10^{-6}	8.2
β subunit	DPC	8.7×10^{-7}	9.8
β subunit	LPPG	8.3×10^{-7}	9.8
Subunit *c*	LMPG	1.9×10^{-6}	9.5
Subunit *c*	LPPG	1.8×10^{-6}	10.8
Subunit *c*	LOPG	1.7×10^{-6}	10.8
Subunit *c*	LPPC	1.4×10^{-6}	n.d.[b]
Subunit *c*	LOPC	8.9×10^{-7}	n.d.
Smr	LPPG	1.2×10^{-6}	12.1
OF4 *c*	LPPG	1.6×10^{-6}	n.d.

[a]The LH1 β subunit was measured at 37 °C and subunit *c* and Smr at 42 °C.
[b]Not determined.

Protein-micelle size and dynamics

Translational diffusion constants for the protein-micelle complexes were measured by pulsed field gradient NMR methods. As shown in Table 2, the self diffusion coefficients varied more than observed previously for diacylglycerol kinase (Vinogradova et al., 1998), but all protein/micelle complexes appeared larger than 100 kDa. The high quality of the 2D ^{1}H-^{15}N HSQC spectra in these solutions implied slower relaxation rates than would be expected for a protein tumbling as part of such a large complex. ^{15}N T_1, T_2, and ^{15}N{^{1}H} NOE relaxation measurements were made to obtain overall rotational correlation times for the proteins within the micelles. The rotational correl-

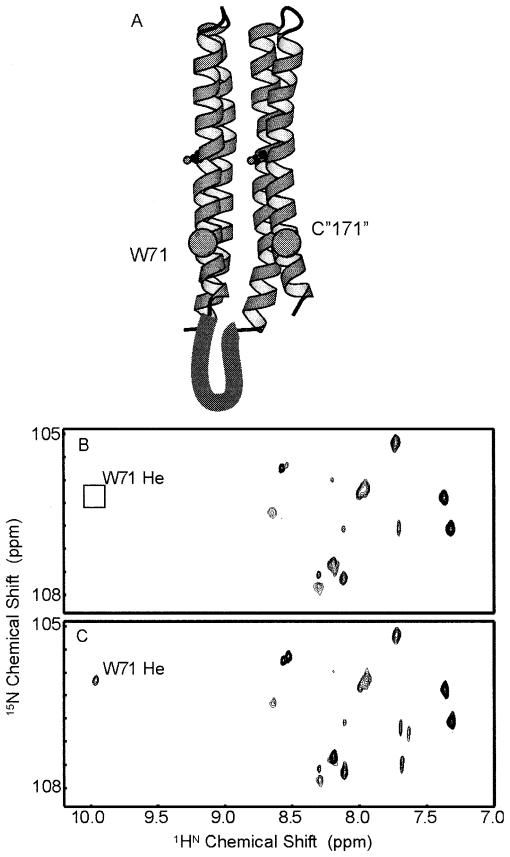

Figure 5. Tertiary folding of subunit *c* dimer in LPPG micelles. (A) Cartoon of genetically fused dimer of *E. coli* subunit *c* with the engineered Cys '171' used to attach a 3-maleimido-PROXYL spin label and Trp 71 used to estimate the distance between the monomers. 2D ^{1}H-^{15}N HSQC NMR spectra of the dimer in LPPG with the spin label (B) oxidized (paramagnetic) and (C) reduced (diamagnetic).

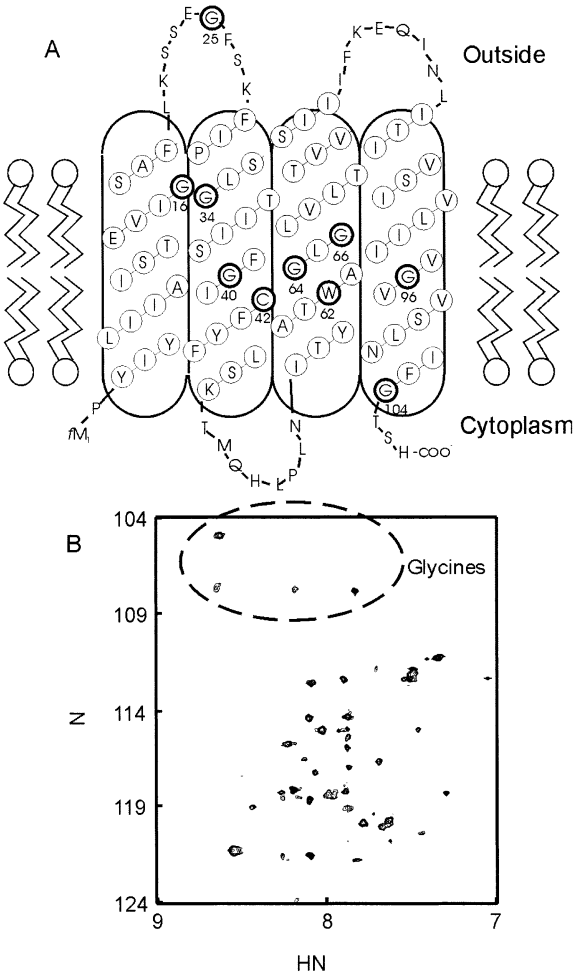

Figure 6. Tertiary folding of Smr in LPPG micelles. (A) Cartoon showing the predicted transmembrane helices of Smr with Cys, Trp and Gly residues numbered. (B) 2D ^{1}H-^{15}N HSQC NMR difference spectrum from C42-3-maleimido-PROXYL Smr. Cross-peaks in this spectrum represent signals that remain after the 2D ^{1}H-^{15}N HSQC spectrum with the spin label in its oxidized (paramagnetic) state was subtracted from the reduced (diamagnetic) spectrum.

ation times were surprisingly short – from 8 to 12 ns, a range expected for a 15–20 kDa protein tumbling isotropically in solution. Hence the detergent appears fluid enough to permit rotation of the proteins within the confines of the micelle. Longer correlation times were observed for the β-barrel PagP in DPC micelles (20 ns at 45 °C; Wang et al., 2002), but translational diffusion measurements were not reported. The overall rotational correlation times and translational diffusion constants for subunit *c* were relatively constant in LMPG, LPPG, and LOPG detergents (τ_c = 9.5, 10.8 and 10.8 nsec, respectively). There was no measurable difference in the overall rotational correlation time for the β subunit in LPPG and DPC although there was a distinct difference in the quality of the spectra collected in these two detergents (compare Figure 1A and Figure 2G). The observed correlation times were consistent with all three proteins being monomeric.

Detergent dependent variation of protein structure

The high quality of the 2D ^{1}H-^{15}N HSQC spectra of subunit *c* in LPPG, LPPC, and SDS, and the sensitivity of amide proton and nitrogen chemical shifts to environment provided a crude method to investigate variations in structure (or detergent-protein interactions) between these three membrane mimics. Figure 7 shows the chemical shift changes for the backbone amides of subunit *c* on changing the detergent from

LPPG to SDS or LPPC. Cross peak identities, based on positions in the LPPG spectrum, were maintained by tracking the signals in a series of 2D ^{1}H-^{15}N HSQC spectra as they shifted in small amounts on increasing the fraction of either SDS or LPPC relative to LPPG. Much larger chemical shift changes were associated with moving from LPPG micelles to SDS micelles than to LPPC micelles.

Discussion

The behavior of membrane proteins in different detergents is difficult to predict. An ideal detergent for solubilizing and stabilizing a membrane protein for structural study should be gentle enough to avoid even partial denaturation, but strong enough to prevent aggregation at high protein concentrations. It would be expected that membrane proteins would have different tolerances and requirements for stability in a detergent, since each protein has different inherent stabilities and structural features. In this study a range of detergents was tested to determine their effectiveness for use in samples for high-resolution NMR and other structural studies. Representatives of the single TMH, two TMH, and multiple (here four) TMH classes of membrane proteins were tested with various detergent types. The surprising result was that a single group of detergents, the lyso-phosphatidylglycerols, stood out as clearly superior for all proteins tested (for a description of their general properties see (Stafford et al., 1989)). While similar success can not be guaranteed for every membrane protein, the results of this study strongly suggest that these lysolipids should be among the detergents tested when working with a new protein. The success of LPPG with OF4 subunit *c* and the LH1 α subunit, two proteins that stubbornly resisted reconstitution into detergents in the past, were encouraging in this regard.

The ability of lyso-phosphatidylglycerols, and to a lesser extent lyso-phosphatidylcholines, to discourage aggregation is apparent from the lifetime of the protein samples – one or more months at room temperature, and from the quality of the 2D ^{1}H-^{15}N HSQC spectra observed over this period. The ability to prevent aggregation is especially challenging for the proteins studied here. The reconstitution method ensured that the proteins were initially solubilized as monomers, or dimers for the fused subunit *c* construct, but all of the tested proteins have a strong tendency to form oligomers. Possible explanations for this stability are: (1) That the long acyl chains reduce the solubility of free detergent monomer, minimizing micelle-micelle exchange, (2) that favorable interactions with the native lipid functional groups (glycerol backbone and native headgroup) stabilize the folded protein, and (3) that the negative headgroup on lyso-phosphatidylglycerols leads to electrostatic repulsion between micelles, reducing the opportunities for aggregation to occur.

Unlike harsh detergents, the lyso-phosphatidylglycerols retard aggregation without denaturing. The negative headgroup on LPPG does not lead to its mimicking the behavior of SDS. Transferring subunit *c* from LPPG to SDS induced large chemical shift changes, indicative of major conformational changes such as denaturing, while transferring it to LPPC caused only minor changes in chemical shifts. It was not possible to track chemical shift changes into DPC, but the pattern of chemical shifts in LPPG and DPC were similar. Lysolipids have been used in the past to isolate large membrane proteins and membrane protein complexes in native, functional form (Foury et al., 1981; Huang et al., 1998; Palmgren et al., 1990; Hennessey and Scarborough, 1988). The present work demonstrates that the expected helix-helix contacts are observed in monomers of Smr and dimers of subunit *c*. Furthermore, if all the associating components are present, native oligomeric species can be reconstituted from monomeric subunits in LPPG, as shown here for the B820 subcomplex of LH1.

Despite these favorable properties, the lysolipids have not been widely used for structural studies. The lysolipids are not included in most tabulations of detergents for membrane protein purification and study (Henry and Sykes, 1994; von Jagow et al., 1994; Michel, 1990), but lysolipids have been used to characterize the folding properties of CFTR segments (Therien and Deber, 2002) and OmpA (Kleinschmidt et al., 1999). Williams et al used LMPG in solving the solution structure of the single TMH IKe coat protein (Williams et al., 1996) – the only NMR study using a lysolipid that we could identify. Most NMR studies of single-helix membrane proteins have used SDS or DPC as the detergent (Damberg et al., 2001). Two outer membrane β-barrel protein structures have been solved using DPC (Arora et al., 2001; Hwang et al., 2002), and one using DHPC (Fernandez et al., 2001). Most of the backbone resonances of the trimeric diacylglycerol kinase have been assigned in DPC micelles (Oxenoid et al., 2002). None of these detergents performed nearly as reliably as LPPG for any of the proteins in the present study.

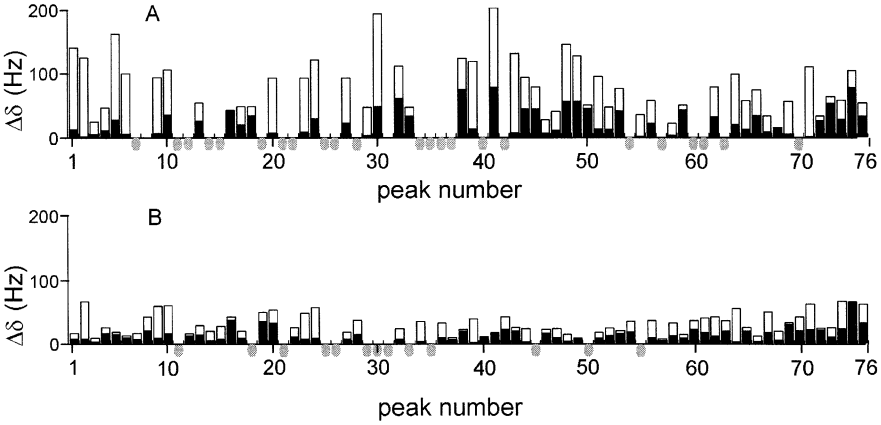

Figure 7. Histograms showing the absolute value of the difference in ^{1}H chemical shift (white) and ^{15}N chemical shift (black), both expressed in Hz, for 76 cross-peaks from the non-proline residues of *E. coli* subunit c in LPPG compared to (A) SDS and (B) LPPC. Peak numbers are from the LPPG spectra and gray ovals indicate cross-peaks that could not be tracked.

This survey used 2D ^{1}H-^{15}N HSQC spectra of ^{15}N labeled proteins (over two grams total) to assess sample quality and stability. The HSQC is an obvious choice because it forms the basis for most three-dimensional NMR experiments. The cross peak properties in a 2D ^{1}H-^{15}N HSQC are sensitive to the conformational stability and the oligomerization state of a protein in solution, and a good spectrum is a prerequisite for a structural study. The variation in 2D ^{1}H-^{15}N HSQC quality between detergents could have arisen from conformational heterogeneity, conformational exchange, or from aggregation. Backbone ^{15}N relaxation measurements were not possible in the poorer detergents, but rotational correlation times measured in intermediate detergents, such as DPC, were similar to those in LPPG. Hence the most likely explanation for moderately poor spectra is conformational heterogeneity and conformational exchange, while the disappearance of cross peaks over time likely corresponds to aggregation. Extrapolating the trend in rotational correlation times in LPPG micelles with increasing molecular weight would predict that a 45 kDa membrane protein would have a rotational correlation time of about 23 ns, well within the range accessible to TROSY based NMR methods. In summary, the lyso-phosphatidyl-glycerols appear to stabilize homogeneous conformations and prevent aggregation of monotopic and polytopic membrane proteins at high concentrations over very long times – conditions which should be useful for most structural and biophysical studies of membrane proteins in micelles.

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