Peptergents: Peptide Detergents That Improve Stability and Functionality of a Membrane Protein, Glycerol-3-phosphate Dehydrogenase†

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ABSTRACT: Toward enhancing in vitro membrane protein studies, we have utilized small self-assembling peptides with detergent properties ("peptergents") to extract and stabilize the integral membrane flavoenzyme, glycerol-3-phosphate dehydrogenase (GlpD), and the soluble redox flavoenzyme, NADH peroxidase (Npx). GlpD is a six transmembrane spanning redox enzyme that catalyzes the oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate. Although detergents such as *n*-octyl-β-D-glucpyranoside can efficiently solubilize the enzyme, GlpD is inactivated within days once reconstituted into detergent micelles. In contrast, peptergents can efficiently extract and solubilize GlpD from native *Escherichia coli* membrane and maintain its enzymatic activity up to 10 times longer than in traditional detergents. Intriguingly, peptergents also extended the activity of a soluble flavoenzyme, Npx, when used as an additive. Npx is a flavoenzyme that catalyzes the two-electron reduction of hydrogen peroxide to water using a cysteine-sulfenic acid as a secondary redox center. The lability of the peroxidase results from oxidation of the sulfenic acid to the sulfinic or sulfonic acid forms. Oxidation of the sulfenic acid, the secondary redox center, results in inactivation, and this reaction proceeds in vitro even in the presence of reducing agents. Although the exact mechanism by which peptergents influence solution stability of Npx remains to be determined, the positive effects may be due to antioxidant properties of the peptides. Peptide-based detergents can be beneficial for many applications and may be particularly useful for structural and functional studies of membrane proteins due to their propensity to enhance the formation of ordered supramolecular assemblies.

Structural and functional studies of membrane proteins are often complicated by difficulties in obtaining active reconstituted material (*1*, *2*). Typical solubilization and purification procedures involve extracting the protein from the native membranes and reconstituting them into detergent micelles. Detergents, consisting of short-chain fatty acid amphiphiles, are routinely used in solubilization of proteins from native membranes (*3*). For crystal structure studies, detergents that form type II micellar structures, characterized by high critical micellar concentration ("CMC"), have been used most successfully for crystallization, although these detergents can have an adverse effect on protein stability. It is thought that the small micelle size allows these protein-embedded micellar surfaces to approach each other so that polar domains of proteins can contact, allowing for enhanced interaction to promote lattice formation (*4*, *5*).

Short peptides possessing detergent properties ("peptergents") have been developed to self-assemble into well-ordered nanostructures and mimic some of the properties of lipid surfactant molecules (*6*). Polar interactions among headgroups are known to be necessary in the stabilization of the crystal lattice (*7*). As these peptergents possess optimizable chemical properties, mixtures of cationic and anionic peptides can result in unusual charge and polarity characteristics, leading to alteration of interactions between the peptides and proteins. Furthermore, peptergents form higher ordered structures in a concentration-dependent manner (*8*, *9*).

Peptergents typically consist of a short hydrophobic tail produced by repeat copies of nonpolar amino acids and a hydrophilic headgroup. The head can be either positively charged or negatively charged so that peptergents are consequently categorized as cationic or anionic detergents, respectively. Negatively charged heads consist of one or two aspartate or glutamate residues, and positively charged heads consist of one or two lysine, arginine, or histidine residues. In this study, V_6D, V_6K, A_6D, A_6K, and mixtures of A_6D:A_6K and A_6D:V_6K were used to solubilize and stabilize the enzymes of interest.

V_6D (VVVVVD) is a peptergent that has biochemical properties that resemble those of lipids and other organic detergents. Structurally, in addition to its hydrophilic head and hydrophobic tail, V_6D resembles a phospholipid molecule in its length of approximately 2 nm (*10*). Functionally, V_6D, along with other peptergents, resembles lipids in their

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self-assembling properties, by sequestering their hydrophobic tails from aqueous solutions and self-organizing to form nanomicelles, nanotubules, and nanovesicles in water (*8*, *9*, *10*).

A_6D (AAAAAAD), like V_6D, is an anionic peptergent, whereas A_6K (AAAAAAK) and V_6K are cationic peptergents. Aside from A_6K's positive headgroup, both A_6D and A_6K have similar physical properties and nanostructure-forming abilities as that of V_6D (*10*). The observation that peptergents self-assemble into distinct and ordered topologies, resembling bilayer formation by native lipids, led us to investigate the application of these peptides for structural and functional studies of membrane proteins. In vitro studies of membrane proteins are complicated by a multitude of obstacles. A common problem is the low stability of detergent-extracted membrane proteins, which can lead to their inactivation (*1*, *2*). Often, the processes required to extract these proteins from their native bilayer environment can impair the stability of membrane proteins. The choice of an appropriate detergent is vital for efficient extraction, for enhancing stability of the solubilized protein, and for successful crystallization (*2*, *4*, *5*). High quality crystals often require weeks of growth, so detergent-solubilized proteins must maintain the native protein fold for at least this amount of time (*3*, *11*).

We investigated the ability of peptergents to extract the integral membrane protein, glycerol-3-phosphate dehydrogenase (GlpD[1]) (*12*, *13*, *14*), from native *Escherichia coli* membrane and maintain its activity over time. We also studied the effect of peptergents on stabilizing the oxidation-labile *Enterococcus casseliflavus* NADH peroxidase (Npx) (*15*). In both cases, the proteins in the presence of peptergents maintained their time-dependent enzyme activities 3 to 10 times longer than in traditional detergents.

MATERIALS AND METHODS

Materials. Chemicals were from Sigma (St. Louis, MO). Nickel nitrilotriacetate (Ni^{2+}-NTA) and XL1-Blue cells were from Qiagen (Valencia, CA). Total phospholipid extract from *E. coli* was from Avanti Polar Lipids (Alabaster, AL).

GlpD Expression. Starter cultures were grown overnight at 37 °C and supplemented with 100 μg/mL of ampicillin and 12.5 μg/mL of tetracycline. These cultures were diluted 100-fold into fresh media and allowed to grow to an optical density at 600 nm (OD_{600}) of ~0.7. Protein expression was induced by addition of IPTG to a final concentration of 1 mM, and the culture was then grown for an additional 4 h. Cells were harvested by centrifugation at 6000*g* for 10 min at 4 °C. Cells were resuspended in a 1:100 culture volume of cold buffer A (10 mM Tris pH 7.5, 2 mM DTT) and lysed by three passages through a French pressure cell (American Instruments Co., Silver Spring, MD). Cell debris and unbroken cells were removed by centrifugation at 9000*g* for 45 min at 4 °C. The supernatant was ultracentrifuged at 100000*g* for 90 min at 4 °C to separate soluble proteins from membrane proteins. The membrane protein containing pellet was resuspended with a homogenizer in buffer A supplemented with 0.2 M KCl at a volume 1:100 of the original culture. The solution was ultracentrifuged for 90 min at 100000*g* at 4 °C to remove peripheral membrane proteins from integral membrane proteins.

GlpD Solubilization and Purification. For solubilization of the hexahistidine-tagged GlpD, the KCl-washed pellet was resuspended in the appropriate detergent or peptergent buffer solution to obtain a final protein concentration of approximately 3−4 mg/mL. To maintain the proper detergent concentration throughout the purification and the assays, all buffers were generally made as 2X and supplemented with the desired 2X detergent solutions in water in a 1:1 ratio. The final concentrations of detergents were 1.75 times the CMC. Concentrations used were as follows: 3.08 mM DM (510.6), 13.68 mM CHAPS (MW 614.9), 42.75 mM OG (MW 292.4), 0.41 mM Triton X-100 (MW 631), and 14.02 mM SDS (MW 288.4). For all peptergents, the final concentration used was 0.5 mg/mL. Pellets obtained after ultracentrifugation were homogenized, incubated with either a detergent or peptergent (or peptergent mixture) at the concentration indicated above, and incubated for 2 h to overnight at 4 °C. After incubation, the solution was ultracentrifuged for 90 min at 100000*g* at 4 °C to separate solubilized membrane proteins from unextracted membrane proteins. The total amount of protein solubilized was determined by a dye-binding assay (Bradford), and the amount of GlpD was estimated as a percentage of this total by visualization of the SDS−PAGE results.

The solubilized GlpD membrane protein was batch-purified using 1 part Ni^{2+}-NTA slurry (composed of 50% resin to buffer) to 4 parts solubilized protein solution. After a 3-h incubation with gentle mixing at 4 °C, excess buffer was allowed to flow out of column and the column was washed twice with two column volumes of wash buffer (10 mM Tris pH 7.5, 300 mM NaCl, 10 mM imidazole, plus detergent or peptergent). The polyhistidine-tagged protein was step-eluted with buffer containing imidazole (10 mM Tris pH 7.5, 300 mM NaCl, 300 mM imidazole, plus detergent or peptergent). The GlpD obtained was >95% pure; as needed, the protein was concentrated using Centricon YM-50 spin filtration units (Amicon, Bedford, MA).

GlpD Enzymatic Assay. A method using PMS/MTT was adapted to quantitate the activity of GlpD (*12*, *16*). Each 250 μL microcuvette contained the following: 50 mM Tris/HCl pH 7.4, 75 mM NaCl, 0.5 mM MTT, 0.2 mM PMS, and 7.2 nM GlpD. This was used as the blank, and the reaction was initiated with the addition of 20 mM DL-glycerol-3-phosphate. The reduction of MTT ($\epsilon = 17\ mM^{-1}\ cm^{-1}$) at 570 nm was continuously monitored on a Cary 50 UV−vis spectrophotometer (Varian, Palo Alto, CA) for 15 min at room temperature. One unit of enzyme activity is defined as the reduction of 1 μmol of MTT per min per mg of protein.

Lipid Content. The amount of lipid phosphate in GlpD samples solubilized and purified with various detergents and peptergents was determined through quantitating the phosphate content using a microdetermination method (*17*).

Npx Enzymatic Assay. Protocols for the expression, purification, and quantification of NADH peroxidase activity

[1] Abbreviations: FAD, flavin adenine dinucleotide; PMS, phenazine methylsulfate; MTT, 3-(4,5-dimethylthiazolyl-2-)-2,5-diphenyltetrazolium bromide; GlpD, glycerol-3-phosphate dehydrogenase; OG, 1-O-*n*-Octyl-β-D-glucopyranoside; G3P, *sn*-glycerol-3-phosphate; DTT, DL-dithiothreitol; DM, 1-O-*n*-Dodecyl-β-D-glucopyranosyl(1→4)α-D-glucopyranoside; SDS, sodium *n*-Dodecyl sulfate; CHAPS, 3[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate.

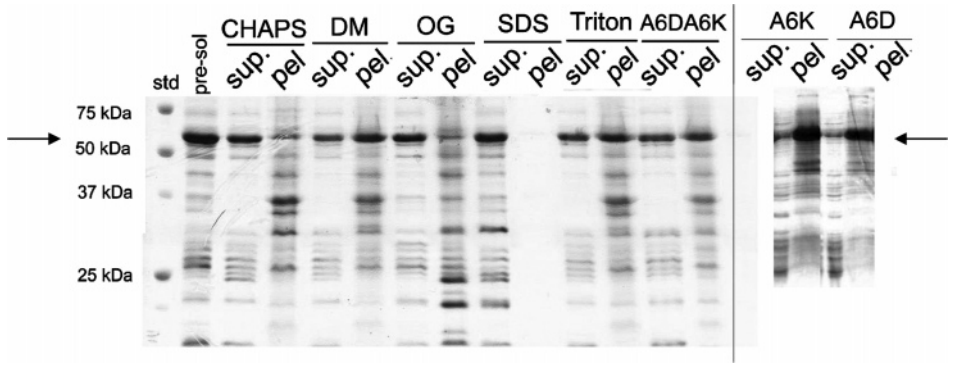

FIGURE 1: Solubilization of GlpD. The pellets containing all integral membrane proteins were incubated with various detergents and a 40:60 peptergent mixture of A_6D:A_6K for 1 h at 4 °C (left panel). Solubilized membrane proteins (lanes "sup") were separated from unsolubilized ("pel") by spinning at 100000*g* for 1 h at 4 °C. In a separate analysis, individual A_6D and A_6K were analyzed for solubilization efficiency (right panel).

have been described elsewhere (*15*, *18*). Quantitation of Npx activity is by a spectroscopic assay that determines the initial velocity (*18*). The assay mixture contained 0.1 M potassium acetate buffer, pH 5.4, 0.3 mM EDTA, 0.16 mM NADH, and 1.3 mM H_2O_2, and enzyme at 20.2 nM. Peptergents, if present, were added stoichiometrically in a molar ratio of peptergent to Npx enzyme. NADH was added just prior to the assay to avoid nonenzymatic oxidation. The reaction was begun by adding 0.010−0.014 unit of enzyme and was followed by the decrease in 340 nm absorbance over 3 min, during which time the rate was linear. Rates were corrected for the spontaneous acid-catalyzed oxidation of NADH. One unit of activity is defined as the amount of enzyme that catalyzes the net oxidation of 1 μmol of NADH/min at 25 °C.

Typically, Npx is oxidized over a period of 3−5 days at 4 °C, resulting in a concomitant decrease in activity. Because oxidation of the recombinantly expressed enzyme can be variable under ambient conditions, all assays were performed in parallel using the same enzyme preparation. The specific activity of the fully active enzyme is ∼130 units/mg (*18*), and this value was used to normalize the specific activity for analysis.

RESULTS

I. GlpD

Membrane Protein Extraction. Each detergent and peptergent studied was able to extract, to varying extents, the membrane-embedded GlpD (Figure 1). For the nonionic detergents, DM and Triton X-100, both extracted approximately 50% of the membrane-associated GlpD and OG extracted approximately 90% of the GlpD enzyme from the membrane. The mild, zwitterionic detergent CHAPS extracted greater than 70% of the GlpD. As expected, the strong ionic detergent SDS extracted nearly all of the GlpD, as well as most other proteins. The peptergents, A_6D and A_6K, each extracted about 25% of GlpD when used as solubilizing agents by themselves (Figure 1, right panel). However, when used in a 40:60 mixture of A_6D:A_6K, the peptergents solubilized about 60% of the GlpD from the membrane comparing amount solubilized in the supernatant versus unsolubilized remaining in the pellet (Figure 1, left panel). All lanes on each gel were loaded to keep the amounts of proteins consistent, about 5 μg of total protein.

Membrane Protein Purification. With the exception of SDS, the different detergents or peptergents yielded similar protein purification results. The polyhistidine-tagged GlpD protein eluted mostly in the first fraction following the addition of 300 mM imidazole, with a purity of about 95%. The protein concentrations of this first eluate ranged between 1.5 and 3 mg/mL. With the exception of the SDS-solubilized samples, which did not bind to the column, little or no protein was detected in the initial flow-through or wash steps (data not shown).

Assay of GlpD Enzymatic Activity. The enzymatic activity of GlpD was determined using a PMS-coupled MTT reduction assay. The reduction of MTT to formazan is coupled to the oxidation of glycerol-3-phosphate to dihydroxyactetone phosphate, which is catalyzed by GlpD. Plots were made to show the increase in absorbance at 570 nm due to MTT reduction vs time. The value for the absorbance was normalized with respect to the concentration of the protein used in the assay (typically around 100 ng per 250 μL of assay volume).

For all curves, the plots show a gradual increase of the absorbance at 570 nm over the time of the assay, 15 min (Figure 2). The absorbance reached its highest value after the 15 min, when the enzyme was in the presence of OG. The value for OG absorbance was closely followed by the values for peptide V_6D, after which Triton X-100, DM, and A_6D:A_6K were clustered. CHAPS yielded the lowest activity of the non-denaturing detergents, approximately a factor of 3 less than OG. Samples with SDS, as expected, showed no activity. The standard deviation in the GlpD enzyme assay is approximately ±10%, indicating that the peptergent-solubilized (V_6D) GlpD activity was similar to that of the OG-solubilized enzyme (Figure 2).

Controls were performed by omitting one of the assay components to investigate possible changes in absorbance caused by changes in solution composition rather than from oxidation of glycerol-3-phosphate to dihydroxyacetone phosphate by GlpD. Negligible absorbance readings were recorded when the enzyme, the substrate, or MTT was omitted (data not shown). When the intermediate electron carrier, PMS, was omitted, the reaction still occurred, but the rate of change of absorbance was lower by a factor of 3, similar to what was previously noted (*12*).

Variation of Salt Concentration. The osmotic strength of solutions can affect the solubilization and stabilization of

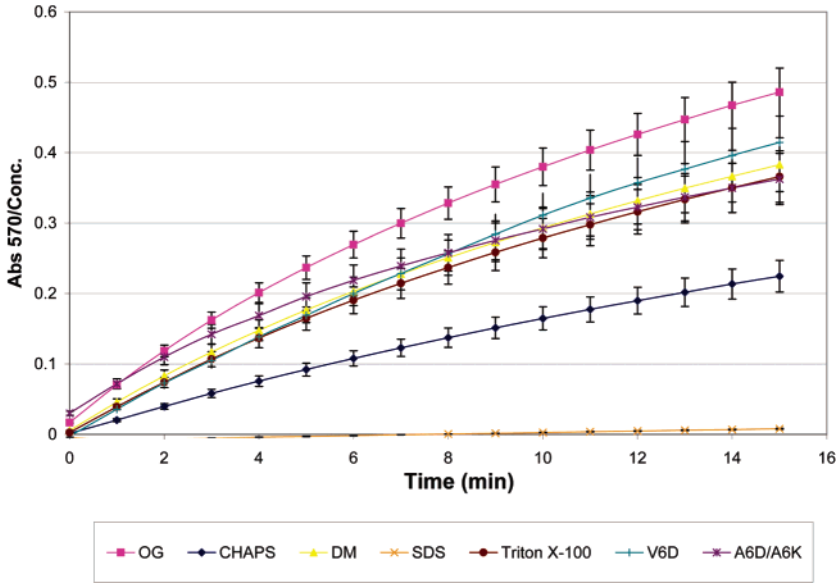

FIGURE 2: Enzymatic activity of GlpD. Changes in absorbance due to MTT reduction as a result of GlpD activity was measured over 15 min (square, OG; diamond, CHAPS; triangle, DM; ex, SDS; circle, Triton X-100; vertical bar, V_6D; diamond with ex, A_6D:A_6K).

Table 1

detergent/peptide	specific activity (±10%)
OG	16.7
CHAPS	5.3
DM	10.1
SDS	0.2
Triton X-100	9.5
A_6D:A_6K	12.8
V_6D	14.3
OG no NaCl	15.7
OG 200 mM NaCl	25.1
V_6D no NaCl	20.5
V_6D 200 mM NaCl	14.6
A_6D:A_6K no NaCl	14.2
A_6D:A_6K 200 mM NaCl	19.0
presolubilized membrane	26.8

membrane proteins (*5*). The salt concentration of the assay was varied to further investigate the mechanism by which membrane protein extraction proceeds in the presence of peptergents. The typical MTT reaction assay contains 75 mM NaCl. To ascertain the effect of salt concentration, the assay was run without NaCl and with NaCl at a concentration of 200 mM. For OG solubilized GlpD, there was no significant difference between the activity at 0 and 75 mM NaCl. However, the activity of the enzyme increased by a factor of about 1.5 at 200 mM NaCl (Figure 3A). The same trend was observed with peptergent mixture, A_6D:A_6K (Figure 3B). In contrast, with V_6D, GlpD exhibited the highest activity in the absence of NaCl (Figure 3C).

Specific Activity. The specific activity of GlpD, solubilized in detergents and peptergents, was calculated under various salt concentrations. One unit of activity of GlpD is defined as equivalent to 1 μmol of MTT reduced per min. Table 1 summarizes the average specific activities for GlpD after 10 min with an error of ±10%. GlpD was most active in its native membrane bilayer ("presolubilized"), with a specific activity of 26.8 units/mg. The lowest enzyme activity was obtained with the zwitterionic CHAPS and anionic SDS. GlpD solubilized with OG in the presence of 200 mM NaCl, had a specific activity of 25.1 units/mg, approaching that of the enzyme in its native membrane. Both V_6D and A_6D:A_6K peptergents maintained the activity of GlpD, approaching up to 77% of the maximum value (i.e. activity of GlpD in presolubilized membrane). Controls, which omitted either enzyme or substrate, possessed specific activities less than 0.3 unit/mg.

Table 2

detergent/peptide	% lipid (±5%)
OG	0.4
CHAPS	2.8
DM	10.1
Triton X-100	14.7
A_6D:A_6K	9.3
V_6D	3.4

Time Dependent Specific Activity. The specific activity of GlpD solubilized in the various detergents and peptergents was measured over two weeks (Figure 4). The activity of GlpD was highest in OG immediately after solubilization, but its activity starts to decrease very rapidly, losing over 40% of its activity within the first day when reconstituted with OG. When solubilized with CHAPS and DM, GlpD's initial activity was lower but the rate at which the enzyme lost its activity was significantly slower than in OG. Using peptergents A_6D:A_6K and V_6D for solubilization, the enzyme's initial activity was 11% to 1% less active than in OG, respectively. However, the rate at which the enzyme lost its functionality was significantly decreased, retaining up to 90% of it initial activity after 2 weeks in both cases.

Lipid Content. Table 2 summarizes the results from microdetermination of phosphates. From the residual phosphate content, it can be seen that OG delipidates the protein to the greatest extent, leaving less than 1% lipid in the purified samples. The other detergents and peptergents permitted greater carry-over of endogenous lipids, varying from 3% to 15%.

II. Npx

Npx Enzymatic Activity Assay. Npx is labile, rapidly losing activity under aerobic conditions via spontaneous oxidation of its secondary redox center, a cysteine sulfenic acid

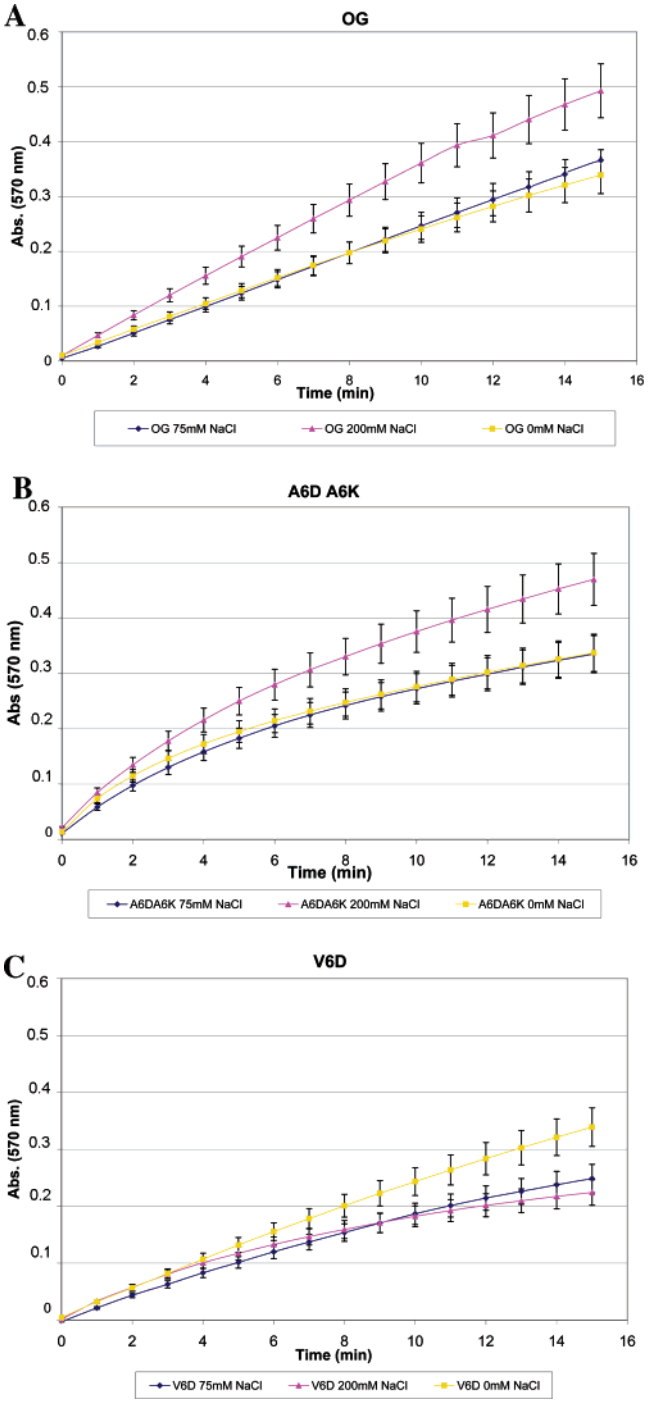

FIGURE 3: Enzymatic activity of GlpD under varying salt concentrations. GlpD retained most activity at high salt concentration in the presence of OG and the peptergent mixture A_6D:A_6K, while in V_6D, activity of the enzyme was decreased at higher salt concentrations (diamond, 75 mM NaCl; triangle, 200 mM NaCl; square, 0 mM NaCl).

(−SOH), to the sulfinic ($-SO_2H$) and sulfonic ($-SO_3H$) forms. Formation of the higher oxidized forms results in the irreversible inactivation of enzyme. The reduction in activity is a time-dependent process under ambient conditions and can be variable in recombinant protein preparations. To help prevent oxidation of the sulfenic acid, reducing agents are added and the enzyme is stored frozen at −20 °C if not used within 24 h. Although the presence of reducing agents can slow the oxidation of the sulfenic acid, the enzyme is nonetheless oxidized over a period of several weeks at 4 °C and at −20 °C. Consequently, in this study, maintenance of enzyme activity over time is taken to be a hallmark of stabilizing effects of various additives. The presence of the peptergents V_6K, A_6D, and a 40:60 mixture of A_6D:A_6K all extended the enzymatic half-life to over 3−5 times longer than in its absence (Figure 5). The stabilizing effects of the peptergents are very marked, extending the peak activity of Npx from 3 days to 15 days. The effects of peptergents were comparable to those of the phosphine reducing agent TCEP and were more effective than glutathione and β-ME. Both glutathione and β-ME become readily oxidized in a pH- and metal-dependent manner.

DISCUSSION

Our results show that small amphiphilic peptides are capable of efficiently extracting integral membrane proteins from endogenous membranes. These amphiphiles appear to be benign for the protein extraction, possibly by mimicking the tight packing of the native membrane environment more closely than detergent micelles. Most promising is their ability to extend the activity of the integral membrane enzyme, GlpD, over time. How peptergents and membrane proteins interact, particularly the topological form adopted by peptergents in the presence of proteins, remains to be elucidated.

Studies to date on the topology of the peptergents have been conducted in the absence of proteins. It is unknown whether peptergents are in particular topological forms when membrane proteins are present. We speculate that it is likely that the membrane proteins embed or partition into the intact topology—nanotube, micelle, or liposome—during solubilization. This is similar to the accepted mechanism by which detergents and lipids interact with membrane proteins. Detergents form micelles in a concentration dependent manner. Once micelles are formed, membrane proteins partition into intact micelles. Peptergents possess properties similar to lipids—i.e., self-assembly and formations of extended amphiphilic topologies—indicating a more membranelike environment.

The specific activity of GlpD solubilized with various detergents and peptergents was followed over two weeks. The peptergents were able to maintain GlpD's enzyme activity longest, losing between 1% to 10% of its initial activity after 14 days (Figure 4). Although the activity of GlpD solubilized in OG was initially the highest, GlpD also lost its activity most rapidly in the presence of OG, losing over 40% of its activity within 24 h. This loss of activity could be due to the small, compact micelles that OG forms, an environment that is distinctly perturbed compared to a membrane bilayer, consequently inactivating the enzyme through conformational destabilization. With DM, the enzyme initially possessed about 65% of its maximum activity when compared to OG (using GlpD's activity in OG as the maximal value) but the enzyme remained active for a longer period of time. This is also likely correlated to the micellar size of the detergent. DM forms larger micelles than OG, which presumably leads to a local microenvironment more closely resembling a planar bilayer than a smaller micelle (e.g. differences in radius of curvature of the micelles). The mechanism by which peptergents confer enhanced functionality over time remains to be fully elucidated but may be

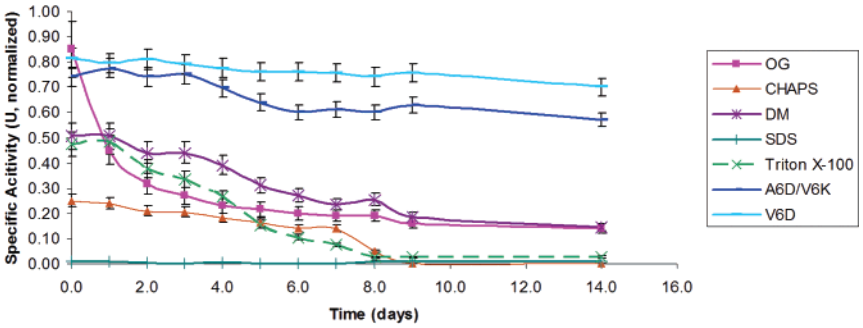

FIGURE 4: Time-dependent enzymatic activity of GlpD. The specific activity of GlpD was measured over two weeks to ascertain the effects of peptergents and detergents on enzymatic activity. Both peptergents extended the activity of GlpD over two weeks, over 10 times longer than OG and other detergents (square, OG; triangle, CHAPS; diamond with ex, DM; vertical bar, SDS; ex, Triton X-100; horizontal bar, A_6D:A_6K; solid line, V_6D).

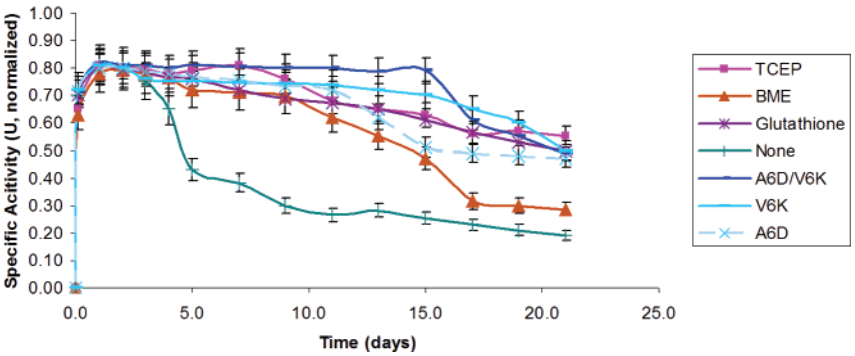

FIGURE 5: Time-dependent enzymatic activity of Npx. The activity of NADH peroxidase (Npx) was measured over three weeks to ascertain the effects of peptergents (square, TCEP; triangle, β-mercaptoethanol; diamond with ex, gluthathione; vertical bar, no additive; solid line, A_6D:V_6K; horizontal bar, V_6K; ex, A_6D). All three peptergents maintained the activity of the oxidation-sensitive Npx, extending its half-life by up to five times compared to without any reducing agents or peptergents, and the A_6D:V_6K mixture conferred the most protection.

due to formation of tighter-packing bilayerlike topologies such as nanovesicles and nanotubes, conformationally stabilizing membrane proteins.

Lipid carry-over from protein solubilization and purification steps can have a significant role in the stability and activity of a membrane protein (*19*, *20*). The amounts of lipid in purified detergent or peptergent solubilized solution were determined (Table 2). The detergent OG solubilized GlpD most efficiently, and it delipidates most extensively. The other detergents and peptergents all maintain some carry-over of endogenous lipids, from 3% to 15%. The stability conferred by the peptergents may have some correlation to the amount of lipid remaining with GlpD as the peptergents seem to be less harsh at removing lipids. However, comparing the stability of GlpD over time when solubilized in peptergents versus a detergent that also has similar lipid carry-over value (e.g. V_6D vs CHAPS; A_6D:A_6K vs DM) indicates that the additional stability conferred is beyond just residual lipid content. This was also the conclusion reached with another membrane protein, bovine rhodopsin, where peptergents were able to extend the activity of the protein over time (Zhang, personal communication). Whether the peptergents reconstitute membrane proteins into a conformation that is more active and stable, or whether the peptergents help to minimize chemical perturbations (e.g. oxidation), or via some other mechanism in extending activity remains to be determined.

The CMCs of the peptergents are 1.5−1.6 mM, and this value is dependent on the ionic strength of the solution, similar to traditional detergents. We have been able to exchange OG with peptergents, by affinity binding of detergent and peptergent exchange through extensive washing. It is likely that mixed detergent:peptergent and peptergent:lipids can be formed, the likelihood of which is indicated by their CMC values and concentrations of detergents/peptergents in solution. In our hands, the ease with which peptergents can be exchanged with detergents is similar to that expected from detergent−detergent exchange, where the CMCs of the detergents/peptergents are the primary determinant in success of exchange. As with detergents, solution parameters such as ionic strength and pH need to be monitored as these can affect the peptergents' propensity to form micelles or nanostructures.

In addition to the results we have obtained with GlpD, other groups have found that peptergents can stabilize and make heat-insensitive bovine rhodopsin when compared to OG and DM. Additional work on photosystem I indicates that peptergents stabilize interactions in protein−pigment complexes (Zhang, personal communication), again suggesting that the membrane protein is stabilized by peptergent−membrane protein interactions.

For the labile Npx enzyme, the presence of peptergents stabilized the enzyme activity over several weeks, extending the half-life from 2 days to >10 days. In the presence of peptergents, the activity of Npx showed two apparent transitions with time, most pronounced with the peptergent mixture, A_6D:V_6K. Our results suggest that the peptergents have a protectant property but that this ability is depleted with time. While the mechanism for the oxidation-protective property remains to be fully elucidated, these peptides may act as a surrogate, sequestering oxygen from the enzyme. Low molecular weight peptides have been reported to possess

antioxidant properties; most noteworthy for this study are peptides containing valines and terminal lysines (*21*, *22*). As our results indicate, both A_6D and V_6K alone confer some oxidation-protective properties but together they retard the time-dependent decrease in activity of Npx. The two-phase component of the activity vs time plots is consistent with a mechanism where the transition to loss of activity occurs upon depletion of the peptergent additive (Figure 5). The eventual loss of protective properties of the peptergents may be due to chemical modification (e.g. oxidation) or degradation of the peptides.

Although "peptitergents" have been proposed for solubilization and crystallization (*23*), these are considered longer peptides of >20 (typically 24) amino acids. Few crystal structures of membrane proteins have been reported using peptitergents. Lipopeptides, formed by linkage of fatty acid groups to peptides, have also been described (*24*). The short heptameric peptides reported here are capable of efficiently extracting membrane proteins without additional incorporation of fatty acid or other lipophilic groups and can maintain the activity of the extracted protein. These short amphiphilic peptides may additionally maximize exposed hydrophilic regions of the membrane proteins, to enhance interaction for lattice formation in crystal structure studies.

For other structural approaches, such as NMR, usefulness of peptergents remains to be studied. NMR methods make use of small-micelle detergents such as SDS and OG because they produce minimal increase in size of the tumbling protein−detergent complex. As discussed earlier, in solution, self-assembling peptides form higher-ordered topologies such as sheets, tubes, and vesicles. The sizes of these assemblies vary from 5 nm to >100 nm; consequently, molecular weight considerations may preclude routine use of some of these peptides in NMR studies as solubilization reagents. It should be noted, however, that the self-assembling properties of these peptides were studied in absence of exogenous molecules—e.g. proteins, DNA—and the presence of these may alter the peptide's self-assembling propensities.

An advantage of peptergents is that they are easily modified to change functionality and size. Variation of tail and head residues can drastically alter their interactions with membranes or proteins, resulting in enhanced solubilization and functionalization. Mixtures of peptergents can confer additional properties, more closely resembling native membranes that are naturally composed of various types of lipids and other components such as cholesterol. Molecules such as cholesterol can affect fluidity of the membrane by loosening the packing of hydrophobic tail regions. Similarly, peptergents can be "doped" with other peptides or compounds to confer different physiochemical characteristics. The self-assembling, amphiphilic nature of peptergents resembles that of membranes, which are capable of forming an effective barrier that is nonetheless fluid and can support the dispersion of various membrane proteins. The observation that peptergents can stabilize soluble proteins highlights their unusual chemical properties. These peptergents appear to be efficient, practical, and economical reagents for membrane protein analysis and as a possible additive for stabilizing soluble proteins.

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