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Solubilization of Native Integral Membrane Proteins in Aqueous Buffer by Noncovalent Chelation with Monomethoxy Poly(ethylene glycol) (mPEG) Polymers

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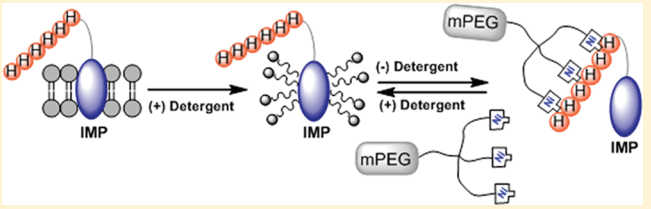
Supporting Information

ABSTRACT: Highly hydrophobic integral membrane proteins (IMPs)are typically purified in excess detergent media, often resulting in rapid inactivation and denaturation of the protein. One promising approach to solve this problem is to couple hydrophilic polymers, such as monomethoxypolyethylene glycol (mPEG) to IMPs under mild conditions in place of detergents. However, the broad application of this approach is hampered by poor reaction efficiencies, low tolerance of detergent stabilized membrane proteins to reaction conditions, and a lack of proper site-specific reversible approaches. Here, we have developed a straightforward, efficient, and mild approach to site-specific noncovalent binding of long-chain polymers to recombinant IMPs. This method uses the hexa-histidine tag (His-Tag) often used for purification of recombinant proteins as an attachment site for mPEGs. Solubility studies performed using five different IMPs confirmed that all tested mPEG-bound IMPs were completely soluble and stable in detergent free aqueous buffer compared to their precipitated native proteins under the identical circumstances. Activity assays and circular dichroism (CD) spectroscopy confirmed the structural integrity of modified IMPs.



INTRODUCTION

Integral membrane proteins (IMPs) comprise about 25−30% of all encoded proteins in genomes.[1] However, *in vitro* structure−function studies of IMPs are technically challenging and require extracting the protein from the lipid membrane and solubilizing with detergents.[2−4] These detergents are dissociating amphiphiles and tend to disrupt the protein−protein, protein−lipid, and lipid−lipid interactions that stabilize the three-dimensional structure of IMPs leading to denaturation and precipitation of protein.[5−7] The poor stability and denaturing effects of detergent solubilized IMPs are the main technical barriers limiting their study.

Considerable efforts have been devoted to developing methods for membrane protein stabilization. One preferred approach is substituting detergents with amphiphilic polymers named amphipols.[8−10] Amphipols are polyacrylamide-based surfactants that bind noncovalently to the transmembrane surfaces of proteins in a quasi-irreversible manner. IMPs have been stabilized in aqueous buffers using zwitterionic, anionic, or neutral amphipols. Although, in some cases addition of such amphipols has been observed to destabilize the protein.[9,11]

Amphipathic α-helical peptides known as peptitergents were also being explored to solubilize IMPs in detergent free buffer. They are predicted to shield the hydrophobic regions of the protein in a rigid, well-ordered, parallel α-helical arrangement. Thus far, this method has had limited success. In previous studies, the method was limited to partially solubilizing only two of the three tested proteins.[12,13] Other studies have shown that covalent modification of IMPs with PEG or PEGylation can stabilize IMPs in an aqueous environment.[14−16] However, covalent PEGylation of IMPs requires significant optimization for each unique protein and can be difficult to localize to a unique site. For example, depending on the PEGylation method, type of protein, site of modification, PEGylated MscL[14] maintained the native structure in aqueous buffer, whereas bacteriorhodopsin[15] and porin[16] resulted in partially denatured water-soluble proteins. Performing covalent modifications to fragile IMPs embedded in detergent micelles requires harsh conditions including low pH, higher temperatures, longer reaction times, and requirement of organic reagents leading to protein denaturation.

Ni(II) doped tris-NTA chelation to His-Tag protein has been successfully applied for many different applications including fluorescence labeling,[17] biotinylation,[18] and protein immobilization on solid support.[19] These studies also have established that

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the stability of tris-NTA chelation to His-Tag protein is ~4-fold stronger than mono-NTA chelation and the resulting derivatized protein solution can be diluted to pM concentration without dissociating the chelation bond.[20]

Herein, we report the reversible attachment of mPEG polymers through nickel (Ni^{2+})-chelated NTA (nitrilotriacetic acid) to solubilize and stabilize recombinant IMPs in their native form either in low-detergent or aqueous buffer solutions. The generality of the noncovalent method was tested using five IMPs of different size, function, and structure. Reactive mPEG-$NTA_3(Ni^{2+})$ polymers of 5 kDa and 10 kDa (average molecular weight) bearing three NTA units (tris-NTA) were synthesized and noncovalently chelated to recombinant IMPs via their His-Tag. We compare our method with the PMAL-12 [Poly(maleic anhydride-alt-1-tetradecene) substituted with 3-(dimethylamino)propylamine] amphipol-stabilized approach.[8] Given the widespread use of His-Tags for Ni-NTA mediated protein purification and the fact that tags are typically located on termini of recombinant proteins where they minimally effect protein structure and function, our tris-NTA(Ni^{2+})-mediated noncovalent polymer chelation approach provides a straightforward means to chemically modify IMPs for the solubilization and stabilization of delicate IMPs in aqueous environment without the need for excessive detergents.

EXPERIMENTAL PROCEDURE

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer. Unless otherwise stated, spectra were acquired at 25 °C in deuterated chloroform ($CDCl_3$).

Pyruvate kinase (PK), lactate dehydrogenase (LDH), phosphoenol pyruvate (PEP), nicotinamide adenine dinucleotide (NADH), 4-(*N*,*N*-dimethylamino)pyridine (DMAP), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDAC·HCl), 2-(trimethylsilyl)ethanol (TMSE), sodium sulfate (Na_2SO_4), trifluoroacetic acid (TFA), magnesium acetate ($Mg(OAc)_2$), and 10% palladium on charcoal (10% Pd/C) were purchased from Sigma-Aldrich; 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-sn-glycerol) (sodium salt) [POPG] from Avanti Polar Lipids; *n*-decyl-β-D-maltoside (DM), 5-cyclohexyl-1-pentyl-β-D-maltoside (CY-5), 6-cyclohexyl-1-pentyl-β-D-maltoside (CY-6), and *n*-octyl-β-D-glucopyranoside (OG), PMAL-12 [poly(maleic anhydride-*alt*-1-tetradecene) substituted with 3-(dimethylamino)propylamine] were from Anatrace; dichloromethane (CH_2Cl_2), hexane, ethylacetate (EtOAc), diethyl ether, and chloroform were from Fisher and used without further purification.

Lys-NTA_3TMSE_9 (compound **1**),[21] mPEG-CH_2-COOH (compound **2**),[22] and dibutyrylglycerol (DBG)[23] were synthesized according to the literature procedure.

Cloning and Expression. *Escherichia coli* diacylglycerol kinase (DGK) was cloned to pSpeedET vector as described previously.[24,25] Expression clones for *Thermotoga maritima* IMPs, TM0561, TM0407, TM1122, and TM0307 were obtained from The Joint Center for Structural Genomic (JCSG) clone collection.[26] Each vector encodes a hexa-histidine (His-Tag) purification tag at the N-terminus of the full-length recombinant protein. Protein expression of all the selected clones was performed using *E. coli* HK100 cells in modified terrific broth media (24 g of yeast extract, 16 g of tryptone, 10 g of bacto-casamino acid, 100 mM Tris-Cl, pH 8, and 2% glycerol per liter culture containing 100 μg/mL ampicillin or 50 μg/mL kanamycin). After approximately three hours at 37 °C, protein expression was induced with 0.02% L-arabinose for an additional four hours. Cells were harvested by centrifugation at 3000*g* for 15 min.

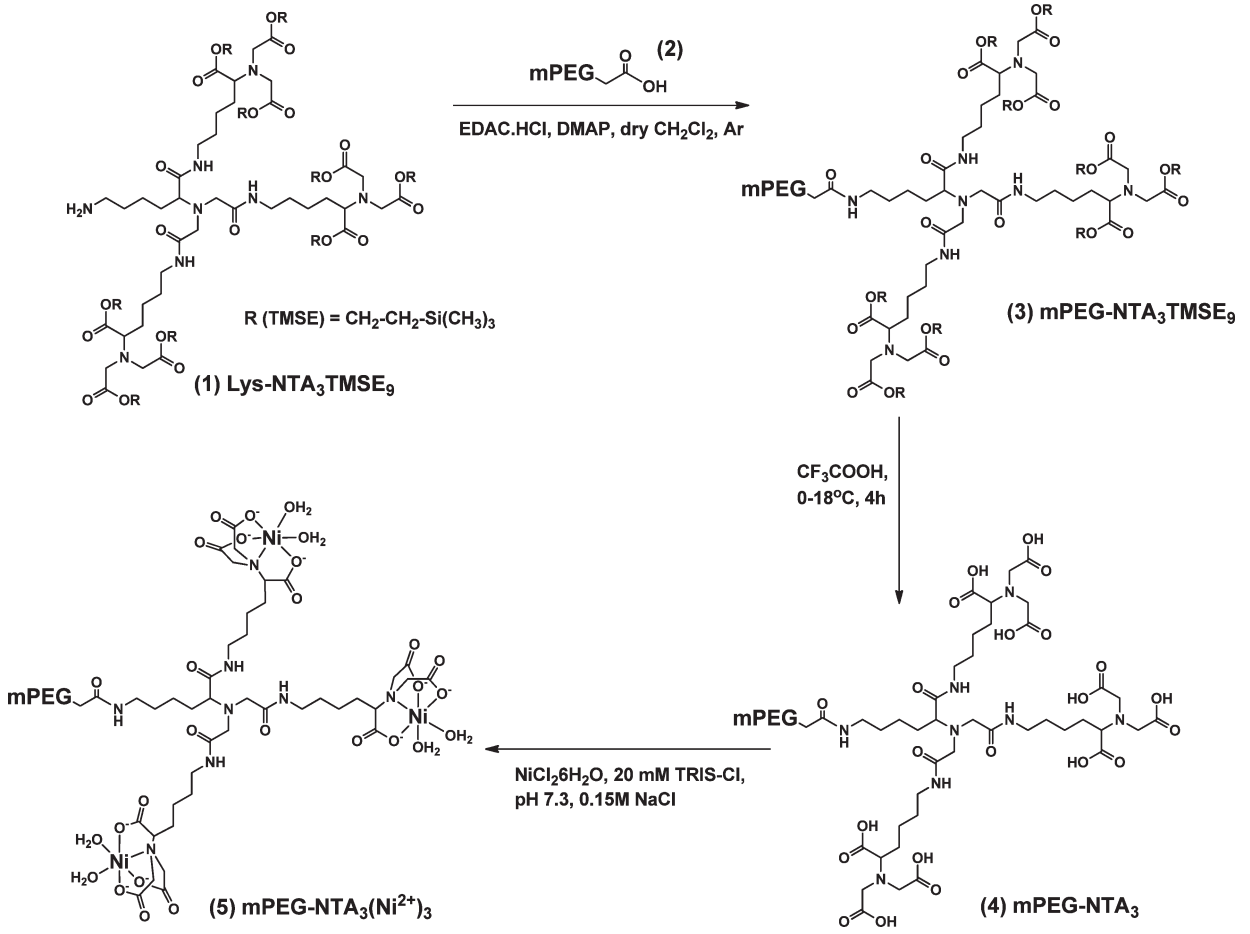
Purification of Integral Membrane Proteins. Cells were resuspended in 50 mL of lysis buffer (50 mM Tris-Cl, pH 7.9, 150 mM NaCl containing one protease inhibitor cocktail [Roche] minitablet per liter of original culture) and then lysed at 4 °C by passage through a M110S Microfluidizer (Microfluidic Corporation, Newtown). The cell lysate was centrifuged at 20 000*g* for 30 min. The clarified supernatant was equilibrated with 0.5% (w/v) detergent (DDM) for 2−3 h at room temperature prior to passage through a Ni-NTA (Qiagen) column (10 mL). The column was washed with 50−100 mL of wash buffer (25 mM sodium phosphate, pH 7.9, 150 mM NaCl, 25 mM imidazole) containing 2× detergent (CY-6/CY-5) CMC. The protein was then eluted with the 10−15 mL elution buffer (25 mM sodium phosphate, pH 7.9, 100 mM NaCl, 400 mM imidazole) containing 1% (w/v) of selected detergents. The protein in elution buffer was concentrated by ultrafiltration using 30 kDa (Millipore) concentrator and the buffer was exchanged to Tris-Cl, pH 7.9, and 150 mM NaCl buffer using PD-10 (GE Healthcare) columns.

Synthesis of Compound 3: mPEG(10K)-NTA_3TMSE_9 (average molecular weight 10 kDa). Compound **2** (1.3 g; 0.13 mmoL; MW ≈ 10 000 Da)[22] and DMAP (28 mg; 0.18 mmol) were dissolved in 200 mL of dry CH_2Cl_2 under argon atmosphere at 0 °C. EDAC·HCl (70 mg; 0.37 mmol) was added under argon and the resulting mixture was maintained at 0 °C for 0.5 h. Then, the compound **1**[21] (0.34 g; 0.18 mmol) solubilized in 10 mL of dry CH_2Cl_2 was added and the ensuing mixture was continuously stirred at 0 °C for an additional 1 h prior to stirring at room temperature for 3 days. Following the reaction, the light yellow organic layer was washed twice with distilled water (400 mL), dried (Na_2SO_4), and concentrated to 20 mL under reduced pressure. The product was precipitated with excess diethyl ether (200 mL) and the precipitate was filtered and washed thoroughly with excess diethyl ether and dried under vacuum overnight to yield compound **3** (1.4 g, 90%). ^1H NMR and negative ninhydrin staining of thin layer chromatography (TLC) analysis of compound **3** confirmed the final product. ^1H NMR 4.10−4.23 (18H, m, −O−CH_2−), 3.10−3.30 (19H, m, -OCH_3 of mPEG, −NH−CH_2−, 4× -NCH−CO-, 2× -NCH_2−CO-NH-, and 3× −CO-NH−CH_2−), 1.21−1.89 (24H, m, 4× -NCH_2−$(CH_2)_3$-), 0.84−1.05 (18H, m, 9× −CH_2Si-), 0.02 (81H, m, 27× -$Si(CH_3)_3$).

Synthesis of Compound 4: mPE(10K)-NTA_3, Average Molecular Weight 10 kDa. Compound **3** (1.5 g, 0.11 mmol) was dissolved in 5 mL of dry CH_2Cl_2. Trifluoroacetic acid (TFA; 5 mL) was added dropwise to the reaction mixture at room temperature. After stirring the reaction at room temperature for 4 h, the solvent was removed under vacuum and the product was precipitated with excess diethyl ether. The precipitate was filtered, washed thoroughly with ether, and dried under vacuum to yield compound **4** (1.2 g, 90%) as a yellowish solid, which gave a negative staining with ninhydrin test. Structure of compound **4** was confirmed by ^1H NMR and MALDI MS. ^1H NMR (D_2O) 3.28 (3H, s, -OCH_3 of mPEG), 1.1−2.0 (24H, m, -$NHCH_2$−$(CH_2)_3$- of tris-NTA), 3.15−3.25 (12H, m, -NCH_2−CO-O−); MALDI: Found, 11225 Da; required 11118 Da (MALDI MS measured against the mPEG(10K)-CH_2COOH starting material: 10230 Da).

Synthesis of Compound 5 (Average Molecular Weight, 10 kDa): mPEG(10K)-$NTA_3(Ni^{2+})_3$. Compound **4** (0.2 g, 0.017 mmol) in 1 mL of 20 mM Tris-Cl, pH 7.5, 150 mM NaCl buffer

Scheme 1. Synthesis of mPEG-NTA$_3$(Ni^{2+})$_3$ (Average Molecular Weight = 5000 or 10 000) Polymers



was stirred with 48 mg of $NiCl_2$ $6H_2O$ (0.1 mmol) at room temperature. After stirring for 30 min, the excess $NiCl_2$ was removed by dialysis to yield the compound **5**. The successive Ni(II) chelated compound **5** in Tris buffer was subsequently reacted with recombinant IMPs.

Synthesis of Compound 5 (Average Molecular Weight, 5 kDa): mPEG(5K)NTA$_3$(Ni^{2+})$_3$. mPEG(5K)NTA$_3$(Ni^{2+})$_3$ was synthesized from mPEG(5K)-CH_2-COOH[22] similar to compounds **3** to **5** (10 kDa) and confirmed by MALDI MS spectrometry. mPE(5K)-NTA$_3$: MALDI MS: Found, 5760; required: 5988 (MALDI MS measured against mPEG(5K)-CH_2COOH: 5100).

Noncovalent Coordination of mPEG Polymers to Recombinant IMPs. One equivalent of purified protein (between 10 and 100 μM concentration) in 20 mM Tris-Cl, pH 7.5, 150 mM NaCl was mixed with 1−1.1 equiv of mPEG-NTA$_3$(Ni^{2+})$_3$ [5 or 10 kDa][5] at 4 °C for 15 min. After mixing, detergent was completely removed from the protein solution by dialysis. The final detergent concentration in solution was measured colorimetrically by following the literature procedure.[27]

Enzyme Activity Assays. Activity of DGK (*E. coli* diacylglycerol kinase) was assayed following published procedures.[23,28] The assay mixture contained 3 mM Na_3ATP, 15 mM Mg(OAc)$_2$, 0.25 mM NADH, 1 mM PEP, 20 units/mL PK, 20 units/mL LDH, 10 mM DBG (substrate), and 0.42 mM POPG (lipid activator) in 0.8 mL of buffer (75 mM PIPES pH 6.9, 0.1 mM EGTA, 0.1 mM EDTA, 50 mM LiCl). The reaction was initiated by adding 0.04−0.1 μM DGK or derivatized DGK to the assay mixture at 30 °C. Activity of mPEG-bound or wild-type DGK was calculated in units/mg from the absorbance (340 nm) vs time plots as described.[23,28]

Circular Dichroism Spectroscopy. Circular dichroism spectra of DGK derivatives were recorded using a JASCO J-720 circular dichroism spectrophotometer in 1 mm optical path length quartz cuvettes between 260 and 190 at 0.1 nm intervals. Spectra were recorded in 20 μM protein concentration in 20 mM Tris-Cl, pH 7.9, and 100 mM NaCl buffer.

RESULTS

Noncovalent Coordination of IMPs with mPEG-NTA$_3$(Ni^{2+})$_3$. Tris-NTA(Ni^{2+})-activated mPEG polymers, mPEG(5K)-NTA$_3$(Ni^{2+})$_3$, and mPEG(10K)-NTA$_3$(Ni^{2+})$_3$ [5 or 10 kDa] employed in this study were synthesized from mPEG−OH as described in Methods (Scheme 1). The recombinant IMPs were purified in excess detergents and noncovalently bound to mPEG-NTA$_3$(Ni^{2+})$_3$ through N-terminal His-Tag chelation (4 °C, 10 min). All the IMPs tested in Table 1 were chelated with mPEG-(10K)NTA$_3$(Ni^{2+})$_3$ polymer. Protein concentration measurements at 280 nm showed that all the mPEG-bound IMPs in detergents have extended shelf lives as compared to the native proteins in detergents alone (Table 1). Polymer (mPEG)

Table 1. Comparison of mPEG-Bound IMPs Stability Vs Their Detergent Counterparts[a]

IMPs	detergent	TM[b]	MW (kDa)	stability[c]	stability[d]	stability[e]
TM0561	Cymal-6	2	43	-	++	++
DGK	Cymal-5	3	15	+	++	++
TM0407	Cymal-5	4	20	-	++	++
TM1122	OG	6	32	-	++	++
TM0317	Cymal-5	7	81	-	++	++

[a] − protein started precipitating in less than two weeks; + protein started precipitating after two weeks; ++ protein didn't precipitate after two months. [b] Number of transmembrane helices (TM) were determined using http://www.cbs.dtu.dk/services/TMHMM/ server. [c] Stability of IMPs in detergent. [d] Stability of mPEG(10K)$NTA_3(Ni^{2+})_3$ chelated IMPs in detergent. [e] Stability of mPEG(10K)$NTA_3(Ni^{2+})_3$ chelated IMPs in aqueous buffer; MW = Molecular weight.

chelated IMPs maintained solubility under detergent depleted conditions as well as in aqueous buffer (20 mM Tris-Cl, pH 7.5, 150 mM NaCl) (Table 1). During dialysis, the detergent distribution was followed by absorbance at 490 nm.[27] The amount of detergent present in the 10 kDa mPEG polymer bound IMPs in aqueous buffer was less than 10 molecules per IMP after dialysis. Derivatized IMPs with the smaller size mPEG-(5K)$NTA_3(Ni^{2+})_3$ with N-terminal His-Tag precipitated partially or fully in detergent free buffer. Depending on the protein, mPEG-(5K)$NTA_3(Ni^{2+})_3$-bound-IMPs required retaining 7−15% of the native protein detergent concentration to sustain solubility. As a negative control, derivatization of IMPs with non-Ni(II)-chelated mPEG(10K)NTA_3 was tested. In the absence of chelated Ni(II), all these tested proteins precipitated out upon detergent removal demonstrating the contribution of Ni(II)-mediated coordination of mPEG for the His-Tag protein stability.

Activity Assays. To investigate the influence of noncovalent tris-NTA(Ni^{2+})-mediated polymer binding toward maintaining 3D structural integrity, we performed activity assays using *E. coli* DGK as a model IMP.[29−32] DGK was purified in CY-5 (CMC = 2−5 mM) and the kinase activity of the protein was measured in the presence of phosphatidylglycerol (PG) lipid activator. PG is the major anionic lipid component of the *E. coli* lipid membrane constituting 15−20% by weight compared to 2−5% of cardiolipin.[33] Kinetic activity of DGK derivatives was studied by coupling their production of MgADP to NADH oxidation measured at 340 nm.[28]

Table 2 compares the kinase activity of different mPEG-chelated DGK derivatives during phase transitions from detergent to aqueous phase under identical conditions.[23,28] DGK-mPEG(5K)$NTA_3(Ni^{2+})_3$ sustains the original activity at its lowest soluble detergent concentration (15% of the original native DGK detergent concentration) confirming that protein activity, which is used as a symbol of structural integrity, is unaffected by the change from detergent media to hydrophilic polymer (Table 2, row 5). Enzyme activity of native DGK was not measured under identical conditions, as it remained predominantly in precipitated form.

Substitution of mPEG(5K)$NTA_3(Ni^{2+})_3$ with the longer chain length mPEG(10K)$NTA_3(Ni^{2+})_3$ allowed completed removal of detergents. Chelation efficiency did not change in comparison to mPEG(5K)$NTA_3(Ni^{2+})_3$. However, the increasing mPEG polymer chain length yielded reduced enzyme activity. DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$ could be solubilized in detergent free

Table 2. Activity of DGK Derivatives in Different CY-5/DGK Ratio

DGK derivative	CY-5/DGK	% of CY-5	*A* (units/mg)	% *A*
[a]DGK-CY-5	220	100%	108 ± 1.5	100%
[b]DGK-PMAL	14	6.3%	86 ± 0.6	80%
[c]DGK-mPEG(5K)$NTA_3(Ni^{2+})_3$	220	100%	108 ± 0.6	100%
[c]DGK-mPEG(5K)$NTA_3(Ni^{2+})_3$	32	14.5%	96 ± 0.6	90%
[c]DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$	220	100%	97 ± 1	90%
[c]DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$	<7	<3.1%	35 ± 1	32%
[d]DGK-CY-5	225	102%	105 ± 2.5	97%

[a] Native DGK protein in the presence of minimum detergent needed to stabilize the protein in aqueous buffer. We normalize the native protein's detergent/protein ratio, 220, and the activity (A) as 100% for comparison. [b] Kinase activity of amphipol-stabilized DGK in trace detergent. [c] Kinase activity of mPEG-bound DGK proteins was shown during different detergent/protein ratio. [d] Kinase activity of DGK after reincorporating back to detergents from mPEG(10K)$NTA_3(Ni^{2+})_3$ media; *A* = Activity in units/mg.

buffer with 32% of residual activity of the DGK native protein (Table 2, row 7).

Tris-NTA(Ni^{2+}) mediated chelation of DGK-mPEG-(10K)$NTA_3(Ni^{2+})_3$ was reversed using imidazole and EDTA and introduced the mPEG free protein back into the detergent phase (see Supporting Information). Activity measured in re-introduced DGK in detergents yielded the original activity (Table 2, row 8). ANSEC analysis of mPEG chelated DGK derivatives suggests that the reduced activity with increasing mPEG chain length can be attributed to the formation of large discrete structures in aqueous media which reverse back to the original protein size distribution upon removal of the mPEG (Figure S1 in Supporting Information). Expansion of the hydrodynamic volume is a general result of protein PEGylation and is widely employed to improve serum half-life of protein-based therapeutics.[34,35] Protein unfolding and aggregation as a cause of the observed increase in hydrodynamic volume can be excluded based on the enzyme activity data and the CD spectroscopy data discussed below.

CD Spectroscopy. The secondary structure of mPEG-bound and unbound DGK derivatives in low detergent concentration or aqueous buffer was analyzed by far-UV CD spectroscopy to provide further evidence of structural integrity of mPEG chelated proteins. As shown in Figure 1, mPEG-bound DGK derivatives have very similar CD spectral patterns to those of native DGK than DGK-PMAL as reflected by the characteristic CD bands around 195, 209, and 223 nm.

DISCUSSION

The noncovalent mPEG-chelating approach presented here provides a successful method to couple mPEGs site-specifically to IMPs in excess detergent media. It has been shown in previous work that the harsh reaction conditions employed in covalent derivatization of IMPs with mPEG polymers (5 kDa) resulted in partially denatured proteins.[15,16] Chelation is an extremely mild, reversible, and efficient coupling method for rapid recovery of active IMPs from detergent micelles. PEGylation of proteins is a well established technique which has many advantages including improved protein solubility, stability, and serum half-life.[36−38] We have demonstrated application of Tris-NTA(Ni^{2+}) mediated mPEG chelation to stabilize active recombinant IMPs in low-detergent or detergent-free environment.

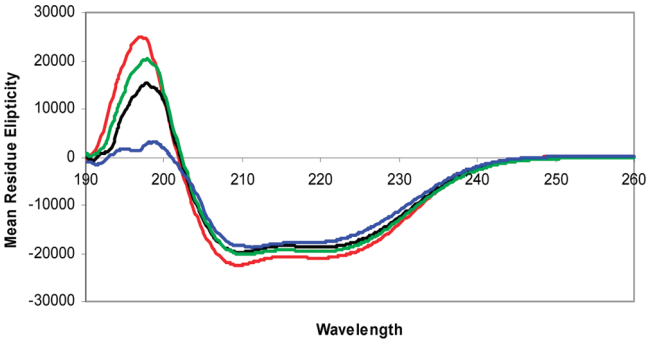


Figure 1. Far-UV CD spectra (selected DGK derivatives in Table 2) of DGK-WT (red; in 100% detergent; Table 2, row 2), DGK-PMAL (blue; in 6.3% residual detergent; Table 2, row 3), DGK-mPEG(5K)$NTA_3(Ni^{2+})_3$ (green; in 14.5% residual detergent; Table 2, row 5), and DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$ (black; in <3.1% residual detergent; Table 2, row 7) in 20 mM Tris-Cl, pH 7.9 and 100 mM NaCl buffer.

Reactive mPEG-$NTA_3(Ni^{2+})_3$ derivatives of different chain lengths were synthesized and confirmed by NMR and MALDI MS. All IMPs tested were successfully chelated with both 5 kDa and 10 kDa mPEGs under mild conditions. It has been shown in this study that approximately 85−90% of the detergent required to stabilize the native proteins could be removed using mPEG(5K)$NTA_3(Ni^{2+})_3$ chelation and practically 100% using mPEG(10K)$NTA_3(Ni^{2+})_3$. Therefore, our current noncovalent coupling method has been able to produce stable mPEG-bound IMPs to significantly improve stability and solubility in aqueous buffer solutions over their detergent solubilized counterparts.

All mPEG-bound DGK derivatives tested were found to be enzymatically active. Observed activity of the protein varied with length of the chelated mPEG chain. Although the detergents could not be removed completely from the DGK-mPEG-(5K)$NTA_3(Ni^{2+})_3$, the derivatized DGK retained full activity while amphipol-stabilized DGK (DGK-PMAL) was less active. Chelation of the protein with a longer 10 kDa mPEG chain supported the solubility of protein in detergent free buffer, but the activity was reduced. DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$ retained 32% (Table 2, row 7) of native DGK protein's activity and regained original enzyme activity upon introduction into detergent media (Table 2, row 8). Further, the similar CD spectra of all DGK derivatives suggest that the backbone configuration of the protein has not changed following the post-translational modification. The least enzymatically active DGK-mPEG-(10K)$NTA_3(Ni^{2+})_3$ yielded a much more similar CD spectrum to that of the native protein than DGK-PMAL (Figure 1, blue spectrum). This leads to the conclusion that the observed activity loss upon increasing mPEG chain length is not the result of structural changes within DGK. Activity loss as a result of PEGylation is well documented in the literature.[34,35] The ANSEC profile of DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$ in aqueous phase and detergent inclusion supports that DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$ formed larger discrete structures in aqueous media that reversed after reintroduction into detergent media (Supporting Information Figure S1). Therefore, the observed activity reduction is likely due to interference of the enlarged PEG chains with substrate access to the active site.

CONCLUSION

The present work establishes that highly hydrophobic recombinant IMPs can be successfully solubilized and stabilized in low detergent or in aqueous buffer by noncovalently coupling with tris-NTA(Ni^{2+})-mediated mPEGs. The reaction is straightforward, efficient, and utilizes a common protein purification tag. The solubility of IMPs can readily be adjusted by varying the size of the mPEG chain. CD spectroscopy and enzyme assay of derivatized DGK confirmed the retention of secondary structure upon chelation with mPEG. This method will be useful for structure−functional studies of IMPs where the use of detergent is deemed problematic. Multiple efforts that encompass crystallization of IMPs in detergent free medium, the attachment of different crystallizable polymers to the IMP backbone, and the introduction of native, detergent free protein into different matrixes (lipid cubic phase, lipid bilayer) for crystallization purposes are currently in progress.

ASSOCIATED CONTENT

Supporting Information. Analytical SEC behavior of DGK in detergent phase and polymer phase, and re-extraction of DGK from DGK-mPEG(10K)$NTA_3(Ni^{2+})_3$ to detergent medium. This material is available free of charge via the Internet at http://pubs.acs.org.

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