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A rapid procedure to isolate isotopically labeled peptides for NMR studies: application to the Disabled-2 sulfatide-binding motif

Shuyan Xiao,[a] Xiaolin Zhao,[a] Carla V. Finkielstein[b] and Daniel G. S. Capelluto[a]*

A procedure for obtaining isotopically labeled peptides, by combining affinity chromatography, urea-equilibrated gel filtration, and hydrophobic chromatography procedures, is presented using the Disabled-2 (Dab2) sulfatide-binding motif (SBM) as a proof of concept. The protocol is designed to isolate unstructured, membrane-binding, recombinant peptides that co-purify with bacterial proteins (e.g., chaperones). Dab2 SBM is overexpressed in bacteria as an isotopically labeled glutathione S-transferase (GST) fusion protein using minimal media containing [^{15}N] ammonium chloride as the nitrogen source. The fusion protein is purified using glutathione beads, and Dab2 SBM is released from GST using a specific protease. It is then dried, resuspended in urea to release the bound bacterial protein, and subjected to urea-equilibrated gel filtration. Urea and buffer reagents are removed using an octadecyl column. The peptide is eluted with acetonitrile, dried, and stored at −80 °C. Purification of Dab2 SBM can be accomplished in 6 days with a yield of ~2 mg/l of culture. The properties of Dab2 SBM can be studied in the presence of detergents using NMR spectroscopy. Although this method also allows for the purification of unlabeled peptides that co-purify with bacterial proteins, the procedure is more relevant to isotopically labeled peptides, thus alleviating the cost of peptide production. Copyright © 2014 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: detergent; disabled-2; isotopes; NMR; recombinant peptide; urea

Introduction

Peptides are involved in a variety of biological processes acting as antimicrobial macromolecules, enzyme inhibitors, and signal transduction triggers. Peptide purification methodologies have improved over the last few years by reducing the amount of time and effort required to obtain intact, biologically active peptides suitable for structural studies. Initially, peptide purification technologies (molecular mass $<10,000$ Da) involved selective precipitations and adsorption of peptides along with ion-exchange and gel filtration chromatography, which led to low yields; more advanced technology involving the use of reversed-phase HPLC has led to higher yields [1]. Reversed-phased HPLC is carried out using sequential chromatography on columns containing different classes of silica-based packing materials, with octadecyl (C_{18}) columns being the most popular because they are suitable for the purification of hydrophilic peptides. For hydrophobic peptides, however, C_4 and diphenyl columns are recommended [1]. These columns use acetonitrile as a solvent because of its low viscosity, low absorbance at 214 nm (which is important for detecting the peptide bonds), and relatively high volatility. However, the HPLC technology has limitations, including low yield in the purification of extremely hydrophilic peptides, which show low retention in most of the HPLC-based columns; acetonitrile-mediated denaturation; and insolubility of polypeptides [2].

Although peptides can easily be produced by chemical synthesis, the large quantities required for structural or functional assays make this method cost-ineffective, resulting in their overexpression in a heterologous system as the default option. Although many peptides can be overexpressed as fusion proteins using *Escherichia coli* and other expression systems, the major limitation is their aggregation propensity, resulting in a low-solubility behavior. Expression, yield, stability, and solubility of peptides were successfully tackled when maltose-binding protein [3], E/K coils [4], and a combination of the B1 immunoglobulin domain of streptococcal protein G (GB1) and His tags were employed [5]. Antimicrobial peptides, which are ~50 amino acids in length, exhibit more unusual properties, such as being hydrophobic with an excess of positive charges, making them difficult to isolate [6]. Thioredoxin, GB1, and glutathione S-transferase (GST) are the most popular carrier proteins for fusion expression with peptides [7,8]. The reason for their popularity resides in their ability to increase the solubility of fusion proteins, with thioredoxin being most favorable because of its small size (11.8 kDa) and because of its role as a chaperone in facilitating protein folding [9].

Nuclear magnetic resonance (NMR) spectroscopy is a powerful technology that provides detailed information about the

* *Correspondence to: Daniel G. S. Capelluto, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA. E-mail: capellut@vt.edu*

a *Protein Signaling Domains Laboratory, Department of Biological Sciences, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061, USA*

b *Integrated Cellular Responses Laboratory, Department of Biological Sciences, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061, USA*

Abbreviations: *Dab2, Disabled-2; DPC, dodecylphosphocholine; HSQC, heteronuclear single quantum coherence; SBM, sulfatide-binding motifs peptide.*

structure, function, and dynamics of macromolecules. To obtain such information, NMR depends on the presence of stable isotopes in peptides, including carbon (^{13}C) and nitrogen (^{15}N). Chemical synthesis of isotopically stable peptides is expensive; the cost is associated with both the amount and length of the peptide to be studied. Thus, isotopic enrichment of media for bacterial growth and peptide overexpression is used, as such media can easily be manipulated by the addition of specific isotopes. Indeed, [^{13}C] glucose and [^{15}N] ammonium chloride are commonly used as the sole source of carbon and nitrogen, respectively, and usually help to generate milligram amounts of isotopically labeled proteins [10].

Here, we report on an optimized protocol to isolate peptides, which, because of their unstructured nature, often co-purify with host bacterial proteins such as chaperones. As an example, we describe the purification of a membrane-binding peptide derived from Dab2, a protein that is best known for modulating the extent of platelet–platelet and heterotypic interactions [11]. Dab2 exerts such functions by interacting with platelet sulfatides [12] *via* a short helical and amphipathic membrane-binding region that we have recently characterized as necessary and sufficient for negatively regulating platelet aggregation events [13]. This region, which we named Dab2 SBM, has been isolated for structural studies by a method that involves the labeling and use of affinity chromatography, gel filtration, and hydrophobic chromatography with yields of ~2 mg peptide/l of culture. The soluble peptide was unstructured but folded in the presence of the detergent dodecylphosphocholine (DPC). Under these conditions, one-dimensional, HSQC, and NOESY NMR spectra of Dab2 SBM indicated that the peptide was structured and active, suggesting that this methodology could be beneficial for NMR analysis of membrane-binding peptides alleviating the cost of synthetic peptide production.

Materials and Methods

Materials

The following is a list of chemicals used and their suppliers: restriction endonucleases, T4 DNA ligase, aprotinin, and acetonitrile (Fisher Scientific; Fair Lawn, NJ, USA); leupeptin and pepstatin (G-Biosciences; St.Louis, MO, USA); DL-dithiothreitol (DTT), isopropyl-β-D-thio-galactopyranoside (IPTG), urea, benzamidine HCl, and Luria Bertani (LB) medium (Research Products International; Mt. Prospect, IL, USA); lysozyme (MP Biomedicals; Solon, OH, USA); Triton X-100, TFA, formic acid, BSA, and sodium azide (Sigma); glutathione Sepharose 4B beads and *PreScission* protease (GE Healthcare; Pittsburgh, PA, USA); Coomassie brilliant blue R-250 (Pierce; Rockford, IL, USA); [^{15}N] ammonium chloride, deuterium oxide, and d_{38}-DPC (Cambridge Isotopes Laboratories; Andover, MA, USA); and brain sulfatides (Avanti Polar Lipids; Alabaster, AL, USA). A peptide representing Dab2 residues 24–58 was chemically synthesized by Biopeptide Co., Inc. (San Diego, CA, USA)

Cloning, Expression, and Purification of Dab2 SBM

A cDNA comprising the human Dab2 SBM (residues 24–58) was cloned into a pGEX6P1 vector (GE Healthcare; Pittsburgh, PA, USA). The cDNA region was amplified using the primers 5′ CGCGGATCCTCAAAGAAGGA-AAAAAAGAAAGGCCC 3′ (forward) and 5′ CCGGAATTCCTAATCA-ATGCCAATCAGCTTGGCC 3′ (reverse) and by ligation of the PCR product to linear pGEX6P1 using sticky ends generated by the restriction enzymes *Bam*HI and *Eco*RI. On the basis of this cloning strategy, the recombinant peptide is expected to have five extra amino acids from the vector at its N- terminus after protease cleavage. The recombinant plasmid was transformed into Rosetta *E. coli* cells (Stratagene). *E. coli* LB pre-cultures were inoculated from a glycerol stock of transformed cells. Preparative LB cultures were obtained by a 1 : 50 dilution of pre-cultures in shaking flasks and incubated at 37 °C until the media reached an optical density of ~0.8. Induction of the GST fusion peptide resulted from the addition of 1 mM IPTG followed by incubation for 4 h at 25 °C. Induced bacterial cells were harvested by centrifugation at 9,600*g* for 10 min at 4 °C and subjected to two freeze–thaw cycles to facilitate the extraction of the fusion peptide during purification. The cell pellet was resuspended in an extraction buffer (50 mM Tris–HCl, 500 mM NaCl, 1 mM DTT, pH 7.3) containing a protease cocktail represented by 1 μM leupeptin, 1 μM pepstatin, 1.7 μg/ml aprotinin, and 1 mg/ml benzamidine and then lysed by the addition of 0.1 mg/ml lysozyme and 0.5% Triton X-100 and incubated for 20 min at 4 °C. The resulting crude extract was subjected to sonication (using a Branson model 250 sonicator; Cleveland, OH, USA) on ice at 30% amplitude with eight 30-s bursts and 30-s cooling intervals between bursts. The lysate was clarified at 9,600*g* for 60 min at 4 °C. The supernatant was stirred with glutathione beads (2 ml of wet beads/l of culture) for 60 min at 4 °C. Beads were then washed four times with a washing buffer (50 mM Tris–HCl, 500 mM NaCl, 1 mM DTT, 1 mM NaN_3, pH 8). The GST fusion peptide, bound to glutathione beads, was cleaved by incubation with *PreScission* protease (0.05 units of protease/μg of protein; GE Healthcare) for 3 h at room temperature. Untagged Dab2 SBM, which was bound to a bacterial protein (likely a chaperone) was then concentrated to dryness using a centrifugal evaporator (Savant SpeedVac; Farmingdale, NY, USA). The dried protein film was then suspended in 6 M urea for 30 min at room temperature and subjected to an fast protein liquid chromatography-driven size-exclusion chromatography using a Superdex 30 column (GE Healthcare), equilibrated with 50 mM Tris–HCl, 250 mM NaCl, and 3 M urea, pH 8.0. Fractions containing free Dab2 SBM were pooled and dried again. The peptide film was then resuspended in a minimum volume of 0.1% TFA, acidified to a pH equal to or less than 3.0 by the addition of a small amount of formic or acetic acid, and loaded onto a C_{18} column (Waters Sep-Pak; Milford, MA, USA) previously equilibrated with 2% acetonitrile and 0.1% TFA. The column was washed twice with 2% acetonitrile and 0.1% TFA to remove salt and urea, and the peptide was eluted with 75% acetonitrile. Dab2 SBM was dried again as described earlier and stored at −80 °C until use. The purity of Dab2 SBM (~1 nM peptide in 75% acetonitrile) was estimated by mass spectrometry analysis using an Applied Biosystems Grand Island, NY, USA; model 4800 MALDI TOF/TOF mass spectrometer at the Virginia Tech Mass Spectrometry Incubator Facility. Peptide concentration was estimated by the bicinchoninic acid method [14]. The molecular mass of the isotopically labeled peptide was estimated using the Expasy Bioinformatics Resource Portal (web.expasy.org/compute_pi/). For the preparation of labeled peptides, the procedure was essentially the same except that bacterial LB cultures were first obtained in shaking flasks by a 1 : 10 dilution of LB pre-cultures to a volume of 100 ml of minimal media incubated for 1 h at 37 °C followed by another 1 : 10 dilution to the rest of the minimal media containing [^{15}N] ammonium chloride as the source of nitrogen and incubated at the conditions described above. Protein electrophoresis analyses were performed using 15% acrylamide Laemmli gels. Bands were identified using Coomassie blue staining and prestained molecular weight markers (Bio-Rad) as protein standards.

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NMR Spectroscopy

Nuclear magnetic resonance measurements of either unlabeled or uniformly ^{15}N-labeled Dab2 SBM in 90% H_2O/10%^2H_2O and 10 mM d_4-citrate (pH 5), 40 mM KCl, and 1 mM NaN_3 with either 40 or 200 mM d_{38}-DPC (NMR buffer) were obtained using 600 MHz Avance NMR spectrometer (Bruker Biospin; Billerica, MA, USA, at the Virginia Tech NMR Facility) equipped with a pulse field gradient triple resonance TBI probe at 25 °C. ^{15}N-edited HSQC spectrum analyses were carried out by preparing 200 μM of the ^{15}N-labeled peptide in an NMR buffer, whereas NOESY spectra were obtained using unlabeled Dab2 SBM with a mixing time of 200 ms. NMR

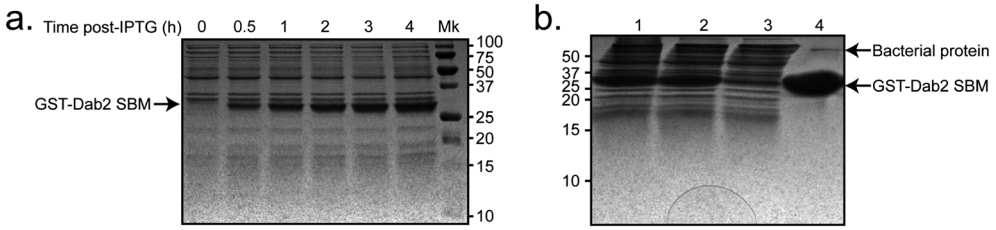

Figure 1. (a) SDS-PAGE analysis of recombinant GST–Dab2 SBM expressed in *E. coli* at the indicated time points after IPTG induction. (b) Analysis of the purification of GST-Dab2 SBM at different stages using SDS-PAGE. *Lane 1*, crude extract; *lane 2*, supernatant after centrifugation of the crude extract; *lane 3*, bacterial proteins that did not bind to glutathione beads; *lane 4*, purified GST–Dab2 SBM that co-purified with a high-molecular-weight bacterial protein.

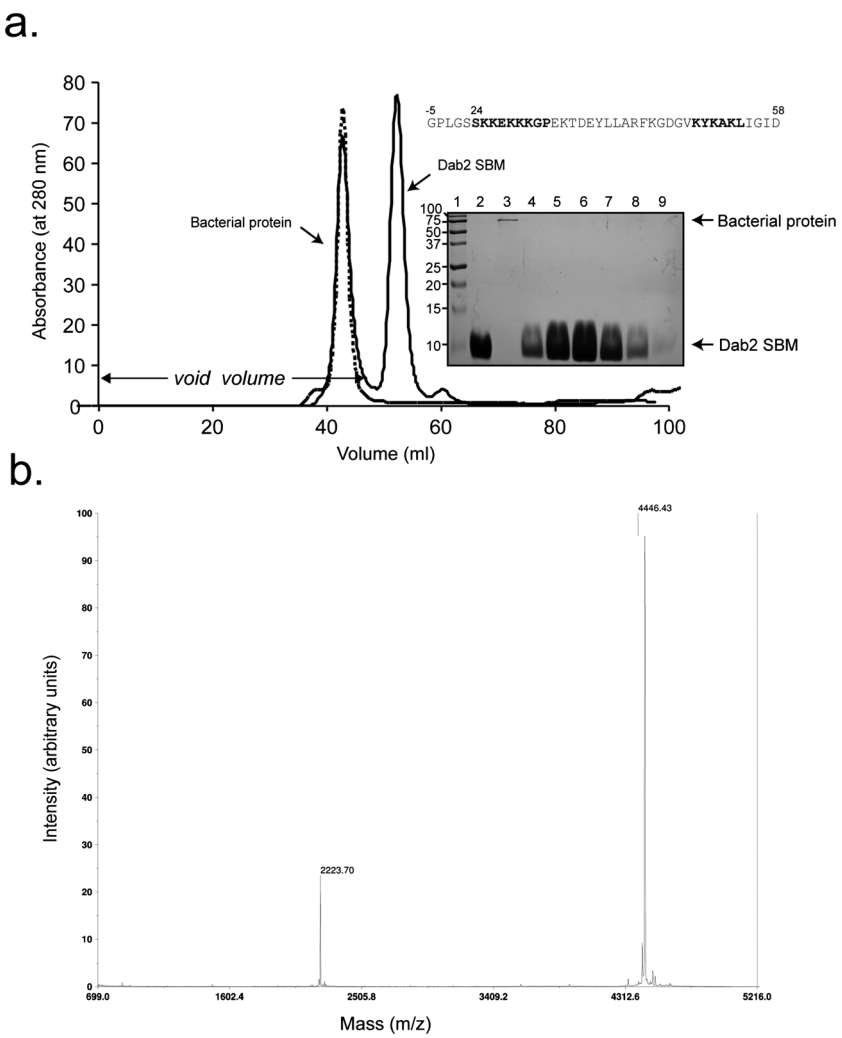

Figure 2. (a) Gel filtration analysis of Dab2 SBM. A Dab2 SBM/bacterial endogenous protein was loaded onto a fast protein liquid chromatography-driven Superdex 30 column under native (dotted line) and denaturing (solid line) conditions using a buffer containing 3 M urea as described in the Materials and Methods section. Inset, top: amino acid sequence corresponding to the recombinant Dab2 SBM. Bolded residues represent the two sulfatide-binding motifs in Dab2. Amino acids from the vector are GPLGS. Inset, bottom: SDS-PAGE analysis of the peptide purification. *Lane 1*, molecular mass markers; *lane 2*, synthetic Dab2 SBM; *lane 3*, endogenous bacterial protein from a fraction of the first peak of elution; *lanes 4–9*, fractions corresponding to the second peak of the chromatogram containing the purified Dab2 SBM peptide. (b) MALDI TOF analysis of purified untagged uniformly ^{15}N-labeled Dab2 SBM.

spectra were processed with Topspin 2.1 (Bruker) and NMRPipe [15] and analyzed using Sparky [16]. Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonic acid. For the sulfatide-binding assay using ^{1}H NMR, 100 μM Dab2 SBM was suspended in an NMR buffer containing 50 mM DPC in the absence and presence of 1.6 mM sulfatides dissolved in the same buffer.

Results and Discussion

Dab2 SBM Expression and Purification

The structural and sulfatide-binding characterization of Dab2 focused on SBM because this region binds sulfatides and associates with the surface of platelets to modulate their aggregation [13]. The Dab2 SBM peptide was expressed as a GST fusion protein in *E. coli* cells after IPTG induction. The SDS-PAGE analysis indicated that the protein stably overexpressed over a course of 4 h at 25 °C (Figure 1a). The GST–Dab2 SBM fusion protein can easily be purified by conventional affinity chromatography using glutathione beads. Purification requires the presence of a cocktail of protease inhibitors given the unstructured nature of the peptide. After glutathione chromatography purification, we observed two bands, one at about 29 kDa, which corresponds to GST–Dab2 SBM, and another at about 80 kDa, which represents a bacterial protein, presumably a chaperone bound to the unfolded peptide (Figure 1b). Because of their high level of expression and the unstructured nature of the peptides, they usually co-purify with bacterial chaperones. Several protocols have been published to avoid purification of recombinant bacterial proteins with endogenous bacterial chaperones. For example, removal of the contaminating chaperone GroEL from GST fusion proteins can be achieved by the addition of denatured proteins to the bacterial lysate prior to the addition of glutathione beads [17]. Similarly, removal of undesired *E. coli* Hsp70 molecular chaperones (e.g. DnaK) can be obtained by a combination of two approaches: (i) choosing the amino acids surrounding and belonging to the cloning site on the basis of an algorithm to decrease the interaction of the fusion protein with the chaperone and (ii) washing the fusion protein bound to the beads with ATP, $MgCl_2$, and soluble denatured *E. coli* proteins before the elution of the protein of interest [18]. In our experiments, size-exclusion chromatography analysis indicated that, after GST removal, the peptide co-eluted in a peak with a high-molecular-weight bacterial protein, with both proteins being found in the void volume of the column (Figure 2, dotted line, and data not shown). However, resuspension of the peptide sample in 6 M urea and further fractionation of the sample using a size-exclusion column, previously equilibrated with a buffer containing 3 M urea, allowed for the release of Dab2 SBM from the bacterial protein (Figure 2a, solid line) with purity at the same level of a peptide that was generated synthetically (Figure 2a, inset).

The purified peptide can be easily subjected to solvent exchange and removal of both urea and salt using a C_{18} column. Dab2 SBM resulted in a very stable peptide with no obvious indication of degradation based on mass spectrometry analysis of the peptide in 75% acetonitrile (Figure 2b). Indeed, the estimated molecular mass of the ^{15}N-labeled peptide from mass spectrometry analysis was 4,446.43, close to that estimated from its amino acid sequence (4,445.46). Of note, there have been some reported cases in which the isolated peptides are accompanied by some impurities. For example, the purification of the endothelin-3 peptide using a C_{18} column cannot be achieved in a single loading of the peptide into the column; instead, a C_{18} rechromatography of the leading edge fractions of the first C_{18} peak is recommended [19]. A work flow diagram of the purification protocol is indicated in Figure 3. Thus, to our knowledge, the methodology shown in this manuscript represents a novel approach for purifying soluble and intact peptides from host endogenous bacterial proteins using a combined urea treatment and sequential size-exclusion and hydrophobic chromatography procedures.

Expression of untagged peptides in bacteria usually does not work well because of their proteolysis, low yield, and/or toxicity. Fusion peptides not only offer protection of recombinant peptides from endogenous proteases during their overexpression in bacteria but also can be fused by a single or combination of a variety of tags and the introduction of proteolytic and/or chemical cleavage sites to further remove the tag from the recombinant peptide. Accordingly, and to alleviate the cost of production of labeled peptides, additional purification strategies of peptides for NMR studies have been reported using His [20–22], maltose-binding protein [23], the RNA-binding domain of human hnRNCP1 [24], GB1 domain [7], ketosteroid isomerase [25], thioredoxin [26,27], and even a combination of tags [20,21,23] yielding high-resolution spectra. Recently, a protocol for the purification of the cysteine-rich heat-stable enterotoxin was developed, in which its three disulfide bonds and proper folding were preserved during purification by the use of human uroguanylin as a tag (Table 1) as demonstrated by the high-resolution HSQC spectrum of the peptide [28]. Protease cleavage often triggers peptide proteolysis and chemical cleavage, such as with cyanogen bromide, and is an alternative option to isolating untagged peptides [29]. However, cyanogen bromide-mediated cleavage is limited to the presence of methionine

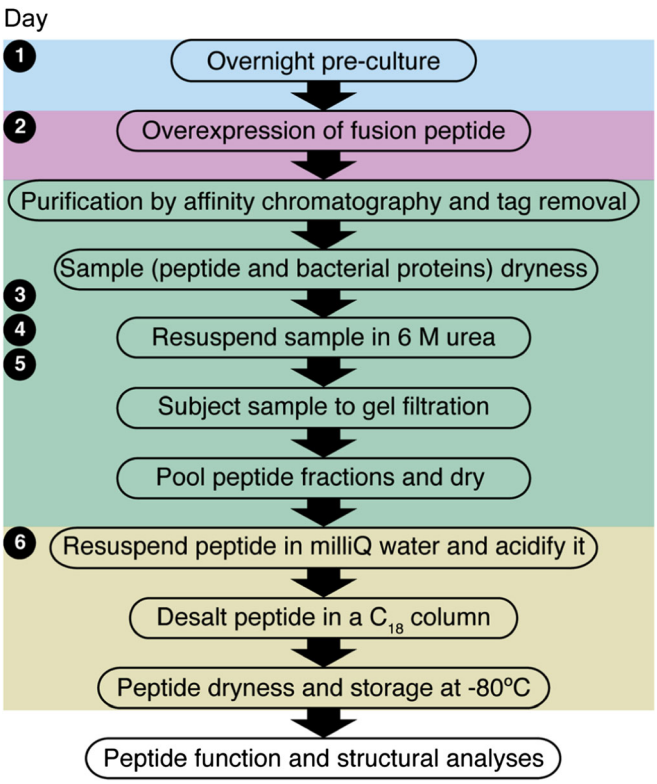

Figure 3. Work flow diagram of key steps described in this report.

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Table 1. Properties of representative peptides purified from bacteria and analyzed using solution NMR spectroscopy

Peptide	Size (amino acids)	Affinity tag	Property	Cleavage reagent	Yield (mg/l)	Reference
Early nodulin	24	Ubiquitin + $[His]_6$	Hydrophilic	Yeast ubiquitin hydrolase	3.4-7.0	[20]
Prostatic acidic phosphatase	39	$[His]_6$+ chitin-binding domain	Hydrophilic	TEV + intein	2.3	[21]
Parathyroid hormone	34	Thioredoxin	Hydrophilic	TEV	20.0	[27]
HIV-1_{MN} V3	23	RNA-binding domain of RNCP1	Hydrophilic	Cyanogen bromide	6.0–13.0	[24]
Phospholamb cytosolic domain	20	MBP + $[His]_6$	Hydrophilic	TEV	1.0	[23]
Coiled-coil trimerization domain of cartilage matrix protein	43	GB1 domain	Hydrophilic	Factor Xa	48.0	[7]
Disabled sulfatide-binding motif	40	GST	Membrane binding	PreScission protease	2.0	This report
Warnericin RK/Nwar	22	$[His]_6$	Membrane binding	—	1.4	[22]
Pituitary adenylate cyclase activating polypeptide	27	Thioredoxin	Membrane binding	Factor Xa	0.8	[26]
p75 neurotrophin receptor transmembrane domain	49	Ketosteroid isomerase	Membrane binding	Thrombin	1.0	[25]
Heat-stable enterotoxin	19	Uroguanylin	Cysteine rich	Trypsin	0.3	[28]

residues at the C- terminus of the tag and the absence of internal methionine residues in the target peptide. There is a good rate of success for the purification of fusion peptides from bacterial inclusion bodies (as reviewed in [29]). Despite their high yield, isolation of fusion peptides from these compartments requires additional work and the use of additional chemical denaturants for peptide isolation. The peptide yield obtained using our protocol (~2 mg/l of minimal media) is comparable with those reported for hydrophilic and membrane-binding peptides (Table 1) making a convenient strategy for the isolation of poorly structured peptides at yields required for NMR studies.

NMR Structural and Functional Analysis

Nuclear magnetic resonance spectroscopy is one of the most sophisticated biophysical techniques that provide details of disordered states of proteins and peptides [30]. For membrane-binding peptides, perdeuterated detergents (such as DPC) can promote peptide folding to improve the dispersion of the peptide NMR resonances [31]. DPC is the default detergent of choice because it contains the most common phospholipid head group found in biological membranes that form small micelles suitable for NMR studies. Detergent concentration should be

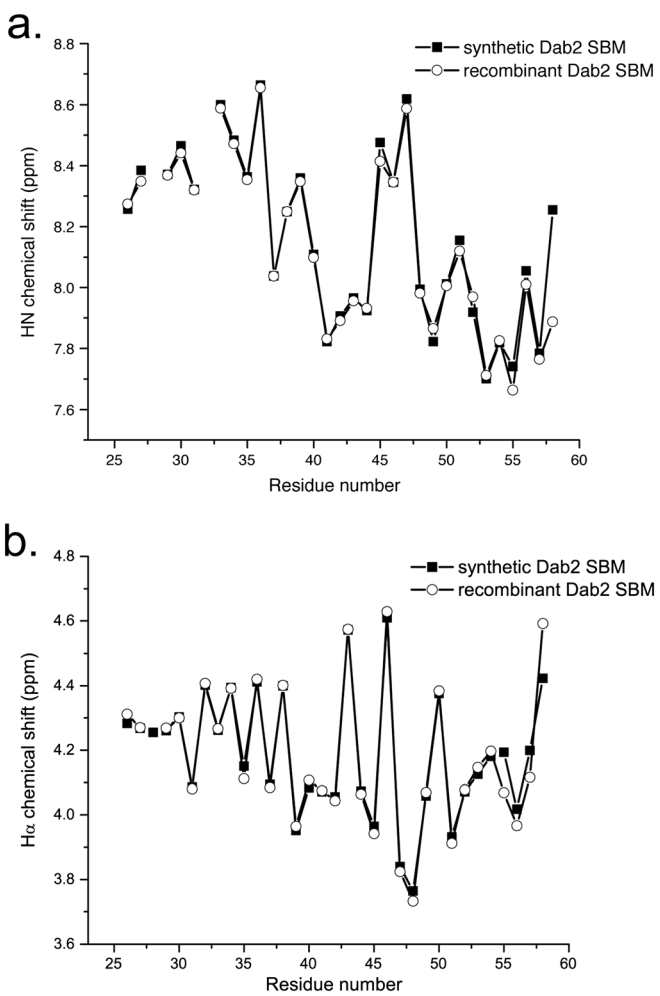

Figure 4. Comparison of the HN (a) and Hα (b) chemical shifts of the recombinant Dab2 SBM (opened circles) with the synthetic version of the peptide (filled squares).

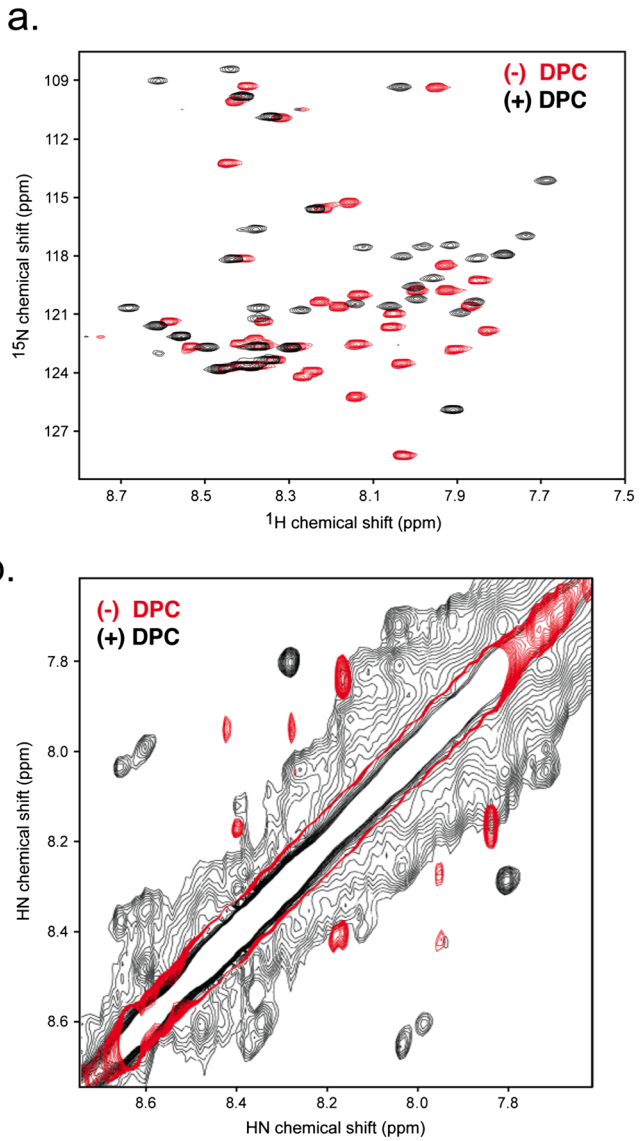

Figure 5. (a) Two-dimensional ^{1}H–^{15}N HSQC NMR spectra of uniformly ^{15}N-labeled Dab2 SBM (200 μM) in the absence (red) and presence of isotopically labeled DPC micelles (black). (b) Superposition of the HN–HN region of 2D NMR ^{1}H–^{1}H NOESY spectra of Dab2 SBM (1 mM) without (red) and with (black) DPC micelles. Mixing time t_m = 200 ms.

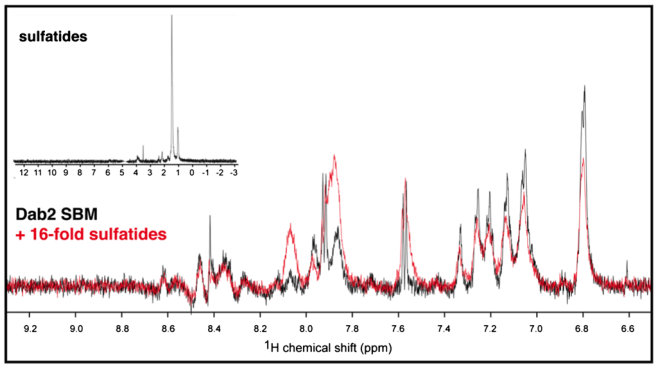

Figure 6. A zoom of the HN region of the one-dimensional ^{1}H NMR spectrum of unlabeled Dab2 SBM showing ^{1}H chemical shift perturbations before (black) and after (red) the addition of 16-fold sulfatides in DPC micelles. Inset: one-dimensional ^{1}H NMR spectrum of sulfatides in 50 mM DPC indicating the absence of any resonances in the HN region.

above its critical micelle concentration [31]. The efficacy of the methodology presented in this report was also demonstrated by comparison of the NH chemical shifts of the recombinant Dab2 SBM with the synthetic version of the peptide in DPC micelles. Under these conditions, both the HN and HA chemical shifts of the recombinant Dab2 SBM are very close to those corresponding to the synthetic peptide (Figure 4). Comparison of the ^{1}H, ^{15}N HSQC spectra of Dab2 SBM in the absence and presence of DPC micelles indicates that Dab2 SBM alone exhibited a random coil conformation in aqueous solution as evidenced by the limited chemical shift dispersion of the backbone resonances of the peptide (7.8–8.6 ppm in ^{1}H), which improved in the presence of DPC (7.65–8.7 ppm in ^{1}H) (Figure 5a), results that are consistent with circular dichroism studies of the Dab2 SBM in the presence of the detergent [13]. Moreover, the superposition of the NH region of the NOESY spectra also showed an improvement in the number of well-resolved resonances when Dab2 SBM was in the presence of DPC micelles (Figure 5b). The presence of NOEs in the NH region is indicative of a helical structure in the peptide. Therefore, the degree of spectral dispersion in the amide region and the presence of additional well-resolved NOESY cross-peaks are evidence of a folded peptide structure. The functionality of the recombinant ^{15}N- Dab2 SBM can be demonstrated by the observation of changes in the NH region of one-dimensional ^{1}H NMR spectra of the DPC-embedded peptide in the presence of a 16-fold excess of sulfatides (Figure 6).

Conclusions

We have demonstrated the feasibility of the isolation of an isotopically labeled recombinant peptide of high purity and good yield. To assess the utility of the protocol presented in this manuscript, N-terminal tagged GST–Dab2 SBM was employed as an example. By using a GST fusion construct, we limited the amount of proteolytic degradation of the peptide in *E. coli*. Because of its unstructured nature, Dab2 SBM co-purified with an endogenous bacterial protein, presumably a chaperone. The peptide could be purified to homogeneity by incubation of the complex with 6 M urea followed by size-exclusion chromatography in 3 M urea, followed by hydrophobic chromatography to remove urea. Peptide quantity is sufficient for NMR structural studies in a volume as modest as 1 l of isotopically labeled media. The relevance of Dab2 SBM arises from its ability to negatively modulate platelet aggregation by binding to the platelet surface [13]. Dab2 interacts with platelet surface sulfatides [32], an association that is mediated by the interaction of a short helical basic region that penetrates sulfatide-enriched membranes [33]. The role of Dab2 SBM in membrane binding becomes evident from structural studies that demonstrate that the peptide is unstructured but folds in the presence of DPC micelles. HSQC NMR spectra of Dab2 SBM in the presence of DPC micelles exhibited better dispersion of the peptide resonances (Figure 5) and, more importantly, bound sulfatides (Figure 6). Overall, the protocol presented here provides an alternative strategy for purifying unstructured peptides with a relatively high yield and purity and simultaneously alleviating the costs of generating isotopically labeled synthetic peptides for structural studies.

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