Published in final edited form as:

Mol Biotechnol. 2011 March ; 47(3): 205–210. doi:10.1007/s12033-010-9330-1.

The Use of the Condensed Single Protein Production (cSPP) System for Isotope-Labeled Outer Membrane Proteins, OmpA and OmpX in *E. coli*

S. Thangminlal Vaipheia^a, Yuefeng Tang^b, Gaetano T. Montelione^b, and Masayori Inouye^a

^aDepartment of Biochemistry, Robert Wood Johnson Medical School and Centre for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, New Jersey, USA 08854

^bDepartment of Molecular Biology and Biochemistry, Rutgers, and Centre for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, New Jersey, USA 08854

Abstract

Gram-negative bacteria consist of two independent membranes, the inner cytoplasmic membrane and the outer membrane. The outer membrane contains a number of β -barrel proteins such as OmpF, OmpC, OmpA and OmpX. In this paper, we explored to use the condensed Single Protein Production (cSPP) system for isotope labelling of OmpA and OmpX for NMR structural study, both of which are known to consist of eight β -strands forming a barrel in the outer membrane. Using a deletion strain lacking all major outer membrane proteins, both OmpA and OmpX were expressed well in a 20-fold condensed SPP (cSPP) system. We demonstrated that outer membrane fractions prepared from the cSPP system in M9 medium containing $^{15}\text{N-NH}_4\text{Cl}$ can be directly used for NMR structural study of the outer membrane proteins without any further purification to get excellent $[^1\text{H-}^{15}\text{N}]$ -TROSY spectra.

Introduction

For NMR structural studies of membrane proteins, one of the major obstacles is that a target membrane protein has to be purified from the cells after they are labeled with isotopes, which is quite tedious and time-consuming. In order to circumvent this problem, it is ideal if one could label only a target membrane protein with isotopes in the cells so that the whole membrane fraction may be used for NMR structural study of the protein without purification of the protein. In our laboratory, we have developed a protein expression system in *Escherichia coli* termed the SPP system, with which *E. coli* cells are used as a bioreactor producing only a protein of interest without producing any other cellular proteins [1]. In this system, MazF, an ACA-specific endoribonuclease or mRNA interferase found in *E. coli* [2] is used. When this enzyme is induced in the cells, almost all cellular mRNAs are degraded. However, if an mRNA is engineered to have no ACA sequences, MazF-induced cells are still able to produce the protein from the ACA-less mRNA. Notably, the RNA sequences of any mRNAs containing ACA sequences can be modified to ACA-less mRNAs without altering amino acid sequences of the proteins encoded by the mRNAs. Importantly, the

growth of the MazF-induced cells is completely arrested, the cell cultures can be highly (up to 100 fold) condensed without affecting the protein yield [3,4]. This condensed SPP (cSPP) system is particularly important as one can achieve extensive cost saving when expensive isotopes and isotope-labeled amino acids or other compounds are used and for preparing perdeuterated protein samples [9].

Approximately 2–3% of the genes in gram-negative bacteria encode β -barrel proteins which constitute majority of the outer membranes [5 Wimley, 2003]. The β -barrel outer membrane proteins draw special attention in structural and functional determination due to the versatility and ambiguity of this protein family. The β -barrel proteins are also found in the outer membranes of chloroplasts and mitochondria [5]. Even though there are many common structural features among them, β -barrel membrane proteins carry out diverse functions in diverse organisms. Proteins destined for the outer membrane of *E.coli* are synthesized in the cytoplasm and transported across the inner membrane through the SecYEG protein secretion machinery [6]. The signal peptide directing the precursor protein through the inner membrane is cleaved off by signal peptidase at the outer face of the inner membrane, and the processed OMP then traverses the periplasmic space to the β -barrel assembly sites in the outer membrane assisted by chaperones [7].

Earlier reports [8,9], have shown the expression of several membrane proteins using cSPP system for NMR studies. However, for NMR measurements the proteins were mostly purified by using Ni-NTA column. In these paper, we elaborate the use of cSPP system for the production of isotope-enriched outer membrane proteins (OMPs) of *E. coli* for NMR structural studies without purification. For this, we chose two outer-membrane proteins from *E.coli* as model proteins. First, the outer-membrane protein A (OmpA) from *E.coli* [10] consists of 325 residues in which the N-terminal (1–171 residues) domain formed an 8-stranded anti-parallel β -barrel in the outer membrane, whereas the C-terminal domain is located in the periplasm [11,12]. OmpA is important for maintaining structural stability of the outer membrane [13], bacterial conjugation [14] and acting as a receptor for bacteriophages [15] and colicins [16]. Second, outer-membrane protein X (OmpX) is homologous to membrane proteins of pathogenic bacteria like PagC of *Salmonella typhimurium* and Ail of *Yersinia enterocolitica* [17,18]. These proteins are responsible for the adherence of bacteria to mammalian cells and protect against the immune system. Both the structures of these two transmembrane beta-barrels OmpA (171 residues) and OmpX (148 residues) have been determined by solution NMR [19,20].

Without following the tedious purification steps of denaturation and renaturation, we directly isolated the outer membranes from the cells followed by NMR measurements in the presence of detergent DPC (Dodecyl-phosphocholine). The [^1H - ^{15}N]-TROSY-HSQC spectra of OmpA and OmpX [19,20] obtained from these methods were comparable to those that have been published for purified OmpA and OmpX samples, suggesting that this system would be highly advantageous for structural analysis of OMPs at low cost and less time.

Materials and Methods

Host Strain for Expression

E. coli BL-21(DE3) was used for the construction of OMP deletion strain (BL *ompA ompF*) from phage lysates of *ompA* and *ompF* deletion strain obtained from the National Institute of Genetics, Japan by using the P1 transduction method [21]. To confirm that BL *ompA ompF* cells does not produce both OmpA and OmpF, the cells were grown overnight in 5 ml LB at 37°C, and 1 ml from the culture was used to prepare cell lysate by sonication in 50 mM Tris buffer, pH 7.4. The cell debris was spun down at 12,000 rpm for 10 min and the supernatant was subjected to centrifugation at 50,000 rpm for 30 min at 4°C. The membrane pellet was then resuspended in the same buffer (0.5 ml) containing 0.5 % sarkosyl and the suspension was incubated at room temperature for 20 min. The sarkosyl insoluble outer membrane pellet was then isolated by centrifugation at 50,000 rpm for 30 min at 4°C. The total membrane and the outer membrane fractions were resuspended in SDS-loading buffer and analyzed by SDS-PAGE. The wild type *E. coli* host strain was used as a control.

Plasmids for Expression

The ACA-less genes for OmpA and OmpX with their respective signal sequences were obtained from GenScript USA Inc. and inserted into pColdIV vector having an ampicillin resistant gene (Takara Bio Inc) as shown in Fig. 1B. The N-terminal (1–176 residues) domain of OmpA (OmpA1-176) was also constructed by introducing a termination codon at position 177 of the full length *ompA* gene using site directed mutagenesis (Stratagene). BL *ompA ompF* cells bearing pACYCmazF were used for expression of these target proteins.

Media composition

M9 medium consists of M9 salts (0.68% Na₂HPO₄, 0.3% KH₂PO₄, 0.05% NaCl, and 0.1% NH₄Cl); 0.2% Casamino acids; 1 mM MgSO₄; 0.4% Glucose and 0.002 mg per ml vitamin B1.

³⁵S-Methionine incorporation

The incorporation of ³⁵S-Methionine was conducted to confirm the endoribonuclease activity of MazF by pulse-chase experiment. Overnight culture of BL *ompAF* cells harboring both pACYCmazF and pColdIVompA was inoculated in M9 medium and grown at 30°C till OD₆₀₀ reached 0.5 to 0.6. After 5 minutes incubation on ice, the cells were transferred back to 30°C for at least half an hour before adding IPTG. For isotope incorporation, at each time point, 1 ml cell culture was transferred into a tube containing ³⁵S-methionine at a final concentration of 10 μ curie. Pulse chase experiment was conducted for 15 minutes at all time points of 30 minutes interval. At the end of each time point cold methionine was added to quench the reaction. Samples were collected and resuspended in SDS-PAGE loading buffer. SDS-PAGE gel was dried and exposed overnight to X-ray film and developed the next day.

Preparation of NMR samples

1. The plasmids pColdIV*ompA* and pColdIV*ompX* were transformed in BL *ompA ompF* containing pACYC*mazF* and the cells were plated on M9 plates containing ampicillin (50 µg/ml) and chloramphenicol (15 µg/ml) at 37°C for 16–20 hours.
2. Inoculate few colonies in 50 ml M9 media using 250 ml flasks containing the same concentration of antibiotics and incubate at 30°C overnight.
3. The cells were spun down at 5000 rpm for 5 min and resuspended in M9 media (without Casamino acids) and transfer the cells in 5000 ml flasks containing 1L M9 (without Casamino acids) with the same amount of antibiotics.
4. The cells were allowed to grow at 30°C shaker until the OD₆₀₀ reached 0.5 – 0.6, in which 0.5 mM IPTG was added.
5. After 30 min the cells were kept on ice for 5 min and were spun down at 4°C and washed with 20 ml M9 (without Casamino acids and NH₄Cl) for 3 times by resuspending and spinning down the cells repeatedly.
6. Finally, the cells were resuspended in 500 ml flasks containing 50 ml (20 times condensed) M9 media containing ¹⁵NH₄Cl, 0.5 mM IPTG and the same amount of antibiotics.
7. The cells were harvested after culturing for 6 – 8 hours at 30°C and stored at –20°C.
8. For outer-membrane preparation, the cells were resuspended in 10–20 ml 50 mM Tris buffer (pH 7.4) and subjected to French-press till the lysates become clear.
9. The cell debris was pelleted down at 12,000 rpm for 10 min and the supernatant was subjected to centrifugation at 50,000 rpm for 30 min.
10. The supernatant was discarded and the total membrane pellet was resuspended in 20 mM potassium phosphate buffer (pH 6.5) containing 0.5% sarkosyl (sodium lauryl sarcosinate) and incubated at room temperature for 20 min. This was followed by centrifugation at 50,000 rpm for 30 min.
11. The outer-membrane pellet was washed with 20 mM phosphate buffer (pH 6.5) by repeated sonications and centrifugations.
12. The pellet was finally resuspended in 20 mM potassium phosphate buffer (pH 6.5) containing 5 % DPC and 5 % D₂O by sonication and heat treatment at 80–90°C for 3 min.
13. The sample was cooled down at room temperature and centrifuged for 30 min to remove the insoluble parts. The soluble portion was transferred into a regular NMR tubes and scanned with 800 MHz Bruker AVANCE spectrometer at 50°C. NMR TROSY – [¹H-¹⁵N] HSQC was recorded as described previously [8, 9].

NMR Measurements

NMR measurements of uniformly ^{15}N -labeled OmpA1-176 and OmpX in 5 % DPC were obtained using a 800 MHz Bruker AVANCE spectrometer with a cryoprobe at 50 °C. [^1H - ^{15}N] TROSY HSQC NMR data were acquired using spectral widths of 14 ppm in ^1H dimension and 34 ppm in ^{15}N dimension. The matrix size of collected spectra was 1024 × 256 total data points.

In all NMR experiments, data processing involved zero-filling and weighting by sine squared window function in acquisition and evolution dimensions. All NMR spectra were processed and examined using the program NMR-Pipe/NMR-Draw software package [22]. The program SPARKY [23] was used for data visualization and analysis. Chemical shifts were referenced to external 2, 2-dimethyl-2-silapentane-5-sulfonic acid (DSS).

Results and Discussion

OMP production in the outer membrane

The outer membrane fraction prepared from BL *ompAFmazF* strain was almost pure since no other major OMP was produced (Fig. 1A). This strain not only allows recovery of extremely pure target protein but also provides enough cellular space for target protein accumulation when over expressed inside the cells. The use of signal sequence directed the precursor protein to localize in the outer membrane rather than becoming insoluble aggregates in the cytosol as shown in Fig. 1C, 1D and 1E. Also, the total amount of OMP being produced after 12 hours incubation reached 8–10 mg per litre for both OmpA1-176 and OmpX. The SDS-PAGE profile clearly showed the difference in mobility shift between the boiled and non-boiled samples, a well known characteristic property exhibited by OMPs termed as heat-modifiability [24,25]. In case of full-length OmpA, the fully denatured protein (boiled sample) moved slower than the non-denatured (non-boiled) sample (Fig. 1C); and vice versa in the case of truncated transmembrane beta-barrel Omp176 (Fig. 1D). In the case of OmpX, the mobility on SDS-PAGE was similar to the truncated OmpA1-176. The difference in boiled and non-boiled sample could be explained by the highly stable conformation of OMPs which even SDS does not unfold completely unless the sample is heated or boiled at 100°C. The non-boiled sample represents a folded or native form of OMP in SDS-PAGE gel whereas the boiled represents the largely unfolded protein. A CD spectrum recorded at different temperatures also showed that OmpX still maintains β -stranded secondary structure in 5 % DPC (data not shown).

Isotope incorporation of OMP

The endoribonuclease activity of MazF as indicated by the result of ^{35}S -Methionine incorporation clearly indicates that essentially all cellular mRNAs were degraded by overproduction of MazF (Fig. 2). The Commassie stained gel shows only the background proteins which were produced before the induction of MazF (Fig. 2A); whereas the autoradiography result shows that no significant amount of cellular proteins are produced following MazF induction since MazF activity results in mRNA degradation (Fig. 2B).

Uniformly ^{15}N -labeled OmpA1-176 and full-length OmpX ($\sim 200\mu\text{M}$ concentration) were employed for NMR measurements using 800 MHz Bruker AVANCE spectrometer at 50°C as done previously [8]. The NMR spectra shown in Fig 3, suggest that the distribution pattern of the amide peaks were similar to that reported for reconstituted purified OmpA1-176 and OmpX [19, 20]. Notably, the total expected numbers of indole N^{H} resonances of tryptophan residues (OmpA1-176 and OmpX contain 5 and 2 tryptophan residues respectively) were observed in the 2D $[\text{}^1\text{H}-^{15}\text{N}]$ -TROSY-HSQC NMR spectra as shown in Fig. 3A and B (inbox). Similar results have been reported for samples of OmpX labeled with three isotopes ($^{15}\text{NH}_4\text{Cl}$, ^{13}C -Glucose and $^2\text{H}_2\text{O}$) at the same time [9].

Hydrogen deuterium back-exchange

Beta-barrels make very stable structures that do not readily unfold in membranes as a result of hydrogen-bonding interactions [26, 27]. The outer membrane pellet containing uniformly ^{15}N -enriched OmpA1-176 produced in M9- H_2O medium was resuspended in 0.5 ml H_2O or 99.9% D_2O both containing 5 % DPC. The pellet was solubilized by sonication and this time not subjected to heat treatment. The NMR spectra recorded from the H_2O -resuspended sample showed maximum numbers of peaks (Fig. 4A) whereas, the D_2O -resuspend sample showed only about one-half of the peaks (Fig. 4B) due to hydrogen-to-deuterium exchange of the most accessible amide sites; the core amide sites remain protonated and observable even in D_2O . Overlapping these two spectra also clearly shows the regions which were missing due to incomplete hydrogen back exchange or inaccessibility of water molecules (Fig. 4C). Under these conditions, little or no back-exchange is observed for the amide sites inside of the barrel. Therefore, no peaks were visible from areas easily exposed whereas visible peaks originated from the buried transmembrane hydrophobic core. This result also supported the previous report that the high rigidity around the barrel core is due to the extensive nonlocal backbone hydrogen bonds between strands and that the barrel is most rigid in the bilayer center and most flexible near the bilayer interfaces [26].

This experiment suggests that a harsher condition such as heat treatment would also be needed to destabilize highly stable beta-barrel transmembrane proteins for applications aimed at providing fully perdeuterated proteins with back exchange. Our preliminary result suggested that fully deuterated OMP sample did not yield as many peaks without heat treatment when compared with the non-deuterated sample (data not shown). However, in our previous report by Schneider et al., we have shown the NMR spectrum obtained from fully deuterated OmpX after heat treatment. Heat treatment destabilized the beta-barrel structure, and it folds back immediately to a native structure by lowering the temperature in the presence of detergent.

Conclusion

We described a concise practical approach and a platform for production of OMPs using *E.coli* cSPP system in NMR application. By using this system, isotope-labeled OMPs could be expressed in high amount and extracted from the outer membrane for direct NMR

measurements. This approach may be helpful for structural studies and other biophysical and spectrometric analysis of bacterial outer membrane proteins.

Acknowledgments

We thank Dr. Sung-Gun Kim for CD analysis of OmpX. This work was supported by National Institutes of Health Grants, 5R01GM085449 (to M. I.), Northeast Structural Genomics (grant U54 GM074958 to G.T.M. and M.I.) and New York Consortium on Membrane Protein Structure (grant U54 GM075026 to W. Hendrickson and M.I.). This work was also supported by a grant from Takara Bio Inc.

References

1. Suzuki M, Zhang J, Liu M, Woychik NA, Inouye M. Single protein production in living cells facilitated by an mRNA interferase. *Mol Cell*. 2005; 18:253–261. [PubMed: 15837428]
2. Zhang Y, Zhang J, Hoeflich KP, Ikura M, Qing G, Inouye M. MazF cleaves cellular RNAs specifically at ACA to block protein synthesis in *Escherichia coli*. *Mol Cell*. 2003; 12:913–923. [PubMed: 14580342]
3. Suzuki M, Rohini R, Zheng H, Woychik N, Inouye M. Bacterial bioreactors for high yield production of recombinant protein. *J Biol Chem*. 2006; 281:37559–37565. [PubMed: 17020876]
4. Suzuki M, Mao L, Inouye M. Single protein production (SPP) system in *Escherichia coli*. *Nat Protoc*. 2007; 2:1802–1810. [PubMed: 17641648]
5. Wimley WC. The versatile beta-barrel membrane protein. *Curr Opin Struct Biol*. 2003; 13:404–411. [PubMed: 12948769]
6. Veenendaal AK, van der Does C, Driessen AJ. The protein-conducting channel SecYEG. *Biochim Biophys Acta*. 2004; 1694:81–95. [PubMed: 15546659]
7. Mogensen JE, Otzen DE. Interactions between folding factors and bacterial outer membrane proteins. *Mol Microbiol*. 2005; 57:326–346. [PubMed: 15978068]
8. Mao L, Tang Y, Vaiphei ST, Shimazu T, Kim SG, Mani R, Fakhoury E, White E, Montelione GT, Inouye M. Production of membrane proteins for NMR studies using the condensed single protein (cSPP) production system. *J Struct Funct Genomics*. 2009; 10:281–289. [PubMed: 19856129]
9. Schneider WM, Tang Y, Vaiphei ST, Mao L, Maglaqui M, Inouye M, Roth MJ, Montelione GT. Efficient condensed-phase production of perdeuterated soluble and membrane proteins. *J Struct Funct Genomics*. 2010; 11:143–54. [PubMed: 20333498]
10. Hindennach I, Henning U. The major proteins of the *Escherichia coli* outer cell envelope membrane. Preparative isolation of all major membrane proteins. *Eur J Biochem*. 1975; 59:207–13. [PubMed: 812694]
11. Chen R, Schmidmayr W, Kramer C, Chen-Schmeisser U, Henning U. Primary structure of major outer membrane protein II (ompA protein) of *Escherichia coli* K-12. *Proc Natl Acad Sci USA*. 1980; 77:4592–6. [PubMed: 7001461]
12. Morona R, Klose M, Henning U. *Escherichia coli* K-12 outer membrane protein (OmpA) as a bacteriophage receptor: analysis of mutant genes expressing altered proteins. *J Bacteriol*. 1984; 159:570–8. [PubMed: 6086577]
13. Sonntag I, Schwarz H, Hirota Y, Henning U. Cell envelope and shape of *Escherichia coli*: multiple mutants missing the outer membrane lipoprotein and other major outer membrane proteins. *J Bacteriol*. 1978; 136:280–5. [PubMed: 361695]
14. Schweizer M, Henning U. Action of a major outer cell envelope membrane protein in conjugation of *Escherichia coli* K-12. *J Bacteriol*. 1977; 129:1651–2. [PubMed: 321438]
15. Morona R, Krämer C, Henning U. Bacteriophage receptor area of outer membrane protein OmpA of *Escherichia coli* K-12. *J Bacteriol*. 1985; 164:539–43. [PubMed: 3902787]
16. Foulds J, Barrett C. Characterization of *Escherichia coli* mutants tolerant to bacteriocin JF246: two new classes of tolerant mutants. *J Bacteriol*. 1973; 116:885–92. [PubMed: 4583254]
17. Meccas J, Welch R, Erickson JW, Gross CA. Identification and characterization of an outer membrane protein, OmpX, in *Escherichia coli* that is homologous to a family of outer membrane

- proteins including Ail of *Yersinia enterocolitica*. *J Bacteriol.* 1995; 177:799–804. [PubMed: 7836315]
18. Heffernan EJ, Harwood J, Fierer J, Guiney D. The *Salmonella typhimurium* virulence plasmid complement resistance gene *rck* is homologous to a family of virulence-related outer membrane protein genes, including *pagC* and *ail*. *J Bacteriol.* 1992; 174:84–91. [PubMed: 1729227]
 19. Arora A, Abildgaard F, Bushweller JH, Tamm LK. Structure of outer membrane protein A transmembrane domain by NMR spectroscopy. *Nat Struct Biol.* 2001; 8:334–338. [PubMed: 11276254]
 20. Fernandez C, Adeishvili K, Wuthrich K. Transverse relaxation-optimized NMR spectroscopy with the outer membrane protein OmpX in dihexanoyl phosphatidylcholine micelles. *Proc Natl Acad Sci USA.* 2001; 98:2358–63. [PubMed: 11226244]
 21. Miller, JH. *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press; New York: 1972.
 22. Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A. NMRPipe: A multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR.* 1995; 6:277–93. [PubMed: 8520220]
 23. Goddard, TD.; Kneller, DG. *SPARKY 3*. University of California; San Francisco: 2004.
 24. Dornmair K, Keifer H, Jahnig F. Refolding of an integral membrane protein OmpA of *Escherichia coli*. *J Biol Chem.* 1990; 265:18907–11. [PubMed: 2229053]
 25. Burgess NK, Dao TP, Stanley AM, Fleming KG. Beta-barrel proteins that reside in the *Escherichia coli* outer membrane in vivo demonstrate varied folding behavior in vitro. *J Biol Chem.* 2008; 283:26748–58. [PubMed: 18641391]
 26. Haltia T, Freire E. Forces and factors that contribute to the structural stability of membrane proteins. *Biochim Biophys Acta.* 1995; 1241:295–322. [PubMed: 7640299]
 27. Rosenbusch JP. Stability of membrane proteins: relevance for the selection of appropriate methods for high-resolution structure determinations. *J Struct Biol.* 2001; 136:144–157. [PubMed: 11886216]

Fig. 1. Expression system for OMP in the cSPP system

(A) SDS-PAGE analysis for comparison of wild type BL-21(DE3) cells and BL *ompAF* total membrane (TM) and outer membrane (OM) fractions. (B) Schematic diagram of pCold IV expression vector used for OMP expression. (C), (D) and (E) Membrane localization of full-length OmpA, OmpA176 and OmpX respectively. Lane 1: total membrane; lane 2: sarkosyl soluble fraction; lane 3: outer-membrane fraction (boiled); lane 4: outer-membrane fraction (non-boiled); lane M: molecular weight markers.

Fig. 2. SDS-PAGE profile for OmpA production by ^{35}S -methionine incorporation
(A) Coomassie stained SDS-PAGE gel of OmpA production at different time points; (B) Pulse chase experiment showing ^{35}S -methionine incorporation specifically in target protein OmpA using cSPP system. Lane 1: before induction; lane 2 and 3: 15 and 30 minutes after induction; lane 4,5,6, 7,8,9 and 10: 1, 2, 3, 4, 6,12 and 24 hr after induction; lane M: molecular weight markers.

Fig. 3. 2D [^1H - ^{15}N] TROSY HSQC NMR spectra of OmpA1-176 (A) and OmpX (B). Uniformly ^{15}N enriched OMP from the outer membrane pellet was resuspended in 20 mM potassium phosphate buffer at pH 6.5, by sonication in the presence of 5 % DPC. The sample was treated at 80°C for 3 minutes and the insoluble parts were removed by centrifugation at 50,000 rpm for 30min. Protein concentration was about $200\ \mu\text{M}$ in final volume of 0.5 ml buffer solution. NMR measurements were conducted with 800 MHz Bruker AVANCE spectrometer at 50°C .

Fig. 4. ${}^2\text{H}$ - ${}^1\text{H}$ back exchange experiment demonstrating the structural stability of OmpA by using NMR buffer constituted with ${}^1\text{H}_2\text{O}$ (A) and ${}^2\text{H}_2\text{O}$ (B)

Uniformly ${}^{15}\text{N}$ enriched OmpA was produced from M9 media in ${}^1\text{H}_2\text{O}$. The outer membrane pellet was resuspended in NMR buffer constituted with ${}^1\text{H}_2\text{O}$ and ${}^2\text{H}_2\text{O}$ containing 5% DPC. [${}^1\text{H}$ - ${}^{15}\text{N}$]TROSY HSQC NMR spectra were recorded from these samples using an 800 MHz Bruker AVANCE spectrometer at 50°C . While essentially all amide protons are observed in (A), only the slowly exchanging core amide protons of OmpA are observed in (B).