Published in final edited form as:

Biomol NMR Assign. 2013 October ; 7(2): 203–206. doi:10.1007/s12104-012-9410-1.

Backbone ^1H , ^{13}C and ^{15}N Resonance Assignments of the α -Helical Membrane Protein TM0026 from *Thermotoga maritima*

Brett M. Kroncke and Linda Columbus*

Department of Chemistry, University of Virginia, Charlottesville, VA, 22904

Abstract

Critical to the use of solution NMR to describe the structure and flexibility of membrane proteins is the thorough understanding of the degree of perturbation induced by the detergent or other membrane mimetic. To develop a deeper understanding of the interaction between membrane proteins and micelles or bicelles, we will investigate the differences in structure and flexibility of a model membrane protein TM0026 from *Thermotoga Maritima* using solution NMR. A comparison of the structural differences between TM0026 solubilized in different detergent combinations will provide important insight into the degree of modulation of membrane proteins by detergent physical properties. Here we report the nearly complete backbone and C_β resonance assignments of the two transmembrane helical model protein TM0026. These assignments are the first step to using TM0026 to elucidate the interaction between membrane proteins and membrane mimetics.

Biological context

Membrane proteins mediate communication and transport of small molecules across the membrane barrier of the cell. In order to study membrane protein structure, the protein of interest typically needs to be extracted and purified from the native membrane environment and solubilized in a membrane mimetic such as detergent micelles or bicelles (Carpenter et al. 2008; Patching 2011). In the absence of a functional assay, detergents are screened based on ability to produce crystals (Wiener 2004) and high-resolution NMR spectra (Sanders and Sonnichsen 2006). However, relatively little is known about how membrane mimetics modulate protein structure, flexibility and function (Columbus et al. 2009; Garavito and Ferguson-Miller 2001). The assignment of the model protein system TM0026 from *Thermotoga maritima* described in this paper provides a model system to investigate the impact detergent composition has on polytopic membrane protein dynamics and structure.

TM0026 from *T. maritima* is an excellent polytopic model system since it contains only two transmembrane helices and has been shown to be sensitive to changes in detergent composition (Columbus et al. 2009). The function of TM0026 is not known; however the gene is located in the BglR operon. In the presence of glucose, the expression of TM0026, along with a putative cellobiose/laminaribiose ABC transporter and other proteins in the BglR operon, is up-regulated (Frock et al. 2012). Thus, TM0026 may act as a regulating protein in glucose transport. Regardless of function, TM0026 has served as a model system for investigating helix-helix interactions in polytopic membrane proteins and the effects of detergents on the structure of membrane proteins (Columbus et al. 2009). The backbone assignment facilitates a more detailed understanding of the perturbing effects of micelle

*To whom correspondence should be addressed. Linda Columbus: University of Virginia, Department of Chemistry, McCormick Rd, Charlottesville, VA, 22904, phone: (434) 243-2123, fax: (434) 924-3710, columbus@virginia.edu.

composition and insight on the interpretation of structures determined in these membrane mimetic environments.

Here we report the backbone ^1H , ^{13}C and ^{15}N resonance assignment of the α -helical membrane protein TM0026 from *T. maritima*, including the two transmembrane helices between residues 5 to 28 and 33 to 53. In addition to structure determination, the assignment provides information required to interpret and compare NMR relaxation, chemical shift and structural perturbations in non-native membrane mimetics.

Methods and experiments

Protein expression and purification

The tm0026 gene with the encoded MGSDKIHSHHHH N-terminal tag was subcloned from the JCSG expression vector (Lesley et al. 2002) (which uses glucose as a repressor) to pET21b using restriction sites NdeI and XhoI. Protein expression and purification was performed as previously described (Kroncke and Columbus 2012). All protein samples were perdeuterated. Briefly, cells were grown at 310 K to an OD₆₀₀ of 0.6–0.8 in Luria-Bertani medium and induced with 1 mM isopropyl β -D-1-thiogalactopyranoside (Research Products International; Mt. Prospect, IL) for 4 hours. The cells were resuspended and lysed in 50 mM Tris-HCl (pH 8.0) and 150 mM NaCl (lysis buffer). After removing cell debris, by centrifugation at $15,000 \times g$ for 60 min, membranes were solubilized with 10 mM n-decyl- β -D-maltopyranoside (DM; Anatrace, Inc., Maumee, OH) for 3 hours at 298 K. The suspension was passed through a Co^{2+} immobilized metal affinity chromatography (IMAC) column equilibrated with 10 column volumes (CV) of lysis buffer and 5 mM DM. The column was washed with 15 CV of 20 mM phosphate buffer (pH 7.8), 150 mM NaCl, 15 mM imidazole and 5 mM DM and eluted in 10 CV of 20 mM phosphate buffer (pH 7.0), 150 mM NaCl, 5 mM n-dodecyl- β -D-maltopyranoside (DDM; Anatrace, Inc., Maumee, OH), 15 mM n-decylphosphocholine (FC-10; Anatrace, Inc., Maumee, OH) and 600 mM imidazole. Samples were dialyzed against 20 mM phosphate buffer (pH 6.2) and 150 mM NaCl three times for two hours each.

NMR spectroscopy

NMR samples were prepared to ~ 0.5 mM TM0026 in 20 mM phosphate buffer (pH 6.2), 150 mM NaCl, and 10% D₂O. Spectra were collected at 313 K on Bruker AVANCE 600 MHz and 800 MHz spectrometers using Bruker 5 mm TXI cryoprobes. Spectra were processed with NMRPipe (Delaglio *et al.* 1995) and assigned using CARA (Keller 2004). For the sequence-specific resonance assignments of backbone atoms, TROSY versions of the following pulse sequences were recorded: 2D ^{15}N , ^1H -HSQC, 3D HNCACB, 3D HN(CO)CA, 3D HN(COCA)CB, 3D HN(CA)CO and 3D HNCO (Bax A 1993; Pervushin *et al.* 1997). Proton chemical shifts were referenced to 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) and used to scale the ^{15}N and ^{13}C resonances.

Extent of assignments and data deposition

The construct of TM0026 used in this study consists of all 68 residue of the native protein and an additional 12 residue N-terminal His₆ tag. Figure 1 shows the ^{15}N , ^1H -HSQC annotated with the assignments of TM0026. Seventy-one peaks were observed out of 78 expected peaks. Backbone and C β assignments were complete except for the N-terminal affinity tag and residues M1, I9, W12, K56–R59, both proline amide nitrogens and L48 C α and C β . The assignment accounts for 90% of the assignable backbone and C β atoms. Figure 2a shows the sequence derived hydropathicity plot (Kyte and Doolittle 1982) and secondary structure of TM0026 as determined through PHDhtm (Rost et al. 1995). The NMR derived ^{13}C chemical shifts (Fig. 2b and c) and chemical shift index (Fig. 2d) also suggest

TM0026 consists of two α -helices separated by an unstructured linker. The chemical shift data is consistent with the predicted secondary structure consistent and strongly suggests TM0026 contains two transmembrane helices. The chemical shift assignments for the backbone of TM0026 have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu/>) under the accession number 18494.

References

- Bax AGS. Methodological advances in protein NMR. *Acc Chem Res.* 1993; 26(4):131–138.
- Carpenter E, Beis K, Cameron A, Iwata S. Overcoming the challenges of membrane protein crystallography. *Curr Opin Struct Biol.* 2008; 18(5):581–586. [PubMed: 18674618]
- Columbus L, Lipfert J, Jambunathan K, Fox DA, Sim AY, Doniach S, Lesley SA. Mixing and matching detergents for membrane protein NMR structure determination. *J Am Chem Soc.* 2009; 131(21):7320–7326. [PubMed: 19425578]
- 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(3):277–293. [PubMed: 8520220]
- Frock A, Gray S, Kelly R. Hyperthermophilic *Thermotoga* species differ with respect to specific carbohydrate transporters and glycoside hydrolases. *Applied and environmental microbiology.* 2012; 78(6):1978–1986. [PubMed: 22247137]
- Garavito M, Ferguson-Miller S. Detergents as Tools in Membrane Biochemistry. *J Biol Chem.* 2001; 276(35):32403–32406. [PubMed: 11432878]
- Keller, RLJ. Optimizing the process of nuclear magnetic resonance spectrum analysis and computer aided resonance assignment. Zurich: Swiss Federal Institute of Technology Zurich; 2004.
- Kroncke B, Columbus L. Identification and removal of nitroxide spin label contaminant: impact on PRE studies of ($E\pm$ -helical membrane proteins in detergent. *Protein science : a publication of the Protein Society.* 2012; 21(4):589–595. [PubMed: 22389096]
- Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol.* 1982; 157(1):105–132. [PubMed: 7108955]
- Lesley S, Kuhn P, Godzik A, Deacon A, Mathews I, Kreusch A, Spraggon G, Klock H, McMullan D, Shin T, Vincent J, Robb A, Brinen L, Miller M, McPhillips T, Miller M, Scheibe D, Canaves J, Guda C, Jaroszewski L, Selby T, Elsliger M-A, Wooley J, Taylor S, Hodgson K, Wilson I, Schultz P, Stevens R. Structural genomics of the *Thermotoga maritima* proteome implemented in a high-throughput structure determination pipeline. *Proceedings of the National Academy of Sciences of the United States of America.* 2002; 99(18):11664–11669. [PubMed: 12193646]
- Patching SG. NMR structures of polytopic integral membrane proteins. *Mol Membr Biol.* 2011
- Pervushin K, Riek R, Wider G, Wuthrich K. Attenuated T2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. *Proc Natl Acad Sci U S A.* 1997; 94(23):12366–12371. [PubMed: 9356455]
- Rost B, Casadio R, Fariselli P, Sander C. Transmembrane helices predicted at 95% accuracy. *Protein science : a publication of the Protein Society.* 1995; 4(3):521–533. [PubMed: 7795533]
- Sanders CR, Sonnichsen F. Solution NMR of membrane proteins: practice and challenges. *Magn Reson Chem.* 2006; 44:S24–S40. Spec No: [PubMed: 16826539]
- Wiener M. A pedestrian guide to membrane protein crystallization. *Methods (San Diego, Calif).* 2004; 34(3):364–372.
- Wishart DS, Sykes BD. The ^{13}C chemical-shift index: a simple method for the identification of protein secondary structure using ^{13}C chemical-shift data. *J Biomol NMR.* 1994; 4(2):171–180. [PubMed: 8019132]

Figure 1.
A ^{15}N , ^1H -HSQC spectrum of TM0026 collected at 600 MHz. Assigned peaks are labeled with the one-letter amino acid code for the backbone atoms and the two tryptophan side chain indole peaks in the lower left corner of the spectrum.

Figure 2.

Sequence and chemical shift derived secondary structure predictions. (a) The membrane-spanning region predicted by the hydropathicity score and secondary structure predicted by PHDhtm (top). (b–c) NMR chemical shifts of (b) $^{13}\text{C}^\alpha$, (c) $^{13}\text{C}^\beta$ and (d) the derived chemical shift index (Wishart and Sykes 1994) for TM0026 are shown. All three chemical shift plots are consistent with a helical membrane protein containing two membrane-spanning helices.