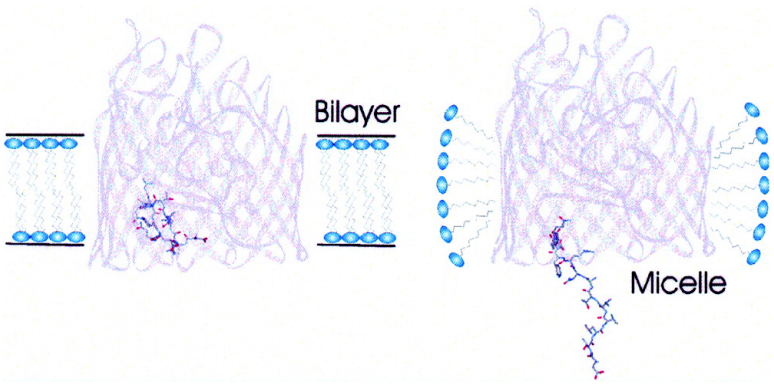
Communication

Membrane Mimetic Environments Alter the Conformation of the Outer Membrane Protein BtuB

Gail E. Fanucci, Jae Y. Lee, and David S. Cafiso

J. Am. Chem. Soc., **2003**, 125 (46), 13932-13933• DOI: 10.1021/ja0376442 • Publication Date (Web): 25 October 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/25/2003

Membrane Mimetic Environments Alter the Conformation of the Outer Membrane Protein BtuB

Gail E. Fanucci, Jae Y. Lee, and David S. Cafiso*

Department of Chemistry, University of Virginia, P.O. Box 400319, Charlottesville, Virginia 22904-4319

Received July 30, 2003; E-mail: cafiso@virginia.edu

Membrane proteins are estimated to constitute nearly 30% of encoding sequences, are targets for the majority of currently used pharmaceuticals, and their structures are critical to understanding many fundamental biological processes.[1−3] As a result, membrane proteins are a focus of the current structural genomics effort. Typically, structure determination using high-resolution NMR or X-ray diffraction employs detergents to solubilize the membrane protein, resulting in a micelle or bicelle phase.[4,5] Although these phases are generally thought to substitute for the native membrane environment, there is little direct evidence comparing the structure and function of membrane proteins in these mimetic systems to those in bilayers.[6,7]

Here we use site-directed spin labeling (SDSL) and EPR spectroscopy to show that the conformation of the N-terminal energy-coupling motif of BtuB in micelles or bicelles differs from its structure in a bilayer phase. BtuB is a 66 kD TonB-dependent outer membrane transporter found in *Escherichia coli* that functions to sequester vitamin B_{12} from the surroundings and transport it into the periplasm.[8,9] The crystal structure of BtuB[10] is similar to other TonB-dependent transporters FepA, FhuA, and FecA and consists of a 22-strand transmembrane β-barrel and a globular N-terminal hatch that occludes the barrel. A highly conserved region called the Ton box is located at the N-terminus. The Ton box is believed to mediate the protein−protein coupling interaction with the transperiplasmic protein TonB, which is necessary for transport. Furthermore, when BtuB binds substrate the Ton box undergoes a conformational change, which may provide a signal to TonB that the receptor is loaded with substrate.[11−13]

SDSL is a powerful technique to study membrane protein structure and function because proteins can be examined in their native membrane,[12,14] in reconstituted lipid bilayer systems,[15−17] or in micelles.[18] SDSL was used previously to show that the energy coupling Ton box motif of BtuB (residues 6−12) exists in two different conformational states.[12,19] In the absence of substrate, the Ton box is structured and docked within the transporter barrel, and upon substrate binding it undergoes a change to an unfolded or undocked state. Residues 1−5 are unfolded in both states, and the conformational change upon substrate binding involves an order to disorder transition up to residue 14 with the region near residues 15−16 acting as a hinge or pivot point.[19]

Single engineered cysteine residues in BtuB are derivatized with a methanethisulfonate spin label[20] to produce the nitroxide side chain R1. BtuB is then reconstituted into palmitoyloleoylphosphatidylcholine (POPC) bilayers. Figure 1A shows EPR spectra in bilayers at selected sites within and surrounding the Ton box in two different conformational states. In the absence of substrate (blue lines), broad spectra are seen for a number of residues, including L8R1 and V10R1. These spectra indicate that in the substrate-free form the Ton box has a defined secondary structure. The relatively narrow line shapes for residues T3R1, L8R1, and V10R1 in the B_{12} bound state (red lines) are similar to those observed for a denatured protein and indicate that this segment is lacking a defined secondary structure.[21] Upon B_{12} addition, the EPR line shapes for F15R1 and E16R1 change only slightly if at all, whereas the line shape for Q17R1 becomes more immobilized and is characteristic of a side chain that is buried in the protein interior in strong tertiary contact with other parts of the protein.

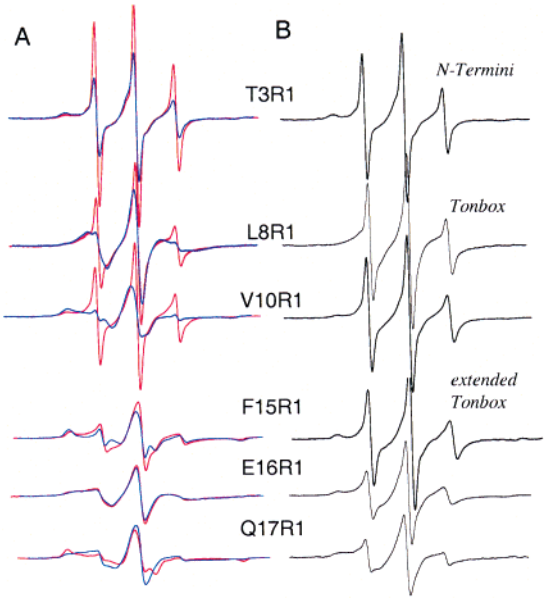


Figure 1. EPR spectra of specifically spin-labeled sites in BtuB. (A) In the presence of POPC bilayers with (red) and without (blue) substrate. (B) In the presence of mixed OG/POPC micelles.

When samples of BtuB in the absence of substrate are placed into a mixed micelle phase by addition of either dodecylmaltoside (DM) or octylglucoside (OG), the conformation of the Ton box changes. This conformational state differs from that induced by substrate binding. Shown in Figure 1B are spectra obtained for samples prepared in a mixed micelle environment of OG:POPC (17:1). The EPR spectra for T3R1, L8R1, and V10R1 are similar to those obtained in the presence of substrate; however, for residues F15R1, E16R1, and Q17R1 the line shapes are dramatically different from those obtained in either the absence or presence of substrate. These EPR line shapes exhibit greater motional averaging than those in Figure 1A and indicate that in this mixed micelle environment the N-terminal region has unfolded past residue 14. This perturbation is reversible, and detergent removal by dialysis restores the Ton box conformation to the docked state. Structural models that are consistent with the EPR data for the substrate-free, B_{12}-bound, and detergent-destabilized states are illustrated in Figure 2.

The mixed micelle environment does not appear to perturb the conformation of BtuB deeper in its core region. In particular, the EPR spectra for R1 placed along the first β-strand in the core,

10.1021/ja0376442 CCC: $25.00

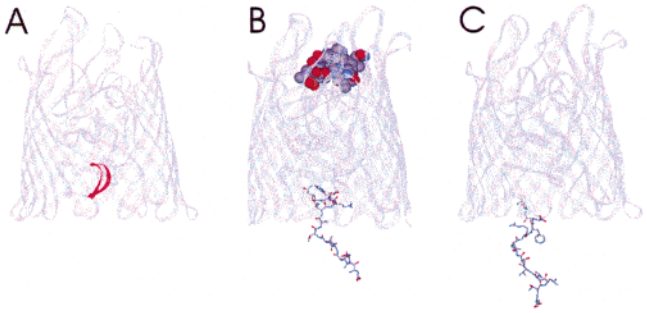


Figure 2. (A) Crystal structure for BtuB in the absence of substrate. Region in red shows positions 6−17 and includes the Ton box (PDB ID: 1NQE). (B) Model for substrate-bound form consistent with EPR data showing residues 6−14 unfolded. (C) Model for detergent unfolded form consistent with EPR data showing residues 6−17 unfolded.

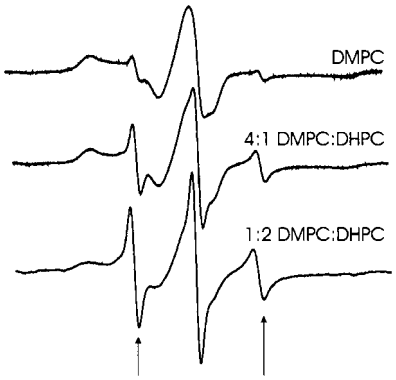


Figure 3. EPR spectra of V10R1 reconstituted into DMPC or DMPC/DHPC bilcelles (100 G scan).

positions 25−30, are nearly identical for samples reconstituted into POPC bilayers or mixed POPC/OG (1:17) micelles (see Supporting Information). Previous work on BtuB using SDSL indicated that the β-barrel retains its fold but becomes more dynamic in the presence of detergents.[18] This increase in dynamics is particularly dramatic toward the periplasmic surface of the protein and may be responsible for weakening interactions between the hatch and barrel that maintain the N-terminal fold.

Bicelles may offer a more appropriate membrane mimetic environment than micelles because they are thought to resemble small bilayer disks. Bicelles can be formed spontaneously in mixtures of short-chain and long-chain phospholipids (usually dihexanoylphosphatidylcholine (DHPC) and dimyristoyl-phosphatidylcholine (DMPC)), and they have been utilized in NMR investigations of membrane proteins[22−24] and in crystallization.[25]

The conformation of the Ton box appears to be preserved when BtuB is reconstituted into some bicelle systems (Figure 3). Bicelles formed from a 4:1 DMPC/DHPC ratio appear to have a perforated lamellar morphology.[24,26] For V10R1 reconstituted into this bicelle system, the native fold is largely maintained, as seen by broad features in the EPR spectrum. The sharper component (arrows) in this spectrum results from the unfolded conformation and represents approximately 5% of the total spin population (as estimated by spectral subtraction). In contrast to these large bicelles, small bicelles, which form with lower DMPC/DHPC ratios, do not preserve the folded Ton box structure, as indicated by the EPR spectrum of V10R1 at a DMPC/DHPC ratio of 2:1. In these small disk-shaped bicelles, the narrower spectral component resulting from an unfolded N-terminus is now the dominant population. Hence, for BtuB, bicelles with high DMPC/DHPC ratios provide a better membrane mimetic system than detergent-based micelles or mixed lipid−detergent micelles.

The energy difference between docked and undocked forms of the Ton box is estimated from the EPR spectra to be about 2 kcal/mol in the absence of substrate.[27] The sensitivity to detergents may be due in part to this modest energy, which may be typical of energy differences for other membrane protein conformational transitions.

In summary, the work presented here demonstrates that relatively mild detergents such as OG and DM can alter the conformation of membrane proteins, and that detergents are not necessarily a substitute for a membrane environment. This work also demonstrates the value of techniques such as SDSL, which can be used to directly compare the conformation of membrane proteins in different membrane mimetic systems and test structural models generated by X-ray diffraction or NMR.

Acknowledgment. This research was supported by NIH Grant GM35215 to D.S.C. and NRSA Fellowship GM20298 to G.E.F.

Supporting Information Available: Detailed experimental protocols and EPR spectra from positions 25, 27, 28, and 30 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Arora, A.; Tamm, L. K. *Curr. Opin. Struct. Biol.* **2001**, *11*, 540−547.
(2) Loll, P. J. *J. Struct. Biol.* **2003**, *142*, 144−153.
(3) Torres, J.; Stevens, T. J.; Samso, M. *Trends Biochem. Sci.* **2003**, *28*, 137−144.
(4) Fernandez, C.; Hilty, C.; Bonjour, S.; Adeishvili, K.; Pervushin, K.; Wuthrich, K. *FEBS Lett.* **2001**, *504*, 173−178.
(5) Caffrey, M. *J. Struct. Biol.* **2003**, *142*, 108−132.
(6) Czerski, L.; Sanders, C. R. *Anal. Biochem.* **2000**, *284*, 327−333.
(7) Chou, J. J.; Kaufman, J. D.; Stahl, S. J.; Wingfield, P. T.; Bax, A. *J. Am. Chem. Soc.* **2002**, *124*, 2450−2451.
(8) Postle, K. *Nat. Struct. Biol.* **1999**, *6*, 3−6.
(9) Faraldo-Gómez, J. D.; Sansom, M. S. P. *Nature Rev. Mol. Cell Biol.* **2003**, *4*, 105−116.
(10) Chimento, D. P.; Mohanty, A. K.; Kadner, R. J.; Wiener, M. C. *Nat. Struct. Biol.* **2003**, *10*, 394−401.
(11) Cadieux, N.; Kadner, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10673−10678.
(12) Merianos, H. J.; Cadieux, N.; Lin, C. H.; Kadner, R.; Cafiso, D. S. *Nat. Struct. Biol.* **2000**, *7*, 205−209.
(13) Cadieux, N.; Bradbeer, C.; Kadner, R. *J. Bacteriol.* **2000**, *182*, 5954−5961.
(14) Jiang, X.; Payne, M. A.; Cao, Z.; Foster, S. B.; Feix, J. B.; Newton, S. M.; Klebba, P. E. *Science* **1997**, *276*, 1261−1264.
(15) Hubbell, W. L.; Altenbach, C. *Curr. Opin. Struct. Biol.* **1994**, *4*, 566−573.
(16) Hubbell, W. L.; Gross, A.; Langen, R.; Lietzow, M. A. *Curr. Opin. Struct. Biol.* **1998**, *8*, 649−656.
(17) Hubbell, W. L.; Cafiso, D. S.; Altenbach, C. A. *Nat. Struct. Biol.* **2000**, *7*, 735−739.
(18) Fanucci, G. E.; Cadieux, N.; Piedmont, C. A.; Kadner, R. J.; Cafiso, D. S. *Biochemistry* **2002**, *41*, 11543−11551.
(19) Fanucci, G. E.; Coggshall, K. A.; Cadieux, N.; Kim, M.; Kadner, R. J.; Cafiso, D. S. *Biochemistry* **2003**, *42*, 1391−1400.
(20) Berliner, L. J.; Grunwald, J.; Hankovszky, H. O.; Hideg, K. *Anal. Biochem.* **1982**, *119*, 450−455.
(21) Klug, C. S.; Feix, J. B. *Protein Sci.* **1998**, *7*, 1469−1476.
(22) Sanders, C. R., II; Schwonek, J. P. *Biochemistry* **1992**, *31*, 8898−8905.
(23) Vold, R. R.; Prosser, R. S.; Deese, A. J. *J. Biomol. NMR* **1997**, *9*, 329−335.
(24) Sanders, C. R.; Prosser, R. S. *Structure* **1998**, *6*, 1227−1234.
(25) Faham, S.; Bowie, J. U. *J. Mol. Biol.* **2002**, *316*, 1−6.
(26) Gaemers, S.; Bax, A. *J. Am. Chem. Soc.* **2001**, *123*, 12343−12352.
(27) Fanucci, G. E.; Cadieux, N.; Kadner, R.; Cafiso, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11382−11387.

JA0376442