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Catalytic activity of MsbA reconstituted in nanodisc particles is modulated by remote interactions with the bilayer

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ABSTRACT

ATP-binding cassette (ABC) transporters couple hydrolysis of ATP with vectorial transport across the cell membrane. We have reconstituted ABC transporter MsbA in nanodiscs of various sizes and lipid compositions to test whether ATPase activity is modulated by the properties of the bilayer. ATP hydrolysis rates, Michaelis–Menten parameters, and dissociation constants of substrate analog ATP-γ-S demonstrated that physicochemical properties of the bilayer modulated binding and ATPase activity. This is remarkable when considering that the catalytic unit is located ~50 Å from the transmembrane region. Our results validated the use of nanodiscs as an effective tool to reconstitute MsbA in an active catalytic state, and highlighted the close relationship between otherwise distant transmembrane and ATPase modules.

1. Introduction

ATP binding cassette (ABC) transporters are a class of integral membrane proteins involved in the uphill translocation of a wide range of bioactive molecules across the plasmatic membrane of the cell [1–3]. Vectorial translocation is powered by the hydrolysis of ATP taking place in specialized nucleotide-binding domains (NBDs) of the transporter. MsbA is an essential membrane protein of *Escherichia coli* that facilitates the movement of lipid A from the inner to the outer leaflet of the plasmatic membrane [4,5]. Structurally, MsbA is composed of a transmembrane region embedded in the bilayer, and an ATP binding domain (NBD) responsible for ATP hydrolysis. Low-resolution crystal structures (>3.5 Å) revealed significant differences in the overall conformation of MsbA dimer with and without nucleotides bound [6]. Biophysical and biochemical data supported the crystallographic models, and together with the structural data demonstrated that MsbA must undergo a large conformational rearrangement during its functional cycle (Fig. 1) [7,8]. An outstanding question is how MsbA converts favorable chemical energy released by hydrolysis of ATP into vectorial transport and conformational change. Ultimately, this energy must be transmitted across remote domains of the protein as suggested from crystal structures and molecular dynamics simulations [9].

We used nanodiscs as a tool to reconstitute functional MsbA. Nanodiscs are particles generated by the self-assembly of phospholipids and membrane scaffold protein (MSP, Supplementary Fig. S1) [10]. These nanoparticles afforded some improved properties over traditional model systems. For example, nanodiscs allow precise control of particle size (Supplementary Figs. S1 and S2), and yield a simplified topology more suitable for analytical techniques such as surface plasmon resonance (SPR) [11–14].

In this report we have characterized ATPase activity of MsbA reconstituted in nanodiscs of varying particle sizes and lipid compositions. We compared this reconstitution system with detergent micelles. We also report a detailed binding analysis of the substrate analog ATP-γ-S to MsbA using SPR technique. Overall, our work revealed functional relationships between distant domains of MsbA.

Abbreviations: ABC, ATP binding cassette; NBD, nucleotide-binding domain; β-DDM, *n*-dodecyl-β-D-maltoside; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol); DLPC , 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DM(9:cis)PC, 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; ATP-γ-S, adenosine-5′-*O*-(3-thiotriphosphate); MSP1, membrane scaffold protein 1; MSP1E1, extended MSP1 with helix 4 repeated; MSP1E2, extended MSP1 with helices 4 and 5 repeated; MSP1E3, extended MSP1 with helices 4, 5 and 6 repeated; SPR, surface plasmon resonance

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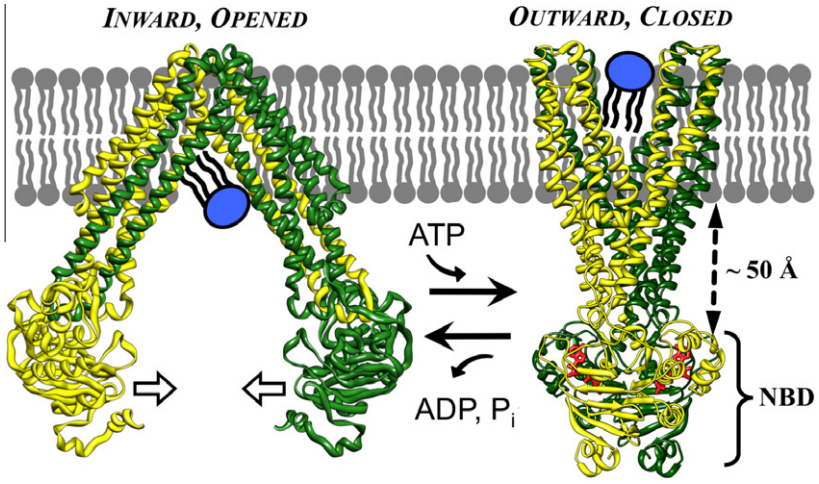

Fig. 1. Structure of MsbA dimer in (A) opened (inward-facing) and (B) closed (outward-facing) conformations. Binding of ATP and lipid A to the opened conformation favors transition of MsbA to an outward-facing structure with ensuing translocation of ligand to the outer leaflet of the membrane. Protein monomers are colored in yellow and green, respectively. Dinucleotide bound to closed form of MsbA is shown in red. Lipid A (blue) and phospholipids (gray) are not part of the crystal structures, but they are shown for illustration purposes. Distance between nucleotide and trans-membrane region is approximately 50 Å. Coordinates were obtained from Protein Data Base under entries 3B5W (*E. coli*, opened form), and 3B60 (*Salmonella typhimurium*, closed form).

2. Materials and methods

2.1. Materials

Lipids 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG), 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (DM(9:cis)PC), and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Detergent *n*-dodecyl-β-D-maltoside (β-DDM) was purchased from Dojindo (Kumamoto, Japan). ATP was purchased from MP Biochemicals (Solon, OH), and analog adenosine-5′-*O*-(3-thiotriphosphate) (ATP-γ-S) was purchased from Merck (Darmstadt, Germany). All other chemicals were purchased from Wako (Osaka, Japan).

2.2. Cloning, expression and purification of membrane scaffold proteins

Expression vector of membrane scaffold protein 1 (MSP1) with an N-terminal heptahistidine and a TEV cleavage site was a gift from the Institute of Genome Research at The University of Tokushima. Enlarged versions of MSP1 with repeated helix 4 (MSP1E1), repeated helices 4 and 5 (MSP1E2), and repeated helices 4, 5 and 6 (MSP1E3) [13] were cloned into expression vectors pET28 with a mutagenesis kit (Toyobo, Osaka, Japan) (Supplementary Fig. S2). Expression and purification of MSP was carried out essentially as described previously [10,13]. TEV protease containing a hexahistidine tag in the N-terminal end was purified using a Ni^{2+} affinity column as described elsewhere [15]. Cleaved MSP was separated from uncut MSP and TEV protease in a HisTrap HP column. The flow-through fractions containing MSP protein were concentrated with a 10 kDa Amicon Ultra-filter (Millipore, Billerica MA) to a concentration of 100–200 μM, frozen by immersion in liquid N_2, and stored at −80 °C until use.

2.3. Cloning, expression and purification of MsbA

MsbA gene from *E. coli* was cloned into expression vector pET26M(+) using forward primer 5′-GGAATTCCATATGCATAACGACAAAGATCTCTCTAC-3′, and reverse primer 5’-ACGCGTCGACTTGGCCAAACTGCATTTTGTG-3′ displaying restriction sites Nde-I and Sal-I (underlined), respectively. C43 (DE3) cells transformed with MsbA plasmid were grown in 2YT medium containing 50 μg ml^{-1} kanamycin at 37 °C. Expression was induced by addition of 0.5 mM IPTG. Cells were harvested 5 h after induction by centrifugation at 7000×*g* for 10 min at 4 °C, suspended in buffer containing 40 mM Tris–HCl, 300 mM NaCl (pH 8.0), and subsequently lysed with an EmulsiFlex C-5 homogenizer (Avestin, Ontario, Canada). Lysate solution was centrifugated at 10000×*g* for 1 h at 4 °C, and the supernatant further centrifuged at 150000×*g* for 1 h at 4 °C. Membrane fraction was resuspended in a buffer composed of 40 mM Tris–HCl, 300 mM NaCl and 5 mM imidazole (pH 8.0). This solution was supplemented with 1% (w:v) β-DDM to allow solubilization of the membranes for one hour at 4 °C, after which it was centrifuged at 150000×*g* for 1 h at 4 °C to discard insoluble material. MsbA was purified in a Talon Co^{2+} affinity column (Takara, Otsu, Japan) with buffer supplemented with 0.1% β-DDM containing up to 10 mM imidazol, after which MsbA was eluted with a solution containing 300 mM imidazole. Purified MsbA was concentrated to 100–200 μM, frozen in liquid N_2 and stored at −80 °C until use.

2.4. Reconstitution of MsbA in nanodiscs

Appropriate amount of dried lipid was solubilized at a concentration of 50 mM in a buffer containing 10 mM Tris–HCl, 100 mM NaCl and 100 mM sodium cholate at pH 7.4. Proteins MSP and MsbA, at a ratio (1:1) were incubated with excess lipid at 20:1, 40:1, 60:1 or 100:1 stoichiometric ratios. Optimal incubation temperature for nanodiscs containing DMPC and DMPG was 25 °C, whereas that for samples containing DLPC, DM(9:cis)PC, and DOPC was 4 °C. Detergents β-DDM and sodium cholate were removed by treatment with 0.6 g ml^{-1} Bio-Beads SM2 (Bio-Rad, Hercules, CA) for 4 h. Nanodiscs were separated from aggregated material in a Superdex 200 10/300 GL column (GE Healthcare) equilibrated with a solution composed of 10 mM Tris–HCl (pH 7.4) and 100 mM NaCl. Samples of MsbA incorporated in nanodiscs were further purified in a Ni–NTA agarose column following the instructions of the manufacturer (Qiagen, Valencia, CA). MsbA concentration was determined from absorbance at 280 nm and densitometry analysis of SDS–PAGE gels calibrated with known concentrations of MsbA or MSP, and analyzed