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Biochimica et Biophysica Acta 1777 (2008) 227–237
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbabio

Interaction of transmembrane helices in ATP synthase subunit *a* in solution as revealed by spin label difference NMR

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Received 22 October 2007; received in revised form 28 November 2007; accepted 29 November 2007
Available online 15 December 2007

Abstract

Subunit *a* in the membrane traversing F_0 sector of *Escherichia coli* ATP synthase is known to fold with five transmembrane helices (TMHs) with residue 218 in TMH IV packing close to residue 248 in TMH V. In this study, we have introduced a spin label probe at Cys residues substituted at positions 222 or 223 and measured the effects on the Trp εNH indole NMR signals of the seven Trp residues in the protein. The protein was purified and NMR experiments were carried out in a chloroform–methanol–H_2O (4:4:1) solvent mixture. The spin label at positions 222 or 223 proved to broaden the signals of W231, W232, W235 and W241 located at the periplasmic ends of TMH IV and TMH V and the connecting loop between these helices. The broadening of W241 would require that the loop residues fold back on themselves in a hairpin-like structure much like it is predicted to fold in the native membrane. Placement of the spin label probe at several other positions also proved to have broadening effects on some of these Trp residues and provided additional constraints on folding of TMH IV and TMH V. The effects of the 223 probes on backbone amide resonances of subunit *a* were also measured by an HNCO experiment and the results are consistent with the two helices folding back on themselves in this solvent mixture. When Cys and Trp were substituted at residues 206 and 254 at the cytoplasmic ends of TMHs IV and V respectively, the W254 resonance was not broadened by the spin label at position 206. We conclude that the helices fold back on themselves in this solvent system and then pack at an angle such that the cytoplasmic ends of the polypeptide backbone are significantly displaced from each other.

Keywords: ATP synthase subunit *a*; Proton transport; Transmembrane helices; Solution NMR; Spin label; Membrane protein structure

1. Introduction

The F_1F_0 ATP synthases of oxidative phosphorylation utilize the energy of a transmembrane electrochemical gradient of H^+ or Na^+ to mechanically drive the synthesis of ATP via two coupled rotary motors in the F_1 and F_0 sectors of the enzyme [1–3]. In the intact enzyme, ATP synthesis or hydrolysis takes place in the F_1 sector at the surface of the membrane, synthesis being coupled to H^+ or Na^+ transport through the transmembrane F_0 sector. Homologous enzymes are found in mitochondria, chloroplasts and many bacteria [4]. In *Escherichia coli* and other eubacteria, F_1 consists of five subunits in an $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ stoichiometry [4]. F_0 is composed of three subunits in a likely ratio of $a_1b_2c_{10}$ in *E. coli* and thermophilic *Bacillus* PS3, or $a_1b_2c_{11}$ in the Na^+ translocating *Ilyobacter tartaricus* ATP synthase [3,5–7]. A 3.9 Å resolution crystal structure of a yeast mitochondrial F_1–c_{10} complex depicts 10 *c* subunits arranged in a ring-like structure [8]. Other bacterial *c*-rings may have as many as 15 *c* subunits [9]. Subunit *c* spans the membrane as a hairpin of two transmembrane helices (TMHs) with the first TMH on the inside and the second TMH on the outside of the *c*-ring [7,10,11]. Helical hairpin-like structures resembling that predicted for the membrane have been solved by NMR using chloroform–methanol solvent mixtures [12–14]. A high resolution X-ray structure of the *I. tartaricus* c_{11}-ring that differs significantly from the NMR structures has revealed the Na^+ binding site at the periphery of the ring with the chelating groups to the Na^+ ion extending from two interacting subunits

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doi:10.1016/j.bbabio.2007.11.011

[7]. In the H^+-transporting *E. coli* enzyme, Asp61 at the center of the second TMH is thought to undergo protonation and deprotonation as each subunit of the *c*-ring moves past a stationary subunit *a*. In the complete membranous enzyme, the rotation of the *c*-ring is proposed to be driven by H^+ transport at the subunit *a/c* interface, with *c*-ring rotation being coupled to rotation of subunit γ within the $\alpha_3\beta_3$ hexamer of F_1 to cause conformational changes in the catalytic sites leading to synthesis and release of ATP [1–3].

Subunit *a* is thought to provide access channels to the proton-binding Asp61 residue in the *c*-ring, and candidate residues lining a possible aqueous access pathway were tentatively identified [15–18]. Subunit *a* is known to fold with 5 TMHs [19–21] with TMH IV packing next to the second TMH of subunit *c* [22], *i.e.* the helix in which Asp61 is anchored. Interaction of the conserved Arg210 residue in *a*TMH IV with *c*TMH II is thought to be critical during the deprotonation–protonation cycle of *c*Asp61 [13,15,23–25]. Cross-linking of Cys residues introduced into subunits *a* and *c*, or *b* and *c*, supports the positioning of subunit *a* and the two *b* subunits at the periphery of the *c*-ring [22,26,27]. Little is known about the structure or three-dimensional arrangement of the TMHs in subunit *a*. Several sets of second site suppressor mutations in one TMH to a primary mutation in a second TMH had suggested possible helix–helix interactions [19,23,28]. More recently, we have introduced pairs of Cys residues into putatively interacting TMHs and tested for cross-linking of the Cys thiol groups with resultant disulfide bond formation. Cross-links were found with eight different Cys pairs and define a juxtaposition of TMHs II–III, II–IV, II–V, III–IV, III–V and IV–V in a four helix bundle [29]. The water accessible residues identified previously in helices II, III, IV and V were proposed to form aqueous half channels to each side of the membrane [16–18,30].

Subunit *a* is 271 amino acids long and is proposed to fold in the membrane as shown in Fig. 1. The NMR spectra of subunit *a* in a chloroform–methanol–water (4:4:1) solvent mixture shows good chemical shift resolution and dispersion, suggesting a mono-disperse and folded protein structure [31]. Nearly complete backbone chemical shift assignments in this solvent system were reported [32], and the single set of backbone resonances supports sample mono-dispersity. The location of α-helical segments in the subunit *a* sequence determined from secondary chemical shift values was generally consistent with the *a priori* predictions and biochemical data [19,33]. Here we used site-directed paramagnetic spin labeling to study the tertiary folding of subunit *a* in chloroform–methanol–water solvent. The large magnetic moment of an unpaired electron causes rapid relaxation of NMR signals of nuclei located in the proximity of the spin label. This effect can be used to identify the reporter groups located within 15–20 Å of the spin label [34–36]. The initial intent of this study was to probe the interactions of TMHs IV and V in this solvent system. TMHs IV and V can be cross-linked in native membranes via disulfide bond formation when cysteines are introduced at positions 218 and 248 [29], and residues in this region are proposed to interact to form parts of the periplasmic H^+ half-channel located within F_0 [16–18]. Initially we studied the effect of a site-specific 1-oxyl-2,2,5,5-tetramethylpyrrolidin-3-yl (PROXYL) spin label attached to cysteines on the indole resonances of tryptophan

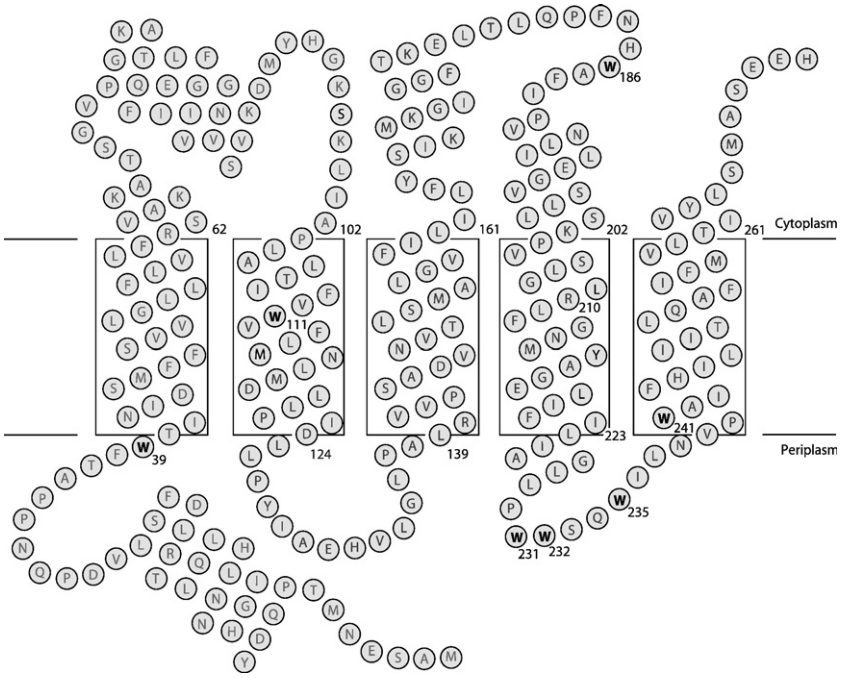

Fig. 1. Topological model for folding of subunit *a* in *E. coli* inner membrane. The biochemical evidence for the insertion of the five TMHs is discussed in the text. The depth of placement of the helices in the membrane is based upon cross-linking studies as described elsewhere [29]. The positions of the seven Trp residues in the wild type protein are highlighted. The helical segments shown in regions peripheral to the lipid bilayer were predicted by backbone chemical shift analysis for the protein dissolved in chloroform–methanol–water solvent [32].