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2.8-Å crystal structure of *Escherichia coli* YidC revealing all core regions, including flexible C2 loop

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ABSTRACT

YidC/Alb3/Oxa1 family proteins are involved in the insertion and assembly of membrane proteins. The core five transmembrane regions of YidC, which are conserved in the protein family, form a positively charged cavity open to the cytoplasmic side. The cavity plays an important role in membrane protein insertion. In all reported structural studies of YidC, the second cytoplasmic loop (C2 loop) was disordered, limiting the understanding of its role. Here, we determined the crystal structure of YidC including the C2 loop at 2.8 Å resolution with $R/R_{free} = 21.8/27.5$. This structure and subsequent molecular dynamics simulation indicated that the intrinsic flexible C2 loop covered the positively charged cavity. This crystal structure provides the coordinates of the complete core region including the C2 loop, which is valuable for further analyses of YidC.

1. Introduction

Membrane proteins are translated by ribosomes and properly integrated into the membrane, which is a fundamental mechanism conserved in all organisms [1]. The bacterial membrane protein YidC, an essential factor for cell growth, is involved in the integration of membrane proteins [2]. YidC proteins are conserved in the mitochondria and thylakoid as Oxa1 and Alb3, respectively [3,4]. Recently, YidC-like proteins were identified in the endoplasmic reticulum membrane [5] and archaea [6]. YidC has been proposed to function as an insertase for membrane protein biogenesis and chaperon promoting the proper folding of membrane proteins in the membrane. During membrane protein integration *via* the SecYEG complex, the protein-conducting channel, YidC cooperates with SecYEG to assist with folding of newly synthesized proteins in the membrane. The translating ribosome directly interacts with SecYEG [7–9] or YidC [10], enabling co-current protein translation and integration into the membrane.

Conserved regions of YidC/Oxa1/Alb3 family proteins contain five transmembrane (TM) regions. Based on currently available high-resolution structures, *Bacillus halodurans* YidC (BhYidC) at 2.4 Å [11], *Escherichia coli* YidC (EcYidC) at 3.2 Å [12], and *Thermotoga maritima* YidC (TmYidC) at 3.8 Å resolutions [13], the architectures of the core five TM helixes are essentially identical (Fig. 1b). The core TMs form a positively charged cavity open to the membrane and cytoplasmic side. The conserved Arg is positioned in the cavity. In contrast, loops of the cytoplasmic region 1 (C1) in the crystal structures of EcYidC and BhYidC show different orientations, but the C1 loop in TmYidC is disordered. The C2 loop is completely disordered in all avertable structures. Hence, the C1 and C2 loops appear to have intrinsically high mobility. Only YidCs from gram-negative bacteria possess the additional TM1 and periplasm region (P1), when compared to other YidC orthologs. TM1 and P1 are not crucial for protein activity [14], which is consistent with the non-identical architecture of the P1 domains in EcYidC and TmYidC. Additionally, TM1 is disordered in each crystal structure. TM1 may be extremely flexible in the membrane and may function as an anchor to the membrane or signal peptide for targeting the membrane. In *Bacillus subtilis*, Arg in the cavity is essential for membrane integration of MifM, a substrate of YidC, and cell growth [11]. In contrast, this residue is not essential for *E. coli* viability [15], but shows a conditional defect in cell growth [12]. Together with molecular dynamics (MD) simulation results, this cavity provides a hydrophilic environment and appears to always be filled by water molecules [11,16]. Mutations that reduce the hydrophilicity of the cavity caused defects in membrane insertion by YidC [17].

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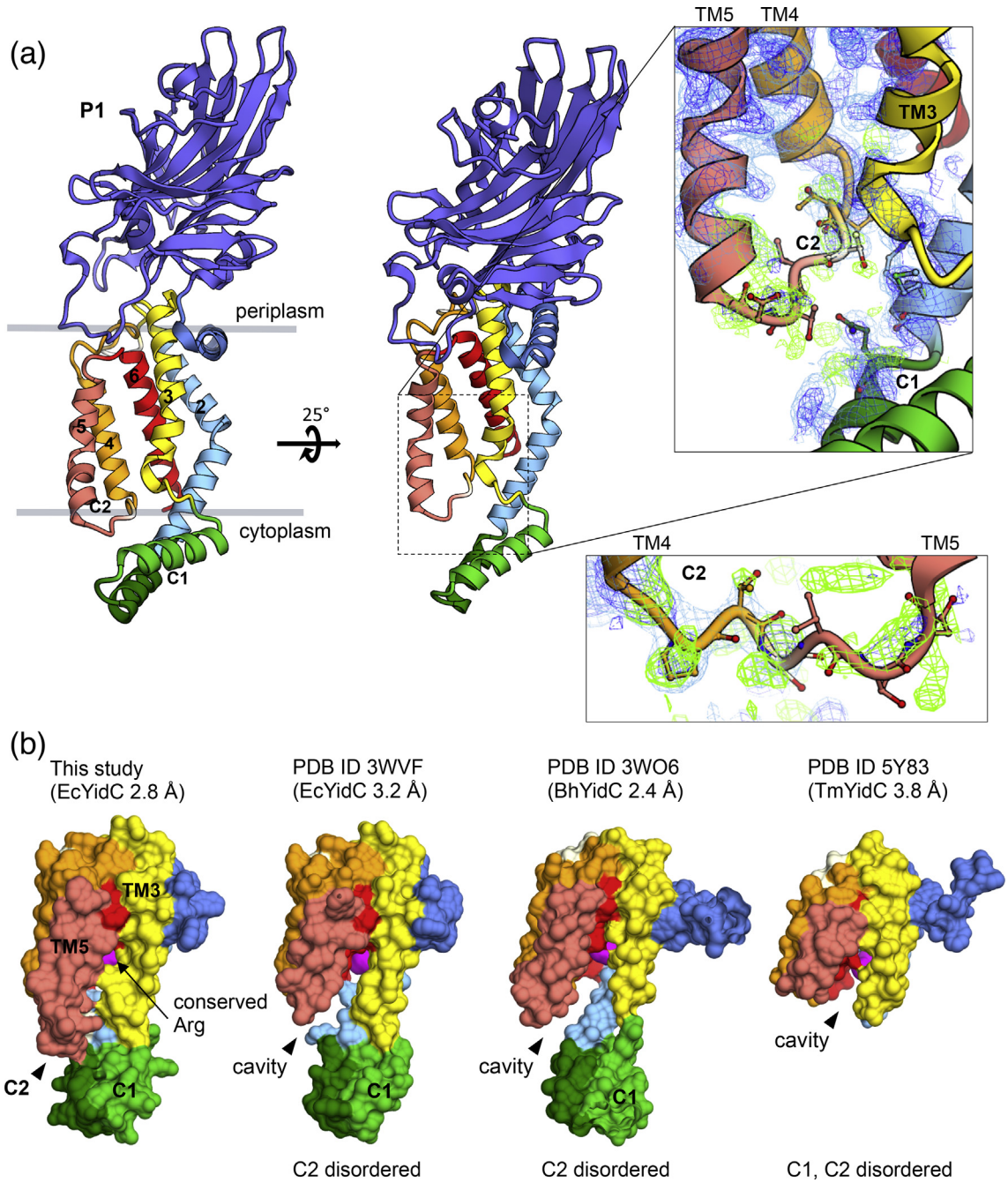

Fig. 1. Crystal structures of YidC.
(a) 2.8 Å crystal structure of EcYidC. P1, TM2, C1, TM3, TM4, and TM5 are colored in blue, light blue, green, yellow, orange, pink, and red, respectively. Magnified views of the C2 area (right panels) including the 2*Fo-Fc* electron density map at 1.0 σ (blue) and the omit map at 1.7 σ (green). (b) Comparison of the 2.8 Å crystal structure with previously reported structures, PDB ID 3WVF, 3WO6, and 5Y83. The structures are represented as surface models and colored as in (a). Each conserved Arg residue is colored in purple.

Additionally, the cavity cross-linked to MifM provides insight into the insertion model of single spanning membrane proteins by YidC [11]. Other studies of cross-linking showed that YidC interacts with the SecYEG and SecDF complex [18,19]. YidC complexed with SecYEG and SecDF was shown to form a Sec holo translocon complex [20,21].

Currently, some information exists regarding the function of YidC, which can be used to perform functional analyses. However, the available structures lack information for the C2 loop, which is important for interacting with ribosomes [22], making evaluation of its molecular mechanism difficult. In this study, we determined the 2.8 Å crystal structure of EcYidC including the C2 loop and conducted MD simulation, which revealed the flexibility of the C2 loop. The complete structure of the core YidC region provides a structural basis for further analysis.

2. Results and discussion

2.1. 2.8 Å-crystal structure of YidC

We used the Helical Data Collection Method at beamline BL32XU at SPring-8 with a microbeam [23] by using more than 100

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