Structure
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ATP-Induced Conformational Dynamics in the AAA+ Motor Unit of Magnesium Chelatase

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SUMMARY

Mg-chelatase catalyzes the first committed step of the chlorophyll biosynthetic pathway, the ATP-dependent insertion of Mg^{2+} into protoporphyrin IX (PPIX). Here we report the reconstruction using single-particle cryo-electron microscopy of the complex between subunits BchD and BchI of *Rhodobacter capsulatus* Mg-chelatase in the presence of ADP, the nonhydrolyzable ATP analog AMPPNP, and ATP at 7.5 Å, 14 Å, and 13 Å resolution, respectively. We show that the two AAA+ modules of the subunits form a unique complex of 3 dimers related by a three-fold axis. The reconstructions demonstrate substantial differences between the conformations of the complex in the presence of ATP and ADP, and suggest that the C-terminal integrin-I domains of the BchD subunits play a central role in transmitting conformational changes of BchI to BchD. Based on these data a model for the function of magnesium chelatase is proposed.

INTRODUCTION

The enzyme magnesium chelatase (Mg-chelatase) is active in the branch point between chlorophyll and heme biosynthesis. It catalyzes the insertion of Mg^{2+} into protoporphyrin IX (PPIX), which is the first committed reaction of the chlorophyll biosynthesis pathway. Mg-chelatase belongs to the class of AAA+-type chelatases (Al-Karadaghi et al., 2006; Reid and Hunter, 2002; Schubert et al., 1999; Willows and Hansson, 2003). This is also true for aerobic cobaltochelatase and nickel chelatase, which are active in cobalamin (vitamin B_{12}) and coenzyme F_{430} biosynthesis, respectively (Fodje et al., 2001; Lundqvist et al., 2009; Raux et al., 2000). Magnesium chelatase is currently the most extensively studied enzyme in this class of AAA+ chelatases. Its activity requires the presence of three subunits, BchH, BchD, and BchI, which have molecular masses of approximately 140, 70, and 40 kDa, respectively (Gibson et al., 1995; Jensen et al., 1998; Walker and Weinstein, 1991; Willows et al., 1996; Willows and Beale, 1998). Biochemical studies have suggested that the enzymatic reaction proceeds in distinct steps (Jensen et al., 1998; Jensen et al., 1999; Reid and Hunter, 2004; Sawicki and Willows, 2008; Walker and Weinstein, 1994). In the first step, the AAA+ motor complex between subunits BchI and BchD is formed in the presence of ATP and Mg^{2+}, while the largest subunit, BchH, binds PPIX by an unknown mechanism. Our recent work has suggested that subunit BchD may serve as a platform for the assembly of the complex (Axelsson et al., 2006). Using the method of single-particle reconstruction from electron microscopic images, we have also demonstrated that binding of PPIX to BchH induces a large conformational rearrangement in this subunit (Sirijovski et al., 2008).

In the reaction step that follows, the BchH:PPIX complex interacts with the BchI:BchD complex, leading to insertion of Mg^{2+} into PPIX. During this part of the reaction, the BchH:PPIX complex is a substrate of the BchI:BchD complex. It has been estimated that around 15 (Reid and Hunter, 2004; Sawicki and Willows, 2008) ATP molecules may be required for each catalytic cycle. The BchI subunit, which contains the characteristic ATP binding Walker A and Walker B motifs (GX_4GKSX_6A and hhhhD(D/E), where h is any hydrophobic residue), is responsible for ATP hydrolysis (Jensen et al., 1999; Lake et al., 2004; Reid et al., 2003; Reid and Hunter, 2004; Walker and Weinstein, 1994). The X-ray crystallographic structure of *Rhodobacter capsulatus* BchI has been determined and it has been shown to belong to the AAA+ family of ATPases (Fodje et al., 2001). The protein was later assigned to the pre-sensor II (PS-II) insert clade of the AAA+ family, which includes the MCM (minichromosome maintenance) family of helicases, the MoxR family of molecular chaperones, and the dynein/midacin family of ATP-dependent motors, the members of which are known to interact with microtubules and the nuclear pore complex (Erzberger and Berger, 2006; Iyer et al., 2004). A characteristic feature of AAA+ proteins is the formation of oligomeric ring structures with the most common ring types consisting of 6 or 7 monomers (Vale, 2000). Electron microscopy (EM) and single-particle analysis has indeed shown that in the presence of ATP, *R. capsulatus* BchI and the corresponding subunit ChlI from *Synechocystis sp.* PCC6830 can form hexameric and heptameric ring structures, respectively (Reid et al., 2003; Willows et al., 2004).

Amino acid sequence analysis has demonstrated that subunit BchD, which is the second in size after BchH, has an AAA+ module at its N terminus with distinct homology to BchI (Figure 1). However, the Walker A and Walker B motifs, which are necessary for ATP hydrolyzing activity, are poorly conserved in this subunit. Despite this, BchD is still capable of forming oligomeric ring structures, even in the absence of ATP (Axelsson et al., 2006). Interestingly, the C-terminal part of BchD was found to contain a domain homologous to a class of proteins termed integrin I domains, which is a subgroup of a larger group of von Willebrand factor A domain proteins (Hynes, 1992; Tuckwell, 1999). These domains are usually found as part of larger complexes, and they are the principal receptors on the surfaces of animal cells, being involved in the binding of most extracellular matrix proteins. Integrin I domains are characterized by the MIDAS motif (metal ion-dependent adhesion site), which constitutes a unique Mg^{2+}/Mn^{2+} binding site. The same type of domain is present in cobalto-chelatase (Fodje et al., 2001) and in the MoxR family of AAA+ proteins (Snider and Houry, 2006). Mutation of residues in the MIDAS motif of *R. capsulatus* BchD (D385A and S387A) was found to abolish Mg-chelatase activity (Axelsson et al., 2006). The integrin I domain and the N-terminal AAA+ module of BchD are linked to each other by a proline and an acidic residue-rich region (Fodje et al., 2001). These types of domains are often involved in protein-protein interactions (Kay et al., 2000). Based on this knowledge, the region was suggested to be involved in the stabilization of the BchI:BchD complex (Fodje et al., 2001).

In an earlier publication, we presented a new method for ab initio single-particle reconstruction from cryo-EM images (Elmlund et al., 2008). The power of the method was demonstrated by the first reconstruction of the BchI:BchD complex (referred to as the ID complex in the following text), which revealed that in the presence of ADP the two subunits form a structure with a C3 point group symmetry. In the present work, this reconstruction was further refined at 7.5 Å resolution and used for a high-accuracy fit of the X-ray crystallographic structure of subunit BchI and the homology models of the integrin I domain and of the AAA+ module of subunit BchD. The resulting first quasi-atomic model was used in the interpretation of the conformations of the complex in the presence of ADP, AMPPNP, and ATP.

RESULTS

Homology-Based Modeling of the D Subunit

The AAA+ module consists of two subdomains, an N-terminal Rossman-type domain that contains the Walker A and B nucleotide binding motifs and a C-terminal helical bundle domain. Alignment of the amino acid sequences of the *Rhodobacter capsulatus* subunit BchI and the AAA+-like module of subunit BchD (residues 1-238) showed 25% and 42% identity for the N-terminal AAA+ core and the C-terminal helical bundle subdomains, respectively. This relatively high degree of overall sequence similarity between the two AAA+ modules suggested that the crystallographic structure of *R. capsulatus* BchI would be a suitable template for homology modeling of the AAA+-like module of BchD. A structure-based sequence alignment between BchI and the N-terminal region of BchD and also a comparison of the topology of BchI with the topology of the modeled BchD are shown in Figure 1. Three major deletions in the *R. capsulatus* BchD sequence, as compared with BchI, can be seen in the alignment: (1) a long hairpin between helix α1 and strand β2 (the numbering of the secondary structure elements is according to [Iyer et al., 2004]), (2) the pre-sensor 1 (PS-I) hairpin between helix α3 and strand β4, and (3) the PS-II insert between helices α5 and α6. Interestingly, these loops are not deleted in the D subunit of some other organisms, like *Synechocystis sp* and *Nicotiana tabacum* (Figure 1). For these organisms the D subunit is much more similar to the I subunit. On the other hand, the hairpin insertion into helix α2 (insertion H2, characteristic of the H2-insert clade [Erzberger and Berger, 2006]) that is present in BchI is also present in BchD. Based on these features, the AAA+ module of *R. capsulatus* BchD can be assigned to a unique clade of AAA+ proteins, which can be placed between the clamp loader and the H2-insert clades. An important observation is that the absence of the PS-II insert in BchD would place the helix bundle domain of the AAA+ module in a position more common for other AAA+ modules, in contrast to BchI in which this domain has a unique position (Fodje et al., 2001).

Overall Organization of the ID_{ADP} Complex

Due to the high quality and the relatively high resolution of the reconstruction (7.5 Å), several domains and even secondary structure elements could be distinguished in the reconstructed density. After manual docking of the X-ray coordinates of subunit BchI and the homology model of the two domains of BchD, the model was verified against mass spectrometric analysis of crosslinking data (see below) and subsequently optimized by least-squares refinement. With the exception of the bundle domain located in the BchI dimer-dimer interface, for which a rotational and translational adjustment was necessary, only small adjustments of the positions of the domains were needed during refinement. At the final stage, C3 symmetry was applied to the model. Details of the resulting structure of the complex in the presence of ADP are shown in Figure 2.

Panels A and B of Figure 2 show top views of the complex, whereas panel C shows a side view. The reconstruction suggests that the complex can be divided into two ring structures separated by a region of extensive interactions. Analysis of the density of the reconstruction indicated that the smaller bottom ring structure (~110 Å in diameter, gray in Figure 2B) is built up by a trimer of dimers. After thorough examination of the volume density and repeated attempts to fit different structures into it, it became clear that the most optimal fit could be achieved when subunit BchI was placed in the bottom ring (Figure 2B). Subsequently, the upper ring was assigned to subunit BchD. Similar to the BchI ring, the BchD ring appears to be built up of a trimer of dimers (Figure 2A and C). However, the reconstruction indicates that unlike the BchI ring, the BchD monomers within each dimer have different conformations, with at least some parts of the structure rotated by about 45° relative to each other (Figure 2C).

For identification of the positions of the different domains within the rings, the ID_{ADP} complex was incubated with the 3,3′-dithiobis(sulphosuccinimidylpropionate) (DTSSP), a thiol-cleavable crosslinker that covalently binds lysine residues. DTSSP reacts with primary amines (side chains of lysine residues and the N terminus of the protein) at pH 7–9 and forms stable amide bonds. After proteolysis, the material was analyzed