Molecular Cell
Article

Structure of a 14-3-3 Coordinated Hexamer of the Plant Plasma Membrane H$^+$-ATPase by Combining X-Ray Crystallography and Electron Cryomicroscopy

Christian Ottmann,[1,5,6] Sergio Marco,[2,5] Nina Jaspert,[1] Caroline Marcon,[1] Nicolas Schauer,[1] Michael Weyand,[3] Caroline Vandermeeren,[4] Geoffrey Duby,[4] Marc Boutry,[4] Alfred Wittinghofer,[3] Jean-Louis Rigaud,[2] and Claudia Oecking[1,*]

[1] Zentrum für Molekularbiologie der Pflanzen, Pflanzenphysiologie, Universität Tübingen, Auf der Morgenstelle 5, 72076 Tübingen, Germany

[2] Institut Curie, Unité Mixte de Recherche 168—Centre National de la Recherche Scientifique and Laboratoire de Recherche 34V-Commissariat Energie Atomique, 11 Rue Pierre et Marie Curie, 75231 Paris Cedex 05, France

[3] Max-Planck Institut für Molekulare Physiologie, Abteilung Strukturelle Biologie, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

[4] Unité de Biochimie Physiologique, Institut des Sciences de la Vie, Université de Louvain, Croix du Sud 5-15, 1348 Louvain-la-Neuve, Belgium

[5] These authors contributed equally to this work.

[6] Present address: Chemical Genomics Centre, Otto-Hahn-Strasse 15, 44227 Dortmund, Germany.

*Correspondence: claudia.oecking@zmbp.uni-tuebingen.de

DOI 10.1016/j.molcel.2006.12.017

SUMMARY

Regulatory 14-3-3 proteins activate the plant plasma membrane H$^+$-ATPase by binding to its C-terminal autoinhibitory domain. This interaction requires phosphorylation of a C-terminal, mode III, recognition motif as well as an adjacent span of approximately 50 amino acids. Here we report the X-ray crystal structure of 14-3-3 in complex with the entire binding motif, revealing a previously unidentified mode of interaction. A 14-3-3 dimer simultaneously binds two H$^+$-ATPase peptides, each of which forms a loop within the typical 14-3-3 binding groove and therefore exits from the center of the dimer. Several H$^+$-ATPase mutants support this structure determination. Accordingly, 14-3-3 binding could result in H$^+$-ATPase oligomerization. Indeed, by using single-particle electron cryomicroscopy, the 3D reconstruction of the purified H$^+$-ATPase/14-3-3 complex demonstrates a hexameric arrangement. Fitting of 14-3-3 and H$^+$-ATPase atomic structures into the 3D reconstruction map suggests the spatial arrangement of the holocomplex.

INTRODUCTION

P-type H$^+$-ATPases of plants are responsible for building up an electrochemical proton gradient across the plasma membrane that provides the driving force for nutrient uptake and maintenance of cell turgor. The fundamental role of the H$^+$-ATPase in plant physiology has stimulated the search for factors involved in its regulation. The enzyme is kept at a low activity level by its C-terminal domain, the autoinhibitory action of which is released upon binding of 14-3-3 proteins (Oecking et al., 1997; Piotrowski et al., 1998; Fuglsang et al., 1999; Maudoux et al., 2000).

Members of the eukaryotic 14-3-3 family are highly conserved proteins that have been implicated in the regulation of diverse physiological processes by protein-protein interactions. Coordination of a client protein within the amphipathic groove of a 14-3-3 dimer most often requires a phosphorylated consensus motif (mode I, RSXpSXP; mode II, RXY/FXpSXP; Yaffe et al., 1997) and can have a range of context-dependent effects such as conformational change or relocalization (MacKintosh, 2004). With respect to the H$^+$-ATPase, phosphorylation of the C terminus (YpTV-COOH) is a prerequisite for 14-3-3 binding in vivo. Although initially termed atypical, such C-terminal motifs are now referred to as mode III motifs ([pS/pT]X_{1-2}-COOH; Ganguly et al., 2005; Coblitz et al., 2005).

The fungal phytotoxin fusicoccin (FC), a diterpene glucoside, is a well-known activator of the H$^+$-pump in vivo and has been shown to bind to the preformed H$^+$-ATPase/14-3-3 complex, thereby stabilizing the enzyme in its activated state (Oecking et al., 1997; Piotrowski et al., 1998; Fuglsang et al., 1999; Maudoux et al., 2000). The crystal structure of 14-3-3 in complex with a phosphorylated pentapeptide of PMA2 (mode III, residues 952–956, QSYpTV-COOH), a H$^+$-ATPase isoform from *Nicotiana plumbaginifolia*, explains the mechanism of the toxin's action (Würtele et al., 2003). In brief, FC closes a gap that remains in the 14-3-3 groove after binding of the phosphopeptide, leading to mutual stabilization of both ligands. As a consequence of permanent activation of the H$^+$-ATPase, stomatal pores are irreversibly opened followed by wilting of plants (Marrè, 1979).

Within the C-terminal domain of the plant H+-ATPase, two autoinhibitory regions (regions I and II) have been identified (Axelsen et al., 1999; Dambly and Boutry, 2001). Since they do not encompass the C terminus, it is difficult to imagine how 14-3-3 association can abolish their inhibitory action. Previous experiments have demonstrated that, in addition to the YpTV-motif, a number of adjacent residues including the autoinhibitory region II contribute to binding of activatory 14-3-3 proteins (Jelich-Ottmann et al., 2001; Fuglsang et al., 2003).

Here, by using X-ray crystallography, it is shown that a 14-3-3 dimer binds two entire binding motifs of PMA2 H+-ATPase (52 amino acids) in an as-yet-unrecognized manner that allows exit of both peptides from the center of the dimer in the same direction. This unusual structure is supported by reduced 14-3-3 binding capability of several H+-ATPase mutants and suggests that the membrane embedded enzyme oligomerizes upon 14-3-3 association. In this regard, Kanczewska et al. (2005) have recently shown that activation of PMA2 by phosphorylation and 14-3-3 binding converts a dimer into a hexamer, which is therefore expected to be the active form of the enzyme. We used single-particle electron cryomicroscopy to reconstruct the 3D structure of the purified hexameric PMA2/14-3-3 complex. A model of the holocomplex was built based upon 14-3-3 and H+-ATPase atomic structures. The fit of the modeled structure into the obtained 3D volume suggests the relative positions of 14-3-3 within the assumed hexameric structure. Three 14-3-3 dimers appear to be located on top of a PMA2 hexamer where they may coordinate six autoinhibitory domains of the active enzyme.

RESULTS AND DISCUSSION

The 14-3-3/CT52 Complex

The entire 14-3-3 binding motif of PMA2 H+-ATPase comprises 52 amino acids (CT52, residues 905–956) including the mode III consensus YpTV-COOH as well as the autoinhibitory region II (Jelich-Ottmann et al., 2001). With the aim of solving the crystal structure of the corresponding complex, its reconstitution using bacterially expressed polypeptides in the presence of FC seems to be of advantage. FC enables 14-3-3 to bind to the unphosphorylated C terminus of the H+-ATPase (Svennelid et al., 1999; Jelich-Ottmann et al., 2001). Nonetheless, due to missing phosphorylation, the complex is probably unstable. Therefore, we first exchanged Thr in the C-terminal tripeptide of PMA2 (YTV) for Asp (YDV), the latter well known to mimic phosphorylation sites. Furthermore, we substituted Val for Ileu (YTI), which—according to the previous crystal structure (Würtele et al., 2003)—seems to fit better into the cavity due to an enhanced contact surface. Since the individual substitutions (YDV, YTI) caused increased 14-3-3 binding to the unphosphorylated C terminus in the presence of FC (data not shown), they were combined yielding the C-terminal tripeptide YDI. This double exchange allowed 14-3-3 association even in the absence of FC (shown in Figure 3D), indicating that the stability of the complex is enhanced.

Subsequently, the CT52 peptide (YTV or YDI) was purified by means of the IMPACT system, which utilizes the inducible self-cleavage activity of intein to separate the target protein from the affinity tag. In addition, we purified the recombinant His-tagged tobacco 14-3-3c isoform (T14-3c) deleted of its C-terminal 18 amino acids (T14-3cΔC). Since the structure of the C terminus is not resolved in any of the available X-ray data, the region is presumably highly mobile and seems to represent an inhibitor of 14-3-3/ligand interactions (Truong et al., 2002; Shen et al., 2003; Obsilova et al., 2004). Indeed, as compared to wild-type T14-3c (dissociation constant K_D, 14 nM, data not shown), deletion of the tail (T14-3cΔC) increased the binding affinity (K_D, 0.85 nM) for the CT52 peptide (YDI) in the presence of 1 μM FC (measured by surface plasmon resonance spectroscopy [SPRS], see Figure 4 and see Figure S1 in the Supplemental Data available with this article online). Furthermore, for the interaction between the T14-3cΔC and the wild-type CT52 peptide (YTV), a K_D of 41 nM (1 μM FC, Figure S1) was calculated, thus confirming that the double exchange (YDI) causes a significantly enhanced affinity.

Following incubation of the peptide CT52(YDI) with T14-3cΔC in the presence of FC, we observed coelution during gel filtration (data not shown). The apparent molecular mass of the complex was 140 kDa, which is indicative of a 14-3-3 tetramer, and its structure was solved at 2.7 Å. Data for crystal structure analysis are summarized in Table 1. Notably, the formation of a 14-3-3 tetramer was exclusively due to intermolecular contacts between the N-terminal parts of the bound PMA2 peptides (approximately residues 906–925, shown in Figure 6A). Thus, two 14-3-3 dimers are connected via the CT52 peptides while the individual 14-3-3 dimers themselves do not contact each other (Figure S2). We limit the structural description to one 14-3-3 dimer since this is the biologically active unit as supported by biochemical data (Oecking et al., 1997; Oecking and Hagemann, 1999; Maudoux et al., 2000). Nonetheless, the intermolecular interaction of the PMA2 peptides constitutes the basis for a model that will be discussed later.

Crystal Structure of the 14-3-3/CT52 Complex

As compared to the pentapeptide including the C-terminal YpTV-motif (Würtele et al., 2003), the corresponding span of the CT52 peptide (YDI, Figure 1D) shows a root-mean-square deviation (rmsd) of the backbone C_α atoms of 0.3 Å (rmsd FC, 0.38 Å, data not shown), indicating that there are no major conformational changes caused by the exchange of the two C-terminal residues (pTV, by DI).

A 14-3-3 dimer is simultaneously occupied by two molecules of FC and two entire H+-ATPase binding motifs (Figure 1). The latter are arranged in an unexpected and unusual conformation since each CT52 peptide forms a loop via its C-terminal 27 amino acids (956–930, Figure 2A) within the typical 14-3-3 binding groove. Consequently,