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Research article

H-bond refinement for electron transfer membrane-bound protein–protein complexes: Cytochrome c oxidase and cytochrome c552

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ABSTRACT

In this study we propose a protocol to evaluate membrane-bound cytochrome c oxidase–cytochrome c552 docking candidates. An initial rigid docking algorithm generates docking poses of the cytochrome c oxidase–cytochrome c552, candidates are then aggregated into a 512-DPPC membrane model and solvated in explicit solvent. Molecular dynamic simulations are performed to induce conformational changes to membrane-bound protein complexes. Lastly each protein–protein complex is optimized in terms of its hydrogen bond network, evaluated energetically and ranked. The protocol is directly applicable to other membrane-protein complexes, such as protein–ligand systems.

1. Introduction

The aim of the present work is to find a protein–protein complex model for cytochrome c oxidase/cytochrome c552 applying a combination of three different methods. First, a rigid docking algorithm is used to efficiently explore the configurational protein–protein space. Second, large scale molecular dynamics is used to embed the protein–protein docking candidates into a membrane model. Finally, a hydrogen bond network refinement algorithm is used for proper energetic ranking of the docking candidates.

Electron transfer is a fundamental process in biology, chemistry and physics and an important stage in many enzymatic cycles (Beratan et al., 1992; Langen et al., 1995). The process involves the flow of electron density from a donor to an acceptor molecule (Kendrew et al., 1958; Dawson, 1988). It plays a central role in many biochemical processes and for this reason, a detailed understanding of electron transfer reactions at the molecular level is of essential importance. Not surprisingly, computational techniques are an attractive tool in mapping electron transfer mechanisms (Balabin and Onuchic, 2000; Gehlen et al., 1996). Obtaining an atomic description of the transfer pathway is however a difficult task, at both the experimental and theoretical levels. The process can involve a short pathway, *e.g.* from a substrate or a cofactor directly bound in the vicinity of the acceptor group, or rather large pathways, *e.g.* across protein–protein complexes, where the donor and acceptor might be relatively distant from each other. Molecular-level structural details are thus extremely relevant to electron transfer processes and should be taken into account in docking algorithms in order to increase the accuracy of the protocols, and to generate more reliable and realistic docking candidates for electron transfer reactions in biological molecules.

Cytochrome c552 is a soluble heme protein playing a crucial role in the mitochondrial respiratory chain and is responsible for electron transfer with its redox partners, such as: cytochrome c reductase, cytochrome c oxidase, and cytochrome c peroxidase (Kim et al., 2000; Bertini et al., 2011). Cytochrome c552 is located in the inter membrane space and weakly binds to the inner mitochondrial membrane. As pointed out in previous studies cytochrome c552 is released from the mitochondria to the cytosol in response to different apopotosis-inducing agents, (Kluck et al., 1997). The role of cytochrome c552 in this important cellular process and the nature of cytochrome c oxidase interactions with model lipid membranes has motivated several studies (El Kirat and Morandat, 2009; Rytömaa et al., 1992).

Some studies have shown that cytochrome c oxidase dissociates from lipid membranes at high ionic strengths, (Rytömaa and

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Kinnunen, 1994), although it is not yet clear whether cytochrome c oxidase binds to the membrane due to sufficient intermembrane ionic strength that obstruct electrostatic protein–protein recognition, (Cortese et al., 1991). Other studies have proposed that cytochrome c oxidase remains partially bound to the membrane even at high ionic strengths (Cortese et al., 1995, 1998), and that the protein interacts with phospholipids *via* hydrophobic forces (Rytömaa et al., 1992; Snel et al., 1994). Yet, other studies have demonstrated that a partially inserted cytochrome c oxidase can be found in the inner mitochondrial membrane with an important role in apoptosis (Ott et al., 2007). Kostrzewa et al. (2000) showed that when cytochrome c552 binds to negatively charged lipids it orients lysine residues toward the membrane surface, exposing the heme group to electron transfer.

To the present the molecular structure of the *Paracoccus denitrificans* protein–protein complex cytochrome c oxidase/cytochrome c552 has no crystallographic solution. However an NMR study by Wienk et al. (2003) showed small chemical shift changes during the interaction of cytochrome c552 and the soluble CuA domain in cytochrome c oxidase. In this work equivalent effects were found for fully reduced and oxidized systems, showing that the protein–protein interaction is practically independent of the redox states of the binding partners. Moreover, the relevance of several positive lysine residues in the long-range electrostatic protein–protein recognition processes was highlighted. The cited study has also agreed with previous works by Witt et al. (1998a) and Drosou et al. (2002) and to previous models proposed for the *P. denitrificans* cytochrome c552 and cytochrome c oxidase system (Witt et al., 1998a,b).

Flöck and Helms (2002) proposed a computational rigid-body docking model based on FTDock followed by energy minimization. The study found cytochrome c552 in two critically different orientations (~95°) when docked against the two or the four subunit structures of the *P. denitrificans* oxidase. Additionally it was suggested that the bound complex exists as a dynamic ensemble of different orientations. However, this docking model predicts that some residues located at the binding interface do not show the chemical shifts reported in the work of Wienk et al. (2003).

2. Materials and methods

In the soil bacterium *P. denitrificans* the unusually fast terminal electron transfer step (Tipmanee and Blumberger, 2012) from heme a in cytochrome bc1 (complex III of the respiratory chain) to heme a3 in cytochrome c oxidase (complex IV of the respiratory chain) is mediated by membrane bound cytochrome c552 (Berry and Trumpower, 1985; Turba et al., 1995). The reduced cytochrome c552 and the subunit II of cytochrome c oxidase hydrophilic domain interact electrostatically through positively charged lysine residues on cytochrome c552 surface and the binuclear Cu_A center in subunit II of cytochrome c oxidase. Lysine residues around the exposed heme edge in cytochrome c552 are thought to be responsible for long range electrostatic protein–protein recognition (Witt et al., 1998a). Previous studies have shown the importance of the interaction of this lysine cluster in cytochrome c552 with negatively charged lipids (Rytömaa et al., 1992). Further analysis (Witt et al., 1998b) has shown minor interfacial conformational changes that allow and extra electron transfer from the reduced heme in cytochrome c552 to the Cu_A center in subunit II of cytochrome c oxidase. Residue tryptophan 121 (the electron transfer entry site) on the interface of subunit II of cytochrome c oxidase mediates the heme-copper electronic transfer process (Witt et al., 1998b). These observations are consistent with previous studies (Briggs and Capaldi, 1978; Millet et al., 1982; Lalla et al., 2001).

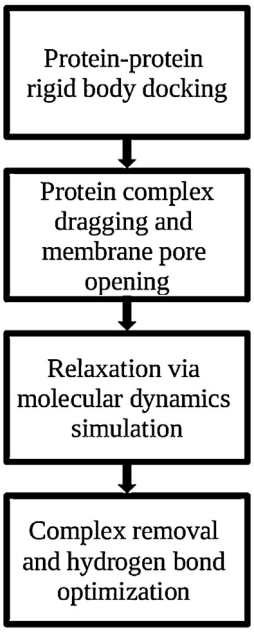

Fig. 1. Block diagram of the computational protocol.

In the present work we describe a computational protocol that allows the analysis of protein–protein complexes bound to a membrane patch. The method can be easily extrapolated to other membrane-bound systems, such as protein–ligand and cell penetrating peptides. Fig. 1 shows a block diagram detailing the process:

2.1. Protein–protein docking of cytochrome c oxidase and cytochrome c552

Using HEX (Ritchie and Kemp, 2000) we have generated 100 initial rigid-body docking poses, in the atomistic space, for the membrane unbound form of the complex (pdb codes 1ar1 and 1ql3). Rigid docking was performed in vacuum. These docking candidates were then geometrically clustered and filtered to fulfill a distance condition for electron transfer to be possible: only docking candidates with heme-copper distance <20 Å were kept for further analysis. From HEX data we kept 19 candidates for further refinement with molecular dynamics, (see Fig. 2).

2.2. Pore opening in the membrane patch

We performed molecular dynamics in GROMACS (Van der Spoel et al., 2005) patched with Plumed (Bonomi et al., 2009) to implement a collective reaction coordinate in order to open a pore in a 512 DPPC membrane patch of 12 nm length (see Fig. 3) solvated in explicit water. We followed Tolpekina et al. (2004) description for pore formation, where the local density of the lipids is lowered by a reaction coordinate defined as:

$$\xi = \frac{\Sigma - \Sigma_0}{\Sigma_{\boldsymbol{M}} - \Sigma_0} \tag{1}$$

with

$$\Sigma = \sum_i \tanh(\boldsymbol{S}\boldsymbol{r}_i) \tag{2}$$

where $\boldsymbol{r_i}$ is the *XY* plane distance projection from every membrane atom to the pore center, $\boldsymbol{S}$ controls the size of the pore, $\Sigma_{\boldsymbol{M}}$ is the total number of atoms in the membrane (25 600) and $\Sigma_{\boldsymbol{0}} = 25\,000$ is the equilibrium value of sigma from an unrestrained simulation. As defined, the reaction coordinate allows for a maximum pore