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In silico construction of HK2-VDAC1 complex and investigating the HK2 binding-induced molecular gating mechanism of VDAC1

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ABSTRACT

Hexokinase 2 (HK2) binds to Voltage-Dependent Anion Channel 1 (VDAC1) on mitochondrial outer membrane (MOM) to facilitate a preferential access of ATP to HK2 for glycolysis, in order to maintain a constant energy source for cell proliferation in cancer especially. While previous studies have discovered that the VDAC1 N-terminal helix is responsible for regulating molecules from within mitochondria to cytoplasm, the molecular mechanism of how HK2 is able to regulate the ATP access remains elusive. We hereby propose a model for the HK2-VDAC1 association. The model is then subjected to molecular dynamics (MD) simulations, where we probe the effect of HK2 binding on the mobility of the VDAC1 N-terminal helix. Results from the simulations show that HK2 binding restricts the movement of the VDAC1 N-terminal helix. As a result, VDAC1 is kept in the open state most of the time and probably allows a constant supply of ATP to HK2 for glycolysis.

1. Introduction

Hexokinase (HK) is one of the critical proteins in the pathway of the conversion of glucose to lactic acid to generate ATP in the cytosol. There are 4 isozymes of HK in mammals, namely HK1, HK2, HK3 and HK4, where they share a high similarity in their sequences (Deeb et al., 1993; Wilson, 2003). HK first binds to Voltage-Dependent Anion Channel 1 (VDAC1) at the mitochondrial outer membrane (MOM). ATP produced at mitochondria then travels through VDAC1 to reach HK where HK catalyzes the conversion of glucose and ATP to glucose-6-phosphate (G6P) in the glycolysis pathway for cell proliferation (Pedersen, 2007; Wilson, 2003). G6P would then be converted to lactic acid in multi steps by other downstream enzymes to generate cytosolic ATPs. It was found that HK2 is overexpressed in cancer cells (Chen et al., 2009; Lemasters and Holmuhamedov, 2006; Mathupala et al., 2006; Pastorino et al., 2002; Porporato et al., 2011; Shoshan-Barmatz and Mizrachi, 2012) and its binding to VDAC1 on the MOM gives HK a preferential access to ATP. At the same time, the association of HK2 to VDAC1 also suppresses apoptosis (Chen et al., 2009; Galluzzi et al., 2008; Porporato et al., 2011; Shoshan-Barmatz et al., 2010a, b).

VDAC is a trans-membrane protein found commonly on the MOM (Benz, 1994). It consists of a β-barrel wall and a N-terminal α-helix found inside the barrel wall. There are 3 isoforms of VDAC, namely VDAC1, VDAC2 and VDAC3 (Shoshan-Barmatz et al., 2010a, b). Of the three isoforms, VDAC1 was found to be the most abundant in human (De Pinto et al., 2010). VDAC1 serves as a channel for ions and charged metabolites such as ATP to pass through, and differentiates them by charge and size like other ion channels. VDAC possesses two states that define selectivity for different ion types. The open state favors anions and the closed state favors cations (Gincel et al., 2000; Shoshan-Barmatz et al., 2010a, b). Inter-conversion between the two states is controlled by the membrane potential, in which the range of −30 mV to 30 mV favors the open state (Gincel et al., 2000; Hodge and Colombini, 1997; Ludwig et al., 1986; Ludwig et al., 1988). Although both states allow the movement of ions passing through the channel, the open state is found to be of high conductance whereas the closed state is of low conductance (Benz et al., 1990; Colombini, 2012; Mertins et al., 2012).

Previous studies have shown that aligning the N-terminal α-helix with the barrel wall in VDAC1 increases the channel conductance and gives the open state (Mertins et al., 2012; Summers and Court, 2010; Ujwal et al., 2008). This observation is then accounted for by the difference in the electrostatic environment when the N-terminal helix translates to different positions within the β-barrel (Benz et al., 1990; Colombini, 2012; Mertins et al., 2012). On the other hand, the N-terminus is also found to control the ATP flux by changing the pore size of VDAC1 (Rostovtseva and Colombini, 1997). All these suggest that the N-terminal helix possesses a molecular gating function in VDAC1.

However, most studies only relate the charge of metabolites and the electrostatic environment of the pore in providing the molecular picture of VDAC1 gating function and focus very little on the variation of the

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pore size. Since ion channels in general differentiate charged molecules via both size and charge, and the VDAC1 pore is found to be able to change its pore size under different membrane potentials, both the electrostatic environment and the pore size are important and assume complementary in regulating the flux of charged molecules (Komarov et al., 2005).

While the X-ray crystal structure of VDAC1 shows that the N-terminus of VDAC1 is found within its pore (Bayrhuber et al., 2008), studies have shown that the N-terminus of VDAC1 does not remain in the pore continuously. Instead, it was found that the N-terminus of VDAC1 would translocate out to the cytosol to interact with HK2 (Geula et al., 2012). Experimental studies also showed that the peptide alone with the sequence of the VDAC1 N-terminal helix was able to bind with HK2 competitively and prevent HK2 from binding to VDAC1 on the membrane (Arbel and Shoshan-Barmatz, 2010; Shoshan-Barmatz et al., 2009). On the other hand, there were studies showing that the VDAC1 N-terminus translocation results in the oligomerization of VDAC1 on the mitochondrial membrane, which would then allow cytochrome c release and trigger apoptosis (Shoshan-Barmatz et al., 2010a, b).

Therefore in this study, a structural model of the HK2-VDAC1 complex is proposed to understand how such an association regulates ATP flux in cells via the modulation of the VDAC1 N-terminal helix movement. While a previous study has constructed a molecular model of HK1-VDAC1 (Rosano, 2011), this model is not suitable for the construction of the HK2-VDAC1 complex because the HK1-VDAC1 association leads to the closing of the channel. Nonetheless, it provides a plausible model for reference in the construction of the HK2-VDAC1 complex.

2. Materials and methods

2.1. Construction of the HK2-VDAC1 complex via molecular modeling

The X-ray crystal structures of VDAC1 (PDB ID: 2JK4) and HK2 (PDB ID: 2NZT) were preprocessed using Maestro program (Maestro, 2011). Appropriate bond orders were assigned and hydrogen atoms were added to the crystal structures. All crystallographic waters were removed. The protonation states of HIS were predicted by PROPKA and assigned with visual inspection to optimize the intra-protein hydrogen bond network at the physiological pH (Bas et al., 2008; Li et al., 2005; Olsson et al., 2011; Søndergaard et al., 2011). The N-terminus half of HK2 was retained and used for constructing the HK2-VDAC1 complex, as it was found to interact with VDAC1.

The geometrical shape of HK2 is observed to complement the VDAC1 pore in a bolt-and-nut manner (Fig. 1), which directs the insertion of HK2 into VDAC1 in our model. The N-terminal helix in VDAC1 defines the HK2 insertion limit. All manual rotations and translations of HK2 in order to reduce steric and electrostatic clashes were carried out in Maestro. The initial structure was then prepared via the LEaP module in AMBER 10 suite for productive MD simulations.

2.2. Productive MD simulations and post-processing

TIP3P water molecules were added in an octahedron box with a distance of 20 Å between the protein and water box surface. Counter-ions were added to maintain a neutral environment in the system.

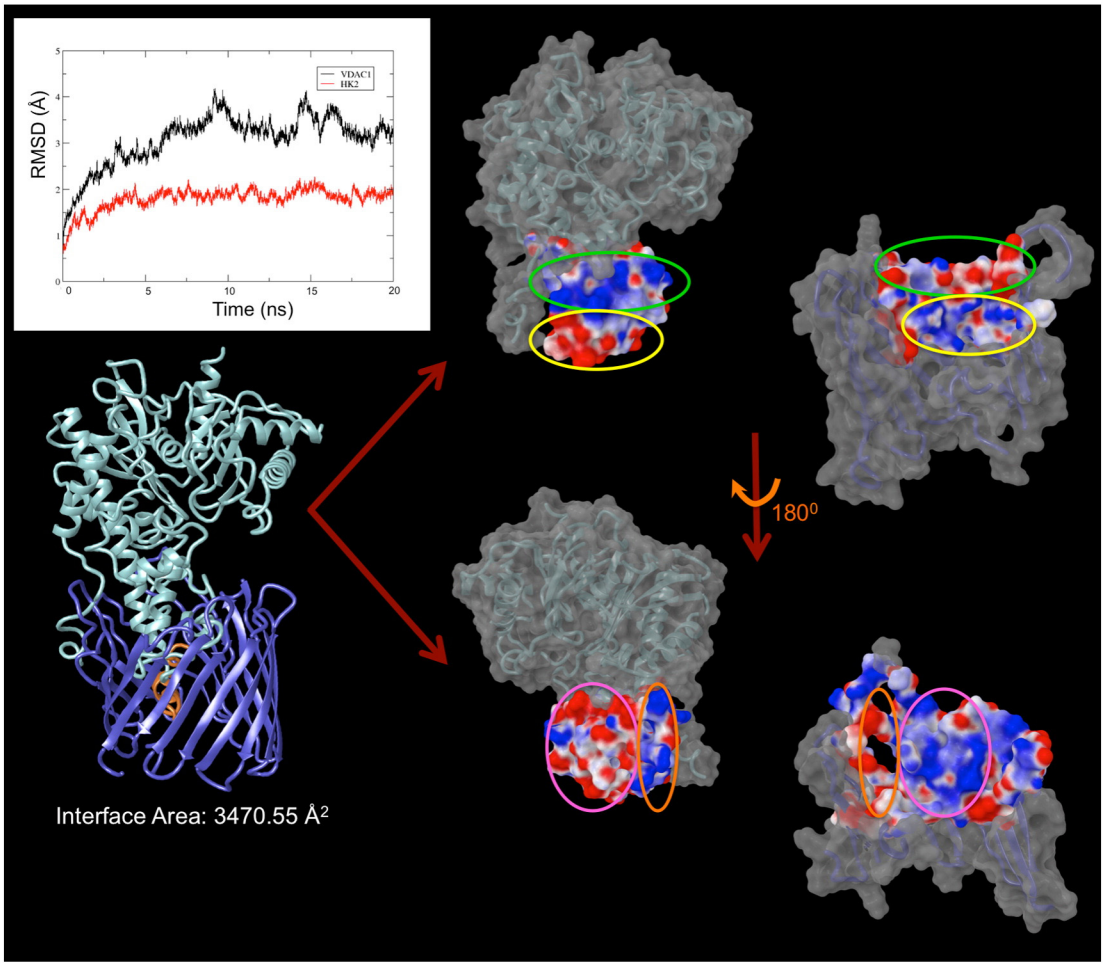

Fig. 1. (Top left) RMSD of HK2 (Red) and VDAC1 (Black). Electrostatic potential surfaces of HK2 and VDAC1 that were in contact. Non-contact surfaces between HK2 and VDAC1 are shown in grey. The electrostatic potential surfaces showed complementary interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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