Mitochondrial Pyruvate Carrier 1 and 2 Heterodimer, In Silico, Models of Plant and Human Complexes: A Comparison of Structure and Transporter Binding Properties

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ABSTRACT

The plant and human mitochondrial pyruvate carrier (MPC) had been studied in the 1970s-1990s providing many predictions on functional protein structure and mechanisms of substrate binding. Genes for human and plant MPC have been identified, but no crystal structure has yet been registered or deposited in a protein data bank. This report describes results for comparisons of structure for human and plant MPC1/2 heterodimer homology models. Key cysteine residues are identified for pyruvate and blocker binding and formation of thiohemiacetal or Michael addition bonds. Evidence is provided for an alternating access model in human, mouse ear-cress, castor and common beans, and corn.

KEYWORDS

Arabidopsis Thaliana, Homo Sapien, In Silico, Phaseolus Vulgaris, Protein Homology, Protein Protein Docking, Pyruvate Docking, Ricinus Communis, Zea Mays

INTRODUCTION

Plant mitochondrial pyruvate carrier (MPC) had been studied for many years, decades ago (Beechey, Brailsford, & Thompson, 1987; Brailsford, Thompson, Kaderbhai, & Beechey, 1986a, 1986b; Day & Hanson, 1977; Laloí, 1999; Proudlove, Beechey, & Moore, 1987b; Walker & Beevers, 1956), but the plant genes have only recently been discovered (C. Li, Wang, Ma, & Zhang, 2014; M. Wang, Ma, Shen, Li, & Zhang, 2014). There is a role of MPC complex in the abscisic acid (ABA) mediated stomata opening and closing during environmental stress (Santelia & Lawson, 2016; Yu & Assmann, 2014), in addition to energy production, primary metabolism and carbon flux in general. Inner mitochondrial membrane proteins are transported across mitochondrial membranes in plants (Alberts, Johnson, & Lewis, 2002; Duncan, Murcha, & Whelan, 2013; Murcha et al., 2014) similar to that in humans (Kühlbrandt, 2015; Neupert & Herrmann, 2007); indicating that the structural characteristics of the functional proteins might be similar. Overall, this information is useful for in silico biosimulations of metabolism in plants (Beckers et al., 2016; Phelix & Feltus, 2014) and humans (Hammack, Perry, LeBaron, Villareal, & Phelix, 2015; Phelix, Bourdon, Villareal, & LeBaron, 2016; Phelix & Dugan, 2016; Phelix, Villareal, LeBaron, Perry, & Roberson, 2014). For Arabidopsis thaliana and Zea mays
this is particularly interesting because full genome-scale metabolic networks are available (Beckers et al., 2016; Saha, Suthers, & Maranas, 2011; Seaver et al., 2015). Understanding membrane transport of pyruvate in plants is critical to engineering plant primary metabolism, in particular for oils and biodiesel production (Lin et al., 2017; Schwender et al., 2015; Weber & Bräutigam, 2013).

To date, since discovery of the MPC1 and MPC2 genes, most detailed molecular information available on the “mitochondrial pyruvate carrier complex” is based upon investigations of yeast and fruit fly MPC gene-products; however, some work on mouse and human MPC1, MPC1Like, and MPC2 is appearing (X. Li et al., 2016; Vanderperre et al., 2016; Vigueira et al., 2017). Of greatest concern is that yeast MPC1 protein (UniProtKB – P53157) has 130 amino acid residues; whereas, human MPC1 protein (UniProtKB – Q9Y5U8) has 109 residues and thus a shorter carboxy-tail. Possibly more importantly, human MPC1 has three cysteine residues, i.e., two adjacent, a doublet, at Cys60 and Cys61 and a singlet at Cys83; whereas, yeast MPC1 has only one cysteine residue, Cys87. Mouse (UniProtKB – P63030) and rat MPC1 (UniProtKB – P63031) have the same three cysteine residues as humans and total of 109. Another animal tissue model used in the early biochemical studies was the bovine heart mitochondria (Nałęcz, Müller, Zambrowicz, Wojtczak, & Azzi, 1990), and the bovine MPC1 amino acid sequence predicted on NCBI (reference sequence XP_001070510.1) has 109 residues with the doublet and singlet cysteine residues at the same locations as human, rat, and mouse MPC1. The fruit fly MPC1_drome (UniProtKB – Q7KSC4) has two singlet cysteine residues, Cys61 and Cys80 with 107 total residues. On the other hand, yeast MPC2 protein (UniProtKB – P38857) has two singlet cysteine residues, Cys86 and Cys111, and 129 residues total; versus MPC2_human (UniProtKB – Q95563) that has one singlet Cys54 residue and 127 total. MPC2_mouse (UniProtKB – Q9D023), MPC2_rat (UniProtKB – P38718), and MPC2_bovine (UniProtKB – E1BD64), each match MPC2_human for these features. Many predicted plant sequences for MPC are available on UniProt and only one has a doublet cysteine on the MPC1 of the common bean, Phaseolus vulgaris. The predicted sequences for Arabidopsis thaliana, Ricinus communis, and Zea mays, i.e., plant species where MPC had been studied to date, were also available. This protein sequence information is the sufficient starting point for human and plant protein homology modeling, as with two other proteins binding pyruvate, i.e., rat MCTs (Manoharan, Wilson, Sessions, & Halestrap, 2006; Nancolas, Sessions, & Halestrap, 2015; Wilson, Meredith, Bunnun, Sessions, & Halestrap, 2009) and human pyruvate kinase (Kalaiarasan et al., 2015).

Additionally, the challenge is to match the model proposed by (Nałęcz, 1994): 1) Binding of the substrate at the outer side of the inner mitochondrial membrane, facilitated by a positively charged guanidyl group of arginine and lower pH, promoting an interaction of pyruvate with cysteine; 2) Movement of the substrate around a guanidyl residue due to the rotation of the pyruvate molecule, followed by interaction with another sulfhydryl (SH) group within the carrier molecule; 3) Breakage of semimercapital bond at high pH inside the mitochondria; 5) Binding of the substrate moves at least one SH group per monomer of the carrier molecule in such a way, that it can interact with an SH group of another monomer; a disulfide, S-S, bridge between them is formed, giving a dimer; 6) This conformational change allows exposure of another internal SH group towards a more hydrophilic environment; the group become more accessible for the internal substrate and less accessible for N-ethyl-maleimide (NEM), which is then unable to inhibit the process of exchange; 8) When SH groups are blocked, binding of the substrate or SH group modifying reagent opens a channel in the carrier protein; an unspecific leak occurs. The cysteine residues within the transporter channel are also critical for Michael addition reactions with the cinnamic acid derivative blockers (Halestrap, 1976, 1978a; Hildyard, Åmmälä, Dukes, Thomson, & Halestrap, 2005). This current study compares human MPC1/2 heterodimer structure to similarly predicted models of these plant species and demonstrates that pyruvate can form thiohemiacetal bonds and the blockers can form Michael addition reactions with a single cysteine residue within the transport channel. Finally, evidence is provided to support the proposed alternating access model for pyruvate transport across the inner membrane of the mitochondrion by MPC (Vanderperre, Bender, Kunji, & Martinou, 2015), and plastid by bile acid sodium symporter 2 or BASS2 (Furumoto et al., 2011; Zhou et al., 2014).
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