Hypothesis

A new model for the membrane topology of glucose-6-phosphatase: the enzyme involved in von Gierke disease

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Abstract Very recently we have proposed [Hemrika et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 2145–2149] that the active site of the vanadate-containing chloroperoxidase from the fungus *Curvularia inaequalis*, of which the tertiary structure is known, is structurally very similar to that of the membrane-bound mammalian glucose-6-phosphatases for which no structural data are available. The proposed active site of glucose-6-phosphatase, however, is incompatible with the six transmembrane–helix topology model that is currently used. Here we present a new topology model for glucose-6-phosphatase which is in agreement with all available data.

Key words: Glucose-6-phosphatase; Vanadate-containing chloroperoxidase; Membrane topology; Glycogen storage disease

1. Introduction

Glucose-6-phosphatase (G-6-Pase) is an enzyme tightly associated with the endoplasmatic reticulum (ER) and nuclear membranes of liver and kidney cells [1]. G-6-Pase catalyzes the last step in both gluconeogenesis and glycogenolysis and as such it is the key enzyme in glucose homeostasis [1]. Glycogen storage disease type 1 (von Gierke disease) is an autosomal recessive disorder — with an incidence of about 1:100 000 in humans — that is the consequence of the absence of G-6-Pase activity. This disorder is characterized by severe clinical manifestations such as hypoglycemia, growth retardation, hepatomegaly, kidney enlargement, hyperlipidemia, hyperuriceamia and lactic acidosis [2,3]. Without dietary treatment patients suffering from this disease die early, usually in their teens, of liver and kidney complications [4].

Research on this disease has received an important impetus by the recent isolation and sequencing of the genes encoding this enzyme in human, rat and mouse [5–7]. The deduced amino acid sequences show that these proteins are highly (>90%) similar. Analysis of their hydropathy profiles indicated the presence of at least six putative membrane-spanning regions and accordingly a topology model for G-6-Pase was proposed in which six membrane spanning helices are incorporated [5] (see also Fig. 2A). This model has been used as the template in several structure–function relation studies on G-6-Pase [8,9]. Mutations, that abolished or greatly reduced G-6-Pase activity, in the *G-6-Pase* gene of von Gierke patients have also been identified [4,8,9]. Arg^{83} of G-6-Pase turned out to be a very important residue for enzyme activity. It was demonstrated that an Arg^{83} to Cys mutation completely abolishes G-6-Pase activity and the structural requirements of this codon were examined by replacing Arg^{83} with the amino acids Glu, Lys, Met, Leu, Asn, Gln, Ser and Thr, respectively [9]. None of these replacements resulted in restoration of G-6-Pase activity. The authors [9] therefore postulated that Arg^{83} of G-6-Pase is involved in positioning of phosphate. Since it is known that a phosphohistidine intermediate is formed during G-6-Pase catalysis [10,11] the authors [9] subsequently mutated the four histidines that, according to the six transmembrane–helix topology model, reside on the same side of the ER membrane as Arg^{83}. Only His^{119} turned out to be absolutely required for G-6-Pase activity and therefore it was suggested that His^{119} is the phosphate acceptor during G-6-Pase catalysis. Very recently, however, based on several lines of evidence, we have proposed that this may be incorrect [12].

2. Evidence for a new membrane topology

Vanadate-containing haloperoxidases catalyze the peroxidation of a halide to the corresponding hypohalous acid. The recent determination of the primary [13] and tertiary [14] structure of one such enzyme, the vanadate-containing chloroperoxidase (V-CPO) from the fungus *Curvularia inaequalis* revealed that the amino acids contributing to the active site of this enzyme are conserved in the bromoperoxidase (V-BPO) from the seaweed *Ascophyllum nodosum* [14] and surprisingly, also in the G-6-Pases and at least two other families of acid phosphatases that were previously considered unrelated [12]. It should be noted that this conservation is only restricted to the domains providing these residues, while overall identity is very low.

Vanadate and phosphate are structurally very similar and many acid phosphatases — including G-6-Pase — are inhibited by vanadate [15]. Further, vanadate-containing haloperoxidases rapidly loose their activity in phosphate-containing buffers and reconstitution of apo-BPO by vanadate is inhibited in the presence of phosphate [16].

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351-EKWEFEFWRPLSGVRDD 395-PFPAYPSGHATFG 488-SRIFLGVHWRFDAAA
 74-FKWILFGQRPYWWVLDT 110-TGPGSPSGHTIFA 168-SRIYLAAHFPHQVVA
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Fig. 1. Alignment of the active site domains of the V-CPO from *C. inaequalis* [14] to the proposed active site domains of G-6-Pase of human, rat and mouse (which are identical in these regions) [5–7]. Residues directly involved in the V-CPO active site are given in bold face. Identical residues are indicated with an asterisk.

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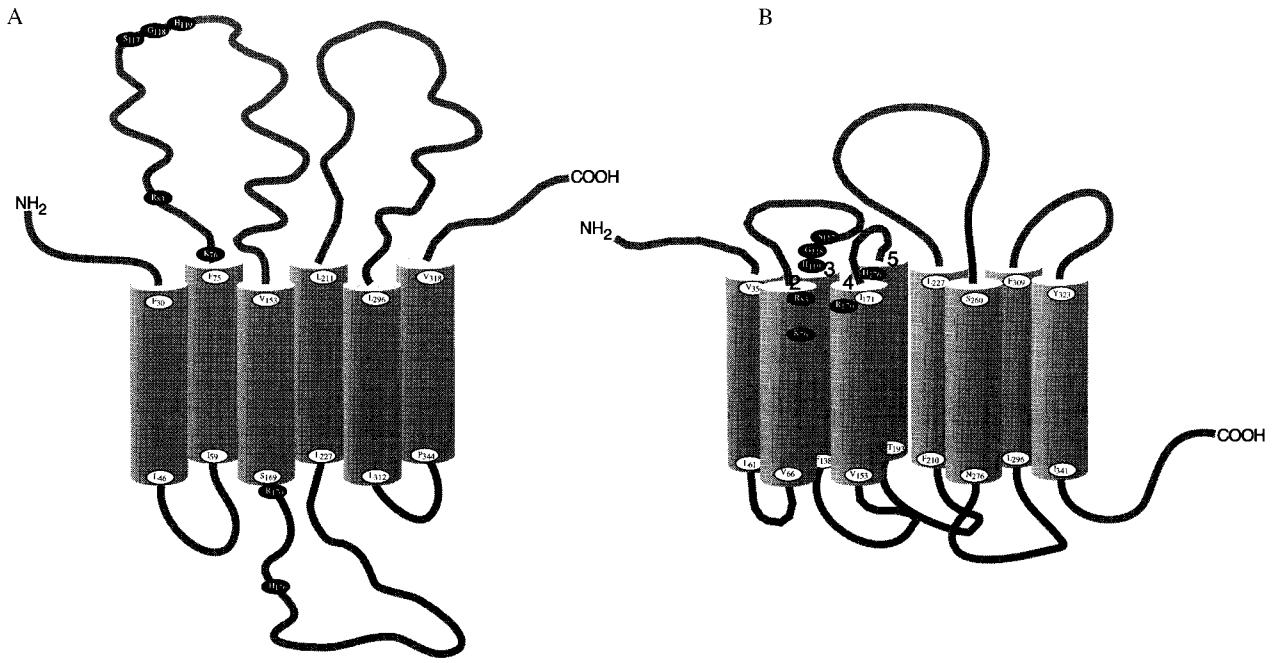

Fig. 2. Membrane topology models for G-6-Pase. A: Current six transmembrane–helix topology model (adapted from [9]). B: Newly proposed nine transmembrane–helix topology model. Residues predicted to be on the membrane interface are depicted as open ovals. Putative G-6-Pase active site residues are depicted as closed ovals.

We have also established that apo-CPO from *C. inaequalis* is able to hydrolyze the commonly used phosphatase substrate *p*-nitrophenyl phosphate (*p*-NPP) and thus can function as a phosphatase [12]. We therefore proposed that the residues involved in the binding of vanadate and/or peroxidative catalysis in holo-CPO are also involved in the binding of phosphate and subsequent *p*-NPP hydrolysis in apo-CPO and as a consequence that the active site of the vanadium-containing haloperoxidases is structurally very similar to that of the aligned acid phosphatases [12].

Fig. 1 shows the alignment of the residues of the active site of the V-CPO to the proposed phosphate binding site of the G-6-Pases. It can be seen that Arg^{83} of G-6-Pase aligns to Arg^{360} of V-CPO, a residue donating hydrogen bonds to vanadate, indeed making Arg^{83} a likely candidate for positioning phosphate in G-6-Pase. His^{119} of G-6-Pase, however, does not align to His^{490} of V-CPO, the residue covalently binding vanadate, but to His^{404}, a proposed acid-base group in catalysis [14]. The histidine of G-6-Pase aligning to His^{490} of V-CPO is His^{176}, but according to the six transmembrane–helix topology model, this residue is situated at the opposite site of the membrane as Arg^{83} and His^{119} (see Fig. 2A). We suggested therefore, that His^{176} resides on the same side of the ER membrane as Arg^{83} and His^{119} and thus that the proposed membrane topology [5,6] is in need of revaluation [12].

Fig. 2B shows our new model for the membrane topology of G-6-Pase which is based on the presented evidence concerning the nature of the G-6-Pase active site and the results of two new algorithms for the prediction of membrane-spanning domains [17–20]. These algorithms independently predict nine transmembrane helices in G-6-Pase as opposed to the previous topology model [5,6]. With this newly predicted topology all residues aligning to the active site residues of V-CPO are situated on the same — luminal — side of the ER-membrane (compare Fig. 2A and B). We propose therefore that helices II, III, IV and V are in close contact and provide the phosphate binding and glucose-6-phosphate hydrolysis site of G-6-Pase.

In our model His^{176} of G-6-Pase is the nucleophile forming the phosphohistidine enzyme–substrate intermediate. The phosphate moiety is positioned by interaction of the negatively charged oxygens with the positively charged Lys^{76}, Arg^{83} and Arg^{170}, while analogous to V-CPO Ser^{117} and Gly^{118} may also donate hydrogen bonds. His^{119} may provide the proton needed to liberate the glucose moiety.

Thus, the common architecture of the active sites of the vanadate containing haloperoxidases and the aligned acid phosphatases [12], for which at present no structural data are available, has important implications for both research in the acid phosphatase and in the peroxidase field. With a new topology model for G-6-Pase and with the designation of the putative residues involved in the binding and the hydrolysis of glucose-6-phosphate the first of these implications may have become evident.

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