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Theory and computation show that Asp463 is the catalytic proton donor in human endoplasmic reticulum α-(1→2)-mannosidase I

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ABSTRACT

It has been difficult to identify the proton donor and nucleophilic assistant/base of endoplasmic reticulum α-(1→2)-mannosidase I, a member of glycoside hydrolase Family 47, which cleaves the glycosidic bond between two α-(1→2)-linked mannosyl residues by the inverting mechanism, trimming $Man_9GlcNAc_2$ to $Man_8GlcNAc_2$ isomer B. Part of the difficulty is caused by the enzyme's use of a water molecule to transmit the proton that attacks the glycosidic oxygen atom. We earlier used automated docking to conclusively determine that Glu435 in the yeast enzyme (Glu599 in the corresponding human enzyme) is the nucleophilic assistant. The commonly accepted proton donor has been Glu330 in the human enzyme (Glu132 in the yeast enzyme). However, for theoretical reasons this conclusion is untenable. Theory, automated docking of α-D-$^3S_1$-mannopyranosyl-(1→2)-α-D-$^4C_1$-mannopyranose and water molecules associated with candidate proton donors, and estimation of dissociation constants of the latter have shown that the true proton donor is Asp463 in the human enzyme (Asp275 in the yeast enzyme).

1. Introduction

Endoplasmic reticulum α-(1→2)-mannosidase I (ERManI, EC 3.2.1.113) belongs to glycoside hydrolase family 47 (GH47).[1] It cleaves the glycosidic bond between two α-(1→2)-linked mannosyl residues by the inverting mechanism[1–3] and trims $Man_9GlcNAc_2$ to $Man_8GlcNAc_2$ isomer B.[2,3] Other GH47 α-(1→2)-mannosidases cleave other mannosyl residues to yield other products.[4]

Members of GH47 have an $(\alpha,\alpha)_7$-barrel fold (Fig. 1) and an active-site calcium ion[5–10] that is necessary for high enzyme activity and thermostability.[11] *Saccharomyces cerevisiae* and human ERManI enzymes are 35% similar in primary sequence,[2] but the amino acids involved in catalysis are practically the same.[6] Both enzymes have essentially the same crystal structure, and inhibitor binding causes little conformational change.[6,10]

In the inverting mechanism, an amino acid residue acting as a nucleophilic assistant/base helps a water molecule to perform a nucleophilic attack on the anomeric carbon. A second amino acid residue aids glycosidic bond cleavage by donating a proton to the glycosidic oxygen atom.[12] However, in ERManI the proton must be relayed by a water molecule, as no active-site carboxyl group is close enough to the glycosidic oxygen for direct proton donation. In addition, all three potential proton-donating groups coordinate water molecules (Fig. 2).[6] This is very unusual.

The conformation of the complexed substrate changes during cleavage. Human ERManI binds the inhibitors kifunensine and 1-deoxymannojirimycin in its subsite −1 in the unusual $^1C_4$ conformation.[6] The glycon of methyl 2-*S*-(α-D-mannopyranosyl)-2-thio-α-D-mannopyranoside (S-Man_2) is bound in the $^3S_1$ conformation, leading to the suggestion that its transition state is a $^3H_4$ conformer, intermediate between $^1C_4$ and $^3S_1$ conformers.[10] An automated docking study indicated that the substrate glycon in yeast ERManI must be in the $^1C_4$ conformation to enter the active site.[13] It then passes through $^3H_2$, $^OS_2$, $^{3,O}B$, and $^3S_1$ conformations to reach the putative ^3E transition-state conformer, structurally adjacent to the $^3H_4$ conformer.[13] After hydrolysis, the β-mannose molecule that had been the glycon finds itself successively in the $^1C_4$, $^1H_2$, and $B_{2,5}$ conformations before being expelled from the enzyme active site.[14]

It has been difficult to identify the ERManI catalytic proton donor and nucleophilic assistant/base. A crystal structure of the yeast enzyme with glycerol in the active site led to two hypotheses: (1) that Glu132 is the nucleophilic assistant to the water nucleophile, and that Asp275 or Glu435, probably the former, is the proton donor; and (2) alternatively and less likely that Glu435 is the nucleophilic assistant, with Glu132 being the proton donor.[5] A companion study with human ERManI again led to two hypotheses similar to those above: (1) that Glu599 (Glu435 in the corresponding yeast enzyme) is the nucleophilic assistant to Water5, with

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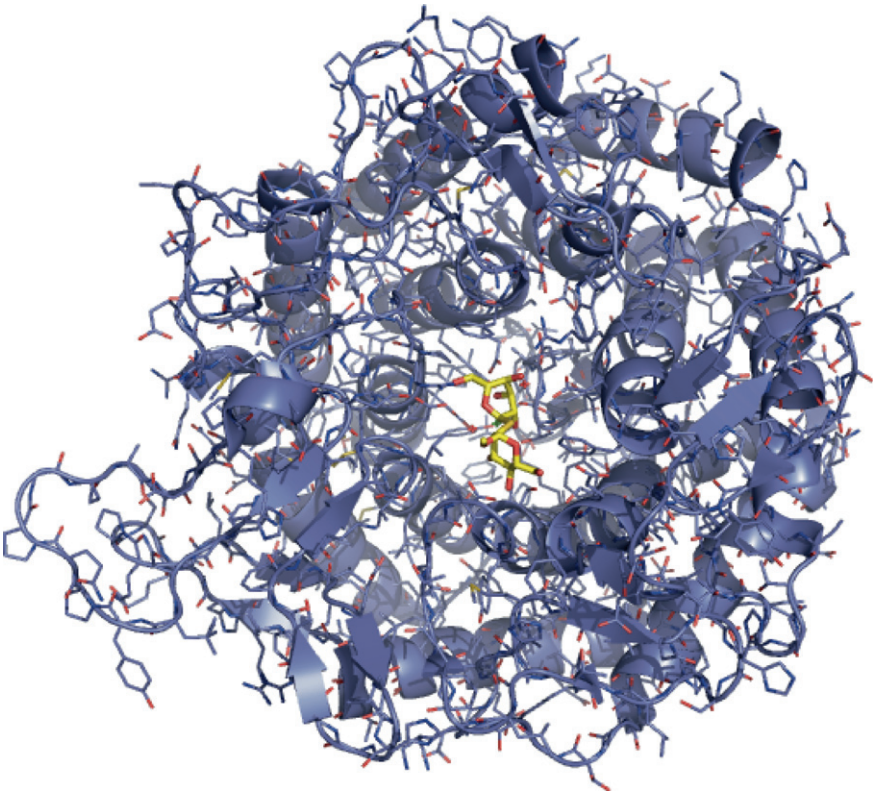

Figure 1. Human ERManI crystal structure 1X9D showing complexed S-Man_2.[10]

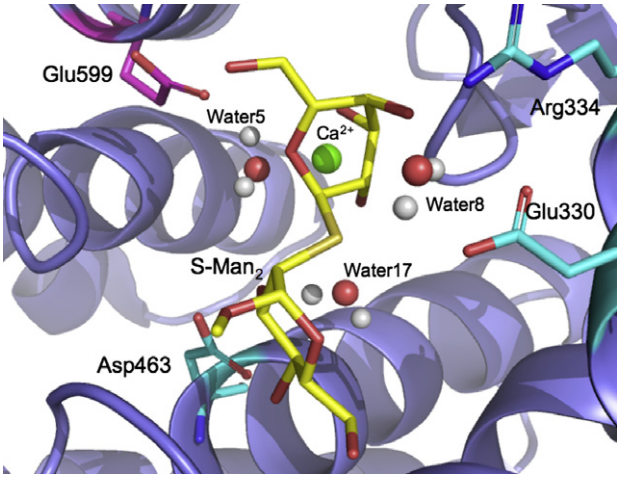

Figure 2. Crystal structure (with best-docked water molecules) of human ERManI active site. Pink: Glu599 nucleophile; blue: possible proton donors, either Asp463 or instead Glu330 paired with Arg334; yellow: S-Man_2; red: oxygen atoms; green: calcium ion. Water5 is between Glu599 and S-Man_2, Water8 is between Glu330 and S-Man_2, and Water17 is between Asp463 and S-Man_2.

Glu330 (Glu132) as the proton donor, transmitting a proton through Water8; and (2) that Asp463 (Asp275) is the nucleophilic assistant, with Water17 being the actual nucleophile, with Glu330 as the proton donor (Fig. 2).[6] A later study on human ERManI adopted the first hypothesis, suggesting that Arg334 (Arg136) contributed to the general acid function.[10] Work on *Hypocrea jecorina* and mouse GH47 enzymes with more capacious active sites yielded the same conclusions about the catalytic residues.[7,9] An automated docking study of yeast ERManI did not challenge Glu132 as the proton donor, acting through Water195, and identified Glu435 rather than Asp275 as the nucleophilic assistant to Water54.[15]

The nine invariant yeast ERManI acidic residues were mutated before any crystal structure was available. E214Q, D275N, E279Q, E435Q, and E503Q were not active, whereas D86N, E132Q, E438Q, and E526Q had <2% of the activity of wild-type ERManI.[11] A similar study on a GH47 enzyme from *Aspergillus saitoi* found the activity of E124Q (E132Q in yeast ERManI), E124D, D269N (D275N), D269E, E411Q (E435Q), and E411D as 0.02%, 0.2%, 0%, 1.9%, 0%, and 0.74% of the wild-type enzyme.[16] A third mutagenesis project conducted on human ERManI gave k_{cat}/K_M values of 3.5%, 0.1%, 0.0005%, 0.006%, and 0.0003% of the wild-type value for E330Q (E132Q in yeast ERManI), D463N (D275N), E599Q (E435Q), E330Q/E599Q, and D463N/E599Q, respectively.[10]

In summary, identification of the ERManI catalytic proton donor and its associated water molecule is uncertain, because all three potential catalytic carboxyl groups coordinate water molecules, and because mutating each of these groups in yeast, *Aspergillus*, and human ERManI causes loss of all or nearly all activity. Therefore we have in this article considered the theory of GH catalysis and then the relative merits of putative proton donor/water systems, and have followed this with extensive use of computation, both by automated docking to determine orientations of substrate and water molecules, and by estimating pK_a's of these groups.

2. Theory

2.1. Electrostatic transition-state stabilization in relation to *syn*- versus *anti*-protonation

Enzyme-catalyzed reactions are mediated by preferential stabilization of the transition state,[17] and electrostatic factors contribute the most to this stabilization.[18–20] At the transition state for the glycoside substitution reaction, the local charge distribution of