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The molecular binding interactions of inhibitors and activators of phosphoenolpyruvate carboxylase

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Abstract

We have performed molecular modelling studies of the binding to maize phosphoenolpyruvate carboxylase (PEPC) of phosphoenolpyruvate (PEP) and a number of representative competitive inhibitors. We predict that all these compounds share a common binding mode and that the differences in inhibitory activity of the various inhibitors arise mainly from either increased hydrophobic interactions of *cis* substituents or small but significant changes in their binding mode arising from steric clashes of *trans* substituents with the active site. We have also performed molecular modelling studies of glucose-6-phosphate (G6P) and a number of other allosteric activators in their putative allosteric binding site in this enzyme. We predict that these molecules share the same binding mode for their phosphate moiety while some of them engage in a variety of hydrogen bonding interactions with residues from different subunits of the enzyme, and others establish hydrophobic and van der Waals interactions with other regions of the allosteric binding site.
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1. Introduction

Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) is an enzyme that catalyzes the highly exergonic and irreversible β-carboxylation of phosphoenolpyruvate (PEP) to form oxaloacetate and inorganic phosphate in the presence of Mg^{2+} or Mn^{2+} as a cofactor [1–3]. This enzyme has primarily an anaplerotic role by replenishing C_4-dicarboxylic acids for both energy and biosynthetic metabolisms. In the case of C_4 plants such as maize, the reaction catalyzed by PEPC constitutes the first step in the assimilation pathway of atmospheric CO_2.

Several competitive inhibitors of PEPC that are structural analogues of PEP have been used to try to elucidate the kinetic mechanism of this enzyme and the properties of its active site [2–12]. However, the detailed mechanism of reaction of PEPC, involving PEP, Mg^{2+} (or Mn^{2+}) and HCO_3^-, has not yet been established unambiguously [1–3,13–22].

PEPC is subject to allosteric regulation by various metabolites. The enzyme is activated by D-glucose-6-phosphate (G6P) and inhibited by L-malate and L-aspartate (L-Asp) [2,3,23–27]. Furthermore, the PEPC isoform involved in C_4 photosynthesis by C_4 monocots such as *Z. mays* is also activated by glycine, alanine and serine [21,24,28–33]. All of these groups of regulators are in fact believed to have separate binding sites on the enzyme [9,32,34]. The allosteric G6P binding site [34] can also bind a large number of hexose and triose phosphates as well as other phosphate esters [16,30,32,35–43]. PEP itself can bind to the G6P binding site, thus behaving as both substrate and allosteric activator of PEPC, although this activating effect seems to be due exclusively to the kinetic mechanism of reaction [16,19]. However, a two-state allosteric transition model has been reported in which PEP binding to the G6P binding site was not required [25].

In recent years, the X-ray crystal structures of the bacterial (*E. coli*) and the maize (*Z. mays*) forms of PEPC have been reported [44–46]. The overall structure of PEPC was described as being a tetramer of four identical subunits arranged as a 'dimer of dimers', with a salt bridge between Arg 438 and Glu 433 in *E. coli* PEPC (Arg 498 and Glu 493 in *Z. mays* PEPC) being responsible for the interaction between the dimers [44–48]. Each monomeric subunit is made up of an eight-stranded β-barrel and approximately 40 α-helices.

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The crystal structures of *E. coli* PEPC (EcPEPC) had L-Asp bound to what is believed to be its allosteric binding site [44,47,48]. It was thus thought that the enzyme was in its inactive state (T state). The binding site of L-Asp was found to be located approximately 20 Å away from the active site. One of the four residues involved in the binding of L-Asp is Arg 587, which is part of a conserved glycine-rich loop essential for the catalytic activity of the enzyme. The crystal structures suggest that L-Asp causes the inhibition of PEPC by shifting this glycine-rich loop and another mobile loop away from the active site, with the formation of an ion pair between the sidechain of Arg 587 and the carboxyl group of L-Asp [44,47,48].

The crystal structures of EcPEPC also revealed the likely position of the active site. Mn^{2+} was found bound to the carboxylate sidechain oxygens of Glu 506 and Asp 543 at the C-terminal end of β5 and β6 strands at the top of the α/β barrel of the enzyme [45,47,48]. The coordination sphere of Mn^{2+} was seen to be approximately octahedral, with four water molecules presumably completing the coordination. After soaking the L-Asp-bound EcPEPC crystal with a solution containing Mn^{2+} and the substrate analogue 3,3-dichloro-2-dihydroxy-phosphinoylmethyl-2-propenoate (DCDP), the likely binding mode of this PEP analogue was also determined [45,47,48]. The phosphate group of DCDP interacts with Mn^{2+}, Arg 396, Arg 699 and Arg 713, all of which presumably stabilise the negative charge of the phosphate group to facilitate the nucleophilic attack by bicarbonate to form carboxyphosphate and enolate of pyruvate in the first step in the proposed reaction mechanism of PEPC [46–48]. A number of hydrophobic residues (Trp 248, Leu 504 and Met 538) were found around the chlorines of DCDP, indicating the presence of a hydrophobic pocket around the binding site of the methylene group of PEP, as suggested earlier [49].

On the other hand, the crystal structure of *Z. mays* PEPC (ZmPEPC) was not bound with L-Asp but with a sulfate anion, which is also an allosteric activator of PEPC [46–48]. Sulfate binds at the dimer interface where two four-α helix bundles are located, in the vicinity of four positively charged residues (Arg 183, Arg 184, Arg 231 and Arg 372). The ion pairing of sulfate with one of these residues, the conserved Arg 372, could be involved in the rearrangement of each bundle. Arg 372 also interacts with the sidechain of Ser 185 in the helix bundle of the neighbouring subunit. It was thus concluded that sulfate binds to the allosteric G6P binding site and that the crystal structure was that of the active state (R state) of ZmPEPC [46–48].

A comparison of EcPEPC and ZmPEPC revealed that the binding of sulfate in the absence of bound L-Asp gives rise to movements of two loops [46–48]. In particular, Arg 647 in ZmPEPC (Arg 587 in EcPEPC), which lies within the conserved glycine-rich loop, moves approximately 15 Å toward the active site. Another conserved loop is also rearranged to interact and stabilise the glycine-rich loop conformation. Specifically, the C-terminal carboxyl group of Gly 970 (Gly 883 in EcPEPC) forms a salt bridge with the side chain of Arg 647. The binding of sulfate also induces the movement of the main chain of the loop comprising residues 174–184 toward the active site. Upon sulfate binding, a hydrogen bond is made between the backbone carbonyl of Arg 184 and the backbone NH group of Gln 188, making the α7 helix longer at its N terminus than in EcPEPC and leading to a dynamic movement of the loop toward the active site. The side chain of His 177 is seen more than 10 Å away from the active site in the EcPEPC structure, whereas in the ZmPEPC structure it is next to the active site, consistent with its possible role in stabilising the carboxyphosphate and abstracting a proton from its carboxyl group in the reaction mechanism of PEPC [46–48,50].

The β barrels of ZmPEPC and DCDP-bound EcPEPC that make up the binding site superimpose rather well [46]. The putative binding mode of PEP in the active site of ZmPEPC was thus suggested on the basis of the structure of the complex of EcPEPC, Mn^{2+} and DCDP [46–48]. In this paper, we report the results of molecular modelling studies of the binding of PEP and a number of representative PEP-analogue competitive inhibitors to ZmPEPC. We also report the molecular modelling of G6P and a number of other allosteric activators of PEPC in the putative allosteric G6P binding site. The interactions of PEP, inhibitors and activators with their binding sites are analysed in detail.

2. Materials and methods

The X-ray crystal structures of ZmPEPC (PDB code 1jqo) and EcPEPC (PDB codes 1jqn and 1qb4) were used. The EcPEPC structures were only used to inspect the nature of the coordination of Mn^{2+} by water molecules and the carboxylate sidechain oxygens of Glu 506 and Asp 543, with and without the bound DCDP inhibitor. For all modelling studies, hydrogen atoms were added to the crystal structure of ZmPEPC assuming a pH of 7.0 (all arginines were protonated). Chain A in the PDB file of ZmPEPC was chosen for modelling the active site. His 177 was modelled so that the tautomeric form chosen had ND1 carrying the hydrogen atom, while NE2 was unprotonated, consistent with the proposed role of this histidine as a proton acceptor in the reaction mechanism of PEPC [46–48,50].

The molecular modelling studies reported here were carried out using Insight 2000 (Accelrys), unless otherwise stated. All energy minimizations were performed with the Discover 3 module using the CFF forcefield [51] and were terminated when the energy gradient reached a value of less than 0.0001 kcal/mol/Å. Conformational changes in a few residues, as described below, were implemented using the Biopolymer module, which uses a rotamer library for generating alternative sidechain conformations [52]. For the energy minimizations, only those residues that had at least one atom within a distance of 40.0 Å from any atom of His 177 were considered. This speeded up significantly the calculations without any loss in accuracy. It is important to mention here that the net charge for the whole crystal structure of ZmPEPC (assuming standard pKa values for all sidechains at a pH of 7.0) is around −40, suggesting the absence of several positively charged residues from the missing loops in the crystal structure and making other kinds of calculations (such as those of the binding

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