Crystal structure of methyl acetylphosphonate activated pyruvate decarboxylase from *Kluyveromyces lactis* - new aspects on the substrate activation mechanism of this enzyme

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The enzyme pyruvate decarboxylase (PDC) from the yeast *Kluyveromyces lactis* (*Kl*PDC) has been crystallized in the presence of the non-convertible substrate surrogate methyl acetylphosphonate (MAP, Fig. 1). From the structural and functional points of view, PDCs are well-characterized enzymes. However, except for the bacterial PDC from *Zymomonas mobilis* (*Zm*PDC) all other examined species are allosterically activated by their substrate pyruvate, resulting in lag phases of the progress curves of catalysis and in sigmoid deviations of the v/S plots [1]. Previous crystallographic studies on PDC from *Saccharomyces cerevisiae* (*Sc*PDC) crystallised in the presence of pyruvamide, another substrate surrogate [2] (Fig.1), indicated that the regulatory site is located in a hinge region between two domains in vicinity to residues Tyr157 and Arg224. However, kinetic studies on the wild type and a number of variants of *Sc*PDC pointed to an important role of residue Cys221 for substrate activation [3-5]. Here, we demonstrate that the substrate surrogate MAP binds covalently to Cys221 resulting in a thiohemiketal structure (Fig 2).

By comparing the crystal structures of KlPDC obtained in the absence and presence of MAP the substrate activation pathway can be described as follows: (i) covalent binding of MAP to Cys221 and its interaction with the three histidine residues (His92, His225 and His310) causes a 4 Å shift of Cys221; (ii) this translocation is then transmitted to the neighbouring residue Ala286, located downstream of the flexible loop 288-304, which is retained; (iii) this structuring again results in the fixation of the loop 104-115; (iv) finally, the side chains of two histidine residues (His114 and His115) important for the interaction with the substrate (or, in our case, surrogate) at the active site are stabilised.

Recently, we could also show that the same activation pathway occurs for pyruvate activated *Sc*PDC (data not shown).

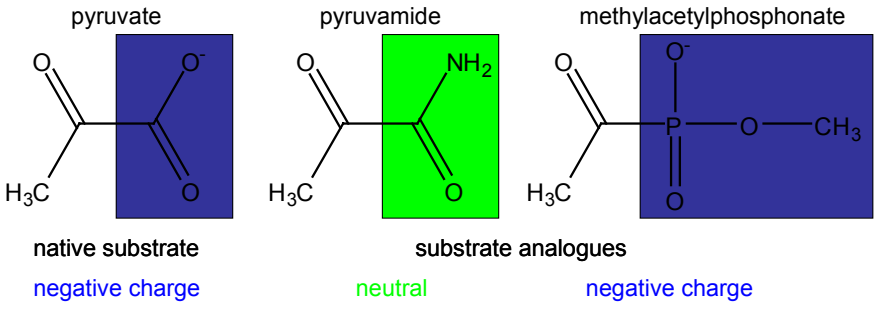

Figure 1: Comparison of the chemical structure of the substrate pyruvate with the non-convertible surrogates pyruvamide and methyl acetylphosphonate.

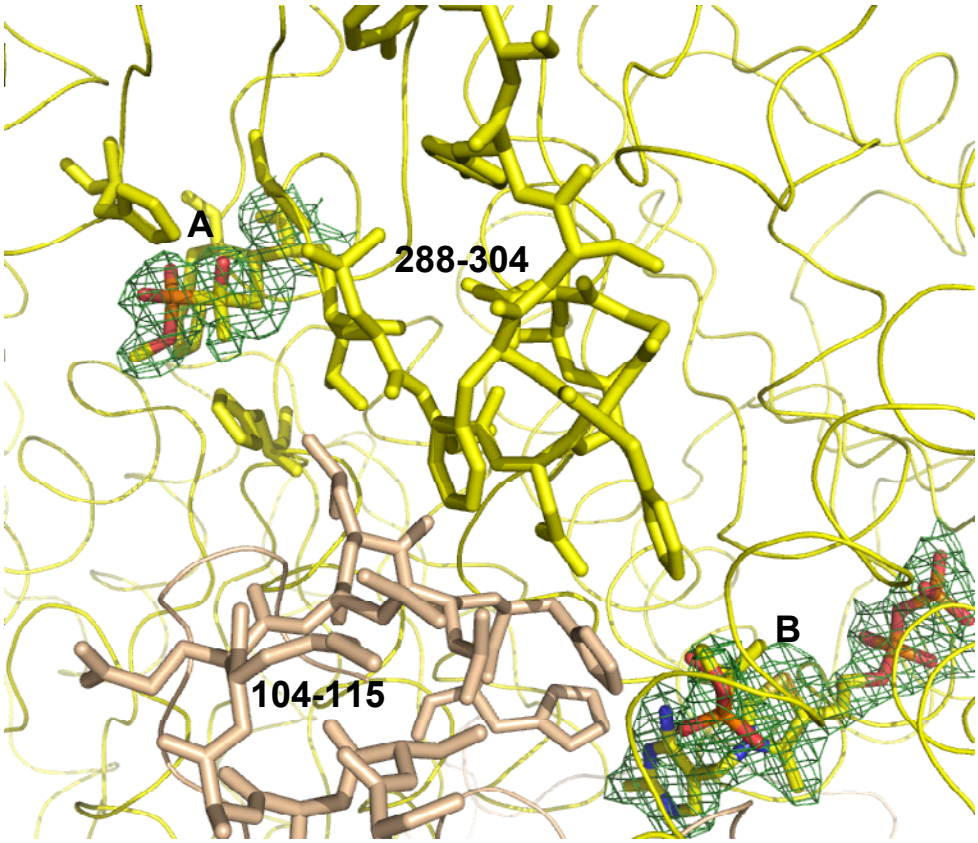

Figure 2: Substrate activation pathway of *Kl*PDC. Electron density is shown for the regulatory site with MAP bound covalently to Cys221 (A) and for the active site with MAP located close to the cofactor thiamine diphosphate (B). Chains (shown as Cα wire) are coloured yellow and light brown, respectively. MAP, Cys221, ThDP (coloured by atom type) regulatory site histidines, and the loop regions important for activation are presented in stick mode.

References

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