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Mini review

Allosteric activation of pyruvate decarboxylases. A never-ending story?

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ABSTRACT

The allosteric substrate activation of pyruvate decarboxylases was studied for more than 30 years using varying techniques and ending up in different hypotheses on the molecular mechanism of substrate activation of this enzyme. Now, a number of high-resolution structures of the pyruvate decarboxylase species from *Saccharomyces cerevisiae* and from *Kluyveromyces lactis* – both wild type and variants in complex with covalently bound substrate or substrate surrogates – provided for the first time structural insights to decipher the mechanism of allosteric activation by describing the signal transduction pathway from the regulatory to the active site in detail. Here, the mechanistic studies on substrate activation of pyruvate decarboxylases are reviewed from a historical point of view, demonstrating that important parts of the different hypotheses got carried away with our new mechanism drawn from latest results of experiments on activation kinetics, small-angle X-ray solution scattering and X-ray crystal diffraction.

1. Introduction

Pyruvate decarboxylases (E.C.4.1.1.1) catalyse the non-oxidative decarboxylation of pyruvate yielding acetaldehyde and carbon dioxide. Together with the enzyme alcohol dehydrogenase (E.C.1.1.1.1), which reduces the acetaldehyde to ethanol with the help of the co-substrate NADH, it constitutes the metabolic pathway of alcoholic fermentation. PDC is localised in the cytosol of cells from yeasts, plant seeds and a few bacteria. In total, 32 gene sequences coding for PDC are deposited (Uni-ProtKB/Swiss-Prot), 18 from yeast, 13 from plants and one from bacteria. PDCs have been purified from all kinds of organisms mentioned above. Purification protocols were published for six species from ascomycetes [1–11], for five from plants [12–16], and for three species from bacteria [17–21]. The catalytic activity of PDC depends on the presence of the cofactor thiamine diphosphate (ThDP, Fig. 1), which is bound mainly via a divalent metal ion (magnesium in most cases) to the protein moiety (Fig. 2) at the interface of two subunits. The chemistry of ThDP catalysis is well understood from a considerable number of kinetic studies of enzyme variants [22–30] along with intermediate analyses [31–33] and studies of the effect of cofactor analogues [34–50]. Consequently a number of very detailed catalytic cycles for the PDC reaction have been proposed [28,30,32,39,51–60] (Fig. 3). Many detailed kinetic studies have been conducted on yeast PDC wild types. A number of *Sc*PDC variants were analysed, too [6,24,28,29,53,57,58,61–64]. Some active site variants (E51A, D28A, E477Q) proved to be almost catalytically inactive. As early as in 1967 Davies [65] found a sigmoidal curvature in the v *vs.* [substrate] plot for the catalysed reaction of PDC from wheat. A similar kinetic behaviour was described for PDC from brewer's yeast (*Sc*PDC) 3 years later [66–68]. As this kinetic phenomenon was induced by the substrate pyruvate itself, this behaviour was interpreted as allosteric substrate activation of PDC. The first quantitative kinetic analysis of this phenomenon was published in 1978 [69]. By using the stopped-flow technique it could be demonstrated that at substrate concentrations around $S_{0.5}$ (the equivalent value to K_m) a considerable time period (10–20 s at 30 °C) elapses before catalysis is accelerated and the final steady state is entered. Assuming a slow isomerisation step from an inactive to an activated enzyme state, it was possible to determine microscopic rate constants. The observed activation rate constant rises hyperbolically with increasing substrate concentration. In a detailed kinetic study on allosteric substrate activation of *Kl*PDC, another yeast PDC, it was demonstrated that this dependence might be even more complex [9]. The existence of different and stable enzyme conformations – a catalytically inactive and an activated state – was proven by chemical cross-linking of *Sc*PDC with bisimidates of varying chain lengths in the absence and the presence of the substrate pyruvate [70]. The yeast enzymes share their substrate activation behaviour with PDCs from plant seeds [11,13,16,71]. In contrast, the PDC from the bacterium *Zymomonas mobilis* (*Zm*PDC, [72]) and indolepyruvate decarboxylase from *Enterobacter cloacae* (*Ec*IPDC, [73]) show Michaelis–Menten type kinetics without any sign of substrate activation. A number of substrate surrogates have been identified which are able to activate PDC as well.

The effects of pyruvamide (PA, Fig. 4) on the activation kinetics have been studied in detail for *Sc*PDC [69] and for *Kl*PDC [9].

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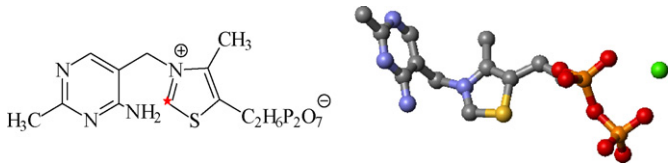

Fig. 1. Structure of the cofactor thiamine diphosphate. Left, chemical structure, the substrate binding site at the C2-atom is marked by a red asterisk; right, ball-and-stick presentation of the crystal structure model of the cofactor representing the V-conformation, carbon atoms are coloured in grey, nitrogens in blue, sulphur in yellow, phosphorous in orange and oxygens in red, the magnesium ion is shown in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Phosphonate analogues of pyruvate (among them methyl acetyl phosphonate, MAP) have been applied to elucidate the catalytic cycle [74–78] or to trap reaction intermediates in crystal structures [79–81]. Chemical modification of PDCs with group specific reagents pointed to an important role of cysteine residues [82]. The number and reactivity of cysteines was determined by modification with 4-hydroxy mercuribenzoate and 3-bromopyruvamide, respectively [83]. Site directed mutagenesis of cysteine residues and the determination of kinetic constants and solvent isotope effects for the corresponding variants demonstrated that modification of residue C221 should be the initial step of enzyme activation [61,84–85]. Furey et al. [86] recognized that C221 is located at a switch point of the middle (R-) domain of the subunit. However, the question remained, how the activating signal is transmitted from the regulatory to the active site. Kinetic studies on a number of variants, which were derived based on the crystal structure model of *Sc*PDC crystallized in the absence of any activator, favoured a direct pathway through adjacent amino acid side chains to the cofactor ThDP [57,63,64]. On the basis of comprehensive analyses of v *vs.* [substrate] plots, Hill coefficients, and pH dependent kinetics of variants along the way, a signal transduction path was postulated, starting from C221 via H92, E91, and W412. The last residue is part of a loop (residues 410–415), which directly interacts with the cofactor ThDP. However, in 2006 Joseph et al. [53] mentioned for the first time that another flexible loop region (residues 288–304) might be involved in the signal transfer pathway.

PDCs are multi-subunit enzymes. The typical molecular mass of one subunit is 59–61 kDa. The tetramer is the catalytically active state of most PDCs. Higher oligomers (octamers) have been described for PDCs from plant seeds [13,16] or fungi [5]. However, studies on structure function relationships of yeast PDCs showed that the dimer is the minimum functional unit of the enzyme with considerable catalytic activity [87,88]. On the other hand, Furey et al. [89] postulated from the crystal structure model of *Sc*PDC that allosteric activation requires a tetrameric structure. Only in this state the middle (R-) domain, which is very flexible and contains C221, can interact with other domains in other subunits.

The first crystal structure of a PDC species was published in 1993 [90] followed by others for *Kl*PDC [91], for *Sc*PDC activated by PA [7] and ketomalonate [89], respectively, and for two non-activated species, *Zm*PDC [92] and *Ec*lPDC [93]. All of these structures display a very high similarity on the basis of monomers and dimers (for an early comparison see Muller et al. [94]) whether or not they are allosterically regulated. Monomers consist of three domains, each with an open α/β topology, 5–6 stranded β-sheets are surrounded by α-helices (Fig. 5). Domains are connected by long, in some cases flexible loop regions. The cofactor ThDP is bound between two monomers. Each N-terminal (PYR-) domain binds the aminopy-

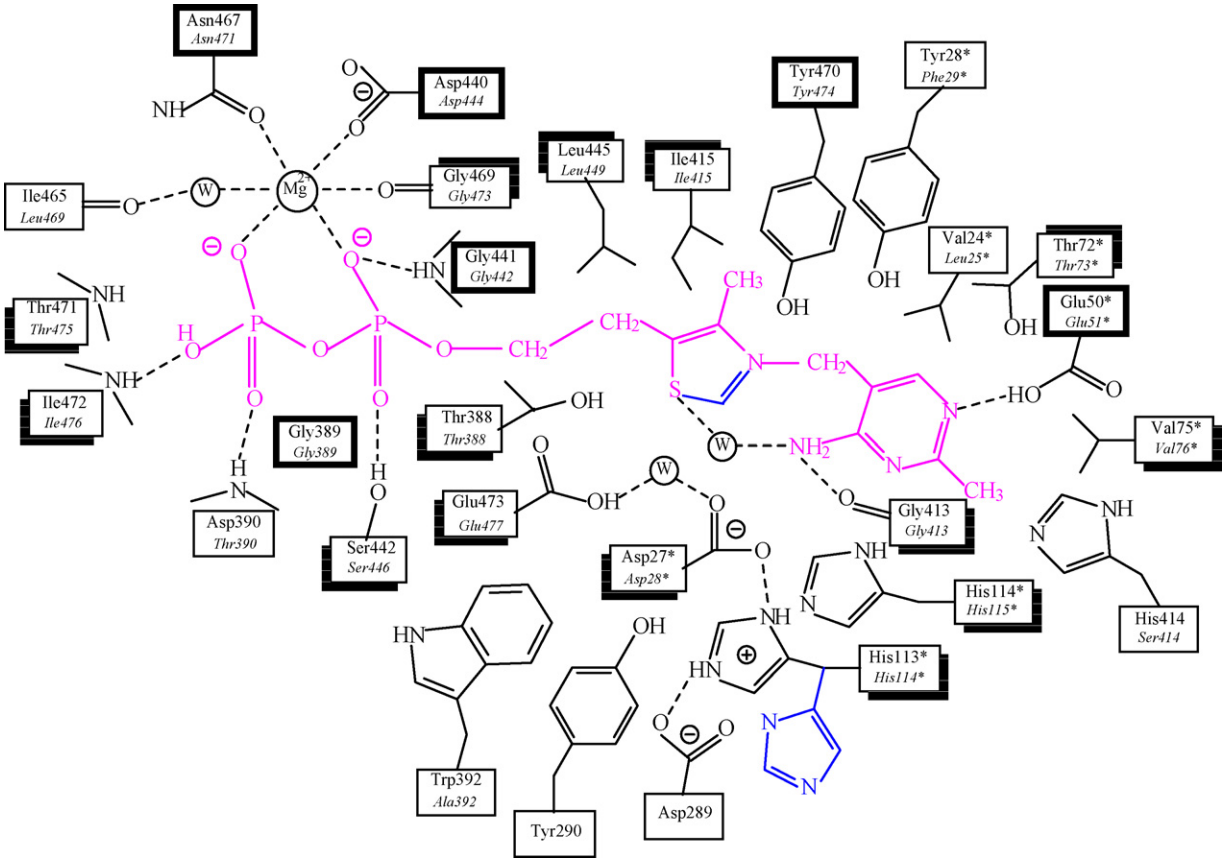

Fig. 2. Schematic view of the structure of the active site of pyruvate decarboxylase. The figure is originally drawn from Dobritzsch et al. [92], first line in the boxes represents numbering of pyruvate decarboxylase from *Zymomonas mobilis*, second line numbering of pyruvate decarboxylase from *Saccharomyces cerevisiae*.