The crystal structure of pyruvate decarboxylase from *Kluyveromyces lactis*

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Pyruvate decarboxylase (PDC) is an enzyme dependent on the cofactors thiamine diphosphate (TDP) and magnesium ions, which catalyses the decarboxylation of α-keto-acids to aldehydes. Apart from PDC from *Zymomonas mobilis* all other investigated species are allosteric enzymes activated by the substrate pyruvate [1].

We solved the crystal structure of the PDC from *Kluyveromyces lactis* (*Kl*PDC) to 2.26 Å resolution. Crystals were obtained by the hanging drop method in 50 mM MES pH 6.45, 5 mM TDP and magnesium sulphate, 20 % (w/v) PEG and 1.75 mg *Kl*PDC/mL. Glycerol (15 % v/v) was used as cryo-protectant. Two datasets were collected at beam lines X31 and X11, where the first one was used for molecular replacement and the second one for refinement of the model. The final R- and free R-factors are 0.163 and 0.230, respectively. The asymmetric unit of the monoclinic unit cell (space group $P2_1$, a=78.72 Å, b=203.09 Å and c=79.78 Å, β=91.80°,) contains one tetramer as a dimer of dimers with one TDP molecule bound per subunit in the so-called "V" conformation. The topology of the dimers is identical to the one observed for non-activated and pyruvamide activated PDC from *Saccharomyces cerevisiae* (*Sc*PDC) [2,3]. Only for the β-domain the C-terminus as well as for the loops 104-115 and 288-302 deviations of more than 3 Å for the positions of the Cα-atoms can be observed. This is not surprising since the sequence identity between PDCs from the two organisms is 86.3 %.

Even though no activator is present, one half of the *Kl*PDC tetramer is in the closed conformation, very much like the pyruvamide activated *Sc*PDC, [3]. This is in contrast to the non-activated *Sc*PDC showing the open conformation at all four active sites [2]. The closed conformation of *Kl*PDC may be stabilised mainly by one amino acid substitution in the dimer interface (Asn143Ala, *Kl*PDC→*Sc*PDC). Small angle X-ray scattering data (SAXS) for *Kl*PDC point to a more compact quaternary solution structure of this species in comparison to *Sc*PDC. Low resolution models calculated from SAXS data indicate a mutual rotation of the dimers within the tetramer. Despite the closed conformation of *Kl*PDC, the two loop regions, 104-115 and 288-302, are flexible and not defined in the final model. The same regions are also not defined in the crystal structure of the non-activated *Sc*PDC with its complete open conformation, but are rigid at the closed site of pyruvamide activated *Sc*PDC tetramer. These two loops are located between the regulatory and the active site, where at least His115 is necessary for substrate binding. So, a defined conformation of these regions (as in the case of pyruvamide activated *Sc*PDC) may be a prerequisite for catalysis. In combination with data on substrate activation kinetics we conclude that substrate activation of *Kl*PDC is not the result of a mutual dimer rotation within the tetramer (as it is the case for *Sc*PDC), but restricting the flexibility of the loop regions 104-115 and 288-302.

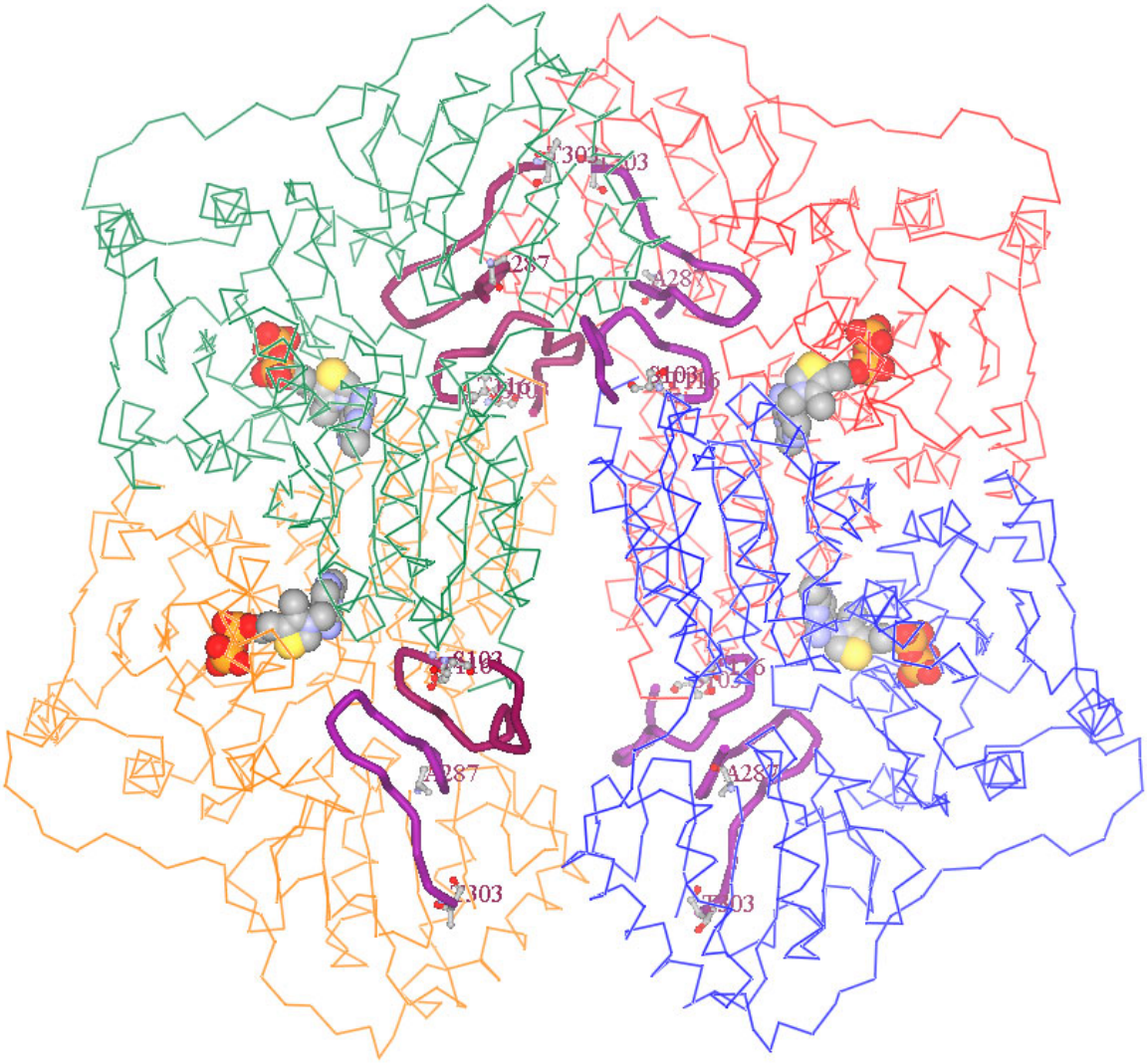

Figure: Crystal structure model of the *Kl*PDC tetramer. Subunits are shown as Cα-trace in different colours and the cofactors thiamine diphosphate and magnesium ions in space filling mode. The flexible loops with poor electron density are presented as tubes in deep purple, the adjacent amino acid residues in ball-and-stick mode.

References

[1] S. Bringer-Meyer, K.L. Schimz, and H. Sahm, Arch. Microbiol. 146, 105-110 (1986)

[2] P. Arjunan, T. Umland, F. Dyda, S. Swaminathan, W. Furey, M. Sax, B. Farrenkopf, Y. Gao, D. Zhang, and F. Jordan, J. Mol. Biol. 256, 590-600 (1996)

[3] G. Lu, D. Dobritzsch, S. Baumann, G. Schneider, and S. König, Eur. J. Biochem. 267, 861-868 (2000)