

The evolution of quaternary structure in a homotetrameric enzyme: dihydrodipicolinate synthase.

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Abstract

In order to probe the functional roles of the quaternary structure of dihydrodipicolinate synthase (DHDPS) we have generated two dimeric variants of the enzyme by site-directed mutagenesis. Rigorous kinetic, biophysical, and structural analysis of the wild-type tetrameric enzyme and the dimeric variants suggests that the oligomeric structure of DHDPS has evolved to facilitate efficient catalysis via a shared catalytic triad. Further we propose that the tetrameric quaternary structure has evolved to restrict flexing of the monomers around the dimer interface.

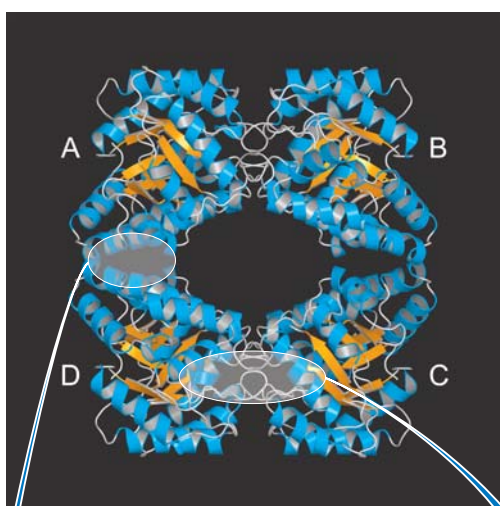
DHDPS catalyses the first committed step of lysine biosynthesis

The enzyme catalyses the condensation of pyruvate and (S)-aspartate semi-aldehyde (ASA) to tetrahydrodipicolinic acid and is feedback inhibited by lysine.

DHDPS is a dimer of dimers

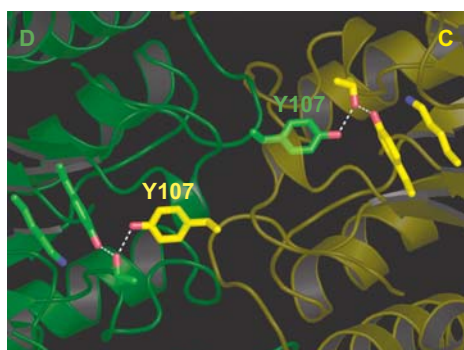
Subunits A and B and subunits C and D associate tightly across a large interface forming 'tight-dimer' units. The inhibitory lysine binding site and an important catalytic motif are formed at this interface.

Subunits A and C and subunits B and D associate only weakly across a far less extensive interface. The role of the association of these dimers is unknown.



Y107 forms part of a hydrogen bonded proton relay triad that is essential for efficient catalysis

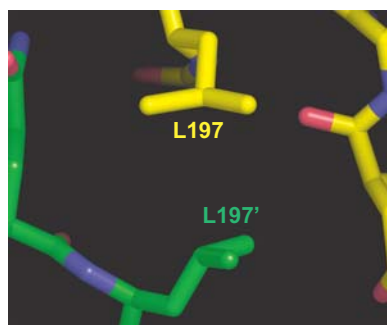
The side chain of Y107 juts into the active site of the neighbouring monomer of the tight-dimer, completing the proton relay to bulk solvent.



This provides a rationale for the formation of the tight-dimer unit of DHDPS. However, the association of two of these dimers to form the wild-type tetramer has no known function.

Production of the dimeric variants

Dimeric variants of DHDPS were produced by site-directed mutagenesis of residues at the dimer-dimer interface in order to probe the functional contribution of the tetrameric structure.



The symmetry of the tetramer is such that L197 of one subunit directly contacts L197 of a neighbouring subunit at the interface between the two tight-dimers.

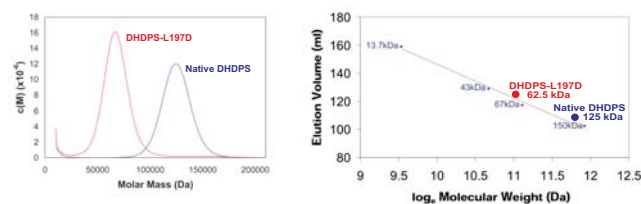
L197 was mutated to aspartate and tyrosine in order to disrupt the dimer-dimer interface, producing variants L197D and L197Y.

The aspartate mutation introduces negative charges and therefore electrostatic repulsion at this site.

The bulky side chain of tyrosine alters the topology of the contact surface and removes surface complementarity.

The DHDPS variants are dimeric in solution, show a subtle change in kinetic mechanism, and are activated by heat

Analytical ultracentrifugation, gel permeation chromatography, and Blue-native PAGE (not shown) all indicate that both variants are stable obligate dimers in solution.

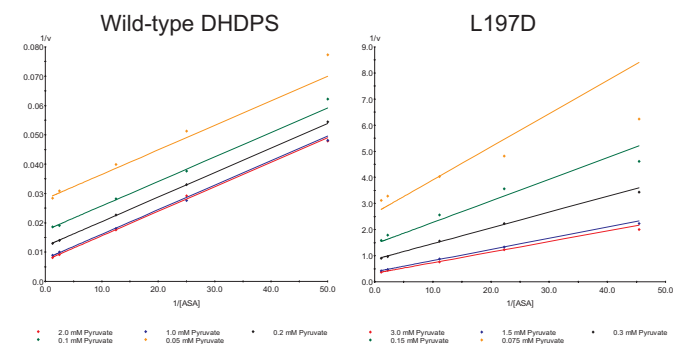


The activity of the variants is greatly attenuated relative to the wild-type

The catalytic efficiency of both dimeric variants is greatly reduced from that of the wild-type enzyme. This attenuation of activity is accompanied by a large increase in the K_m for pyruvate. In contrast other kinetic constants describing catalysis and inhibition of the dimeric DHDPS variants are largely consistent with those of the wild-type.

	WT DHDPS	DHDPS-L197D	DHDPS-L197Y
k_{cat} (sec ⁻¹)	78.3 +/- 0.6	2.1 +/- 0.03	1.0 +/- 0.02
K_m pyruvate (mM)	0.16 +/- 0.003	0.71 +/- 0.03	0.93 +/- 0.06
K_m ASA (mM)	0.12 +/- 0.002	0.15 +/- 0.006	0.16 +/- 0.009
K_i wrt pyruvate (mM)	0.14 +/- 0.007	0.22 +/- 0.03	0.12 +/- 0.03
K_i wrt ASA (mM)	0.40 +/- 0.05	0.30 +/- 0.04	0.16 +/- 0.02

Kinetic analysis of wild-type DHDPS shows the characteristic parallel lines of the ping-pong mechanism on a double reciprocal plot. In contrast, DHDPS dimers display the characteristic diverging lines of the ternary-complex mechanism. This indicates that the order in which the reaction steps occur is subtly different in the variants with respect to the wild-type.

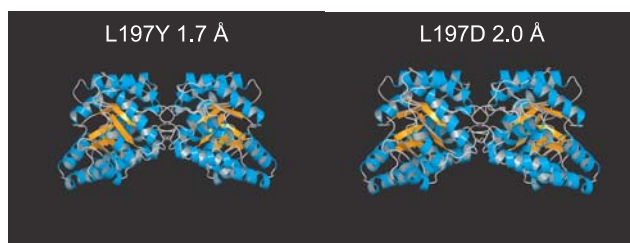


The dimeric variants are heat activated

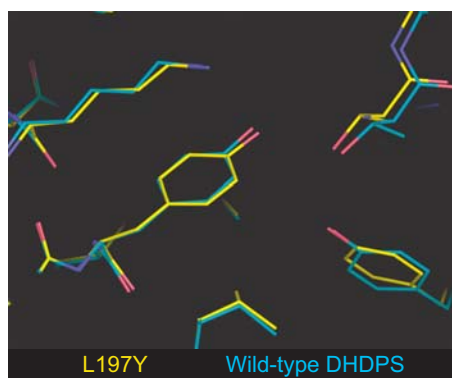
When incubated at high temperature in the presence of pyruvate, the catalytic turnover of both dimeric variants increases dramatically.

The crystal structures of the dimeric variants

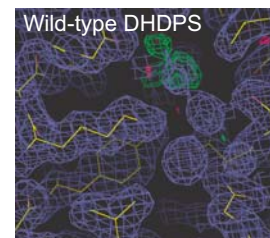
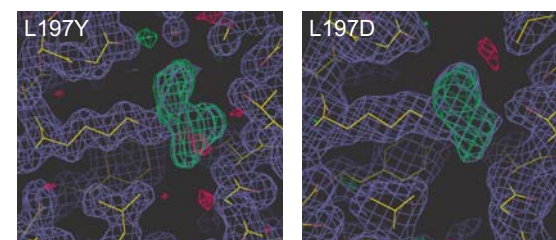
The crystal structures of both dimeric variants have been solved. The overall architecture of the tight-dimer unit is unchanged from that of wild-type DHDPS.



Overlay of the active sites of the dimeric variants with that of the wild-type enzyme shows no gross rearrangement that may account for the reduction in activity observed in the dimers.

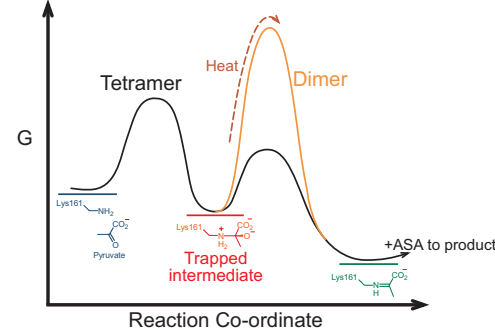


Electron density maps of the dimers crystallised in the absence of pyruvate show positive density at the pyruvate binding active site residue, K161, indicating that an enzyme-pyruvate complex has been trapped.



This electron density is not seen in the wild-type enzyme when crystallised in the absence of pyruvate.

This suggests that the activation barrier for the dehydration of the tetrahedral intermediate formed between K161 and pyruvate has been increased in the dimeric variants.

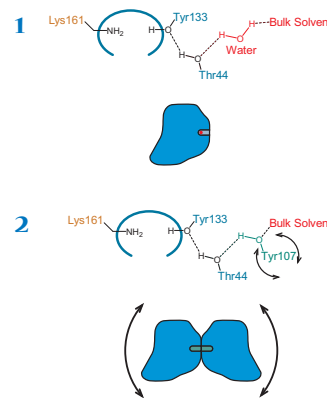


Our model

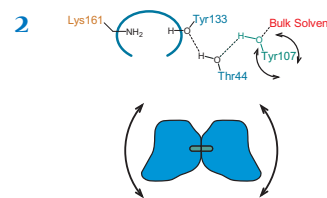
Observed results can be explained by impaired function of the proton relay triad caused by flexing of the dimer about the contact between the two monomers. As this flex would alter the position of Y107 in the hydrogen bonded proton relay, efficiency of proton movement into and out of the active site would be reduced.

Impaired movement of protons at the active site will result in attenuation of the catalytic turnover of the enzyme. Further, we propose that trapping of an enzyme-pyruvate intermediate results in a shift in kinetic mechanism from the ping-pong to the ternary complex mechanism as the binding of the second substrate, ASA, promotes dehydration of the trapped intermediate, allowing the reaction to proceed. Additionally, heat activation of the enzyme can be explained by release of trapped pyruvate at higher temperatures.

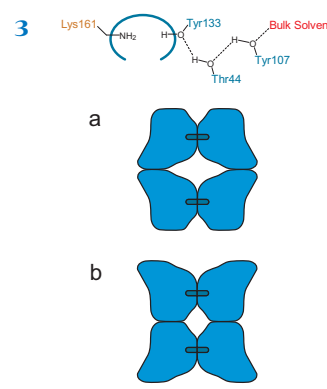
This suggests that the tetrameric structure of DHDPS has evolved to facilitate optimal proton movement into and out of the active site via a proton relay triad.



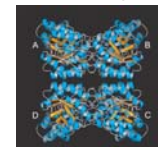
An ancestral monomeric DHDPS may have had a bound water molecule, which facilitated proton donation to bulk solvent. This water molecule is observed in the the DHDPS-Y107F mutant. See Dobson poster.



Introduction of a constrained tyrosine residue to complete the proton shuttle by association of two identical monomers increased the catalytic rate of the enzyme by increasing efficiency of proton movement. However, hinging at the interface results in suboptimal placement of the tyrosine side chain for hydrogen bonding, as the dimeric structure can breathe in solution. DHDPS from the ancient thermophile *Thermotoga maritima* is dimeric (PDB, 105K), and lends evidence for the dimer as an ancestral structure.



Breathing is overcome by association of two dimer units allowing the enzyme to reach optimal catalytic efficiency. Association of the dimers to constrain breathing can occur in two orientations: (a) shows the configuration of the *E. coli* enzyme. Alternatively, the configuration shown in (b) will have a similar effect. This configuration has been found in plant DHDPS!



References

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Dobson, R. C., Griffin, M. D., Jameson, G. B., and Gerrard, J. A. (2005) *Acta Cryst.* submitted.
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