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Original paper

Exploring the dihydrodipicolinate synthase tetramer: How resilient is the dimer–dimer interface?

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ABSTRACT

Dihydrodipicolinate synthase (DHDPS, E.C. 4.2.1.52) is a tetrameric enzyme that catalyses the first committed step of the lysine biosynthetic pathway. Dimeric variants of DHDPS have impaired catalytic activity due to aberrant protein motions within the dimer unit. Thus, it is thought that the tetrameric structure functions to restrict these motions and optimise enzyme dynamics for catalysis. Despite the importance of dimer–dimer association, the interface between subunits of each dimer is small, accounting for only 4.3% of the total monomer surface area, and the structure of the interface is not conserved across species. We have probed the tolerance of dimer–dimer association to mutation by introducing amino acid substitutions within the interface. All point mutations resulted in destabilisation of the 'dimer of dimers' tetrameric structure. Both the position of the mutation in the interface and the physico-chemical nature of the substitution appeared to effect tetramerisation. Despite only weak destabilisation of the tetramer by some mutations, catalytic activity was reduced to ~10–15% of the wild-type in all cases, suggesting that the dimer–dimer interface is finely tuned to optimise function.

Introduction

In the biosynthetic pathway leading to *meso*-diaminopimelate (DAP)[1] and (*S*)-lysine in plants and bacteria, dihydrodipicolinate synthase (DHDPS, E.C. 4.2.1.52) catalyses the branch point reaction: the condensation of (*S*)-aspartate-β-semialdehyde [(*S*)-ASA] and pyruvate to form (4*S*)-4-hydroxy-2,3,4,5-tetrahydro-(2*S*)-dipicolinic acid. Since (*S*)-lysine biosynthesis does not occur in animals, DHDPS is an attractive target for rational antibiotic and herbicide design [1].

The crystal structures of a number of bacterial DHDPS enzymes [2–13] and a plant enzyme [14] have been solved. With the exception of the recently reported dimeric *Staphylococcus aureus* enzyme [4], all functionally and structurally characterised DHDPS enzymes are homotetramers. The *Escherichia coli* DHDPS tetramer can be described as a dimer of tight-dimers (Fig. 1a), where there are many interactions between monomers A and B (or C and D), but relatively few between the two tight-dimers (A–B and C–D) [6]. Interestingly, the arrangement of the tight-dimers is different within the bacterial and plant tetramers, with dimer–dimer contacts formed on the opposite face of each subunit [3,15].

Each subunit is composed of an $(\beta/\alpha)_8$-barrel containing an active site, situated in the centre of the barrel, and each tight-dimer contains two complete active sites and an inhibitory-(*S*)-lysine binding site within the cleft between the subunits. A tyrosine residue from one subunit of the tight-dimer protrudes into the active site of the adjacent subunit and forms part of a catalytic triad that is essential for activity [16]. This suggests that the tight-dimer is the minimum unit necessary for DHDPS catalysis. However, mutation of a central residue in the dimer–dimer interface (L197) produced dimeric variants that displayed severely impaired catalytic function [15], due to aberrant dynamics occurring between the subunits of the dimer that were not present in the tetramer. These movements were proposed to be responsible for a reduction in substrate specificity that resulted in the covalent trapping of a substrate analogue, α-ketoglutarate, at the active site of the dimer. This suggests that the tetrameric structure of *E. coli* DHDPS evolved to optimise the dynamics within the tight-dimer unit.

In DHDPS from *S. aureus*, which occurs naturally as a dimer [4], the tight-dimer interface is significantly more extensive than in DHDPS from other species, in what is hypothesised to be an alternate evolutionary solution to optimising dynamics across this critical interface [4].

Strikingly, despite its role in enzyme function, the dimer–dimer interface of DHDPS is not conserved across species, suggesting that the key feature for function is the interface itself, rather than any specific amino acids. In this study, we probe the resilience of the

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[1] *Abbreviations used:* DHDPS, dihydrodipicolinate synthase; DHDPR, dihydrodipicolinate reductase; CD, circular dichroism; (*S*)-ASA, (*S*)-aspartate-β-semialdehyde; DAP, *meso*-diaminopimelate.

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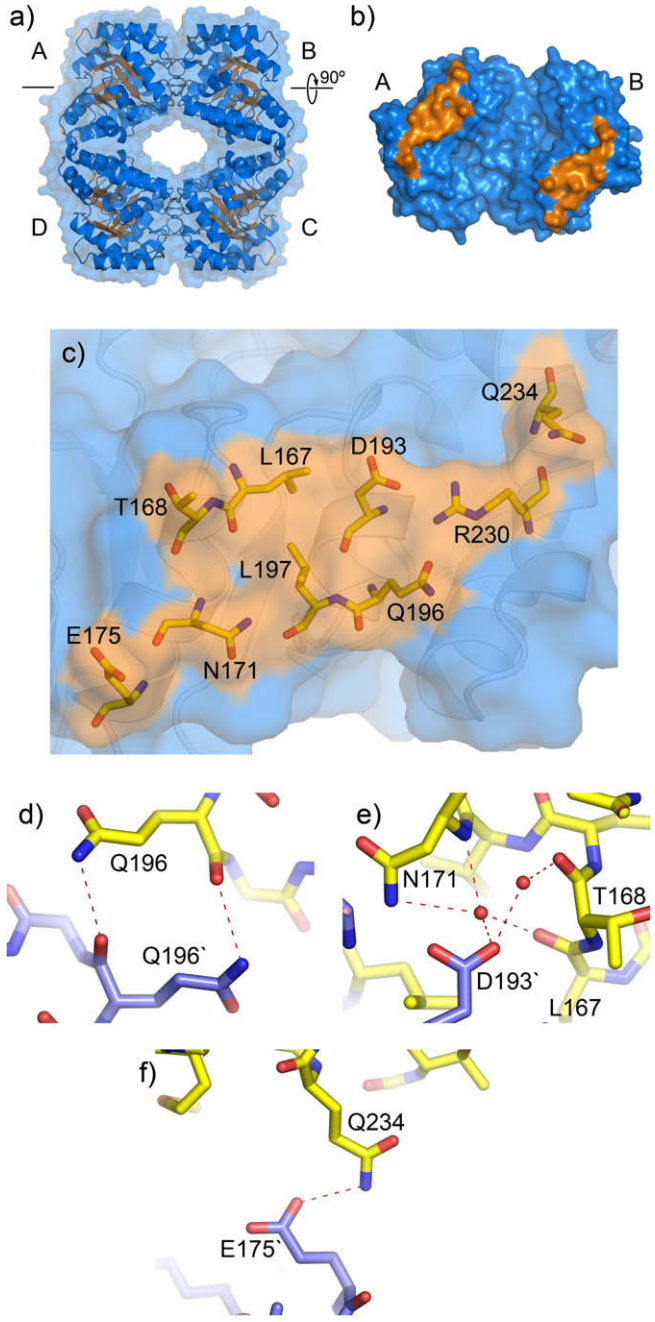

Fig. 1. The dimer–dimer interface of *E. coli* DHDPS. (a) The DHDPS tetramer consists of a dimer of dimers in which subunits A and B (and C and D) associate *via* an extensive interface, and two of these dimers (A–B and C–D) associate *via* two smaller interfaces to form the tetramer. Throughout this report 'tight-dimer interface' refers to the contact between monomers A and B and between monomers C and D. Likewise, 'dimer–dimer interface' refers to contacts between monomers A and D and between monomers B and C. (b) Tight-dimer A–B is tilted 90° away from the viewer to expose the dimer–dimer interface contact areas (orange). (c) Residues contributing to the dimer–dimer contact area. (d–f) The details of contacts formed across the interface by residues Q196, D193 and Q234, respectively. The two interacting monomers are coloured yellow and blue, hydrogen bonds are represented by red dotted lines, and water molecules are shown as red spheres. For detailed description of these contacts see Table 1 and 'Design of the dimer–dimer interface mutants' section. For further analysis of the dimer–dimer interface see [6].

dimer–dimer interface to mutation. We report that mutations at positions 193, 196, and 234, incorporating charge–charge repulsion (Q196D, Q234D), removal of hydrogen bonds (D193A, D193Y) or introducing steric bulk (D193Y), significantly attenuate the tetrameric quaternary structure and/or catalytic competency of *E. coli* DHDPS.

Materials and methods

All materials were obtained from Sigma–Aldrich. Enzymes were manipulated at 4 °C or on ice and were stored in 20 mM Tris–HCl, pH 8.0 at −20 °C.

Site-directed mutagenesis and protein purification

Mutations in the *E. coli dapA* gene (encoding DHDPS, Accession No. M12844), contained on pBluescript based plasmid pJG001 [17], were introduced using the Quikchange site-directed mutagenesis kit (Stratagene). Successful mutation was confirmed by DNA sequencing.

Wild-type DHDPS was expressed as described previously [16]. DHDPS mutants were expressed in *E. coli* strain AT997r− [15] harbouring the appropriate mutant DHDPS expression plasmid. Purification of wild-type DHDPS, and the coupling enzyme, DHDPR, was carried out as described previously [15]. For DHDPS mutants, an ammonium sulphate fractionation, retaining the protein fraction precipitating between 30% and 40% (w/v) ammonium sulphate, was used in place of the initial heat-shock step.

Circular dichroism spectroscopy

Far UV circular dichroism (CD) spectra were recorded using an Aviv 62DS CD spectrometer (NJ, USA). Spectra were recorded between 195 and 250 nm, using a 1 mm path length quartz cuvette, 0.5 nm step size, 1 nm bandwidth and 2 s averaging. Raw spectra were corrected for buffer contribution and subsequently smoothed using a third-order polynomial with window size ranging from 8 to 16.

Analytical ultracentrifugation

Analytical ultracentrifugation experiments were performed in a Beckman Model XL-A analytical ultracentrifuge equipped with UV/Vis scanning optics and An-60 Ti 4-hole rotor. Protein sample (380 μl) and reference (400 μl, 20 mM Tris–HCl, 150 mM NaCl, pH 8.0) solutions were loaded into 12 mm double sector cells with quartz windows. Prior to sedimentation, the protein samples were purified to homogeneity by size-exclusion liquid chromatography on a Sephacryl S400 column (GE Healthcare). For sedimentation velocity experiments, samples at an initial protein concentration of 1.0 mg/ml (32 μM) were centrifuged at 40,000 rpm and 20 °C and data were collected at 280 nm every 6 min without averaging. Sedimentation velocity data were fitted to a continuous size-distribution model [18] using the program SEDFIT [19]. The partial specific volume of the protein, buffer density and viscosity of the reference solution were computed using the program SEDNTERP [20]. For sedimentation equilibrium experiments, samples at initial protein concentrations of 0.1, 0.3 and 1.0 mg/ml (3.2 μM, 9.6 μM and 32 μM, 100 μl) and reference solution (120 μl) were centrifuged at 10,000 or 16,000 rpm until sedimentation equilibrium was attained (∼24 h). Radial absorbance scans were taken at 280 nm with 10 averages and the data obtained at both rotor speeds and all three concentrations were fitted globally using the program SEDPHAT [21].

Enzyme kinetic measurements

DHDPS kinetic measurements were performed using a coupled enzyme assay incorporating DHDPR as previously described [16].