Dehydrogenation through the looking-glass

Sir — During enzymatic nicotinamide adenine dinucleotide (NAD^+)-dependent dehydrogenation a hydride anion leaves the substrate and attacks the C4 atom of the NAD^+ pyridine unit, converting NAD^+ to NADH. A subset of these reactions is the oxidation of 2-hydroxy acids to 2-keto acids (Fig. 1) in which a hydride anion leaves the C2 atom and a proton leaves the hydroxy group of the substrate. An internal property of this reaction is its double stereospecificity; the 2-hydroxy acid may have either the L- or D-configuration and the NAD^+ C4 atom may accept hydride from either the A or B side[1]. This reflects the fact that L- and D-substrates are simply mirror images of one another.

The classic examples of enzymes catalysing the NAD^+-dependent transformation of 2-hydroxy acids to 2-keto acids are L-specific lactate (LDH) and malate (MDH) dehydrogenases, on which biochemical and structural studies were begun long ago[1]. These proteins have topologically similar structures[2]. Their active sites contain a histidine/aspartate pair which forms a 'charge relay system', similar to that first observed in chymotrypsin[3], providing proton transfer, and an arginine whose guanidine unit plays the role of an anchor in binding the substrate carboxylate group (Fig. 1).

The D-specific NAD^+-dependent enzymes, catalyzing dehydrogenation of D-2-hydroxy acids, are homologous to one another but have no sequence homology to the L-specific family. The former family includes D-specific lactate, glycerate, 3-phosphoglycerate, 2-hydroxy isocaproate and erythronate-4-phosphate dehydrogenases[4,5]. While various models derived from three-dimensional crystal analyses for L-specific enzymes are known[6], no structure is yet available for a D-specific enzyme. However, the D-specific dehydrogenases have sequence homology[5] to NAD^+-dependent formate dehydrogenase (FDH), whose three-dimensional structure has recently been determined[7,8]. In spite of the fact that formate is not a chiral substrate and catalysis occurs at the C1 position (compared to C2 in 2-hydroxy acids) the FDH active site contains several residues conserved throughout the D-specific enzymes[8].

The structures compared here are the two L-specific enzymes: LDH complexed with NAD^+ and oxamate, PDB coordinate set 1LDM (J.P. Griffith & M.G. Rossmann, unpublished), MDH complexed with NAD^+ and sulphate, set 4MDH[9] and the 'D-specific' bacterial FDH complexed with NAD^+ and azide, set 1NAD^+ (ref. 8). The use of FDH as a representative of the D-specific enzyme family is appropriate due to the properties assigned to the three catalytic residues[8]. The role of Arg 284 is to bind substrate and is similar to the role of arginine in other dehydrogenases. His 332 is hydrogen bonded to Gln 313, that is, an amide but not an acid. In FDH this 'catalytic' histidine is locked by the acid/amide replacement and loses its ability to switch between charged and uncharged forms, which is required in mechanisms involving proton transfer.

This fits into a scheme where dehydrogenation of formate does not need a base, in contrast to dehydrogenation of 2-hydroxy acids. The glutamine residue in FDH is assumed to be spatially equivalent to glutamate in D-specific enzymes. This is supported by three pieces of information. First, from the sequence alignment[5,8] there is a fully conserved glutamine residue in formate dehydrogenases, in contrast to the fully conserved glutamate within the family of the D-specific enzymes. Second, from the three-dimensional structure of FDH[8] this glutamine residue is located in the active site as

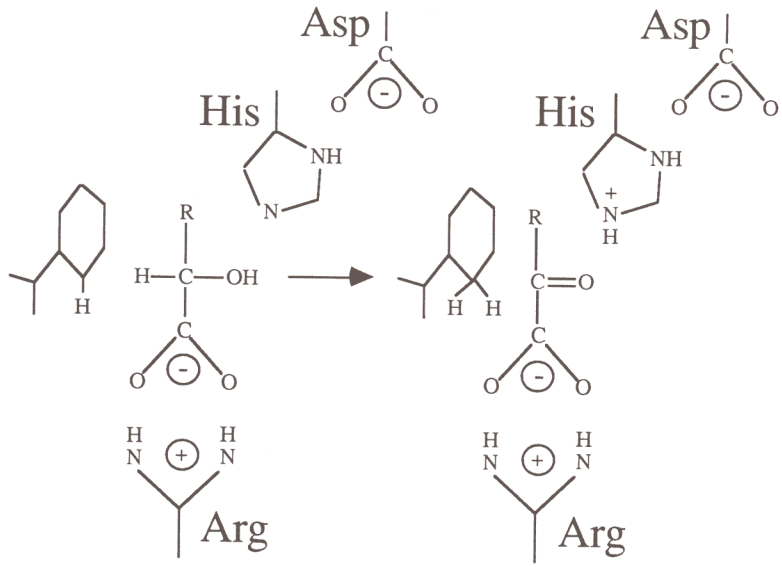

Fig. 1 Reaction mechanism. Scheme of NAD^+-dependent dehydrogenation of 2-hydroxy acids.

described above. Third, on the basis of site-directed mutagenesis the same glutamate residue was proposed to be essential for catalysis and paired with a catalytic histidine in D-specific LDH[10].

As for L-specific LDH and MDH, the reaction catalyzed by FDH occurs on the A-side of the NAD^+ nicotinamide[8,11]. The three structures were superimposed, using only the ten atoms of the nicotinamide moiety (Fig. 2*a*). The superposition reveals several interesting features. The inhibitor molecules occupying the substrate binding site appear to be located in the same place for all three enzymes because they catalyze reactions on the same side of the nicotinamide. The three catalytic residues in LDH and MDH overlap very closely as these structures are topologically similar[2]. The histidine residues in all three proteins (apart from His 332 in FDH, where the imidazole plane is rotated to form a hydrogen bond to Gln 313 as mentioned above) also closely overlap.

An important conclusion from the superposition is that the catalytic residues in FDH and LDH/MDH are related by a mirror (Fig. 2*a*). When the three catalytic residues as well as the inhibitor molecules in the L-specific LDH and MDH are inverted by this mirror plane they superimpose on those in the D-enzyme FDH (Fig. 2*b*)in a respectable manner for enzymes with no sequence homology. The mirror plane passes approximately through the catalytic point as the distance from the NAD^+ C4 atom to the plane is 0.3 Å. The three catalytic residues in both L- and D-specific dehydrogenases are essentially placed in identical positions on the two sides of the looking-glass.

In spite of the intrinsic asymmetry of amino acids, the positions of the side chains of the three catalytic residues in enzymes from the two families are related by mirror symmetry. Thus evolution has resulted in these L- and D-specific enzymes converging on mirror-related active sites, solving the same, but also mirror-inverted, catalytic problem.

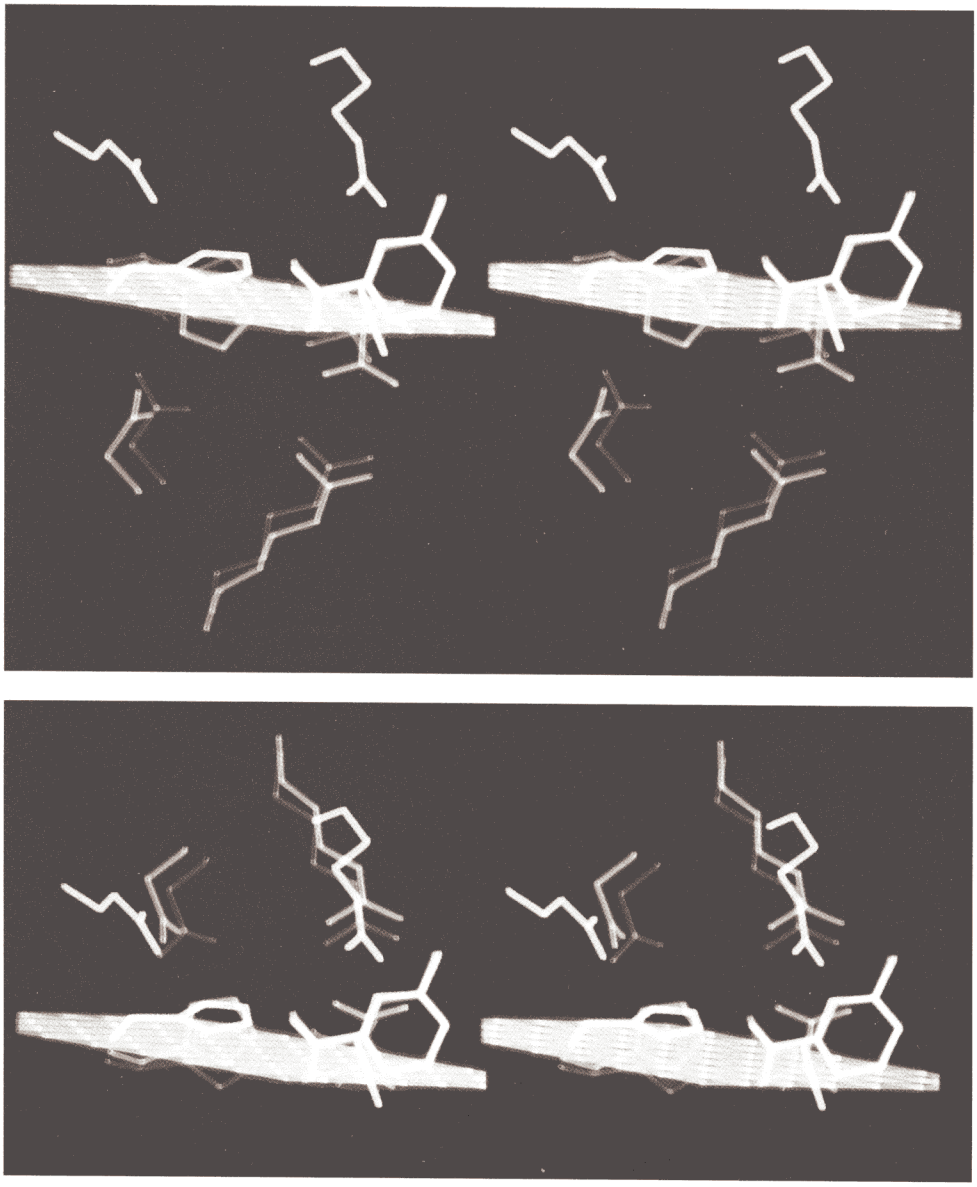

Fig. 2 Mirror-image related active sites. *a,* Superposition of the NAD nicotinamide moiety in L- (LDH in magenta, MDH in red) and D-specific (FDH in green) 2-hydroxy acid dehydrogenases. The catalytic triad (arginine, histidine and aspartate/glutamate), inhibitors occupying the substrate binding site (oxamate in LDH, sulphate in MDH and azide in FDH) and the mirror plane relating the catalytic triad in L- and D-specific enzymes are shown. *b,* The catalytic triad and inhibitors in LDH and MDH are inverted by the mirror and fit well onto those of FDH.

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