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A Close-Packed Planar 4-Atom Motif Serves as a Variable-Pathway Mechanistic Switching Device in Enzymatic Catalysis

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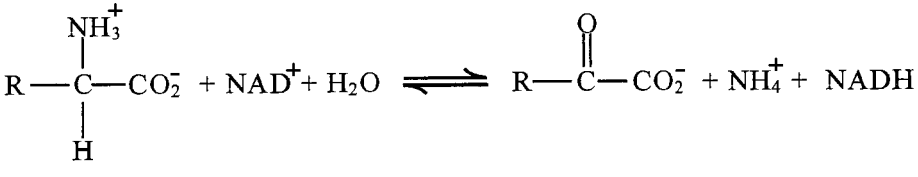
We have found a new kind of structural motif that appears to be highly conserved among the pyridine nucleotide-linked α-amino acid dehydrogenases. This feature is comprised of four atoms closely packed in a planar form. Two of the atoms are donated by the enzyme, one is provided by the substrate (or reactive intermediate), a bound water molecule constitutes the fourth. This tetrad, by virtue of its spatial connectivity, constitutes a two-dimensional machine in contrast to the one-dimensional charge-relay system commonly observed at enzyme active sites. As such, this new motif is capable of more complex behavior permitting a wide variety of possible bonding patterns. Modulation of these potentially variable patterns can lead to qualitative differences in mechanism between structurally similar enzymes, and, in the case of a given enzyme, may constitute the core of its catalytic machinery. We offer a conjecture as to how such a structure may participate in enzymatic catalysis.

Key Words: hydrogen bond; catalytic motif; α-amino acid dehydrogenase.

INTRODUCTION

The α-amino acid dehydrogenases constitute a class of pyridine nucleotide-linked enzymes that carry out the oxidative deamination of a variety of specific L-α-amino acids, including glutamate, alanine, leucine, and phenylalanine. The general stoichiometry of these reactions is:



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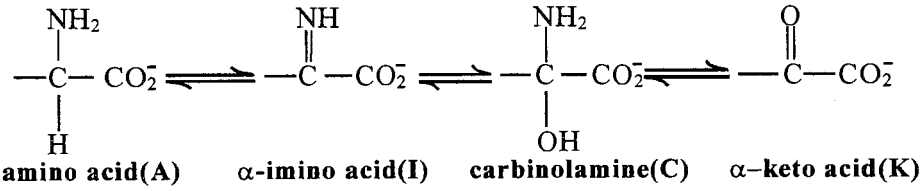
The properties of this group of enzymes have been reviewed by Hummel and Blanchard (*1*). The mechanism of the reaction catalyzed by bovine liver glutamate dehydrogenase (blGDH) has been the most extensively studied of the group (*2*). Evidence of the occurrence of a kinetically competent α-imino acid intermediate in this reaction was provided by O^{18} exchange studies from this laboratory (*3*). Based on steady-state kinetic and isotope effect studies, Rife and Cleland (*4*) proposed a detailed mechanism including the following sequence of major intermediate substrate forms:[2]



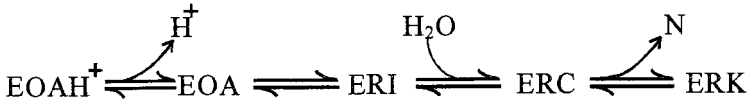
[2]

Their mechanism (proposed long before any crystal structures of this class of enzymes had been obtained) suggested that a hydrogen atom of the substrate α-amino nitrogen atom must be abstracted by an enzyme carboxylate group prior to hydride transfer. They also proposed that the oxygen atom of a water molecule hydrogen bound to an enzyme amino nitrogen atom must be located in a position favorable for a nucleophilic attack on the substrate α-carbon atom, forming α-carbinolamine immediately after hydride transfer.

The first crystal structure of a tertiary complex of an α-amino acid dehydrogenase (which we will describe later) confirmed the prescient proposal of Rife and Cleland, showing an aspartyl residue within H-bonding distance of the substrate α-amino group and a water molecule bound to a lysyl residue within the van der Waals distance of the substrate α-carbon atom!

Some years ago, we established the fact that the 340-nm band of the reduced nicotinamide moiety of NADH or NADPH undergoes different readily characterizable shifts without undergoing significant changes in shape or extinction coefficient in various pyridine nucleotide-linked dehydrogenase complexes (*5*, *6*). More recently, we have used this feature to resolve transient-state multiwavelength time course spectroscopic and spectrofluorometric arrays of dehydrogenase reactions into resolved component time courses. Applying our recently developed theory of transient-state kinetic isotope effects to corresponding experiments using α-deutero-L-glutamate (*7*), we were able to demonstrate the occurrence of the following sequence of identifiable intermediates and to prove the kinetic competence of each of them (*8*):

[2] We will use the symbols A, I, C, and K as defined above to denote the amino acid substrate, its α-imino form, the carbinolamine, and α-keto-acid, respectively, along with R, O, and N, which represent the reduced coenzyme, the oxidized coenzyme, and the ammonia moiety as defined in Eq. [1] throughout the text.



[3]

Where $EOAH^+$ is colorless, EOA is a weakly absorbing, red-shifted, highly fluorescent-charge-transfer complex, ERI is highly blue-shifted and nonfluorescent, ERC is highly red-shifted and highly fluorescent.[3] Similar studies of the stable complexes and transient-state kinetic reactions of two other enzymes of this class; Clostridium symbiosum glutamate dehydrogenase (csGDH) and phenylalanine dehydrogenase (PaDH) showed a strong agreement between the spectroscopic and spectrofluorometric properties of all observable corresponding complexes of the three dehydrogenases. Recent X-ray crystallographic structures of various forms of the α-amino acid dehydrogenases (*9–13*) have lead to the emerging realization of the occurrence of a highly conserved active-site structure in which 9–12 enzyme functional groups have nearly identical geometric conditions. Most of the obvious minor differences between these structures are easily explained on the basis of their need to accommodate different amino acid substrates. Steady-state kinetic studies of this group of enzymes can be accounted for by the same kinetic scheme, with nearly identical catalytic p*K* requirements. This convergence of observations of a number of different laboratories on a variety of enzymes of this class has quite logically led to the assumption of an obligatorily identical detailed chemical mechanism. As new findings on specific enzymes have required modifications to the currently existing mechanism, these changes have reasonably been assumed to apply to the chemistry of the reactions catalyzed by all of the other members of the class.

RESULTS AND DISCUSSION

Evidence of mechanistic diversity in α-amino acid dehydrogenase reactions. While our own transient-state studies of beef liver L-glutamate dehydrogenase (blGDH), Clostridium symbiosum L-glutamate dehydrogenase (csGDH), and L-phenylalanine dehydrogenase (PaDH) have supported the commonality of the sequence of complexes among these three enzymes, certain observations are difficult to explain on the basis of a detailed common chemical mechanism. For example, the transient-state time courses of resolved components shown in Fig. 1 indicated substantial phenomenological differences that cannot be accounted for by variations in specific rate constants or by altered p*K*s. A more significant difference arose from our stopped-flow indicator dye study of the transient-state kinetic isotope effects on the location of the proton release step (*14*). This study proved conclusively that a single proton is released *prior* to the hydride transfer step in the blGDH reaction, while a proton with an identical p*K* is released *after* the hydride transfer step in the csGDH reaction.

Possible explanations of mechanistic diversity. We take it as an obvious truism that, while small variations in distant regions of an enzyme molecule can cause significant effects on the catalyzed reaction, any such change must, in some manner, be transmitted to the active site to do so. Indeed, they must result ultimately in some

[3] These "shifts" refer to the displacement of the normal 340-nm band to higher or lower wavelengths (*8, 21*).

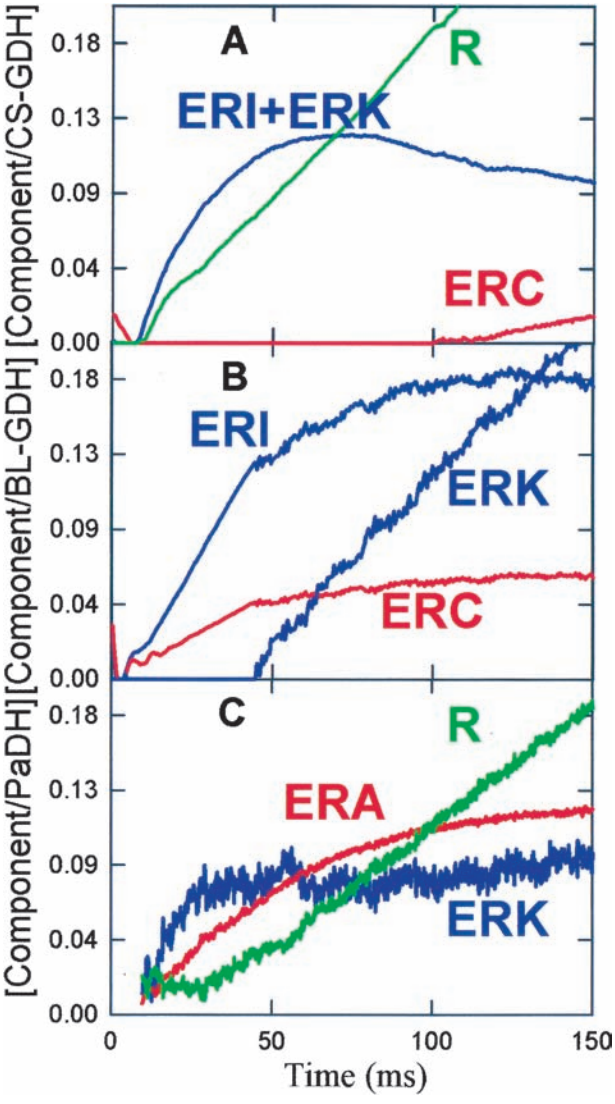


FIG. 1. Transient-state time courses of spectroscopically-resolved components of three α-amino acid dehydrogenase catalyzed oxidative deamination reactions. R is reduced coenzyme. ERI is an enzyme-reduced coenzyme-α-iminoglutarate complex. ERC is an enzyme-reduced coenzyme-α-carbinolamine complex. ERA is an enzyme-reduced coenzyme -L α-amino acid complex. ERK is an enzyme-reduced coenzyme-α-keto-acid complex. The reactions were followed spectroscopically and resolved into components as described previously (23). (A) The csGDH reaction; (B) the blGDH reaction; (C) the PaDH reaction.

change in electron density at a distance of no more than 2Å from some part of the bound substrate. That being so, we must then look to the active sites themselves for the causes of mechanistic diversity.

One source of structural variations which could cause mechanistic variability might lie in differences in the timing of active-site cleft opening and closing events. There is now ample evidence of the occurrence of such events as obligatory steps in enzymatic mechanisms.[4] Cleft closure would certainly create changes within 2Å of the substrate and could account for the difference in the point of proton release in the two enzymatic reactions. Direct evidence that a series of alternating active-site opening or closing events is an integral part of the catalytic blGDH reaction has been obtained from "pH jumps" (*17*) and "ammonia jumps" at various points in time in the transient-state time course of the reaction. The proton abstracted from the substrate α-amino group in the Rife and Cleland mechanism could thus be released to the solvent at different points in the reaction time courses. However, whether the abstracted proton remains on the original enzyme carboxylate oxygen atom or is transferred elsewhere until it is released, its continued presence anywhere in the active site must certainly force the chemical mechanism into a very different pathway. While such alternative mechanisms can certainly be devised, it is somewhat difficult to imagine how two totally different mechanisms for the same stoichiometric reaction could operate with comparable efficiency using essentially the same extensive and geometrically similar set of active-site functional groups. Thus, the problem of reconciling clear phenomenological differences with strongly conserved structural similarities remains to be solved.

The tetradic motif. A detailed examination of the superposition of the X-ray crystal structures of all currently available complexes of this class of α-amino acid dehydrogenases has revealed the existence of a highly conserved active-site motif. The geometry of this motif provides a unique set of bonding characteristics whose mechanistic flexibility appears capable of providing a solution to the problem we have described. The superposition of the crystal structure of five forms of α-amino acid dehydrogenases shown in Fig. 2 reveals a geometric pattern of active-site atoms whose relative positions differ only minutely between the various proteins. Expanding a portion of three of these structures, as in the van der Waal's presentation of Fig. 3, shows a recurring motif consisting of four atoms closely packed into a planar form. The occurrence and position of the necessarily out-of-plane hydrogen atoms have been calculated. The nature of this motif, as shown by the stylized form in Fig. 4, involves a single oxygen atom from the carboxylate group of a highly conserved aspartyl residue, a nitrogen atom from the ε-amino group of a highly conserved lysine residue, an oxygen atom from a specifically bound water molecule, and the nitrogen atom from the α-amino group of the bound amino acid substrate. The latter pair of atoms necessarily shows a variable degree of occurrence in the motif, depending on the nature of the individual complex. Each of the four atoms have been explicitly implicated in

[4] X-ray crystal structures of both open and closed forms of a bacterial glutamate dehydrogenase have been obtained (*11*). Calorimetric studies from this laboratory have demonstrated two-state behavior for a number of different complexes of a variety of pyridine nucleotide-linked dehydrogenases (*16*). The behavior of the set of 38 such complexes could be accounted for by a two-state ligand binding process involving a constant intrinsic ΔH° of $+22$ Kcal. M^{-1} and a widely varying ΔG°. Based on this finding it was concluded that the apo form of beef liver glutamate dehydrogenase (blGDH) is 90% in the closed form at 25°C, while that of Clostridium symbiosum glutamate dehydrogenase (csGDH) is largely open.

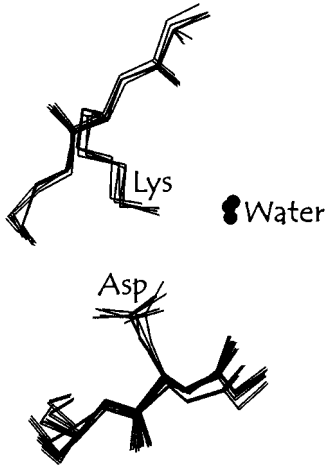


FIG. 2. A superposition of the highly conserved portions of the X-ray crystal structures of six α-amino acid dehydrogenase forms and of a specifically bound water molecule that occurs in only three of the forms. Structures shown include: blGDH (23); csGDH (22); csGDH L-glutamate complex (20); Bacillus sphaericus leucine dehydgrogenase (12); and Rhodococcus sp. PaDH –L-phenylalanine complex (18).

the chemical events which comprise the heart of the catalytic reaction in all of the various mechanisms which have been proposed for this class of enzymes (*1, 4, 18–20*).

The motif shown in Fig. 4 has some interesting properties that appear to permit some novel forms of behavior. The interatomic center distances of the tetrad in most of the observed enzyme forms are such that hydrogen bonding could occur in any

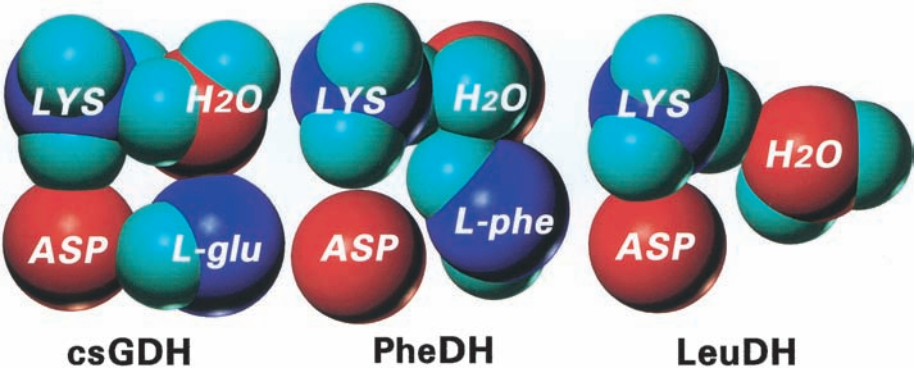


FIG. 3. A space-filling representation of the geometric relationships between the four critical atoms of the conserved motif in three α-amino acid dehydrogenase complexes. The nitrogen atom of the ε-amino group of the enzyme's lysyl residue and that of the substrate's α-amino group are shown in dark blue. The oxygen atoms of the bound water molecule and one of the enzyme's aspartyl carboxyl oxygen atoms are shown in red. The hydrogen atoms whose positions have been calculated using the Biopolymers module of the Tripos SYBYL program are shown in cyan.

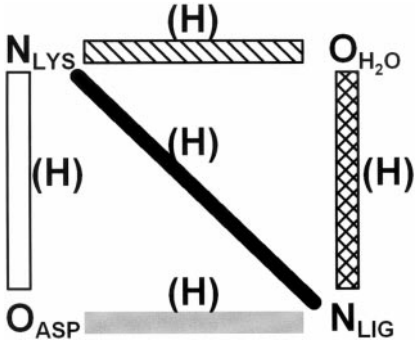


FIG. 4. A stylized scheme of the motif identifying the potential internal hydrogen bonding sites (H) and defining the individual graphical representation of each.

of the five different places indicated in the figure. Given the fact that the number of hydrogen atoms actually available for hydrogen bond formation must be limited, and that a competition between the individual atom pairs for hydrogen bond formation must exist, it follows that the structure shown in Fig. 4 provides the possibility of 32 differently hydrogen bonded structures. It is this feature, the potential for high degree of variability in contrast to the single pathway available to a charge-relay motif that distinguishes the properties and presumably, the potential importance of the tetrad motif.

The potential role of the tetrad as a set of mechanistic switching elements. Chemical mechanisms for enzyme-catalyzed reactions typically involve a number of changes in hydrogen bonding among active-site functional groups interspersed with covalent bond forming and breaking events. Given the apparent ability of the tetrad to afford a wide variety of hydrogen bonding patterns, and to shift from any one pattern to an alternative one with only a very minimal change in atomic position, we may now inquire as to whether this characteristic is capable of serving such a mechanistic role. Our ability to answer this question definitively is precluded by the paucity of complete series of crystallographic structures of any given α-amino acid dehydrogenase, as well as, by the fact that most of the structures available are necessarily those of stable forms not easily relatable to reactive intermediates. We do, however, now have on hand high-resolution structures for four pertinent complexes of phenylalanine dehydrogenase that may provide a suggestive (although, by no means conclusive) answer to this question. Since, as we have seen, the specific H-bonding pattern, which the tetrad assumes in any given complex, depends on very small differences in the center-to-center distances of its four constituent atoms, we can best compare the variation in hydrogen-bonding patterns using a set of bar-graphs. A number of such representations for a series of high-resolution crystallographic structures of phenylalanine dehydrogenase complexes are shown in Fig. 5. Here, the center-to-center distance between each pair of atoms in the tetrad for any given dehydrogenase complex is shown as a vertical bar patterned according to its counterpart in the stylized motif shown in Fig.4. The presence of the symbol "H" immediately under a bar indicates the presence of a hydrogen between the atoms of the pair indicated by that particular

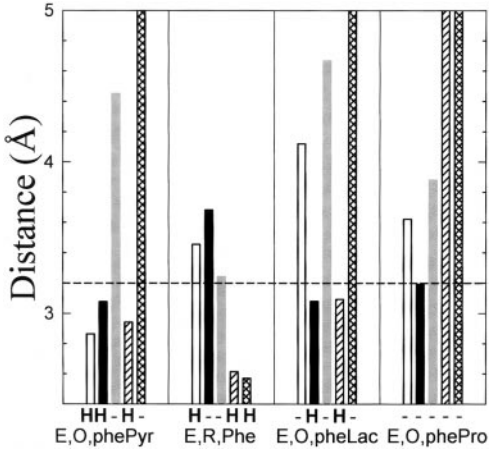


FIG. 5. Inter-atom center-to-center distances and H-bond assignments in the tetrad motif of four phenylalanine dehydrogenase complexes. Each vertical bar indicates the atom-to-atom center distances for the particular bond designated by its shading in accordance with the schematic motif described in the legend of Fig. 4. These distances are plotted from 2.4 Å, the distance required for the strongest possible hydrogen bond. The dashed line at 3.2Å indicates the largest distance at which an N-H-O bond of significant strength would be anticipated. Increasing length indicates decreasing hydrogen bond strength. The occurrence and location of Hydrogen atoms were calculated using the Biopolymers module of SYBYL (Tripos). The symbol "**H**" beneath a given bar denotes the assignment of a hydrogen bond to that particular atom pair. (A) is the enzyme-NAD-phenylpyruvate complex; (B) is the enzyme-NADH-L-phenylalanine complex; (C) is the enzyme-NAD-L-phenyllactate complex; (D) is the enzyme-NAD-phenylproprionate complex.

bar as calculated by the SYBYL Biopolymers program, and the symbol "-" indicates the absence of such a bond. It can be seen that while the tetrad remains intact in all four phenylalanine dehydrogenase complexes, the bond distance of any given atom pair varies by as much as 2Å from one enzyme complex to another. Furthermore, it can be seen that the pattern of H-bonding in the motif (shown by the "**H**" and "-" symbols) is different in each complex, and that the protons would have to shuffle around the tetrad for any one complex to convert to another.[5] It is precisely this sort of alternation in H-bonding between atoms of enzyme functional groups and those

[5] The structure of the catalytically inactive *E-O*-phenylproprionate complex establishes a point of some significance. As can be seen from Fig. 5, the replacement of the customary position in the tetrad of a nitrogen or oxygen atom (contributed by the substrate, intermediate, or product) the methyl group of the proprionate moiety has some dramatic effects. The hydrophobic nature of the methyl group distorts the tetradic structure by repelling both oxygen atoms by distances of several Ångstoms; leaving a tetrad which is still structurally recognizable but completely devoid of any internal hydrogen bonding. This demonstrates the finite but limited flexibility of the structure. The *E-O*-phenyl proprionate complex possesses a second rather curious feature: the terminal methyl group appears to be forced into unexpectedly close proximatry with the tetradic lysine ε-amino nitrogen atom, having a C-N atom distance of only 3.2 Å. It must be presumed that the amino group is unprotonated in this complex. The driving force for this energetically unfavorable local interaction must be provided by the tight binding of the phenyl ring of the ligand.

of the reactants and of water that constitutes the principal features of enzymatic mechanisms as they are currently understood and portrayed.

Thus, the differences in H-bonding in the catalytic tetrad predicted by the corresponding variations in interatomic distances observed in X-ray structures of the various PaDH ternary complexes would appear to provide a competent basis for understanding the detailed chemistry involved in the interconversions of the complexes depicted in Eq. 2. Similar differences between the tetradic structures of corresponding complexes of the various α-amino acid dehydrogenases would therefore appear to offer a plausible explanation for the mechanistic differences exemplified in Fig. 1. Indeed, the results of Tonge and Carey (*15*) have shown that sub-Ångstrom differences in the length of a hydrogen bond involve very substantial energy changes, and that even a change as small as 0.1Å can constitute a mechanistically significant event. Without direct evidence linking a specific change in tetradic H-bonding to some defined chemical step, the role we have ascribed to tetrad H-bonding must be considered a conjecture. The collection of high-resolution X-ray crystal structures is thus far insufficient both in number and in structural relevance to realistic intermediates to permit us to establish such relationships in most cases. We can, however, provide one example that may provide some plausible evidence in support of that conjecture.

Evidence that small differences in bond distance in the catalytic tetrad of two highly homologous enzymes lead to different mechanistic pathways. Based on steady-state p*K* and transient-state spectroscopic kinetic findings, it is generally agreed that amino acid substrates in enzymes of this class bind in the protonated α-amino nitrogen form, and that this proton must be removed before hydride transfer can occur. The prediction of Rife and Cleland (*4*) that this proton is transferred to a carboxylate at the active-site residue is born out by the csGDH crystal structure shown in Fig. 3, and is consistent with the mechanistic step shown in Fig. 6A. As we have noted earlier, the conclusions inferred from this crystal structure of a bacterial GDH fit nicely with the Rife and Cleland mechanism based on data from a mammalian GDH, including our own transient-state kinetic studies. This agreement quite reasonably has been accepted as evidence for a single basic chemical mechanism applicable to α-amino acid dehydrogenases in general. However, the high-resolution crystal structure of the PaDH-R-phenylalanine complex exhibits some small but mechanistically significant differences in the interatomic distances of the tetrads of the two enzymes. The distance between the aspartate oxygen atom and the lysine α-amino nitrogen atom in the PaDH complex is 0.5Å larger than that in the corresponding csGDH complex, as can be seen from Fig. 3, weakening that hydrogen bond significantly and possibly abolishing it. Of even more importance is the 0.5Å increase in the distance between the aspartate oxygen atom and the substrate α-amino nitrogen atom, which abolishes that hydrogen bond as well. Thus, as can be seen more clearly in the van der Waal representation of Fig 3, the aspartyl oxygen atom which plays such a critical role in the csGDH reaction (Fig. 6A) is not bonded to either of its tetradic neighbors in the PaDH complex. It clearly cannot participate in the required removal of a hydrogen atom from the substrate α-amino nitrogen atom in the PaDH reaction. However, the bond distances of the upper- and the right-hand sides of the motif of Fig. 4 are relatively unchanged, and the water molecule remains hydrogen bonded to both the substrate and enzyme nitrogen atoms of the tetrad. Noting these points,

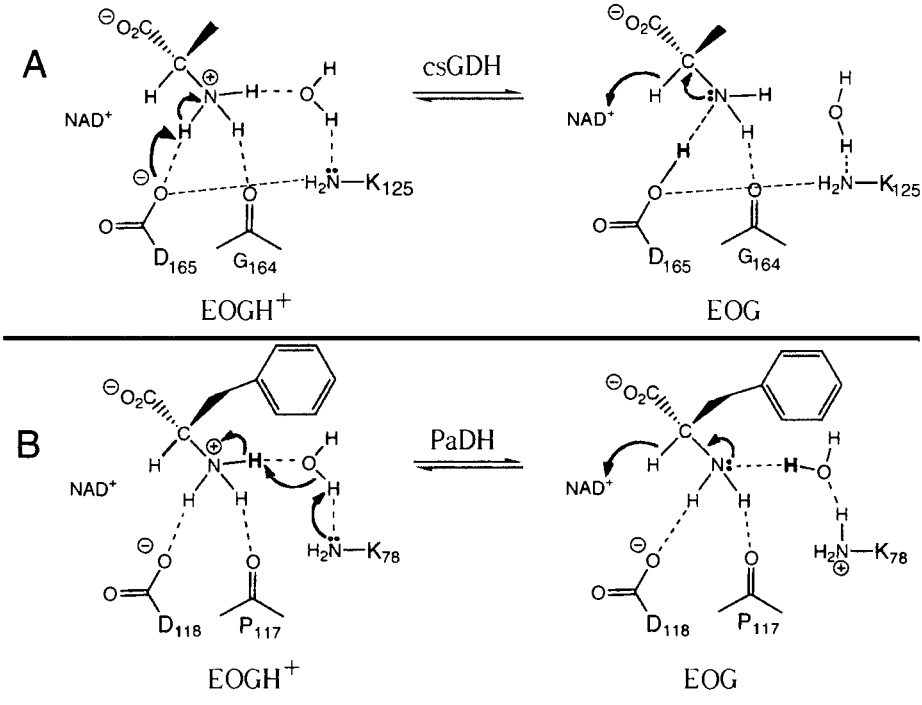


FIG. 6. A comparison of the mechanisms of a single step in the reactions of two homologous α-amino acid dehydrogenases. (A) csGDH. (B) PaDH.

Vanhooke *et al.* proposed a mechanistic step similar to that shown in Fig. 6B (*18*). Here the substrate α-amino hydrogen atom is abstracted by the oxygen atom of the bound water molecule and thence transferred to the adjacent lysine nitrogen atom to which it is already hydrogen bonded. Thus, comparing the mechanisms as portrayed in Fig. 6, it can be seen that the pattern of the electron flow is clockwise in the csGDH reaction. While this single example hardly provides proof of the conjecture we have advanced here, it may serve to demonstrate the degree of mechanistic versatility that the peculiar geometry of the tetrad can provide. This demonstrated variability of internal hydrogen bonding between the atoms of the tetrad may also provide a plausible explanation of the abnormal and varying p*K* values of active-site functional groups, as well as the difference in proton release points in the mechanisms of the bl- and csGDH catalyzed reactions.

Causal bases of bond distance variations among tetrads. These variations may arise from two factors: (1) Intrinsic structural differences between apo forms of different members of the enzyme class. Such small differences may be ascribed in part to the needs of the overall active-site structure to accommodate the binding of different substrate side chains. Examination of the set of available structures of apo forms of α-amino acid dehydrogenases does appear to reveal differences of $\pm$ 0.5Å in the tetradic bond distances, which, if taken literally, predict intrinsic differences in hydrogen-bonding patterns among the various members of the enzyme class.

However, most of these apo enzyme structures have been obtained at relatively low resolution, and the differences observed are only slightly greater than the probable error. (2) The major source of variations in distance (and, therefore, in predicted hydrogen-bonding patterns) can be ascribed to differences in the atom of the tetrad contributed by the bound ligand portrayed at the lower right-hand corner of Fig. 4. This ligand may be a substrate, product, or reactive intermediate. Therefore, this atom of the tetrad may be either a nitrogen atom from an α-amino or α-imino group, an oxygen atom from an α-keto or α-carbinol group; or, in some cases, it may even be unoccupied. The presence or absence of the bound water molecule which can contribute the oxygen atom (upper right-hand corner of the tetrad in Fig. 4) is also variable, occurring in about one half of the pertinent structures available with no discernible preference for either apo-, binary, or ternary complex forms.

CONCLUSIONS

The limited amount of high-resolution structural data available at this point in time permit us to reach only two firm conclusions: (1) a close-packed four atom motif constitutes the catalytic core of the pyridine nucleotide-linked α-amino acid dehydrogenases and (2) small but energetically significant differences in the distances between the atoms of that tetrad lead to individual patterns of internal hydrogen bonding that differ between the various enzymes; and, more significantly, between the various complexes of a given enzyme. On the other hand, the statement made in the title of this article that such a "motif serves as a variable-pathway mechanistic switching device" lacks substantive proof. It is advanced here, therefore, in the form of a conjecture.

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REFERENCES

1. Brunhuber, N. M., and Blanchard, J. S. (1994) *Crit Rev. Biochem Mol. Biol.* **29,** 415–467.
2. Fisher, H. F. (1985) *Methods Enzymol.* **113,** 16–27.
3. Fisher, H. F., and Viswanathan, T. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2747–2751.
4. Rife, J. E., And Cleland, W. W. (1980) *Biochemistry* **19,** 2328–2333.
5. Fisher, H. F., and Cross, D. G. (1966) *Science* **153,** 414–415.
6. Fisher, H. F., Adija, D. L., and Cross, D. G. (1969) *Biochemistry* **8,** 4424–4431.
7. Fisher, H. F., and Saha, S. K. (1996) *Biochemistry* **35,** 83–88.
8. Fisher, H. F. (1998) *in* Enzymatic Mechanisms (P. A. Frey and D. B. Northrop, Eds.) IOS Press, pp. 264–277, Amsterdam.
9. Baker, P. J., Britton, K. L., Engel, P. C., Farrants, G. W., Lilley, K. S., Rice, D. W., and Stillman, T. J. (1992) *Proteins: Struct. Funct. Genet.* **12,** 75–86.
10. Rossman, M. G., Liljas, A., Branden, C. I., and Banaszak, L. J. (1975) *in* The Enzymes, 3rd ed., Vol. 11, pp. 62–102.
11. Stillman, T. J., Baker, P. J., Britton, K. L., and Rice, D. W. (1993) *J. Mol. Biol.* **234,** 1131–1139.
12. Baker, P. J., Turnbull, A. P., Sedelnikova, S. E., Stillman, T. J., and Rice, D. W. (1995) *Structure* **3,** 693–705.
13. Scapin, G., Reddy, S. G., and Blanchard, J. S. (1996) *Biochemistry* **35** (42): 13540–13551.
14. Maniscalco, S. J., Saha, S. K., Vicedomine, P., and Fisher, H. F. (1996) *Biochemistry* **35,** 89–94.

15. Tonge, P. J., and Carey, P. C. (1992) *Biochemistry* **31,** 9122–9125.
16. Fisher, H. F. (1988) *Adv. Enzymol.* **61,** 1–46.
17. Saha, S. K., and Fisher, H. F. (1999) *Biochim. Biophys. Acta.* **1431,** 261–265.
18. Vanhooke, J. L., Thoden, J. B., Brunhuber, N. M. W., Blanchard, J. S., and Holden, H. M. (1999) *Biochemistry* **38** (8), 2326–2339.
19. Baker, P. J., Britton, K. L., Engel, P. C., Farrants, G. W., Lilley, K. S., Rice, D. W., and Stillman, T. J. (1992) *Proteins* **12,** 75–86.
20. Stillman, T. J., Baker, P. J., Britton, K. L., Rice, D. W., and Rodgers, H. F. (1992) *J. Mol. Biol.* **224,** 1181–1184.
21. Maniscalco, S. J., Saha, S. K., and Fisher, H. F. (1998) *Biochemistry* **37,** 14585–14590.
22. Yip, K. S., Stillman, T. J., Britton, K. L., Artymiuk, P. J., Baker, P. J., Sedelnikova, S. E., Engel, P. C., Pasquo, A., Chiaraluce, R. and Consalvi, V. (1995) *Structure* **3,** 1147–1158.
23. Peterson, P. E., and Smith, T. J. (1999) *Structure* **7,** 769.