Haar, W., Fermandjian, S., Vicar, J., Blaha, K., and Fromageot, P. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 4948.
Kotelchuck, D., Scheraga, H. A., and Walter, R. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 3629.
Manning, M., Coy, E., and Sawyer, W. H. (1970), *Biochemistry 9,* 3925.
Meraldi, J. P., Hruby, V. J., and Brewster, A. I. R. (1977), *Proc. Natl. Acad. Sci. U.S.A. 74,* 1373.
Merrifield, R. B. (1965), *Science 150,* 175.
Nicolas, P., Camier, M., Dessen, P., and Cohen, P. (1976), *J. Biol. Chem. 251,* 3965.
Nicolas, P., Di Bello, C., and Cohen, P. (1977), in preparation.
Pearlmutter, A. F., and McMains, C. (1977), *Biochemistry 16,* 628.
Pradelles, P., Morgat, J. L., Fromageot, P., Camier, M., Bonne, D., Cohen, P., Bockaert, J., and Jard, S. (1972), *FEBS Lett. 26,* 189.
Smith, I. C. P., Deslauriers, R., Saito, H., Walter, R., Garrigou-Lagrange, C., McGregor, W. H., and Sarantakis, D. (1973), *Ann. N.Y. Acad. Sci. 222,* 597.
Smith, I. C. P., Deslauriers, R., and Schaumburg, K. (1975), in Peptides: Chemistry, Structure and Biology, Walter, R., and Meienhofer, J., Ed., Ann Arbor, Mich., Ann Arbor Science Publishers, p 97.
Tran-Dinh, S., Fermandjian, S., Sala, E., Mermet-Bouvier, R., Cohen, M., and Fromageot, P. (1974), *J. Am. Chem. Soc. 96,* 1484.
Urry, D. W., and Walter, R. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 956.
Walter, R., Prasad, K. U. M., Deslauriers, R., and Smith, I. C. P. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2086.
Walter, R., Schwartz, I. L., Darnell, J. H., and Urry, D. W. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1355.
Walter, R., Smith, I. C. P., and Deslauriers, R. (1974), *Biochem. Biophys. Res. Commun. 58,* 216.
Wolff, J., Alazard, R., Camier, M., Griffin, J. H., and Cohen, P. (1975), *J. Biol. Chem. 250,* 5215.

Convergence of Active Center Geometries†

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ABSTRACT: Comparisons have been made between the active center geometries of lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, chymotrypsin and papain, and glyceraldehyde-3-phosphate dehydrogenase and papain. In the dehydrogenases, orientation of the nicotinamide ring about the glycosidic bond is determined by the substrate stereochemistry. The proper positioning of the carboxyamide moiety allows for the close approach of the C4 atom on the nicotinamide and the reactive carbon of the substrate. It follows that, once the conformation of the substrate or substrate intermediate has been established with respect to the functional groups in the enzyme, the A- or B-side specificity of the nicotinamide ring is predetermined. Hence, dehydrogenases which are divergently evolving from a common precursor must maintain the nicotinamide specificity if the protein fold of the catalytic domain is conserved. The tetrahedral intermediates produced during acylation of chymotrypsin and papain are found to be of opposite hand, while those of papain and glyceraldehyde-3-phosphate dehydrogenase can be regarded to be of the same hand. Thus the serine proteases, subtilisin and those of the chymotrypsin family, are of one hand while the cysteine enzymes, glyceraldehyde-3-phosphate dehydrogenase and papain, are of the other.

The similarity of the active centers of subtilisin and chymotrypsin (Kraut et al., 1971) is the prime example of convergence of active center geometries in enzymes, although Drenth et al. (1976a) and Polgár (1977) have recently commented on further possible similarities of catalytic sites. While chymotrypsin and subtilisin have totally different foldings of their polypeptide backbones, the residues involved in catalysis (serine, histidine, aspartic acid) have the same spatial relationships. Superposition of the atoms in the active centers shows a root-mean-square difference of only 1.0 Å (Kraut et al., 1971).

It will be shown, by comparing various active center regions, that convergence is a common occurrence. The selected enzymes were specific for a substrate with a carbonyl group which undergoes a trigonal-tetrahedral carbon transition during catalysis. Such substrates and the respective active center moieties will mutually impose chemical and geometrical constraints on the reaction mechanisms. Thus, a comparison of analogous enzymes can determine the essential features of catalysis. Furthermore, the interrelationship of the chirality of the substrate and coenzyme for dehydrogenases can be established.

Experimental Section

A computer program was written based on the superposition techniques of Rao and Rossmann (1973) and Rossmann and Argos (1975). The program was informed of the atoms to be equivalenced which then provided an initial rotation matrix and translation vector. The Eulerian angles and translational components were refined to provide the best weighted least-squares fit between equivalenced atoms. Atoms separated by

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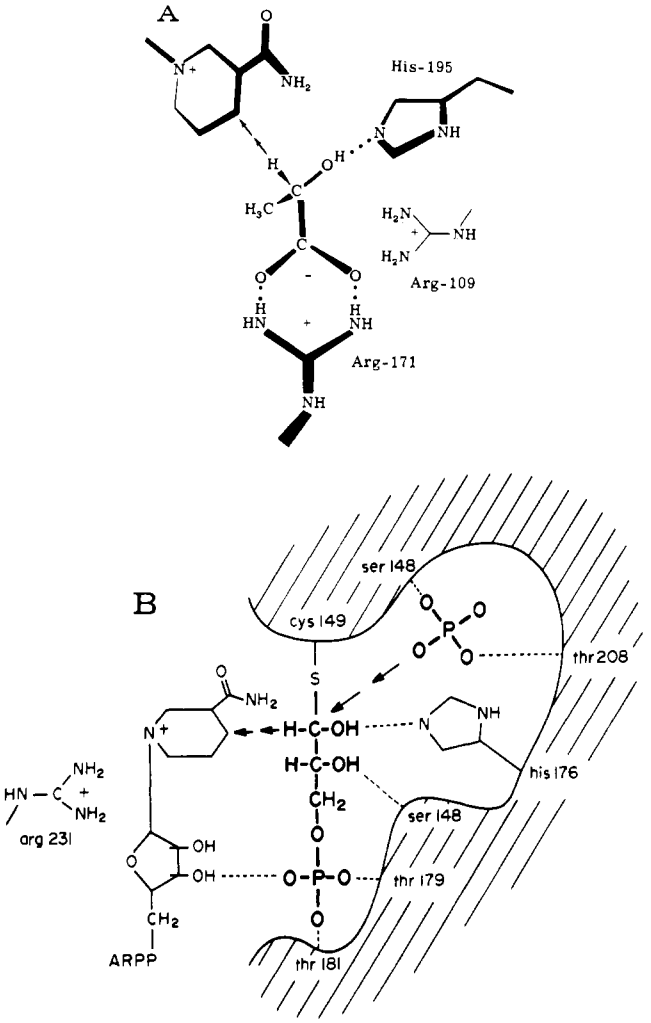


FIGURE 1: Diagrammatic comparison of the active centers of (A) LDH and (B) GAPDH showing substrate binding and residues involved in catalysis. Note the similarity of function of Arg-171 in LDH and Cys-149 in GAPDH.

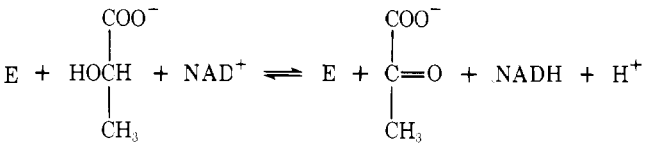
more than a prestated distance were then rejected and the least-squares procedure was repeated.

The coordinates for glyceraldehyde-3-phosphate dehydrogenase and its coenzyme were taken from Moras et al. (1975), those for lactate dehydrogenase from Holbrook et al. (1975), and those for chymotrypsin (Birktoft and Blow, 1972) and for papain (Drenth et al., 1976b) were obtained from the Brookhaven Protein Data Bank. The coordinates for the L-lactate substrate with the LDH[1] molecule were based on a LDH:NADH:oxamate electron density map (White et al., 1976). The thiohemiacetal intermediate of GAPDH was determined from the substrate phosphate binding site (Moras et al., 1975) and the direction of hydride transfer (Moras et al., 1975). The coordinates of the alkylated GAPDH were obtained from Garavito et al. (1977).

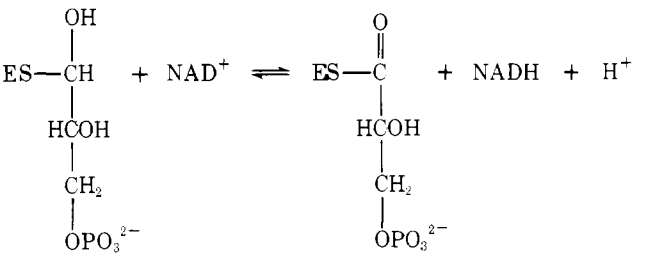
Comparison of LDH and GAPDH Active Centers. It has long been recognized (Levy and Vennesland, 1957) that the reactions catalyzed by NAD^+-dependent dehydrogenases involve the transfer of a hydride ion between the substrate and the nicotinamide ring of the coenzyme. For a given dehydrogenase, the reaction specificity for either the A or B side of the nicotinamide ring and the absolute hand of the hydride transfer have been established (Cornforth et al., 1962). The remarkable conservation of the nicotinamide specificity for a given dehydrogenase over a vast variety of species (Popják, 1970) has been taken as evidence for a highly conserved evolutionary property, and hence of great importance in catalysis. Such observations on A–B specificity have been enhanced by recent results obtained from nuclear magnetic resonance techniques (Arnold et al., 1976).

The structural similarity of the NAD^+-binding domains has also been the subject of much discussion (Rossmann et al., 1974, 1975; Ohlsson et al., 1974). The conformation of the bound cofactors is exceedingly similar, except for a rotation of approximately 180° about the nicotinamide–ribose glycosidic bond. The substrate is required to be positioned on the "outside" of the nicotinamide ring as the "inside" is mostly occupied by residues of the conserved NAD^+-binding fold. Thus, the rotation about the glycosidic bond permits attack by the nicotinamide on the substrate from either the A side (as in LDH) or the B side (as in GAPDH) of the ring.

The dehydrogenation catalyzed by LDH and GAPDH may be respectively written as



and



The end product of the GAPDH dehydrogenation step is an acyl-enzyme intermediate which is deacylated by a subsequent phosphorylation. Despite this extra reaction step in GAPDH, the enzymes have a similar mechanism for dehydrogenation, particularly with respect to the function of the essential histidines, the coenzyme, and the substrate binding residues Arg-171 (LDH) and Cys-149 (GAPDH) (Figure 1).

The active centers of LDH and GAPDH were initially compared by superimposing the NMN moieties of the coenzyme and the functionally equivalent histidines. It was soon apparent that the AMP portions of the coenzymes could not be aligned if the histidines were superimposed, an unexpected result in light of the structural equivalence of the NAD^+-binding domains. In Table I are shown the distances between equivalent atoms in the active centers of LDH and GAPDH, while Figure 2 shows the corresponding superpositions in space. Included in Table I are the guanidinium groups of arginines-109 (LDH) and -231 (GAPDH) which were found to superimpose. Arginine-109 (LDH) is attached to a flexible loop, which undergoes large conformational changes during catalysis (Rossmann et al., 1971; Holbrook et al., 1975), such that this residue is moved into the active center during the formation of the enzyme–coenzyme–substrate ternary complex. Hence the superposition of arginine-109 (LDH) onto arginine-231 (GAPDH) suggests that these guanidinium groups have similar functions. The presence of a positive charge near the similarly charged nicotinamide ring or imidazole ring must cause a destabilization of the Michaelis complex in favor of the

[1] Abbreviations used: LDH, lactate dehydrogenase; GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase.

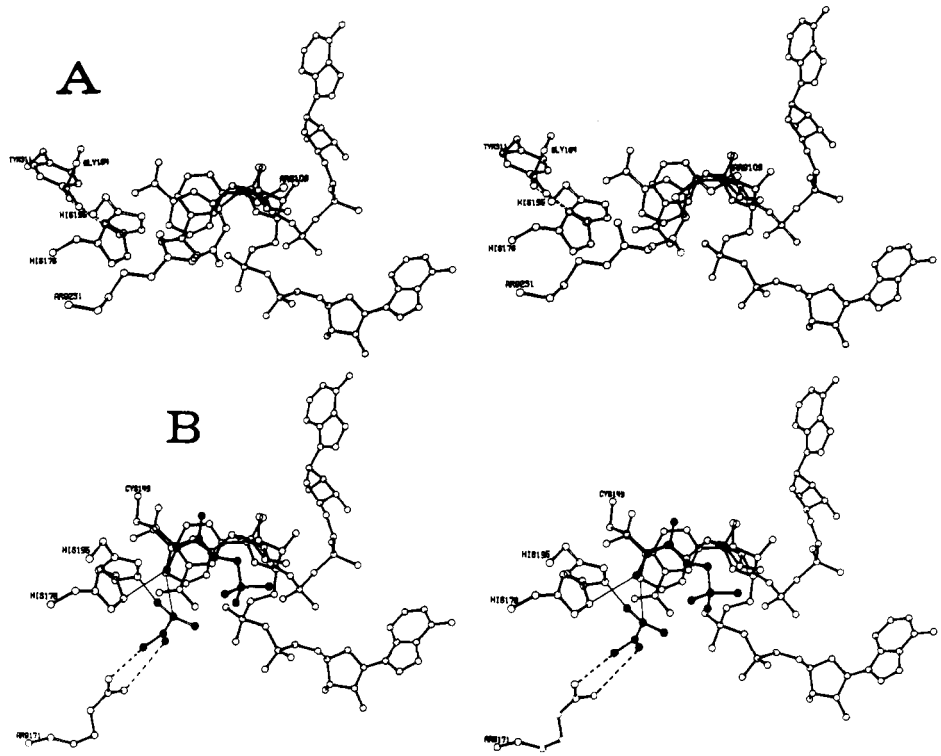


FIGURE 2: Stereo views of the superimposed LDH and GAPDH active centers. The GAPDH molecule is shown with filled in bonds. The L-lactate substrate and D-hemiacetal glyceraldehyde 3-phosphate intermediate are also shown. This view was obtained by superimposing the functionally equivalent NMN moieties, histidine residues as well as the guanidinium groups of Arg-171 (LDH) and Arg-231 (GAPDH). A shows the essential residues, while in B are shown the bound substrates.

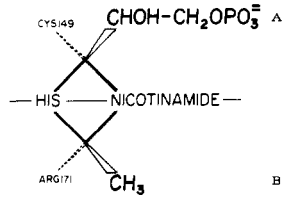


FIGURE 3: Diagrammatic representation of the relationship of the L-lactate and D-hemiacetal tetrahedral intermediate as they occur in (A) GAPDH and (B) LDH.

transition state thus causing a lowering of the activation energy. This observation is supported in a study of liver alcohol dehydrogenase where a charged histidine residue occupies the position of Arg-231 (GAPDH) or Arg-109 (LDH) (Argos, P., Garavito, R. M., Eventoff, W., Rossmann, M. G., and Brändén, C. I., manuscript in preparation).

Striking features in the comparison shown in Figure 2 are the nonalignment of the NAD^+ molecules, lack of superposition of the substrates, and the different directions of approach for the substrate binding residues arginine-171 of LDH and cysteine-149 of GAPDH. This establishes that the catalytic environments within LDH and GAPDH are of opposite hands (Figure 3).[2] Nevertheless, the coenzyme must attack the substrate from a similar direction. Thus when the nicotinamide rings and the reactive carbon atoms of the substrates are superimposed, then Arg-171 (LDH) corresponds spatially to His-176 (GAPDH) and His-195 (LDH) to Cys-149 (GAPDH). Appropriate atoms in these groups were, therefore, used in an alternate equivalencing of the active sites (Figure 4 and Table II). As shown in Figure 4, the exchange of the two pairs of functional residues places the substrate in a similarly related position to the nicotinamide again demonstrating that the environment of the two active centers are of the opposite hand. A small difference in the rotation of the nicotinamide ring about the glycosidic bond still does not permit complete NAD^+ superposition.

It is seen in Figure 2 that, in order to position itself optimally for hydride transfer, the substrate avoids the carboxyamide

TABLE I: Equivalences Used in Comparison of LDH and GAPDH to Obtain Superposition of Functional Groups.[a]

LDH		GAPDH		Separation (Å)
His-195	N_δ	His-176	N_δ	1.5
His-195	N_ϵ	His-176	N_ϵ	1.2
Nicotinamide	C_3	Nicotinamide	C_5	2.1
Nicotinamide	C_4	Nicotinamide	C_4	1.9
Nicotinamide ribose	$C_{1'}$	Nicotinamide ribose	$C_{1'}$	1.6
Nicotinamide ribose	$O_{1'}$	Nicotinamide ribose	$O_{1'}$	1.6
Nicotinamide ribose	$O_{2'}$	Nicotinamide ribose	$O_{2'}$	0.5
Nicotinamide ribose	$O_{3'}$	Nicotinamide ribose	$O_{3'}$	2.8
Nicotinamide phosphorus	P	Nicotinamide phosphorus	P	2.4
Arg-109	C_ζ	Arg-231	C_ζ	1.5

[a] The selected ribose atoms were used to provide maximum information on the orientation of this group.

[2] This statement is based on the equivalence of function for the coenzyme, the histidines, and the substrate binding residues Arg-171 (LDH) and Cys-149 (GAPDH). Nevertheless, the Cahn-Ingold-Prelog convention would signify the chirality of the reactive carbon as S in both cases due to the different weights assigned to the groups surrounding the carbon atom.

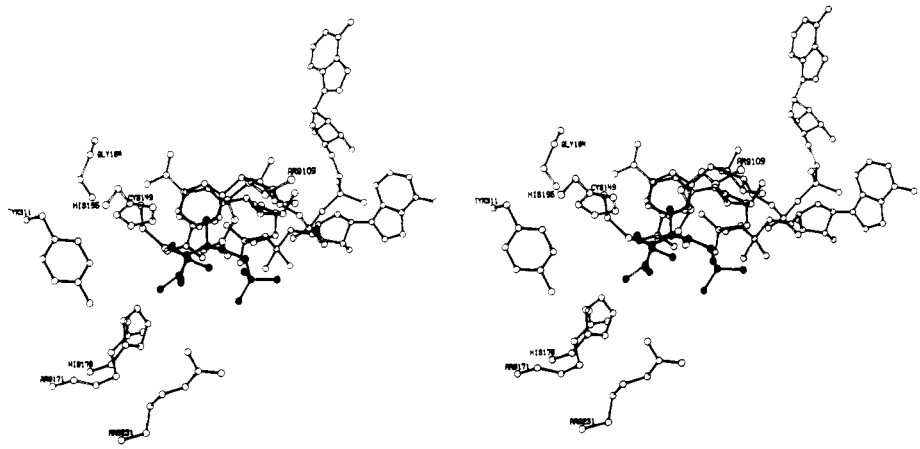


FIGURE 4: Superposition of functional groups His-195 and Arg-171 of LDH on Cys-149 and His-176 of GAPDH, respectively, thus exchanging hands of the active center regions.

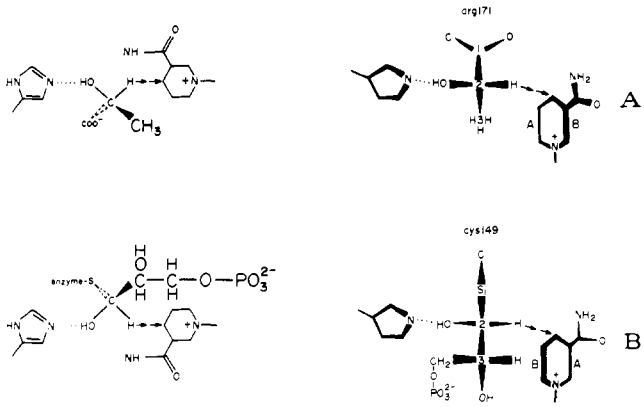


FIGURE 5: Diagrammatic representation of the relationship between substrate binding positions and the orientation of the carboxyamide group on the C3 atom of the nicotinamide ring. When the L-lactate molecule is positioned, only the A side of the nicotinamide ring can receive a hydride ion. If the B side were exposed, then there would be steric hindrance between the substrate and the carboxyamide group. Similarly the ligand positions in GAPDH require a D tetrahedral intermediate with the B side of the nicotinamide ring toward the substrate for an approach sufficiently close to permit hydride transfer.

TABLE II: Equivalences Used in the Comparison of LDH and GAPDH to Obtain Spatial Superposition of the Substrates.

LDH		GAPDH		Separation (Å)
Nicotinamide	C_3	Nicotinamide	C_5	0.4
Nicotinamide	C_4	Nicotinamide	C_4	0.4
Nicotinamide	C_5	Nicotinamide	C_3	0.4
Nicotinamide	N_1	Nicotinamide	N_1	0.7
Substrate	C_2	Substrate	C_1	0.5
His-195	N_ϵ	Cys-149	C_β	0.9
Arg-171	C_ζ	His-176	C_γ	1.0

side group of the nicotinamide ring. This is shown diagrammatically in Figure 5. Thus, the specificity of the nicotinamide ring is determined by the mode of binding of the substrate (Arg-171 in LDH and Cys-149 in GAPDH). It can be shown by model building that an A–B switch would require total rearrangement of the functional residues in the catalytic domain. Such an event is inconceivable during the divergent evolution of a given enzyme in different organisms. Hence, the specificity of the nicotinamide ring must be completely conserved during the divergent evolution of each pyridine-nucleotide linked enzyme. This argument can be extended to the divergence of related enzymes such as LDH and malate dehydrogenase (Banaszak and Bradshaw, 1975). It can also be predicted that the catalytic domain in D-specific LDHs (Tarmy and Kaplan, 1968; LéJohn, 1971; Long and Kaplan, 1973), where the hydride transfer is to the A side of the nicotinamide ring, must have a different structure than L-specific LDHs. The above argument is not altered by the occurrence of hydrogen bonding networks involving the carboxyamide groups as in LDH (Eventoff et al., 1977) or in GAPDH (Moras et al., 1975). While such hydrogen bonding will undoubtedly provide an optimal orientation for the nicotinamide ring (Biellmann, J. F., Marchal-Rosenheimer, N., Samama, J. P., Rossmann, M. G., and Eventoff, W., manuscript in preparation), there is no reason to assume that it would not be altered by evolutionary events.

Figures 2 and 5 illustrate the specificity of GAPDH for the D configuration around the C2 atom. The opposite hand of C2 would result in steric hindrance between the phosphate on atom C3 and the nicotinamide ring. This analysis is supported by the observations of Armstrong and Trentham (1976) that the enzyme not only fails to oxidize the L substrate but that the latter does not even inhibit the oxidation of the D substrate.

Comparison of Chymotrypsin and Papain. The proteases chymotrypsin, subtilisin, and papain cleave a peptide bond of the bound substrate by a nucleophilic attack on a carbonyl group, using a histidine activated "charge relay system". The enzymes then form covalent adducts with the amino end of the substrate's polypeptide chain (Figure 6). The structure of the various intermediates has been studied for trypsin by Rühlmann et al. (1973) and Krieger et al. (1974); for chymotrypsin by Segal et al. (1971) and Henderson et al. (1971); for subtilisin by Robertus et al. (1972); and for papain by Drenth et al. (1976b). The serine and cysteine proteases have the same mechanism, although they differ in their specificity by suitable changes in the substrate binding pockets.

The superposition of the active centers of chymotrypsin and papain is shown in Figure 7 and Table III. The functional histidines were superimposed by equivalencing the N_δ onto the opposite N_ϵ atom as suggested by Figure 6. In addition, the histidine orienters Asp-102 (chymotrypsin) and Asn-175 (papain), as well as the substrate binders Ser-195 (chymotrypsin) and Cys-25 (papain), were used to obtain the best relative orientation. Equivalent atoms were found to differ by a root-mean-square distance of 0.8 Å. Figure 7 shows that the

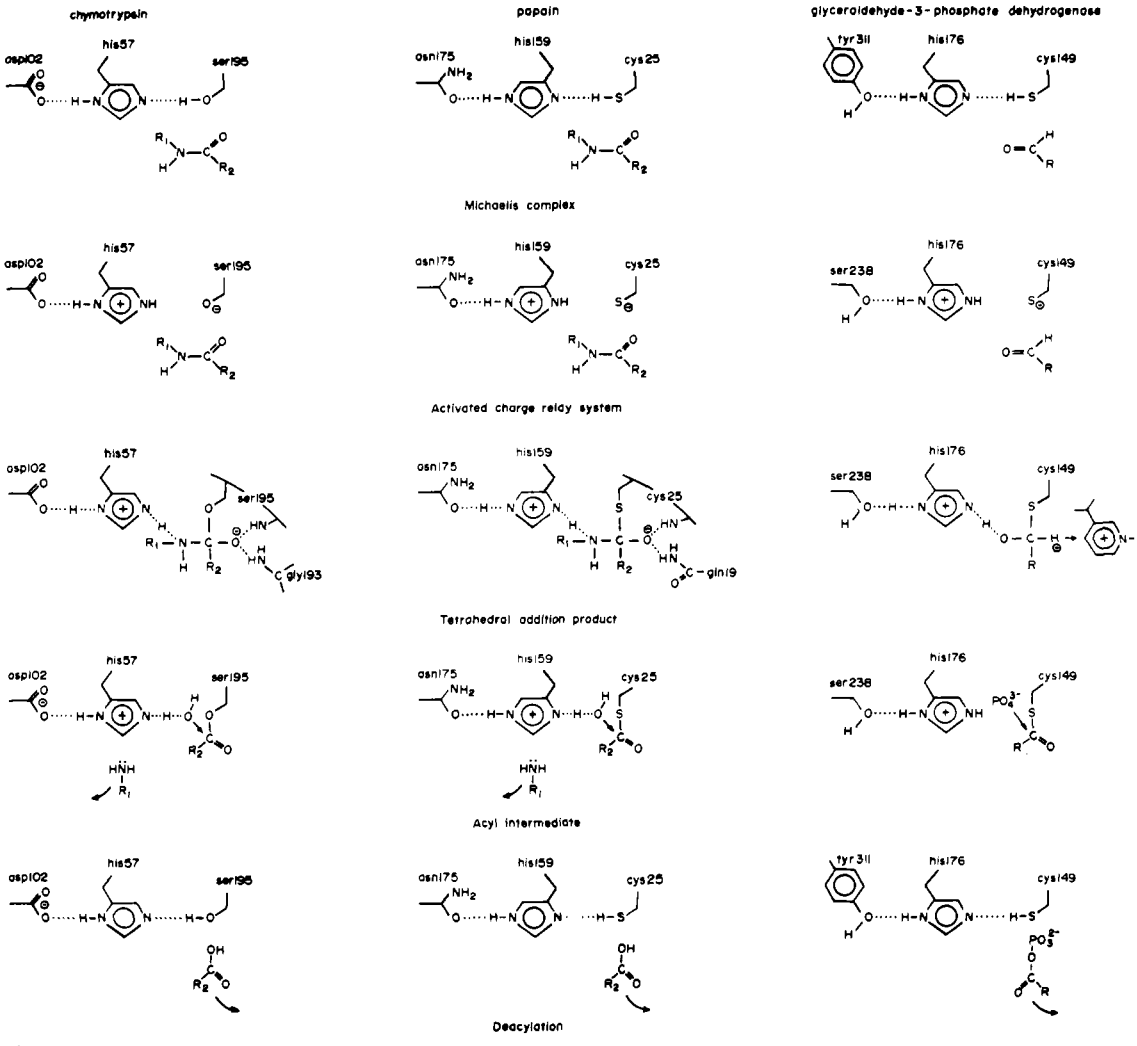


FIGURE 6: Comparison of steps in the reaction catalyzed by chymotrypsin, papain, and GAPDH.

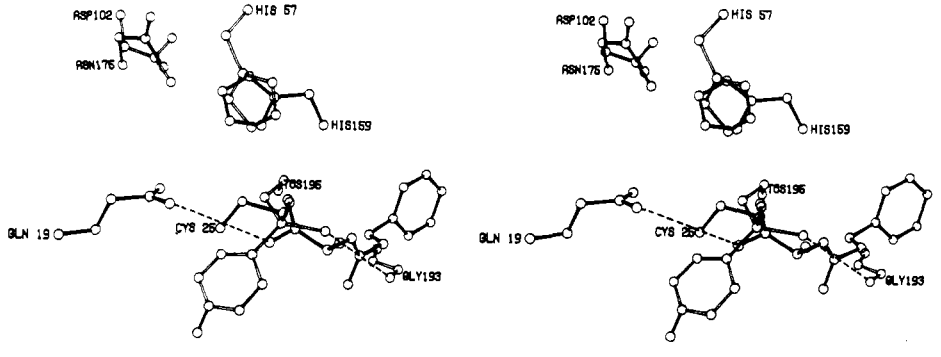


FIGURE 7: Stereo view of the superimposed active centers of Ala-Phe-methyl ketone-papain (filled bonds) and tosylchymotrypsin (open bonds).

tetrahedral intermediate formed in the serine protease chymotrypsin is of the opposite hand as that in the cysteine protease papain as indicated by the position of the oxyanion holes generated by Gln-19 (papain) and Gly-193 (chymotrypsin). The same conclusion could have been derived by comparing the diagrams of Rühlmann et al. (1973) with those of Drenth et al. (1976b).

Comparison of GAPDH and Papain. Both GAPDH and papain form thioester intermediates, by way of a nucleophilic attack on a carbonyl moiety. In addition, both enzymes have a charge relay system operating through a histidine residue (Figure 6). However, the hydride in GAPDH and the RNH_2 group in papain leave the tetrahedral carbon in different directions. It can also be seen in Figure 6 that the histidines may have different roles during catalysis after the activation of the essential thiol. Thus, superposition of "chemically equivalent" groups (e.g., histidine on histidine) does not imply "functional equivalence".

The comparison described in Figure 8 and Table IV is based on a superposition of chemically equivalent substrate ligands. In this experiment Cys-149 (GAPDH) has been compared with Cys-25 (papain), His-176 (GAPDH) with His-159 (papain), and Ser-231 (GAPDH) with Asn-175 (papain). The root-mean-square distance between the selected atoms was

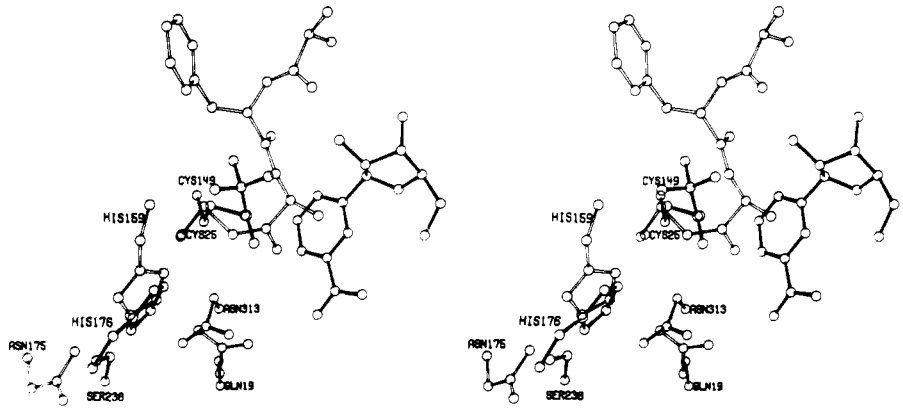


FIGURE 8: Stereo view of the superimposed active centers of GAPDH (dark bonds) on papain (open bonds). The respective substrate analogues shown for GAPDH and papain are trifluoroacetonyl and Ala-Phe-methyl ketone.

TABLE III: Equivalences Used in the Comparison of Chymotrypsin and Papain to Obtain Superposition of the Functionally Similar Groups.

Chymotrypsin		Papain		Separation (Å)
His-57	N_δ	His-159	N_ϵ	0.4
His-57	N_ϵ	His-159	N_δ	0.8
Asp-102	C_γ	Asn-175	C_γ	0.9
Asp-102	$O_{\delta_1}/O_{\delta_2}$	Asn-175	$O_{\delta_1}/N_{\delta_2}$	0.7
Ser-195	O_γ	Cys-25	S_γ	1.0

1.06 Å. Although fewer atoms were used to achieve this superposition compared with the subtilisin–chymotrypsin case (Kraut et al., 1971), it is apparent that the catalytically important groups have the same spatial relationship to their substrate. The tetrahedral intermediates are seen to be of the same hand, while a functional comparison would have shown opposite handedness. The amino group of Gln-19 (papain), which orients the carbonyl of the substrate, then superimposes on Asn-313 (GAPDH), suggesting a similar function for these orienters in their respective enzymes.

The hand of the tetrahedral intermediate is thus found to be the same for the two cysteine enzymes, papain and GAPDH (assuming chemical equivalence), and is opposite to that of the serine proteases, chymotrypsin and subtilisin. It would be reasonable to conclude that the difference in hand of the arrangement of catalytic groups between serine and cysteine enzymes has no significance with respect to a substrate of a known hand; yet questions might now be raised concerning differences in the mode of action between these two types of enzymes.

Conclusions

The convergence of active center geometries of chymotrypsin (and other enzymes in the same family), papain, GAPDH, and LDH is not as extensive as the convergence between chymotrypsin and subtilisin; nevertheless, the correspondence between functional groups is equally precise. Meaningful mechanistic relationships were discernable since the catalytic geometries in enzymes are determined by the substrate stereochemistry. The interrelationship between the mode of binding of the substrate and the specificity of the nicotinamide ring in dehydrogenases has been recognized. The results readily suggest numerous other active center comparisons which are likely to lead to further insights into the catalytic properties of enzymes.

TABLE IV: Equivalences Used in the Comparison of GAPDH and Papain to Obtain Superposition of Chemically Similar Groups.

GAPDH		Papain		Separation (Å)
His-176	N_ϵ	His-159	N_δ	1.1
His-176	N_δ	His-159	N_ϵ	0.9
Cys-149	C_β	Cys-25	C_β	1.3
Cys-149	$1s_\gamma$	Cys-25	S_γ	0.4
Asn-313	$N_{\delta_2}/O_{\delta_1}$	Gln-219	$N_{\epsilon_2}/O_{\epsilon_1}$	0.9
Asn-313	$O_{\delta_1}/N_{\delta_2}$	Gln-219	$O_{\epsilon_1}/N_{\epsilon_2}$	1.3
Asn-313	C_γ	Gln-219	C_δ	1.2
Ser-238	O_γ	Asn-175	$N_{\delta_2}/O_{\delta_1}$	1.0

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References

Armstrong, J. McD., and Trentham, D. R. (1976), *Biochem. J. 159,* 513–527.

Arnold, L. J., Jr., You, K., Allison, W. S., and Kaplan, N. O. (1976), *Biochemistry 15,* 4844–4849.

Banaszak, L. J., and Bradshaw, R. A. (1975), *Enzymes, 3rd Ed. 11,* 369–396.

Birktoft, J. J., and Blow, D. M. (1972), *J. Mol. Biol. 68,* 187–240.

Cornforth, J. W., Ryback, G., Popják, G., Donninger, C., and Schroepfer, G., Jr. (1962), *Biochem. Biophys. Res. Commun. 9,* 371–375.

Drenth, J., Enzing, C. M., Kalk, K. H., and Vessies, J. C. A. (1976a), *Nature (London) 264,* 373–377.

Drenth, J., Kalk, K. H., and Swen, H. M. (1976b), *Biochemistry 15,* 3731–3738.

Eventoff, W., Rossmann, M. G., Taylor, S. S., Torff, H. J., Meyer, H., Keil, W., and Kiltz, H. H. (1977), *Proc. Natl. Acad. Sci. U.S.A. 74,* 2677–2681.

Garavito, R. M., Berger, D., and Rossmann, M. G. (1977), *Biochemistry* (in press).

Henderson, R., Wright, C. S., Hess, G. P., and Blow, D. M. (1971), *Cold Spring Harbor Symp. Quant. Biol. 36,* 63–70.

Holbrook, J. J., Liljas, A., Steindel, S. J., and Rossmann, M. G. (1975), *Enzymes, 3rd Ed. 11,* 191–292.

Kraut, J., Robertus, J. D., Birktoft, J. J., Alden, R. A., Wilcox, P. E., and Powers, J. C. (1971), *Cold Spring Harbor Symp. Quant. Biol. 36,* 117–123.
Krieger, M., Kay, L. M., and Stroud, R. M. (1974), *J. Mol. Biol. 83,* 209–230.
LéJohn, H. B. (1971), *J. Biol. Chem. 246,* 2116–2126.
Levy, H. R., and Vennesland, B. (1957), *J. Biol. Chem. 228,* 85–96.
Long, G. L., and Kaplan, N. O. (1973), *Arch. Biochem. Biophys. 154,* 711–725.
Moras, D., Olsen, K. W., Sabesan, M. N., Buehner, M., Ford, G. C., and Rossmann, M. G. (1975), *J. Biol. Chem. 250,* 9137–9162.
Ohlsson, I., Nordström, B., and Brändén, C. I. (1974), *J. Mol. Biol. 89,* 339–354.
Polgár, L. (1977), *Int. J. Biochem. 8,* 171–176.
Popják, G. (1970), *Enzymes, 3rd. Ed. 2,* 115–215.
Rao, S. T., and Rossmann, M. G. (1973), *J. Mol. Biol. 76,* 241–256.
Robertus, J. D., Alden, R. A., Birktoft, J. J., Kraut, J., Powers, J. C., and Wilcox, P. E. (1972), *Biochemistry 11,* 2439–2449.
Rossmann, M. G., Adams, M. J., Buehner, M., Ford, G. C., Hackert, M. L., Lentz, P. J., Jr., McPherson, A., Jr., Schevitz, R. W., and Smiley, I. E. (1971), *Cold Spring Harbor Symp. Quant. Biol. 36,* 179–191.
Rossmann, M. G., and Argos, P. (1975), *J. Biol. Chem. 250,* 7525–7532.
Rossmann, M. G., Liljas, A., Brändén, C. I., and Banaszak, L. J. (1975), *Enzymes, 3rd Ed. 11,* 61–102.
Rossmann, M. G., Moras, D., and Olsen, K. W. (1974), *Nature (London) 250,* 194–199.
Rühlmann, A., Kukla, D., Schwager, P., Bartels, K., and Huber, R. (1973), *J. Mol. Biol. 77,* 417–436.
Segal, D. M., Powers, J. C., Cohen, G. H., Davies, D. R., and Wilcox, P. E. (1971), *Biochemistry 10,* 3728–3738.
Tarmy, E. M., and Kaplan, N. O. (1968), *J. Biol. Chem. 243,* 2579–2586.
White, J. L., Hackert, M. L., Buehner, M., Adams, M. J., Ford, G. C., Lentz, P. J., Jr., Smiley, I. E., Steindel, S. J., and Rossmann, M. G. (1976), *J. Mol. Biol. 102,* 759–779.

Influence of Substituent Ribose on Transition State Affinity in Reactions Catalyzed by Adenosine Deaminase†

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ABSTRACT: Adenosine deaminase from calf intestine hydrolyzes adenine at a limiting rate four orders of magnitude lower than that for adenosine, while K_m values for these substrates are about the same (Wolfenden, R., et al. (1969), *Biochemistry 8,* 2412–2415). Reactivity of 6-substituents, toward nucleophilic displacement, is found to be affected only slightly by removal of ribose as a 9-substituent, in model reactions. Substituent ribose thus appears to stabilize, selectively, the transition state for enzymatic deamination. In contrast with the small influence of substituent ribose on the apparent binding affinity of substrates, removal of substituent ribose from a potential transition state analogue, 1,6-dihydro-6-hydroxymethylpurine ribonucleoside, results in a lowering of its affinity for the enzyme by several orders of magnitude. The synthesis of the analogue and related compounds is described, and their properties compared with those of other photoadducts and of the naturally occurring inhibitors covidarabine and coformycin. Binding of these inhibitors is found to result in the appearance of ultraviolet-absorbing bands in the neighborhood of 323 nm.

Hydrolytic deaminases, that act on adenosine and related compounds, also catalyze the hydrolytic removal of a variety of leaving groups other than the normal product, ammonia (Cory and Suhadolnik, 1965). That such disparate leaving groups as ammonia and chloride are hydrolyzed from purine ribonucleosides at similar limiting rates, despite vast differences in the stability of their bonds to carbon, provided an early indication that the rate of reaction was at least partly determined by some step other than cleavage of the scissile bond in substrates (Wolfenden, 1966; Bär and Drummond, 1966). It was at first believed that this step might be the hydrolysis of a common intermediate, formed as a result of displacement of the leaving group by enzyme, but it was later found that the rate of this reaction was hardly affected by substituting deuterium oxide for substrate water (Wolfenden, 1969). This suggested that the transition state might be reached very early during the reaction, and a mechanism involving direct water attack on the substrate (Figure 1) was considered as a possibility. A route to a very approximate analogue of a tetrahedral intermediate, formed by water attack on the substrate adenosine, was provided by photoaddition of methanol to purine ribonucleoside, and the adduct proved to be a strong inhibitor of deaminase activity, as described in a preliminary communication (Evans and Wolfenden, 1970).

Pteridine, earlier shown to be covalently hydrated in aqueous solution (Perrin, 1962; Albert et al., 1966), had been found to be a good inhibitor of adenosine deaminase, consistent with the activity of the enzyme on 4-aminopteridine as a substrate (Wolfenden et al., 1969). When an attempt was made to compare pteridine and its hydrate as inhibitors, the enzyme proved unexpectedly to be a *catalyst* of the reversible hydration of pteridine (Evans and Wolfenden, 1972). The hydratase activity of the enzyme provided support for a mechanism in-

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