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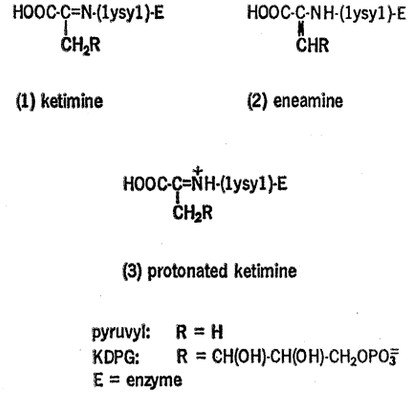
Aldolase Catalysis: Single Base–Mediated Proton Activation

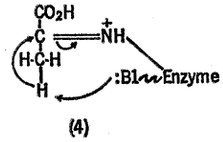
Abstract. *The enzyme, 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, catalyzes several reactions, the natural ones being (i) the exchange of hydrogen atoms of the methyl groups of pyruvate with protons of the solvent (C-H synthesis) and (ii) the reversible condensation of pyruvate with* D*-glyceraldehyde-3-phosphate (C-C synthesis). Previous work has provided chemical evidence for the occurrence of a protein-bound carboxylate group adjacent to the Schiff's base-forming lysine in the active site geometry. This carboxylate could provide the basic group postulated to participate in proton activation catalyzed by aldolases. With the use of three-dimensional models, it is shown that simple rotation about a carbon-carbon bond of the side chain will allow the base to assume the two positions necessary for proton activation in either the C-H synthesis or the C-C synthesis catalyzed by KDPG aldolase. This single base hypothesis provides a model wherein all reagents can approach a single face of the active site and is consistent with the stereochemistry thought to occur in the aldolase reaction.*

Aldolases have long been of great interest to biochemists because such enzymes provide a mechanism by which organisms can make and break carbon-carbon bonds. In this report we propose a model for the active site of 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase, which catalyzes two mechanistically equivalent reactions: (i) the reversible condensation of pyruvate and D-glyceraldehyde-3-phosphate (G3P) to form KDPG and (ii) an exchange reaction between the methyl hydrogen atoms of pyruvate and protons of water (*1*). We show that it is possible for the base-assisted catalytic proton activation, thought to be required for the two substrates, to be accomplished by a single enzyme-bound carboxylate (instead of two different basic groups). This single base hypothesis provides a model wherein all reactants can approach a single face of the active site and is consistent with our present knowledge of the stereochemistry of aldolase reactions.

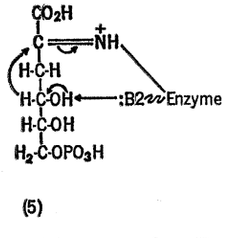
In 1964, Meloche and Wood (*1*) proposed a Schiff's base mechanism for KDPG aldolase. An amino group, provided by an enzyme-bound lysyl group, interacted with the carbonyl group of the substrate (pyruvate or KDPG). The intermediate Schiff's bases, specifically azomethines, represented by the tautomeric ketimine (**1**) and eneamine (**2**) forms, are shown below. A protonated ketimine (**3**) provided the electron sink necessary for catalysis.



In the presence of pyruvate the enzyme was inactivated by borohydride, since the intermediate lysylpyruvylazomethine (*1*, *2*) was reduced to form a secondary amine. Thus KDPG aldolase was considered a class 1 aldolase in the terminology of Rutter (*3*). It was suggested (*1*) that both pyruvate deprotonation and KDPG cleavage led to a common intermediate, a lysylpyruvyl eneamine (**2**) [analogous to the enolate formed when a metal-activated—that is, a class 2—aldolase, acts on its substrate (*3*, *4*)]. Specific proton activation for each substrate was considered (*3*–*5*) to be mediated by basic groups (visualized as B1 and B2 in **4** and **5**) in the active site of the enzyme. Such catalytic steps in KDPG



(4)



(5)

aldolase would cause for B1, deprotonation of the methyl group of pyruvate (**4**) thus leading to the formation of a lysylpyruvyl eneamine (**2**); and, for B2, deprotonation of the hydroxyl group at C-4 of KDPG (**5**) converting C-4 into a leaving group and allowing a rearrangement to G3P and the lysylpyruvyl eneamine (**2**).

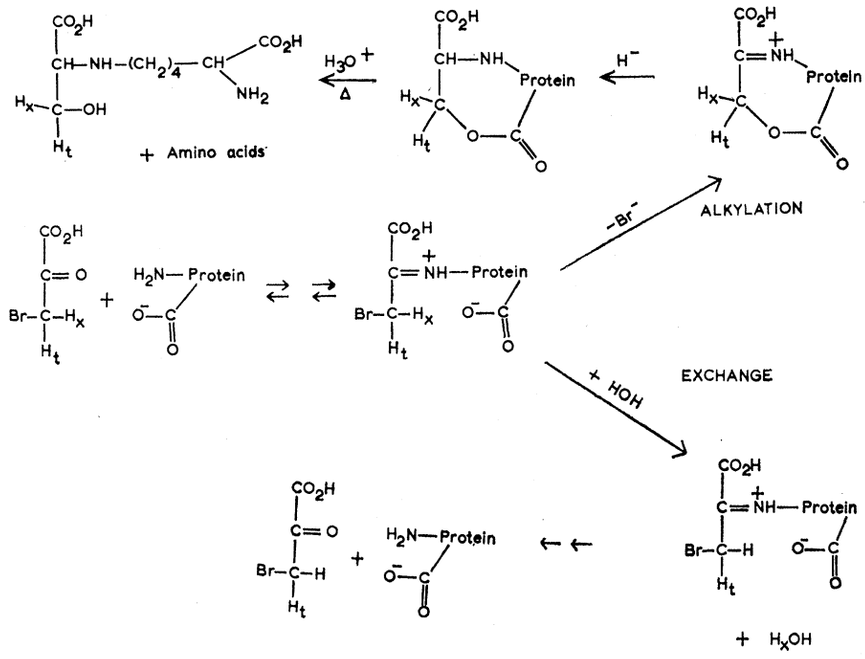


Fig. 1. The interaction of bromopyruvate with KDPG aldolase. This reagent behaves both as an inactivator, by alkylation of an enzyme-bound carboxylate group (upper right), and as a substrate so that one hydrogen atom (H_t) is exchanged with the protons of water (lower right). The rate of alkylation to exchange is 1:50 (*7*). The various reactions illustrated are alkylation ($-Br^-$), borohydride reduction (H^-), acid hydrolysis (H_3O^+ and Δ), and exchange (HOH). The hydrolysis product of inactivated, reduced enzyme—that is, N^{6}-(1′-carboxy-2′-hydroxy)ethyllysine—is shown at the top left. The exchange reaction is shown at the lower left.

Thus the catalysis by KDPG aldolase could be described simply as the attack of either a proton or the carbonyl carbon atom of G3P on the eneamine intermediate (**2**) that is formed as a result of base-mediated deprotonation of the substrates.

In order to locate and identify the basic groups, the substrate analog bromopyruvate was used. Details of the interaction of this reagent with KDPG aldolase are summarized in Fig. 1. Bromopyruvate is both a substrate and inactivator of the enzyme (*4, 6, 7*), thus showing that both catalysis and inactivation occur at the same protein site. Furthermore, the basic group is a carboxylate, since chemical reduction of the enzyme, inactivated by bromopyruvate, and subsequent hydrolysis yields the unique amino acid N^{6}-(1′-carboxy-2′-hydroxy)ethyllysine (*8*). This shows that, in the active site environment, a carboxylate (presumably from an aspartate or glutamate residue) has the geometry that allows its interaction with the β-carbon atom of bound bromopyruvate (as an azomethine). By analogy, this carboxylate could be the basic group (B1) involved in the deprotonation of pyruvate.

From a consideration of three-dimensional models we suggest that it is not necessary to invoke two different bases (B1 and B2) for the specific proton activation envisioned for pyruvate and KDPG, because one basic group (represented as glutamate) could, by rotation about a single carbon-carbon bond, assume the two conformations that allow a specific interaction both with the methyl group of pyruvate and with the hydroxyl group on C-4 of KDPG. Figure 2, A and B, shows scale stick models that are redrawn as line diagrams in Fig. 2, C and D. The interactions of either a proton (parts A and C) or of G3P (parts B and D) with pyruvate, represented as an enzyme-bound lysylpyruvyl eneamine, are shown. The carboxylate-mediated proton transfer involved in C-H synthesis is shown as an interaction of C, H, and O in Fig. 2C. In Fig. 2D the case of C-C synthesis is shown as attack by a G3P carbonyl-carbon atom on the eneamine together with carboxylate-mediated proton transfer to the carbonyl oxygen atom of G3P, viewed as an interaction of O, H, and O. It is evident from Fig. 2 that rotation about the bond between C-2 and C-3 of glutamate allows both proton activations to be catalyzed by the same carboxylate group. Although the model can accommodate either an aspartate or a glutamate residue, it is better fit by the latter (with aspartate

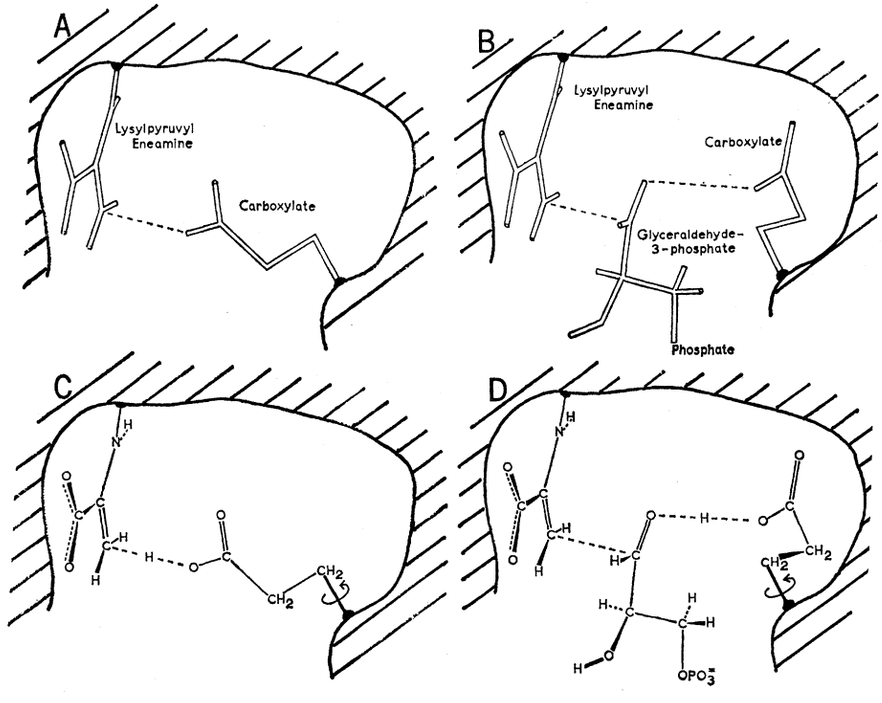


Fig. 2. The models for participation of a glutamyl residue with the substrates of KDPG aldolase (with retention of configuration at C-3 of KDPG). The upper diagrams are stick models, drawn to scale, showing the interaction of the carboxylate with the lysylpyruvyl eneamine (A) and with the G3P which is interacting with the lysylpyruvyl eneamine (B). In exactly the same orientations, line diagrams are given in (C) and (D), respectively. The postulated rotation of the glutamate C-C bond is shown.

some movement of the backbone, less than 1 Å in distance, is required). Experiments to distinguish between these two amino acids in KDPG aldolase are now in progress. It is also evident that other amino acids which could serve as bases, such as lysine or arginine, could participate in such a mechanism in other aldolases. The critical requirement is a chain length adequate to allow a carbon-carbon bond rotation which results in proper orientation of the basic function.

We have been able to build models in which either retention or inversion of configuration at C-3 of KDPG would occur. In these two sets of models, completely different locations of the glutamate in the active site and, presumably, in the peptide sequence are required. Thus the two cases in which a single base interacts with both substrates to give either inversion or retention are entirely different and independent on an evolutionary scale. It has been observed with muscle and yeast aldolase (*9*), L-rhamulose-1-phosphate aldolase (*10*) and two other aldolases active on DL-2-keto-4-hydroxyglutarate (*11*), that both the exchanging proton and the aldehyde approach the same face of the enzyme-bound enolate. In addition we have evidence that retention of configuration is also the case for KDPG aldolase. From this evidence, and on the assumption that a two-step rather than a concerted process is occurring, only the case for retention of configuration is shown in Fig. 2. Thus it seems that single base–mediated proton activation in aldolases contributes to an active site model allowing all exchanging reagents to approach a single (solvent) face of the bound enolate in the active site.

From the concept of a single base one might predict observable intramolecular proton transfer among aldolases, as in the case of phosphoglucose isomerase (*12*), which transfers protons between adjacent carbon atoms so that both exchanging protons approach a single face of the enolate intermediat (*13*), and which has in its active site a glutamate residue that reacts with a substrate analog (*14*). Triose phosphate isomerase transfers protons in the same way (*13*) and also has a glutamate residue in its active site (*15*). It now becomes apparent that a carboxylate, in which the oxygen atoms bridge a 2.2-Å distance, might be an ideal reagent for transferring protons between adjacent carbon atoms as catalyzed by isomerases, providing the single base required for *cis* hydrogen transfer generally seen among isomerases (*16*). The distinction between a carboxylate serving as the base for either an aldolase or an isomerase might be viewed as rotation of a C-C bond in the side chain as compared to the rotation of the carboxyl group itself.

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Mucopolysaccharidosis: Secondarily Induced Abnormal Distribution of Lysosomal Isoenzymes

Abstract. *Total activities of acid hydrolases in liver of two patients with mucopolysaccharidosis are decreased for β-galactosidase, α-galactosidase, and arylsulfatase A; total activities of four other hydrolases are normal or increased. The isoenzyme distribution of five hydrolases (β-glucuronidase, α-glucosidase, β-galactosidase, N-acetyl-β-glucosaminidase, and α-galactosidase) is abnormal in that the isoelectric points (by isoelectric focusing) of these enzymes are more acid than in control liver. Along with the isoenzyme abnormalities different kinds of glycolipids were stored in kidney, liver, and brain. The isoenzyme abnormalities can be reproduced in vitro by addition of chondroitin sulfate to a homogenate of normal liver, suggesting that stable binding occurs between mucopolysaccharides and the hydrolase molecules. After the addition of chondroitin sulfate, the total activity of β-galactosidase is inhibited, whereas other hydrolases are affected only slightly or not at all.*

Patients with mucopolysaccharidoses type 1, 2, and 3 show a striking reduction in the β-galactosidase activity in different organs (*1–3*). The diminished value for the β-galactosidase is rather specific as a number of other lysosomal enzymes—especially *N*-acetyl-β-hexosaminidase, β-glucuronidase, and α-fucosidase—show greatly enhanced values (*1*). The reduction of the β-galactosidase activity is correlated to the deficiency of one thermolabile, slow-moving isoenzyme fraction (*4*). Owing to a lack of experimental evidence for a relation between mucopolysaccharide accumulation and this rather specific deficiency of the β-galactosidase, no logical explanation so far was available. It seemed unlikely however that the deficiency of the β-galactosidase activity was the fundamental defect, because (i) it was found in genetically different diseases, and (ii) patients with GM_1 gangliosidosis, a severe disorder in which β-galactosidase is almost totally lacking, do not store or excrete excessive amounts of chondroitin sulfate B or heparan sulfate (*5*).

We now describe a generalized abnormality in the isoenzyme distribution of several lysosomal hydrolases, including β-galactosidase, and provide experimental evidence that this abnormality might be due to storage of excessive amounts of mucopolysaccharides. The patients included one (D.J.) with Hunter's disease and one (V.D.K.) with Sanfilippo disease type B (*6*). From patient D.J., autopsy material which had been stored at −20°C for about 2 months was examined; from patient V.D.K., a surgical biopsy piece of liver was studied. Total enzyme activities of