Supplementary Material Available: A listing of structure factor amplitudes, phases, and thermal parameters for individual atoms (4 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) Department of Biochemistry, University of Washington. Supported by National Science Foundation Grants GB 32233 and GB 18016 and by a grant of computer time from the University of Washington Computer Center. (b) Department of Chemistry, University of Washington.
(2) (a) G. Cilento and P. Guisti, *J. Am. Chem. Soc.*, **81**, 3801 (1959); (b) I. Isenberg and A. Szent-Gyorgyi, *Proc. Natl. Acad. Sci. U.S.A.*, **45**, 1229 (1959).
(3) E. M. Kosower, *J. Am. Chem. Soc.*, **78**, 3497 (1956).
(4) D. A. Deranleau, R. A. Bradshaw, and R. Schwyzer, *Proc. Natl. Acad. Sci. U.S.A.*, **63**, 885 (1969).
(5) R. A. Bradshaw and D. A. Deranleau, *Biochemistry*, **9**, 3310 (1970).
(6) F. M. Robbins and L. G. Holmes, *J. Biol. Chem.*, **247**, 3062 (1972).
(7) L. M. Hinman, C. R. Coan, and D. A. Deranleau, *J. Am. Chem. Soc.*, **96**, 7067 (1974).
(8) D. A. Deranleau, L. M. Hinman, and C. R. Coan, *J. Mol. Biol.*, **94**, 567 (1975).
(9) L. M. Hinman, C. R. Coan, and D. A. Deranleau, *Biochemistry*, **15**, 2212 (1976).
(10) H. Bosshard, Thesis, Swiss Federal Institute of Technology (E.T.H.), Zurich, 1970; S. Shifrin, *Biochim. Biophys. Acta*, **81**, 205 (1964).
(11) J. R. Herriott, A. Camerman, and D. A. Deranleau, *J. Am. Chem. Soc.*, **96**, 1585 (1974).
(12) R. Foster, "Organic Charge Transfer Complexes", Academic Press, New York, N.Y., 1969, pp 320 ff.
(13) J. M. Stewart, F. A. Kundall, and J. C. Baldwin, Computer Science Center Report, University of Maryland, Baltimore, Md., 1970.
(14) J. Berghuis, I. M. Haanappel, M. Potters, B. O. Loopstra, C. H. MacGillavary, and A. L. Veendaal, *Acta Crystallogr.*, **8**, 478 (1955).
(15) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(16) See paragraph at end of paper regarding supplementary material.
(17) D. Voet, *J. Am. Chem. Soc.*, **95**, 3763 (1973).
(18) J. L. Courbeils, B. Pullman, and Ph. Courriere, *Biochem. Biophys. Res. Commun.*, **44**, 1131 (1971).
(19) I. L. Karle, *Acta Crystallogr.*, **14**, 497 (1961).
(20) H. Koyama, *Z. Kristallogr. Kristallgeom. Kristallphys. Kristallchem.*, **118**, 51 (1963).
(21) J. D. Wright and G. S. D. King, *Acta Crystallogr.*, 7, 283 (1954).
(22) Charge distributions were taken from B. Pullman and A. Pullman, "Quantum Biochemistry", Interscience, New York, N.Y., 1963; G. Karreman, *Bull. Math. Biophys.*, **23**, 135 (1961); G. Maggoria, H. Johnson, and L. L. Ingraham, *Arch. Biochem. Biophys.*, **131**, 352 (1969).
(23) D. W. Jiles and D. W. Urry, *J. Biol. Chem.*, **243**, 4181 (1968); N. J. Oppenheimer, L. J. Arnold, and N. O. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 3200 (1971); J. R. Barrio, J. A. Secrist III, and N. J. Leonard, *ibid.*, **69**, 2039 (1972).

Proton Transfer Reactions of Methylglyoxal Synthase

Michael C. Summers and Irwin A. Rose*[1]

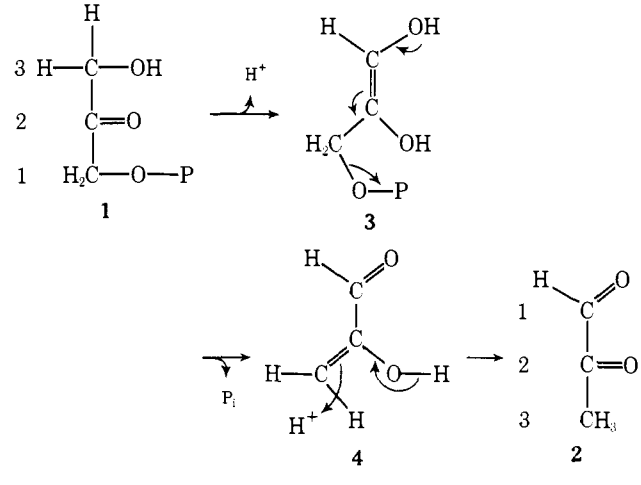
Contribution from The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

Received February 9, 1977

Abstract: *Escherichia coli* methylglyoxal synthase, which catalyzes the conversion of dihydroxyacetone-P to methylglyoxal, was shown to catalyze a stereospecific deprotonation of the *pro-S* hydrogen at C-3 of dihydroxyacetone-P (see Scheme II). Nonstereospecific protonation in the formation of the C-3 methyl group of methylglyoxal suggests that the true product of the enzymatic reaction is the enol form of methylglyoxal which is ketonized in solution. In agreement with this, methylglyoxal does not inhibit the enzyme.

Methylglyoxal synthase (also dihydroxyacetone-phosphate phospho-lase, EC 4.2.99.11) was discovered, purified, and studied in detail kinetically by Cooper and coworkers.[2-4] The enzyme, originally found to be apparently constitutive in *Escherichia coli* and other Enterobacteriaceae and also found in *Pseudomonas*, has recently been crystallized from *Proteus vulgaris*.[5] The role of this enzyme is not obvious since methylglyoxal is not known to have a function and may well be toxic. However, since the enzyme is very strongly inhibited by P_i it may be supposed that it serves to produce P_i from accumulated glycolytic stores in order that glyceraldehyde-3-P dehydrogenase can function.[3] With the well-known glyoxalase system, methylglyoxal synthase forms a bypass for the triose-P → lactate segment of the glycolytic path. Methylglyoxal synthase is specific for dihydroxyacetone-P and does not act on (*R,S*)-glyceraldehyde-3-P.[3] Under alkaline conditions dihydroxyacetone-P is known to produce methylglyoxal and P_i by way of an initial enolization followed by a 1,4 elimination (Scheme I).

Scheme I



If a similar reaction path is followed in the enzymic conversion of dihydroxyacetone-P to methylglyoxal it is of interest to ask the following questions. First, is the initial deprotonation to give the enediol intermediate **3** stereospecific? (The enediol of triose-P is believed to occur as an intermediate in both triose-P isomerase and metal-dependent aldolase reactions.) If so, what is the stereospecificity and does an isotope effect provide a suggestion of a rate-limiting step? Second, is there a stereochemical relationship between the first enolization step and formation of the methyl group of methylglyoxal which results from protonation of the intermediate **4**, i.e., is there a 1,3-proton transfer, and is formation of the methyl group stereospecific?

Experimental Section

E. coli methylglyoxal synthase was prepared from an ammonium sulfate fraction of glucose-grown cells available in this laboratory using steps similar to the procedure of Hopper and Cooper.[3] The enzyme

Table I. Stereospecificity of Proton Activation[a]

	[^{3}H]Dihydroxyacetone-P labeled by	
	Isomerase	Aldolase
[3-^{3}H]Dihydroxyacetone-P	21 000	57 300
CH_3COCHO	19 000	
CH_3CO_2H	100	1 050
HCO_2H	19 500	250

[a] One milliliter with (3*R*)-[3-^{3}H]dihydroxyacetone-P (0.55 mM), imidazole (50 mM, pH 7.0), and 0.1 unit of synthase were incubated for 20 min. With (3*S*)-[3-^{3}H]dihydroxyacetone-P (1.0 mM), 0.9 unit of synthase was used in 1.0 mL for 15 min. The isolated methylglyoxal was cleaved with a 5× excess of $NaIO_4$. The formic and acetic acids were adsorbed onto a column of silicic acid (1 × 6 cm); 60 mL of 2.5% 1-butanol/$CHCl_3$ followed by 50 mL of 5% 1-butanol were passed through the column. Acetic acid normally eluted between 35 and 45 mL of 2.5% 1-butanol/$CHCl_3$, while formic acid eluted between 15 and 30 mL of 15% 1-butanol/$CHCl_3$. The overall recovery of formic acid and acetic acid was 80–90%.

Table II. Rate of Labeled Product Formation from (3*R*)-[3-^{3}H] Dihydroxyacetone-P[a]

Time, min	^{3}H counts in EtOAc, cpm	Extent of reaction, %	CH_3COCHO formed, μmol	Expected counts in EtOAc if *pro-S* H activated
0	60	0	0	0
1	3000	17.0	0.14	2 900
5	7800	47.0	0.39	8 200
10	9000	54.0	0.45	9 400
20	9900	62.0	0.51	10 300

[a] In a total volume of 1.5 mL of imidazole (50 mM, pH 7.0) was incubated (3*R*)-[3-^{3}H]dihydroxyacetone-P (0.825 μmol) and methylglyoxal synthase (0.44 unit). After 0, 1, 5, 10, and 20 min, 0.05 mL was removed and treated with 2,4-dinitrophenylhydrazine solution (1% solution, 0.2 mL) and water (0.25 mL). After 15 min, the reaction mixture was treated with 10% sodium hydroxide (0.5 mL) and extracted with ethyl acetate (2.0 mL). The drastic slowing of the reaction results from product inhibition by P_i.

used had a specific activity of 15–40 units/mg. The preparation contained no detectable triose-P isomerase or aldolase activities.

The synthase reaction was monitored either colorimetrically by treating samples with 2,4-dinitrophenylhydrazine and determining the absorbance of the 2,4-dinitrophenylosazone in alkali[4] or by measuring the appearance of $^{32}P_i$ from dihydroxyacetone [^{32}P] phosphate by extraction of the molybdate complex.[6] The conditions of the latter assay caused release of less than 1% of the radioactivity associated with dihydroxyacetone [^{32}P]phosphate.

Tritiated forms of dihydroxyacetone-P were prepared in four ways: (a) isotope exchange with triose-P isomerase in ^{3}HOH; the labeled equilibrium mixture, 95% of which is (3*R*)-[3-^{3}H]dihydroxyacetone-P,[7] was purified on Dowex-1 Cl^- by elution with 0.03 N HCl; (b) (3*S*)-[3-^{3}H]dihydroxyacetone-P was prepared by exchange in ^{3}HOH with muscle aldolase previously treated with glycidol-P to inactivate the triose-P isomerase activity present in the preparation;[8,9] (c) (3*R*,*S*)-[3-^{3}H]dihydroxyacetone-P was obtained (as in Table IV) by reaction of glycerol-P dehydrogenase on (3*R*,*S*)-[3-^{3}H]glycerol-P prepared by reduction of glyceraldehyde-P with NaB^3H_4; (d) [1*S*-^{2}H,^{3}H]dihydroxyacetone-P was prepared by the series: [1-^{3}H]glucose-6-P was treated with phosphoglucose isomerase in D_2O (98.5 atom %) followed by phosphofructosekinase and ATP to give [1*S*-^{2}H,^{3}H]fructose-1,6-P_2,[10] and further reaction with aldolase and glyceraldehyde-P dehydrogenase in the presence of 10 mM arsenate.

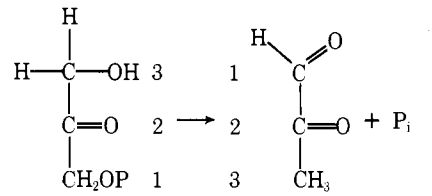
Dihydroxyacetone [^{32}P]phosphate was prepared by phosphorylation of dihydroxyacetone by glycerol kinase with [γ-^{32}P]ATP and was purified on Dowex-1 Cl^- by elution with 0.03 N HCl.

The distribution of tritium in methylglyoxal was determined by passing a reaction mixture through a Dowex-1 Cl^- column and oxidation of the radioactive peak in the effluent with a fivefold excess of $NaIO_4$ at pH 5.0. Formic acid from C-1 and acetic acid from C-2,3 of methylglyoxal were isolated on a silicic acid column by elution with $CHCl_3$ containing 2.5 and 5% 1-butanol, respectively.[10] They were assayed by titration with NaOH solution.

Results

Stereospecificity of C-3 Proton Abstraction. (3*R*)-[3-^{3}H]Dihydroxyacetone-P, synthesized by isotope exchange with triose-P isomerase in tritiated water, was converted to methylglyoxal with only slightly lower specific activity than starting material, Table I. The resulting methylglyoxal was cleaved with $NaIO_4$ to give the corresponding formic and acetic acids which were separated by column chromatography on silicic acid. Importantly, no radioactivity coeluted in the acetate peak indicating no "intramolecular" transfer of the *pro-R* hydrogen of dihydroxyacetone-P to the methyl group of methylglyoxal. In addition, the specific activity of the isolated formic acid approximated that of the methylglyoxal suggesting that proton activation at C-3 of dihydroxyacetone-P

Scheme II



is specific for the *pro-S* hydrogen (see Scheme II). When (3*R*)-[3-^{3}H]dihydroxyacetone-P was incubated with methylglyoxal synthase and the methylglyoxal, and after various time intervals converted to the dinitrophenylosazone, it was found that >95% of the radioactivity was ethyl acetate extractable, becoming so at the same rate as product was formed, Table II. This indicates the absence of a secondary isotope effect. However, this did not preclude the possibility of a nonstereospecific proton activation at C-3 with a large isotope discrimination. The latter possibility was examined further with the complementary sample of [3-^{3}H]dihydroxyacetone-P, i.e., (3*S*)-[3-^{3}H]dihydroxyacetone-P, prepared by isotope exchange with muscle aldolase in tritiated water, Table I. Timed samples were passed through short columns of Dowex-1 Cl^- to remove unreacted [3-^{3}H]dihydroxyacetone-P, and the methylglyoxal in the effluent was converted to the dinitrophenylosazone and extracted into ethyl acetate.

As shown in Table III, counts appeared in the water at the same rate as methylglyoxal was found indicating the absence of an appreciable isotope effect. Thus, at 1.0 min 47% of the tritium was found in the water at a time that 46% of the starting dihydroxyacetone-P was found as methylglyoxal. It follows from this that the activation at C-3 must be stereospecific for the *pro-S* hydrogen. A small amount of radioactivity was associated with the recovered methylglyoxal indicating the possibility of some "intramolecular" tritium transfer. When the methylglyoxal was degraded with $NaIO_4$ the radioactivity was associated with both formic and acetic acid. This is shown in Table I.

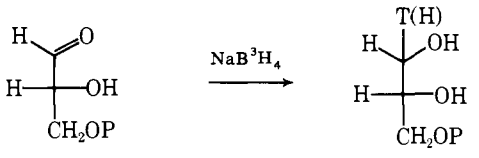
Further Test of Tritium Transfer. The recent report by Lowe and Pratt[12] that the muscle aldolase-activated labeling of dihydroxyacetone-P in D_2O introduces deuterium at C-1 at about 0.2% the rate found at C-3, in what is presumed to be a nonproductive side reaction, makes it likely that the label residing in the methyl group was already present at C-1 of dihydroxyacetone-P. This possibility was confirmed by two experiments. First, the previously prepared (3*S*)-[3-^{3}H] dihydroxyacetone-P was degraded with $NaIO_4$ to formalde-

Table III. Rate of Labeled Product Formation from (3*S*)-[3-^{3}H] Dihydroxyacetone-P[a]

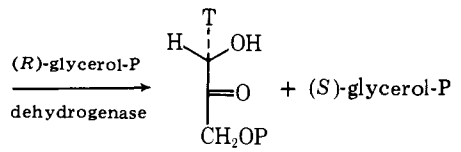
Time, min	μmol of CH_3COCHO	% reaction	Counts in water	Total % counts	Expected counts in water, if no isotope effect
0			600	1	
1	0.464	46.4	27 000	47	26 600
5	0.606	60.6	33 100	58	34 700
10	0.616	61.6	35 280	62	35 300
20	0.67	67	38 640	67	38 400

[a] The same conditions described in Table II except 1.0 μmol of (3*S*)-[3-^{3}H]dihydroxyacetone-P and 0.90 unit of methylglyoxal synthase were used. After derivitization and extraction of CH_3CO-CHO into ethyl acetate, the aqueous phase was vacuum transferred and the volatile counts were determined.

hyde and phosphoglycolate; the latter was isolated on Dowex-1 Cl^-. This material, in turn, was converted to glycolate with acid phosphatase at pH 5.4. The resulting glycolic acid was isolated by column chromatography on silicic acid eluting with $CHCl_3$ containing 15% 1-butanol.[11] The glycolic acid was assayed using 2,7-dihydroxynaphthalene.[13] The specific activities of the phosphoglycolate and glycolate were about 1% of the (3*S*)-[3-^{3}H]dihydroxyacetone, thus confirming the additional labeling of dihydroxyacetone-P at C-1 during treatment with muscle aldolase in tritiated water. In a second experiment, (3*R*)-[3-^{3}H]-dihydroxyacetone-P was generated by the sequence:



(*R,S*)-glyceraldehyde-P (*R,S*)-glycerol-P



The [3-^{3}H]glycerol-P was first isolated by ion exchange and then was treated with glycerol-P dehydrogenase together with lactate dehydrogenase, pyruvate, and NAD^+ to displace the equilibrium toward dihydroxyacetone-P for reaction with the synthase at pH 7.0. The isolated methylglyoxal was treated with $NaIO_4$ and the tritium distribution in the formate and acetate determined after purification on silicic acid. This is shown in Table IV. The acetate, representing the methyl group of methylglyoxal, had only ~0.02% the counts of formic acid indicating essentially no intramolecular transfer.

Stereospecificity of Formation of the CH_3 Group of Methylglyoxal. Dihydroxyacetone-P was prepared, labeled with both tritium and deuterium at C-1, to give an *S* configuration. The aforementioned material with synthase was subsequently converted in H_2O to [3-^{2}H,^{3}H]methylglyoxal which was cleaved with $NaIO_4$ to give [2-^{2}H,^{3}H]acetate. The chirality of the methyl group of the acetate was determined by taking advantage of the isotope effect of the malate synthase reaction with acetyl-CoA, followed by analysis of the malate for distribution of its tritium using the fumarase reaction.[14,15] As reported in Table V, the radioactivity was almost equally distributed between the 3*R* and 3*S* positions of [3-^{3}H]malate, ~2.2% favoring the *pro-R* position. Previously, the reaction

Table IV. Test for Intramolecular Proton Transfer in Formation of the CH_3 Group[a]

Sample	Sp act. cpm/μatom
Glycerol-P (before dilution)	5.9×10^7
CH_3COCHO (114 × diluted)	5×10^5
HCO_2H	5×10^5
CH_3CO_2H	110

[a] In 1.0 mL (2*RS*,3*RS*)-[3-^{3}H]glycerol-P (8.3 mM, 0.18×10^8 cpm/μmol), imidazole (pH 7.0, 50 mM), NAD^+ (7 mM), sodium pyruvate (5 mM), glycerol-P dehydrogenase (10 mL), lactate dehydrogenase (2 units), and methylglyoxal synthase (0.5 unit) were incubated for 30 min at 25 °C. The 0.28 μmol of methylglyoxal formed, 1.6×10^7 cpm at C-3 and an unknown number at C-1, was diluted 114-fold with 32 μmol of methylglyoxal, passed through Dowex-1 Cl^-, and treated with HIO_4 as usual.

Table V. Stereochemistry of Methyl Group Formation

CH_3COCHO formed[a]	Time 15 min	Time 60 min
μmol	0.15	0.21
% reaction	26%	36%
% counts in H_2O	5.3%	4.5%
Chirality analyses[b]		
Malate (cpm/μmol)	88 000	
Fumarase (volatile)	1770	1821
Fumarase (nonvolatile)	1810	1860

[a] (1*S*)-[1-^{2}H,^{3}H]Dihydroxyacetone-P (105 000 cpm/μmol, 0.58 mM), imidazole (pH 7.0, 50 mM), and 0.1 unit of methylglyoxal synthase were incubated in 1.0 mL. The methylglyoxal formed at 15 and 60 min had a specific activity of ~102 000 cpm/μmol and was analyzed as indicated. [b] The column purified malate samples were treated in 1.0 mL with 30 units of fumarase at pH 7.0 (KH_2PO_4 buffer, 0.1 M, pH 7.0) for 1 h, volatile counts were removed, and the procedure was repeated twice. Recovery of counts was 95 and 97%.

of chiral CH_3 of acetyl-CoA in the malate synthase reaction has given asymmetries of 77 and 79%[16,17] due to a rate preference in the cleavage of the C-H bond relative to the C-D bond of about 3.3-fold. The apparent slight dominance of the ^{3}H in the 3*S* position of malate, if indeed all of the unlabilized radioactivity is due to this, may therefore represent at most a 2.2/0.78 ≃2.8% chirality in the methyl group. Therefore, it would seem that ~97% of the methylglyoxal is produced by nonstereospecific protonation, and on the assumption of stereochemical consistency of enzyme reactions it is likely that formation of the methyl group is completely nonstereospecific. The slightly higher radioactivity observed in the residue after fumarase would then represent a 2% impurity.

The explanation usually favored for finding such nonstereospecificity is that the ketonization occurs after dissociation of an unstable product from the enzyme. Thus, the real product of the enzymatic reaction in the present case is the enol form **4** as shown in Scheme I. In previous examples[18,19] it was shown that the assumed product, the keto form, was not recognized by the enzyme as shown by a failure to undergo enzyme-catalyzed enolization, and in some cases not to act as an inhibitor. As will be noted in Table V, the counts in water do not increase between 15 and 60 min, suggesting that there is no detritiation of the [3-^{3}H]methylglyoxal subsequent to its formation, and probably not during its formation. Finally, the ability of methylglyoxal to act as an inhibitor was tested with concentrations up to 100 mM. At pH 7.0 and with 0.4 mM dihydroxyacetone [^{32}P]phosphate, the rate of appearance of $^{32}P_i$ was unaffected by methylglyoxal. Thus, the failure of the

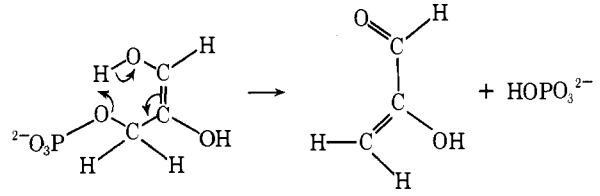
enzyme to recognize the end product as an inhibitor is consistent with the interpretation that the nonstereospecific formation of the methyl group is due to a postcatalytic event. Such an event, very likely the ketonization step shown in Scheme I, may be considered evidence that the proposed reaction mechanism is correct.

Discussion

Although showing the same stereospecificity for the α-CH_2 proton as both yeast and muscle aldolase, methylglyoxal synthase shows neither the metal requirement nor Schiff's base nature,[3] respectively, of these enzymes. This in itself does not argue against an enol mechanism, since many enzymes known to catalyze enolization processes do not require a metal cofactor nor use Schiff's base intermediates. The intermediate formation of the enol of triose phosphate **3** is inferred as perhaps the simplest chemical process. The absence of isotope discrimination suggests that formation of this intermediate and its irreversible conversion to product, Scheme I, are much more rapid than return of this intermediate to free dihydroxyacetone-P. The phosphorylated intermediate is probably not the form that dissociates from the enzyme because in this case nonstereospecific reversal to dihydroxyacetone-P would be expected and the apparent stereospecificity in the proton abstraction step would be lost. Likewise the formation of (*R,S*)-glyceraldehyde-3-P might be expected, but is not found.

The proton abstraction step is likely to require an amino acid, acting as general base, in common with many previously studied enolization reactions. The fate of this proton was of particular interest because many examples are known in which, by proton transfer, the enzyme is restored to its initial state for recycling as a catalyst: $E \rightleftharpoons E{\cdot}SH \rightleftharpoons H\text{-}E{\cdot}S^- \rightleftharpoons E{\cdot}HS \rightleftharpoons E$. In this case, a $1 \rightarrow 3$ allylic proton transfer would be required following the elimination of the $-OPO_3^{2-}$. Because neither a metal nor Schiff's base is involved in polarizing the carbonyl of dihydroxyacetone-P, it is likely that an electrophilic proton derived from an amino acid residue of the enzyme is required in the first step. Because of this, perhaps the enol intermediate, resulting after phosphate expulsion, would be completely uncharged. It would therefore be held to the highly polar active site only by hydrogen bonds derived from the catalytic groups and may dissociate from the enzyme too rapidly for stereospecific ketonization to take place. In what may be a very similar case[19] the conversion of phosphoenolpyruvate to oxalacetate by a bacterial enzyme is known to produce pyruvate as a side product without stereospecificity in formation of the CH_3 group. Pyruvate itself was not a substrate for enolization by the enzyme. It was concluded that the reaction intermediate, E·enolpyruvate, similar to enolpyruvaldehyde (**4**) in the present case, dissociated readily, and that ketonization occurred off the enzyme.

According to Scheme I, C-2 remains sp^2 throughout the reaction. Therefore, the carbon skeleton of the substrate would remain fixed except for slight changes as the other carbons go from sp^3 to sp^2. A preferred geometry of the enediol and the phosphate group might be defined with respect to each other if the original alcohol proton of the dihydroxyacetone-P absorbed the charge of the departing oxygen anion in a monomolecular process:



In this case the enediol would have to be a *trans*-enediol, as shown, rather than as drawn in **3** or as observed to occur in all the aldose-ketose isomerases.[20]

Acknowledgment. This work was supported by grants from the National Institutes of Health (GM-20940, CA-06927, and RR-05539) and also by an appropriation from the Commonwealth of Pennsylvania.

References and Notes

(1) Author to whom correspondence should be addressed.
(2) R. A. Cooper, *FEBS Lett.*, **13**, 213 (1971).
(3) D. J. Hopper and R. A. Cooper, *Biochem. J.*, **128**, 32 (1972).
(4) R. A. Copper, *Eur. J. Biochem.*, **44**, 81 (1974).
(5) P.-K. Tsai and R. W. Gracy, *J. Biol. Chem.*, **251**, 364 (1976).
(6) I. Berenblum and E. Chain, *Biochem. J.*, **32**, 295 (1938).
(7) S. V. Reider and I. A. Rose, *J. Biol. Chem.*, **234**, 1007 (1959).
(8) I. A. Rose and S. V. Reider, *J. Am. Chem. Soc.*, **77**, 5764 (1955).
(9) I. A. Rose and E. L. O'Connell, *J. Biol. Chem.*, **244**, 6548 (1969).
(10) I. A. Rose, *Methods Enzymol.*, **41**, 110 (1975).
(11) J. E. Varner, *Methods Enzymol.*, **3**, 397 (1959).
(12) G. Lowe and R. F. Pratt, *Eur. J. Biochem.*, **66**, 95 (1976).
(13) R. F. Lewis and S. Weinhouse, *Methods Enzymol.*, **3**, 272 (1957).
(14) J. W. Cornforth, J. W. Redmond, H. Eggerer, W. Buckel, and C. Gutschow, *Nature (London)*, **221**, 1212 (1969).
(15) J. Luthy, J. Retey, and D. Arigoni, *Nature (London)*, **221**, 1213 (1969).
(16) H. Lenz and H. Eggerer, *Eur. J. Biochem.*, **65**, 237 (1976).
(17) C. A. Townsend, T. Scholl, and D. Arigoni, *J. Chem. Soc., Chem. Commun.*, 921 (1975).
(18) H. P. Meloche and W. A. Wood, *J. Biol. Chem.*, **239**, 3505 (1964).
(19) J. M. Willard and I. A. Rose, *Biochemistry*, **12**, 5241 (1973).
(20) I. A. Rose, *Adv. Enzymol.*, **43**, 491 (1975).