Failure To Confirm Previous Observations on Triosephosphate Isomerase Intermediate and Bound Substrate Complexes†

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When ^{32}P-labeled dihydroxyacetone phosphate (DHAP) and rabbit muscle triosephosphate isomerase are mixed, a slow production of [^{32}P]P_i is observed that is enzyme catalyzed (Iyengar & Rose, 1981a). This finding has been confirmed and is attributed to instability of an enzyme-bound enediol phosphate intermediate, E·X, of the catalytic reaction (Scheme I). The proportion of the three enzyme-bound species at equilibrium when enough enzyme was used to complex all of the substrate was reported to be E·DHAP/E·X/E·D-G3P ≅ 1/0.1/1 on the basis of an analysis of the acid-quenched solution (Iyengar & Rose, 1981a,b; Rose & Iyengar, 1982, 1983). Bound substrates were analyzed by a mixed enzymatic and isotopic technique as free DHAP and D-glyceraldehyde 3-phosphate (G3P), and E·X was assayed as additional [^{32}P]P_i formed in the acid quench. The latter is based on the fact that β-elimination of the enediol phosphate is favored over conversion to DHAP and DL-G3P (Richard, 1984).

Recent attempts to reproduce these results have been unsuccessful; see Table I. The amount of G3P found was unexpectedly low, ~4% instead of ~50%. No [^{32}P]P_i was found greater than a control sample in which a 150-fold dilution of the incubation mixture was made to dissociate ligands from the enzyme prior to acidification.

Although concentrations of enzyme and substrate in the incubation were as reported previously and the enzyme was fully active in the standard assay, the failure to repeat results that were easily obtained earlier could be attributed to an unexpected inhibitor of the stoichiometric interaction necessary for the formation of enzyme-bound species. To test for this, we have compared the rate of the *catalyzed* $-OPO_3^{2-}$ elimination reaction as a function of the E_T/DHAP ratio at pH 7.5 with values reported earlier [Figure 3 of Iyengar & Rose (1981b)]. In fact, the rates obtained were at least 10-fold greater than those reported earlier, indicating that the active complex must have been formed.

The source of the enzyme used in these experiments, rabbit muscle triosephosphate isomerase, from Boehringer Mannheim, was the same as used in all previous papers. The methods of mixing acid with the incubation mixture were also the same. The use of a variety of mixing methods was investigated, but we have been unable to increase either [^{32}P]P_i or [^{32}P]G3P above the values reported in Table I. Dr. Iyengar, working recently in this laboratory, has been unable to reproduce these experiments either.

It remains to ask whether the apparent absence of the high G3P/DHAP ratio on isomerase and the lack of evidence for the expected unstable enediol phosphate intermediate in the acid-quenched reaction of triosephosphate isomerase represent the true state of the enzyme–ligand equilibria or if an enzyme with an intrinsic catalytic capacity as high as ~10^4 s^{-1} can be trapped in its neutral equilibrium state by rapid mixing with acid (Ray & Long, 1976a). Acid quench studies of enzymes with possibly somewhat less rapidly interconverted internal complexes have been reported in which central equilibria close to unity have been observed under a variety of quench conditions, suggesting that quenching may very easily preserve the native distribution of ligands (Ray & Long, 1976b; Barman et al., 1978; Wilkinson & Rose, 1979).

An additional correction worth citing concerns the pH dependence of the catalytic fragmentation of DHAP. Much higher rates are obtained in the neutral range, Figure 1, than reported previously. Using an estimate of 4 μM isomerase in rat muscle (Veech et al., 1969), one calculates that triose-

Scheme I

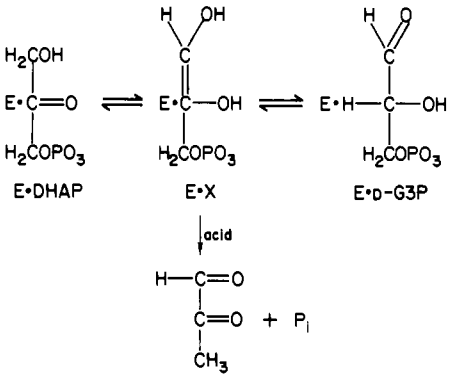

Table I: Relative Concentrations of Isomerase-Bound Species

	% of total ^{32}P present		
	DHAP	P_i	G3P
Iyengar & Rose, 1981a[a]			
pulse quenched directly	40.6	4.9	49.1
pulse diluted, then quenched	94.8	0.2	0.2
this report[b]			
pulse quenched directly	94.5	1.6	3.9
pulse diluted, then quenched	96.9	1.5	1.4

[a] Calculated from the data of Table IV of Iyengar & Rose (1981a). [b] Conditions similar to those in Table IV of Iyengar & Rose (1981a): A 20-μL pulse solution at 4 °C (25 nmol of rabbit muscle isomerase and 5 nmol of [^{32}P]DHAP, 8000 cpm/nmol in 0.06 M sodium cacodylate, pH 6.5) was quenched after 20 s either by injection into 0.4 mL of stirred Cl_3CCO_2H (0.5 N final concentration) or after 150-fold dilution in cacodylate buffer followed within 5 s by Cl_3CCO_2H (0.6 N final concentration). Unlabeled DHAP and G3P, 0.2 μmol each, were included in the quench acid. After removal of protein by centrifugation [^{32}P]P_i was determined on a sample, and the remainder was extracted with ether (1 volume, 3 times) and assayed for DHAP with glycerol-3-phosphate dehydrogenase and for G3P for which isomerase was then added. Samples were taken after each step for determination of additional [^{32}P]P_i found after treatment with alkali (0.5 N NaOH, 15 min, 37 °C).

† From the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received June 6, 1984.* This work was supported by U.S. Public Health Service Grants GM-20940 (I.A.R.), CA-06927, and RR-05539 (Institute for Cancer Research) and also by an appropriation from the Commonwealth of Pennsylvania.

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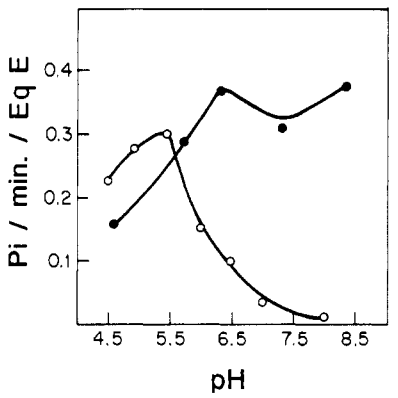

FIGURE 1: Effect of pH on fragmentation of DHAP by triosephosphate isomerase, repeating Figure 3 of Iyengar & Rose (1981a) (symbol O represents $[^{32}P]P_i$ formed in that report as calculated from a 1-h incubation). In the current experiments, represented by symbol ●, the mixture (0.5 mL) contained DHAP (0.94 mM, 5×10^4 cpm) and triosephosphate isomerase subunits (20 μM) in buffer (pH 4.5, 0.02 M sodium acetate; pH 5.5–6.5, 0.02 M sodium cacodylate; pH 7.5–8.5, 0.1 M triethanolamine hydrochloride).

phosphate isomerase would be a significant source of methylglyoxal, ~0.25 μmol h^{-1} (g of wet muscle)$^{-1}$ over the whole range of muscle pHs.

The results of an additional study reporting the ability of triosephosphate isomerase, methylglyoxal synthase, and K_3-$Fe(CN)_6$ to utilize enediol phosphate generated in situ from L-G3P (Iyengar & Rose, 1983) can also not be repeated. A separate report of our inability to confirm these observations is to appear elsewhere (Rose, 1984).

I deeply regret the confusion and evident misinformation introduced into the literature by these papers.

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