BBA 63295

Kinetics of triose phosphate isomerase

Although the glycolytic enzyme triose phosphate isomerase (D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) is one of the most active enzymes known[1], there have been few estimates of its kinetic parameters. We now report provisional values for the maximum rates and Michaelis constants, and draw attention to a snag hitherto overlooked. This is that in the assay[2] with glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12) as auxiliary enzyme, the arsenate used inhibits triose phosphate isomerase. It is only when this inhibition is allowed for that the Haldane relation between the kinetic parameters is satisfied. From the values given in Table I, the estimated equilibrium constant is 22 (the equilibrium being in favour of dihydroxyacetone phosphate); this agrees satisfactorily with the values of 24 (ref. 3) and of 28 (ref. 4) obtained by determination of the amounts of substrates at equilibrium. This agreement suggests that the values given in Table I are probably reliable, despite the fact that the estimation of kinetic parameters from coupled assays is theoretically somewhat uncertain. The Michaelis constant for glyceraldehyde phosphate in Table I is based on the concentration of the D isomer; the presence of the L isomer is assumed to have no effect. The values of the parameters can be used to obtain lower limits for the unimolecular and bimolecular rate constants[5]; the order of magnitude of the latter is 10^7 $M^{-1} \cdot sec^{-1}$, and so the rate of combination of enzyme with substrate is diffusion-controlled. This estimate is based on the assumption that a sub-unit of molecular

TABLE I

KINETIC PARAMETERS OF TRIOSE PHOSPHATE ISOMERASE

	Glyceraldehyde 3-phosphate to dihydroxyacetone phosphate	*Dihydroxyacetone phosphate to glyceraldehyde 3-phosphate*
Maximum rate (units/mg of protein)	6700*	590*
Michaelis constant (mM)	0.46*	0.87*
	0.39**	
	0.35***	
	0.5†	
K_i, arsenate (mM)		5.5*
K_i, phosphate (mM)	6*	
K_i, DL-α-glycerophosphate (mM)	0.23*	

* Present work. Rabbit muscle triose phosphate isomerase, pH 7.8, 25°. The reaction mixtures contained (final concentrations) in the left-hand column: NADH (83 μM), DL-glyceraldehyde 3-phosphate (0.21–1.04 mM), α-glycerophosphate dehydrogenase (2 μg/ml), triose phosphate isomerase (20 mμg), triethanolamine hydrochloride (20 mM), and EDTA (5.4 mM); in the right-hand column: NAD^+ (1 mM), dihydroxyacetone phosphate (0.2–2 mM), glyceraldehyde 3-phosphate dehydrogenase (33 μg/ml), sodium arsenate (6 mM), triose phosphate isomerase (0.2 μg), triethanolamine hydrochloride (21 mM) and EDTA (5.4 mM).

** Calf muscle[8].

*** Human etythrocytes[9].

† Bovine lens[10].

weight 30 000 (ref. 6) has one active site, and that there is no interaction between active sites.

Table I also records inhibition constants for several substances, all of which behaved as competitive inhibitors. The L isomer of α-glycerophosphate gave little or no inhibition at low concentrations; thus the inhibition by DL-α-glycerophosphate is due to the 'unnatural' D isomer, which is configurationally related to the substrate D-glyceraldehyde 3-phosphate, and the value for K_i given in Table I should be halved. The inhibition by high concentrations of substrate[7] does not operate in the concentrations used here.

We thank the Medical Research Council for financial support.

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Received November 17th, 1967