The pK of the Carboxyl Group at the Active Centre of Triose Phosphate Isomerase

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To obtain a reliable estimate of the pK of a group at the active centre of an enzyme is not easy, yet such information is quite valuable, for it provides information about the environment of the active centre. Schmidt & Westheimer (1971) suggested that the pH–rate profile of a specific inactivation could with advantage be used to provide such an estimate. The present communication describes the application of this method to triose phosphate isomerase (EC 5.3.1.1).

Rose & O'Connell (1969) found that the rabbit muscle enzyme was specifically inactivated by glycidol (2,3-epoxypropanol) phosphate. The epoxide reacts with the carboxylate group of a glutamic acid residue (Waley *et al.*, 1970; Miller & Waley, 1971). The same group also reacts specifically with bromo- or chloro-hydroxyacetone phosphate (Coulson *et al.*, 1970; Hartman, 1971). This group fulfils the usual criteria for being considered to be at the active centres of the enzyme, and may well be the base that (in catalysis) abstracts a proton from C-1 of dihydroxyacetone phosphate or C-2 of D-glyceraldehyde 3-phosphate (Rose, 1970).

The pH–rate profile for the reaction of the enzyme with glycidol phosphate is shown in Fig. 1. The solid line is calculated from the equation:

$$k_{\text{obs.}} = k_{\text{A-}} / (1 + [\text{H}^+] / K_{\text{A}})$$

where $k_{\text{obs.}}$ is the rate constant obtained from the measured rates and $k_{\text{A-}}$ is the rate constant for the anionic species of the acid HA whose ionization constant is K_{A} . The values of $k_{\text{A-}}$ and K_{A} were obtained from a plot of $k_{\text{obs.}}$ against $k_{\text{obs.}} \cdot [\text{H}^+]$ (Hofstee, 1960); the theoretical line is then most easily obtained from a plot of $\log[(k_{\text{A-}}/k_{\text{obs.}}) - 1]$ against pH. The value of the pK_A used for the line in Fig. 1 is 5.64. The rate of reaction was proportional to the concentration of glycidol phosphate over the range tested (0.05–0.3 mM); the rate of inactivation is half maximal at 1.2 mM-glycidol phosphate (Rose & O'Connell, 1969). Schmidt & Westheimer (1971) have shown that, for several plausible mechanisms, the apparent pK of the reactive group is directly obtained from the pH–rate profile as long as the kinetics are first-order in reagent. The ionic strength in my experiments was maintained at 0.2 M and the true pK would be higher than the value given. Thus this work suggests that the pK of the carboxyl group is about 6. An unperturbed carboxyl group (of

glutamic acid) is expected to have pK about 4.5 (Nozaki & Tanford, 1967) and the pK of glutamic acid residue 35 in lysozyme, which is in a non-polar environment, is 5.9 (Parsons & Raftery, 1970), and so the carboxyl group in triose phosphate isomerase may also be in a non-polar environment. The pK is consistent with the group's being the base that abstracts

Fig. 1. pH–rate profile for the reaction of glycidol phosphate with triose phosphate isomerase

Rabbit muscle enzyme [from Boehringer Corp. (London) Ltd., London W.5, U.K.] was diluted 50-fold with, and then dialysed against, assay buffer (20 mM-triethanolamine hydrochloride–5.4 mM-EDTA adjusted to pH 7.8) and then diluted 30-fold with the buffer to be used (final protein concn. 7 µg/ml). Glycidol phosphate (Rose & O'Connell, 1969) (kindly provided by Dr. I. A. Rose) (0.05 ml of 1.5 mg/ml solution of the barium salt) was added to 1 ml of the enzyme solution, and the mixture was kept at 25°C. Samples (5 µl) were withdrawn for assay, which was carried out as described by Burton & Waley (1966), except that the concentration of NADH was 83 µM and the α-glycerophosphate dehydrogenase was dialysed to remove SO₄²⁻ ions. A separate portion of enzyme was kept without glycidol phosphate, to check its stability. Duplicate experiments were carried out at each pH. The buffers were 0.1 M both in triethanolamine hydrochloride and in lutidine, adjusted to the required pH with diluted BDH Chemicals Ltd. (Poole, Dorset, U.K.) Aristar HCl, and enough 1 M-NaCl was added to make the ionic strength 0.2 M. The solid line is calculated as described in the text.

a carbon-bound proton during catalysis by the enzyme; the occurrence of isotopic exchange with the medium during catalysis provides an upper limit to the value of the pK of the catalytic group (Rose, 1970). This limit is about pK 6, and indeed B. Plaut & J. R. Knowles (personal communication) find a pK value of 6 from the kinetics of catalysis (in both directions) by the enzyme from chicken muscle. The difference between this value and the higher value (pK 7.35) reported by Wolfenden (1970) for the enzyme from rabbit muscle seems unexpectedly large.

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