HALSALL and C. H. DOY, unpublished results) we are not yet prepared to state that separate, functionally independent isoenzymes exist. In *Bacillus subtilis* control differs in that the deactivating modifiers are chorismate and prephenate[13]. Work concerning the nature of DAHP synthase in micro-organisms is at present under review[3].

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Isocitrate lyase: Determination of K_m values and inhibition by phosphoenolpyruvate

Published accounts of isocitrate lyase (*threo*-D$_s$-isocitrate glyoxylate-lyase, EC 4.1.3.1) give diverse values for the K_m of the enzyme and for the extent of the inhibition by phosphoenolpyruvate (PEP). SMITH AND GUNSALUS[1] cited a K_m value of 0.45 mM *threo*-D$_s$(+)-isocitric acid for the enzyme from *Pseudomonas aeruginosa* and similar values have been reported from other micro-organisms[2–4]. In contrast, K_m values some 10 times smaller have been found chiefly by KORNBERG and his colleagues[5–7]. It is noticeable that nearly all the low values were obtained using a continuous spectrophotometric assay[8] while the higher values were obtained by methods that estimated the glyoxylate formed from isocitrate during a standard period of incuba-

Abbreviation: PEP, phosphoenolpyruvate.

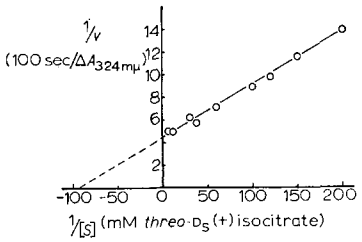
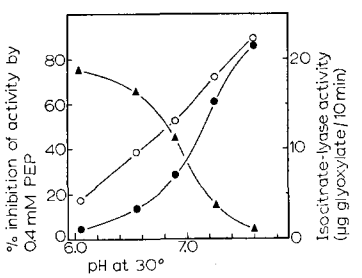


Fig. 1. Lineweaver–Burk plot of the relationship between substrate concentration and the activity of isocitrate lyase from *P. indigofera*. The organisms were grown as described by SHIIO, SHIIO AND MCFADDEN[15] and broken ultrasonically. The 100 000 × *g* supernatant was taken and the fraction precipitating between 45 and 55% saturation with ammonium sulphate dissolved in 0.05 M triethanolamine buffer, pH 7.4, containing 1 mM EDTA and 5 mM dithiothreitol, and used as the enzyme. Enzyme assays were carried out by the method of KORNBERG[8] at pH 6.8 using *threo*-$D_S(+)$-isocitric acid (*i.e.*, $L_S(+)$-isocitric acid) as substrate. The K_m value calculated from extrapolation of the graph is 0.011 mM.

tion. Using partially purified isocitrate lyase from *Chlorella pyrenoidosa*[9] we determined K_m values by both methods. The continuous assay showed clearly that the enzyme had a high affinity for the substrate and that at low substrate concentrations a constant rate of activity continued for less than 60 sec before the rate declined. From such assays, the K_m value was 0.023 mM *threo*-D_S-(+)-isocitrate[9]. From assays in which glyoxylate formed during 10-min incubation was determined, higher but divergent and hence unreliable, K_m values were obtained. We determined by continuous spectrophotometric assay, the K_m value of isocitrate lyase from *Pseudomonas indigofera* (NCIB 9441, strain 1M) which MCFADDEN AND HOWES[4] cited as 0.82 mM. Our value was 0.011 mM (Fig. 1). Clearly K_m values determined other than by continuous spectrophotometric assay are suspect.

KORNBERG and his colleagues[6,10] found that isocitrate lyase was very sensitive to inhibition by PEP; others[11,12] have found the enzyme much less sensitive.



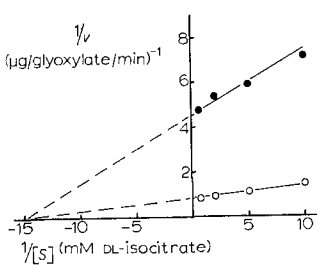


Fig. 2. The effect of pH on the activity of isocitrate lyase from *C. pyrenoidosa* in the presence (●—●) and absence (○—○) of 0.4 mM PEP. The % inhibition of activity by 0.4 mM PEP is also shown (▲—▲). The assay mixture contained 25 mM imidazole; 5 mM $MgCl_2$; 1 mM EDTA; and 10 mM DL-isocitrate. Glyoxylate formed during 10-min incubation at 30° was estimated by the method of KRAMER, KLEIN AND BASELICE[16]. The enzyme used was partially purified by ammonium sulphate precipitation[9].

Fig. 3. Lineweaver–Burk plot of relationship between velocity of isocitrate lyase (from *C. pyrenoidosa*) reaction and substrate concentration in presence (●—●) and absence (○—○) of 0.3 mM PEP. The assay mixture contained 25 mM imidazole, pH 6.8; 5 mM $MgCl_2$ and 1 mM EDTA. Glyoxylate formed during 10-min incubation at 30° was estimated by the method of KRAMER, KLEIN AND BASELICE[16].

KORNBERG[8] routinely assayed the enzyme at pH 6.8, other workers generally used about pH 7.4. This difference in pH markedly affects the degree of inhibition by PEP of the enzyme from *C. pyrenoidosa* (Fig. 2). At pH 6.6, 0.4 mM PEP inhibited enzyme activity by almost 70% while at pH 7.25, it inhibited by only 15%. The activity of the enzyme was also lower at pH 6.6; consequently in the presence of 0.4 mM PEP, the activity of the enzyme at pH 6.6 is only 25% of its activity at pH 7.25 (Fig. 2). Inhibition by PEP showed non-competitive kinetics as others[6,10] have shown (Fig. 3).

Inhibition by PEP was half-maximal at around pH 6.9 (Fig. 2). This is sufficiently above the p*K* of ionization of the phosphate group of PEP, which KIESSLING[13] gives as 6.38, to make it unlikely that pH is affecting inhibition through this ionization. It is clear that in considering the extent of isocitrate lyase inhibition by PEP, pH is of prime importance. The effect of pH on the inhibition of *Escherichia coli* isocitrate lyase may differ from that of Chlorella enzyme since ASHWORTH[14] found the enzyme from *E. coli* B_mRS became less sensitive to PEP only at pH values above 8.0.

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