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Purification and regulatory properties of isocitrate lyase from *Escherichia coli* ML308

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Isocitrate lyase was purified to homogeneity from *Escherichia coli* ML308. Its subunit M_r and native M_r were 44670±460 and 177000–180000 respectively. The kinetic mechanism of the enzyme was investigated by using product and dead-end inhibitors of the cleavage and condensation reactions. The data indicated a random-order equilibrium mechanism, with formation of a ternary enzyme–isocitrate–succinate complex. In an attempt to predict the properties of isocitrate lyase in intact cells, the effects of pH, inorganic anions and potential regulatory metabolites on the enzyme were studied. The K_m of the enzyme for isocitrate was 63 μM at physiological pH and in the absence of competing anions. Chloride, phosphate and sulphate ions inhibited competitively with respect to isocitrate. Phosphoenolpyruvate inhibited non-competitively with respect to isocitrate, but the K_i value suggested that this effect was unlikely to be significant in intact cells. 3-Phosphoglycerate was a competitive inhibitor. At the concentration reported to occur in intact cells, this metabolite would have a significant effect on the activity of isocitrate lyase. The available data suggest that the K_m of isocitrate lyase for isocitrate is similar to the concentration of isocitrate in *E. coli* cells growing on acetate, about one order of magnitude higher than the K_m determined *in vitro* in the absence of competing anions.

INTRODUCTION

During growth of micro-organisms on acetate, the precursors necessary for biosynthesis are generated by carbon flux through the glyoxylate bypass, comprising the enzymes isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2) [1]. In *Escherichia coli* and other enteric bacteria, the competition between the bypass and the tricarboxylic acid cycle at the level of isocitrate is resolved by partial phosphorylation and inactivation of isocitrate dehydrogenase (EC 1.1.1.42) [2–5].

The control of flux at this branch-point has been analysed in great detail both theoretically and experimentally [4–9]. Briefly, the phosphorylation of isocitrate dehydrogenase is thought to render this enzyme rate-limiting in the tricarboxylic acid cycle and thus to raise the intracellular concentration of isocitrate; this in turn would increase the flux through isocitrate lyase (e.g. [9,10]). The phosphorylation state of isocitrate dehydrogenase is regulated by a number of metabolites, including isocitrate, several biosynthetic precursors and the adenine nucleotides [11,12]. These or other metabolites might also affect the control of flux by virtue of effects on isocitrate lyase or malate synthase. Indeed, studies of crude extracts of *E. coli* showed that phosphoenolpyruvate, one of the effectors of the phosphorylation state of isocitrate dehydrogenase, is an inhibitor of isocitrate lyase [13]. It was suggested that the effects of phosphoenolpyruvate on isocitrate lyase might be physiologically significant [13], though later measurements of the intracellular concentration of this metabolite argued against the possibility [14]. However, until very recently [15], no details of the purification of *E. coli* isocitrate lyase had been reported, and its kinetic and regulatory properties have not been examined systematically.

In this paper we report the purification and physical characterisation of isocitrate lyase from *E. coli* ML308. We also present a detailed analysis of its kinetic mechanism and its regulatory properties, and assess whether changes in the intracellular concentration of isocitrate and other metabolites could affect flux through the enzyme.

EXPERIMENTAL

Materials

The active form of isocitrate dehydrogenase was prepared from *E. coli* ML308 grown on glycerol as described previously [16]. Glyoxylic acid, carbonic anhydrase, catalase, ferritin, fumarase and ovalbumin were from Sigma Chemical Co., Poole, Dorset, U.K. Other proteins used as M_r markers, Sephacryl S-300, phenyl-Sepharose and Mono Q columns were from Pharmacia, Milton Keynes, Bucks., U.K. Lactate dehydrogenase (from pig heart, in glycerol) and nicotinamide nucleotides were from Boehringer Corp. (London), Lewes, Sussex, U.K. Phenylhydrazine and succinic acid were from BDH Chemicals, Poole, Dorset, U.K. Other materials were obtained as described previously [16,17].

Assay methods

During the purification and for some kinetic experiments, isocitrate lyase was assayed by coupling the formation of glyoxylate to the oxidation of NADH by using lactate dehydrogenase as described previously [18]. In some kinetic experiments, isocitrate lyase was assayed

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Table 1. Purification of isocitrate lyase from *E. coli* ML308

Step		Protein (mg)	Enzyme activity (μmol/min)	Specific activity (μmol/min per mg)	Yield (%)	Purification factor
1.	Crude extract	1650	1250	0.76	100	1.0
2.	Protamine sulphate treatment	1468	1223	0.83	98	1.1
3.	$(NH_4)_2SO_4$ precipitation	461	1096	2.4	88	3.2
4.	Acid treatment	217	920	4.2	74	5.5
5.	Gel filtration	72	792	11.0	63	14.5
6.	Hydrophobic chromatography	19	531	27.9	42	36.7
7.	Mono Q chromatography	12	450	37.5	36	49.3

by using a modification of the assay of Dixon & Kornberg [19]. Each cuvette contained, in a final volume of 1 ml, 50 mM-Mops/NaOH, pH 7.3, 5 mM-$MgCl_2$, 1 mM-EDTA, 4 mM-phenylhydrazine hydrochloride, isocitrate as indicated and enzyme. Formation of the phenylhydrazine derivative of glyoxylate was monitored at 334 nm. In some experiments the assay buffer was changed to 50 mM-Mops/NaOH, pH 6.8, or 50 mM-Mes/NaOH, pH 6.3.

The condensation reaction catalysed by isocitrate lyase was measured by coupling the formation of isocitrate to the reduction of $NADP^+$ by using isocitrate dehydrogenase. Each cuvette contained, in a final volume of 1 ml, 50 mM-Mops/NaOH, pH 7.3, 5 mM-$MgCl_2$, 1 mM-EDTA, 0.4 mM-$NADP^+$, 6 μg of isocitrate dehydrogenase (0.6 μmol/min in these conditions), succinate and glyoxylate as indicated and enzyme.

In all kinetic experiments except Fig. 7, assays were carried out in duplicate, or in quadruplicate at low isocitrate concentrations; where only a single point is shown, the results obtained were identical. Line fitting was as described previously [20]. The substrate of isocitrate lyase is *threo*-D_s-isocitrate, and *threo*-L_s-isocitrate is neither a substrate nor an inhibitor of the enzyme (see, e.g., [21]). *threo*-DL-Isocitrate was used throughout these studies, and all quoted concentrations refer to those of the true substrate, *threo*-D_s-isocitrate.

Protein concentrations and alkali-labile phosphate content were estimated by published methods [22,23].

Purification of isocitrate lyase

E. coli ML308 (A.T.C.C. 15224) was grown on a mineral-salts solution supplemented with 40 mM-sodium acetate [24] and harvested as described previously [16]. Steps 1–6 were carried out at 0–4 °C and Step 7 at room temperature.

Steps 1 and 2: extraction and treatment with protamine sulphate. These were carried out as described for the purification of isocitrate dehydrogenase kinase/phosphatase [17].

Step 3: fractionation with $(NH_4)_2SO_4$. To the supernatant from step 2, powdered $(NH_4)_2SO_4$ was added slowly to give 30% saturation while the pH was maintained in the range 6.3–7.3 by addition of 5 M-NH_3. The material was stirred for 30 min and centrifuged at 40000 *g* for 10 min, and the pellet was discarded. Further $(NH_4)_2SO_4$ was added to the supernatant to give 45% saturation, and the material was stirred and centrifuged as above. The pellet was dissolved in a small volume of Buffer B (50 mM-Mops/NaOH, pH 7.3, 1 mM-EDTA, 1 mM-benzamidine hydrochloride, 1 mM-dithiothreitol, 1.2 mM-phenylmethanesulphonyl fluoride).

Step 4: acid treatment. The redissolved pellet was gradually adjusted to pH 4.5 by the careful addition of 1 M-acetic acid with stirring. The material was centrifuged at 100000 *g* for 15 min, and the supernatant was re-adjusted to pH 7.3 by the careful addition of 1 M-KOH with stirring.

Step 5: gel filtration. The acid-soluble material was chromatographed overnight on a column (2.2 cm × 71 cm) of Sephacryl S-300 (superfine grade), equilibrated with Buffer B, at a flow rate of 10 ml/h. Fractions (3 ml) containing isocitrate lyase activity were pooled, up to and including the fraction after the peak (Fig. 1).

Step 6: chromatography on phenyl-Sepharose. Powdered $(NH_4)_2SO_4$ was added to the pooled fractions to give a concentration to 0.6 M. The solution was loaded on to a column (1.4 cm × 7 cm) of phenyl-Sepharose equilibrated with Buffer B containing 0.6 M-$(NH_4)_2SO_4$. The column was washed with this buffer and developed with a linear gradient of 0.6–0 M-$(NH_4)_2SO_4$ in Buffer B (total volume 100 ml). The flow rate was 30 ml/h, and 2.5 ml fractions were collected. Active fractions were pooled and dialysed for 4 h against 2 × 2 litres of Buffer B without phenylmethanesulphonyl fluoride.

Step 7: ion-exchange chromatography on Mono Q. The dialysed material was chromatographed in four batches on an HR5/5 Mono Q column with the Fast Protein Liquid Chromatography apparatus. The column was developed with a 0–0.5 M-NaCl gradient in Buffer B without phenylmethanesulphonyl fluoride (0–0.22 M over 10 min, 0.22–0.5 M over 20 min) at a flow rate of 1 ml/min; 0.5 ml fractions were collected. Isocitrate lyase was eluted from the column at 0.4–0.45 M-NaCl. Fractions containing homogeneous enzyme, as assessed by polyacrylamide-gel electrophoresis, were pooled and dialysed overnight at 4 °C into Buffer B without phenylmethanesulphonyl fluoride but containing 40% (v/v) glycerol. The enzyme was stored at −20 °C.

Polyacrylamide-gel electrophoresis

Electrophoresis in 10% polyacrylamide slab gels in the presence of 0.1% SDS was carried out by the method

of Laemmli [25]. The subunit M_r markers were: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; trypsin inhibitor, 20100; α-lactalbumin, 14400.

Estimation of native M_r

Sedimentation equilibrium centrifugation was performed at 8000 rev./min as described previously [16] with isocitrate lyase that had been dialysed exhaustively against 50 mM-Mops/NaOH (pH 7.3)/1 mM-EDTA/1 mM-dithiothreitol. The partial specific volume of isocitrate lyase was estimated from its amino acid composition [26] to be 0.73 ml/g (C. MacKintosh & H. G. Nimmo, unpublished work).

Gel filtration was carried out with a 1.6 cm × 75 cm column of Sephacryl S-300 equilibrated in 100 mM-potassium phosphate (pH 7.5)/2 mM-dithiothreitol at a flow rate of 20 ml/h. The M_r markers were: ferritin, 450000; catalase, 240000; fumarase, 194000; aldolase, 158000; bovine serum albumin, 67000; carbonic anhydrase, 30000; chymotrypsinogen A, 25000; cytochrome *c*, 12500.

RESULTS AND DISCUSSION

Purification and characterization of isocitrate lyase

A typical purification of isocitrate lyase from *E. coli* ML308 is summarized in Table 1. The procedure is rapid, convenient and reproducible. The Sephacryl S-300 step is carried out overnight and 10–12 mg of enzyme can be obtained from 20–25 g (wet wt.) of cells in 2 days. The yield and final purification factor were in the ranges 25–46% and 47–60 respectively. The final material can be stored at −20 °C for at least 6 months with no loss of activity.

The results of a typical Sephacryl S-300 gel-filtration column are shown in Fig. 1. Careful pooling of fractions at this step is essential, in order to remove a contaminating protein of subunit M_r 80000 which co-migrated with isocitrate lyase on many other chromatographic systems. This protein was eluted after isocitrate lyase on gel filtration, so only the isocitrate lyase-containing fractions up to and including the fraction after the peak were pooled. The purified isocitrate lyase was homogeneous, as judged by the criteria of denaturing (Fig. 2) and non-denaturing (results not shown) polyacrylamide-gel electrophoresis, analytical ultracentrifugation and gel filtration (results not shown).

The subunit M_r of isocitrate lyase, determined by SDS/polyacrylamide-gel electrophoresis, was 44670±460 ($n = 7$). Its M_r under non-dissociating conditions was 177000 as judged by gel filtration on a calibrated column of Sephacryl S-300, and 180000 as judged by sedimentation-equilibrium ultracentrifugation (results not shown). These results indicate that, like the enzyme from several other species [27], the isocitrate lyase of *E. coli* is a tetramer of subunits that are probably identical.

Robertson & Reeves [15] have reported the purification of isocitrate lyase from another strain of *E. coli*. The M_r values that they reported (48000 and 188000 under dissociating and non-dissociating conditions respectively) are similar to the values found in our study. However, they reported a specific activity for the purified enzyme at 2.4 μmol/min per mg at 25 °C, considerably lower than the value of 37.5 μmol/min per mg found by us (Table 1).

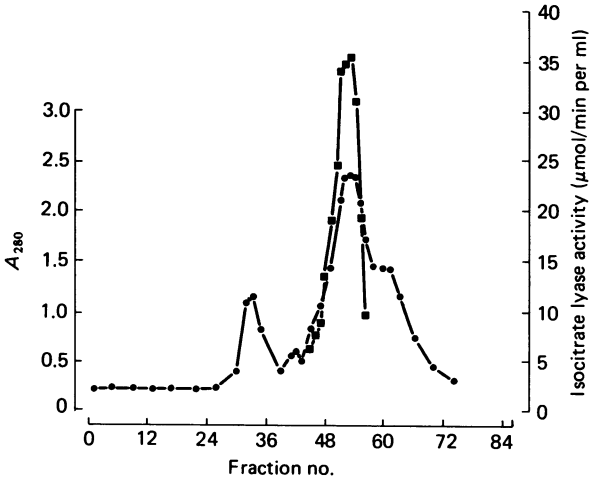

Fig. 1. Gel filtration of isocitrate lyase from *E. coli* ML308

The column was run as described in the Experimental section. ■, Isocitrate lyase activity; ●, A_{280}. Fractions 47–54 were pooled (see the text).

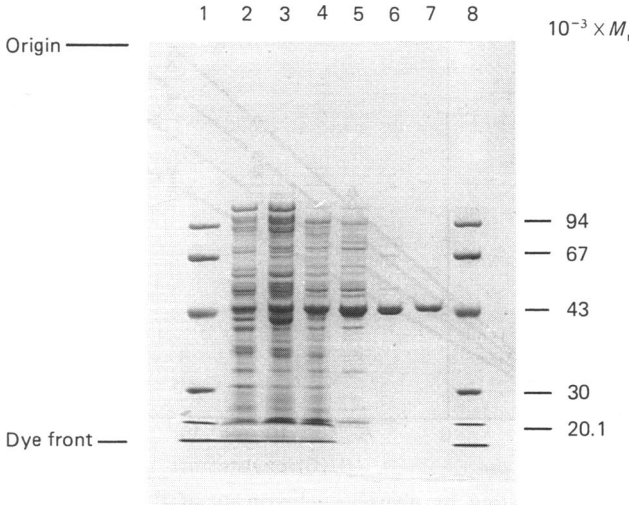

Fig. 2. Purification of isocitrate lyase from *E. coli* ML308

Tracks 1 and 8, M_r markers. The numbered bars indicate the M_r values ($\times 10^{-3}$). Tracks 2–7, enzyme after steps 1, 3, 4, 5, 6 and 7 respectively.

This difference could reflect a strain difference, or could be due to the low ionic strength (10 mM-Tris/HCl, pH 7.5) in the assays carried out by Robertson & Reeves [15].

The alkali-labile phosphate content of our purified enzyme was very low, below the limits imposed by the sensitivity of the assay procedure (about 0.1 mol/mol of subunits). Robertson *et al.* [28] have suggested that active isocitrate lyase might contain an acid-labile phosphate group. Our results do not bear on this question, because only acid-stable, alkali-labile, phosphate groups (the derivatives of serine and threonine) are detected by our method.

Kinetic mechanism of isocitrate lyase

Linear double-reciprocal plots were obtained for the condensation reaction when either glyoxylate or succinate

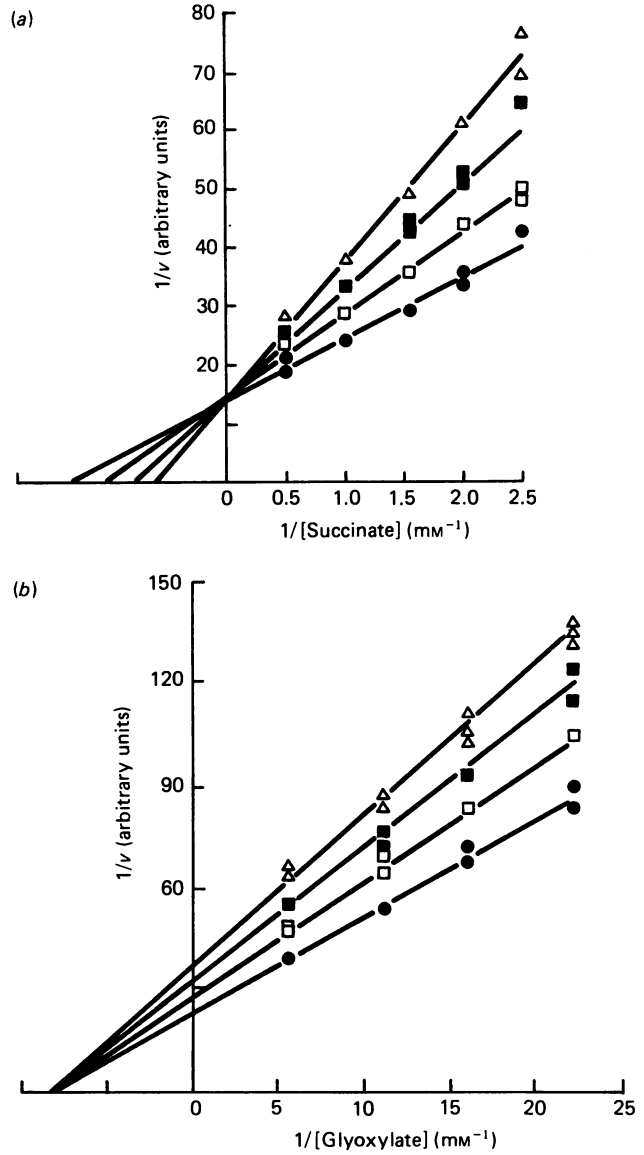

Fig. 3. Inhibition by phosphoenolpyruvate of the condensation reaction catalysed by isocitrate lyase

(*a*) Double-reciprocal plots of initial velocity against succinate concentration at 0.045 mM-glyoxylate. The phosphoenolpyruvate concentrations were 0 (●), 0.2 mM (□), 0.4 mM (■) and 0.6 mM (△). (*b*) Double-reciprocal plots of initial velocity against glyoxylate concentration at 0.39 mM-succinate. The phosphoenolpyruvate concentrations were 0 (●), 0.2 mM (□), 0.4 mM (■) and 0.6 mM (△).

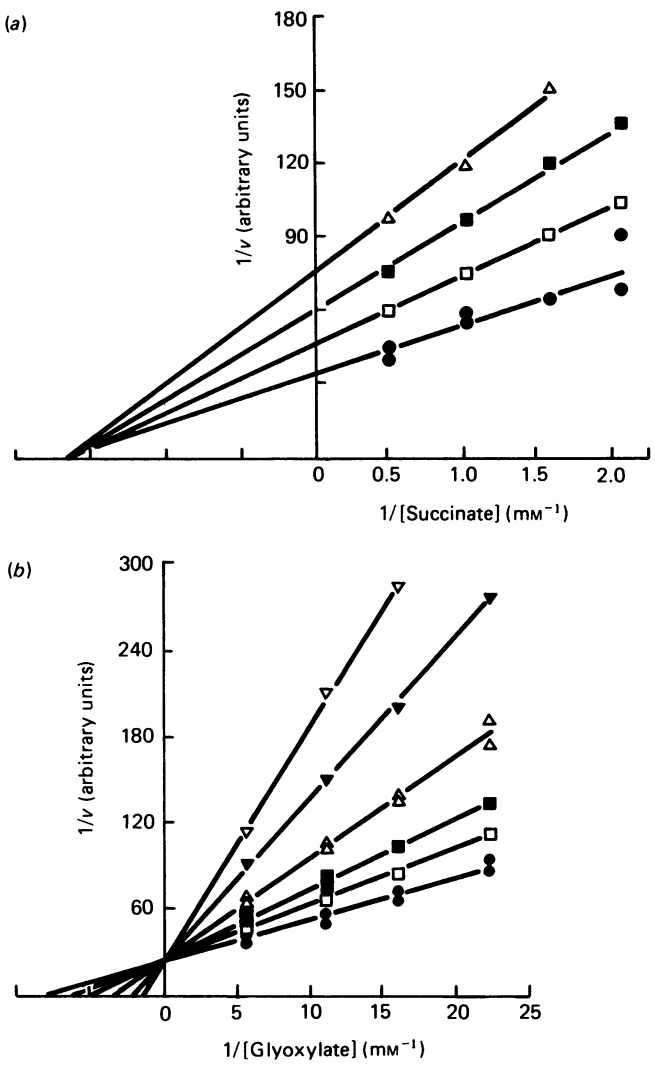

Fig. 4. Inhibition by glycollate of the condensation reaction catalysed by isocitrate lyase

(*a*) Double-reciprocal plots of initial rate against succinate concentration at 0.045 mM-glyoxylate. The glycollate concentrations were 0 (●), 0.011 mM (□), 0.022 mM (■) and 0.033 mM (△). (*b*) Double-reciprocal plots of initial rate against glyoxylate concentration of 0.39 mM-succinate. The glycollate concentrations were 0 (●), 0.06 mM (□), 0.12 mM (■) 0.24 mM (△), 0.48 mM (▼) and 0.72 mM (○).

was varied at fixed concentrations of the other substrate. In both cases all the lines intersected on the abscissa, i.e. neither substrate affected the K_m for the other (results not shown). The kinetic mechanism was probed further by using dead-end inhibitors. Phosphoenolpyruvate has been used as an analogue of succinate in studies of other isocitrate lyases [21,29,30]; it inhibited the *E. coli* enzyme competitively with respect to succinate and non-competitively [31] with respect to glyoxylate (Fig. 3). Glycollate, an analogue of glyoxylate, gave competitive inhibition with respect to glyoxylate and non-competitive inhibition with respect to succinate (Fig. 4). These results are inconsistent with the ordered mechanism (glyoxylate binding first) that has been proposed for other isocitrate lyases [21,29,30]. The ordered mechanism predicts that phosphoenolpyruvate should be an uncompetitive inhibitor with respect to glyoxylate. The data are, however, consistent with a random-order equilibrium mechanism in which neither glyoxylate nor succinate affects the affinity of the enzyme for the other substrate.

The cleavage reaction gave rise to linear double-reciprocal plots of rate against isocitrate concentration. Both succinate and phosphoenolpyruvate gave non-competitive inhibition with respect to isocitrate (Fig. 5). Inhibition by glyoxylate could not be tested, because of interference with the assays. The observed patterns can be accommodated within a random-order equilibrium

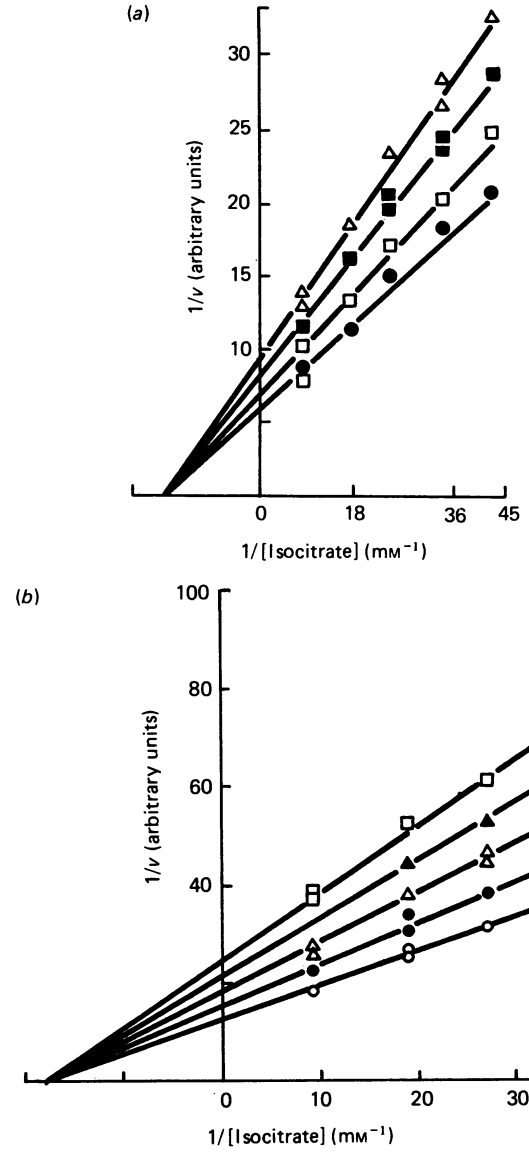

Fig. 5. Inhibition by phosphoenolpyruvate and succinate of the cleavage reaction catalysed by isocitrate lyase

Double-reciprocal plots of initial velocity against isocitrate concentration, obtained by the phenylhydrazine assay. (*a*) The phosphoenolpyruvate concentrations were 0 (●), 0.2 mM (□), 0.4 mM (■) and 0.6 mM (△). (*b*) The succinate concentrations were 0 (○), 0.2 mM (●), 0.4 mM (△), 0.6 mM (▲) and 0.8 mM (□).

mechanism by postulating the existence of a ternary enzyme–isocitrate–succinate complex, but not the existence of the analogous enzyme–isocitrate–glyoxylate complex, as shown in Fig. 6. Some inhibition patterns and K_m and K_i data are summarized in Table 2.

The kinetic mechanism of *E. coli* isocitrate lyase is thus different from the ordered mechanism reported for this enzyme from *Pseudomonas indigofera* and *Neurospora crassa* [21,29]. For the enzyme from *Linum usitatissimum* (flax), however, the inhibition patterns given by succinate, glyoxylate and phosphoenolpyruvate were similar to those shown here, even though the authors favoured a compulsory-order mechanism [30]. Phosphoenolpyruvate is an inhibitor of all the isocitrate lyases that

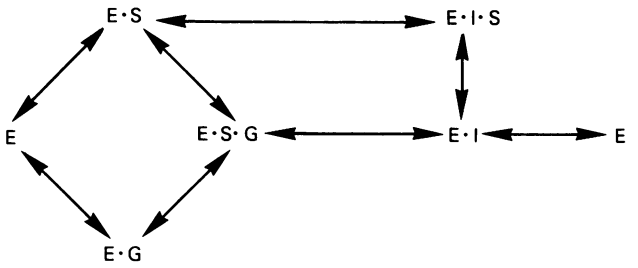

Fig. 6. Postulated kinetic mechanism of isocitrate lyase

E, enzyme; S, succinate; G, glyoxylate; I, isocitrate. Interconversions between the E·S·G and E·I complexes are slow relative to binding steps.

have been studied so far, apparently because it acts as an analogue of succinate. There is no reason to suppose that it inhibits the enzyme allosterically, as suggested in previous work [13].

Effect of anions and pH on the K_m of isocitrate lyase for isocitrate

Current views of the control of flux between the glyoxylate bypass and the tricarboxylic acid cycle have involved the assumptions that the K_m of isocitrate lyase for isocitrate is high relative to that of isocitrate dehydrogenase and that growth on acetate requires maintenance of a high intracellular concentration of isocitrate to sustain the necessary flux through the glyoxylate bypass [5,6,8–11]. However, there has been little agreement about the K_m of *E. coli* isocitrate lyase for isocitrate. Reported values include 8 μM at pH 7.5 [15], 18 μM at pH 6.8 [13], 0.604 mM at pH 7.5 [6] and 3 mM (conditions not stated) [32]. The K_m of the enzyme from other sources is very sensitive to pH and to the presence of competing anions (see, e.g., [21]). We therefore monitored the effects of pH and anions on purified *E. coli* isocitrate lyase in order to facilitate estimation of its K_m under cellular conditions.

KCl gave competitive inhibition of the cleavage reaction catalysed by isocitrate lyase (Fig. 7). The slope replots were non-linear, concave upwards (Fig. 7), which suggests that the inhibitor could interact with the enzyme in at least two ways. NaCl gave essentially identical results, indicating that the inhibitory species was probably the chloride anion. Sulphate and phosphate ions as the potassium salts gave linear competitive inhibition of the cleavage reaction, with K_i values of 20 mM and 11 mM respectively (results not shown). Qualitatively similar results were obtained for the isocitrate lyases of *Neurospora crassa* and *Pseudomonas indigofera* [21].

The K_m values of *E. coli* isocitrate lyase for isocitrate determined by using the two assay methods described in the Experimental section were identical. The K_m decreased with pH in the neutral pH range, being 63 ± 4 μM ($n = 4$) at pH 7.3, 32 ± 5 μM ($n = 3$) at pH 6.8 and 7 μM (single determination) at pH 6.3. As has been shown for isocitrate lyase from other sources [33,34], the interactions of other effectors with isocitrate lyase were also markedly dependent on pH; for example, the K_i for phosphoenolpyruvate in the cleavage reaction was 0.91 mM at pH 7.3, but only 0.1 mM at pH 6.8, and the corresponding values for succinate were 1.12 mM and 0.3 mM.

Table 2. Kinetic parameters of isocitrate lyase

Values are either single determinations or means ± S.D.

Reaction	Parameter	Type of inhibition	Value (mM) pH 7.3	pH 6.8
Cleavage	K_m for isocitrate		0.063 ± 0.004 ($n = 4$)	0.032
	K_i for phosphoenolpyruvate	Non-competitive	0.91 ± 0.25 ($n = 4$)	0.10
	K_i for 3-phosphoglycerate	Competitive	0.80	0.36
	K_i for 2-oxoglutarate	Non-competitive	1.35	
	K_i for succinate	Non-competitive	1.19	0.30
Condensation	K_m for succinate*		0.59	
	K_m for glyoxylate*		0.13	
	K_i for phosphoenolpyruvate versus glyoxylate†	Non-competitive	0.86 ± 0.37 ($n = 5$)	
	K_i for phosphoenolpyruvate versus succinate‡	Competitive	0.59 ± 0.26 ($n = 5$)	
	K_i for glycollate versus glyoxylate†	Competitive	0.16	
	K_i for glycollate versus succinate‡	Non-competitive	0.26	

* Independent of the concentration of the second substrate.
† At 0.33 mM-succinate.
‡ At 0.2 mM-glyoxylate.

The K_m of 32 μM for isocitrate at pH 6.8 reported here for isocitrate lyase purified from *E. coli* ML308 is similar to the values of 18 μM and 24 μM reported for the enzyme in crude extracts of *E. coli* strains Bm and W respectively [13]. However, our values are far lower than the value of 604 μM at pH 7.5, reported by LaPorte *et al.* [6]. This discrepancy can be attributed to the inclusion of 200 mM-KCl in the assays carried out by LaPorte *et al.* [6]; chloride ions at this concentration would increase the K_m for isocitrate by about one order of magnitude (Fig. 7).

The internal pH of *E. coli* is thought to be in the region pH 7.3–7.6 (e.g. [35]). Our results therefore suggest that the K_m of isocitrate lyase for isocitrate in intact cells would be 63 μM or higher, depending on the anionic composition of the intracellular milieu. In our laboratory *E. coli* ML308 is grown on a mineral-salts medium containing 12 mM-sulphate ions (as the magnesium and ammonium salts) and 40 mM-phosphate ions (as the potassium salt) but no chloride [24]. We have no data for the intracellular concentrations of sulphate and phosphate ions in these conditions, but it is known that *E. coli* can take up these species actively (e.g. [36,37]). This suggests that the presence of these inorganic anions may have a significant impact on the K_m of isocitrate lyase for isocitrate in intact cells.

Potential regulators of isocitrate lyase

Assuming that the intracellular water content of *E. coli* is 2.7 ml/g dry wt. [38], the intracellular concentration of phosphoenolpyruvate in cells growing on acetate is 0.22 mM [14]. Comparison of this value with the K_i of 0.91 mM at pH 7.3 (Table 2) suggests that phosphoenolpyruvate has little effect on the flux through isocitrate lyase in intact cells. Other potential regulators were screened by using the phenylhydrazine assay. When tested at 1.5 mM, in the presence of 0.15 mM-isocitrate,

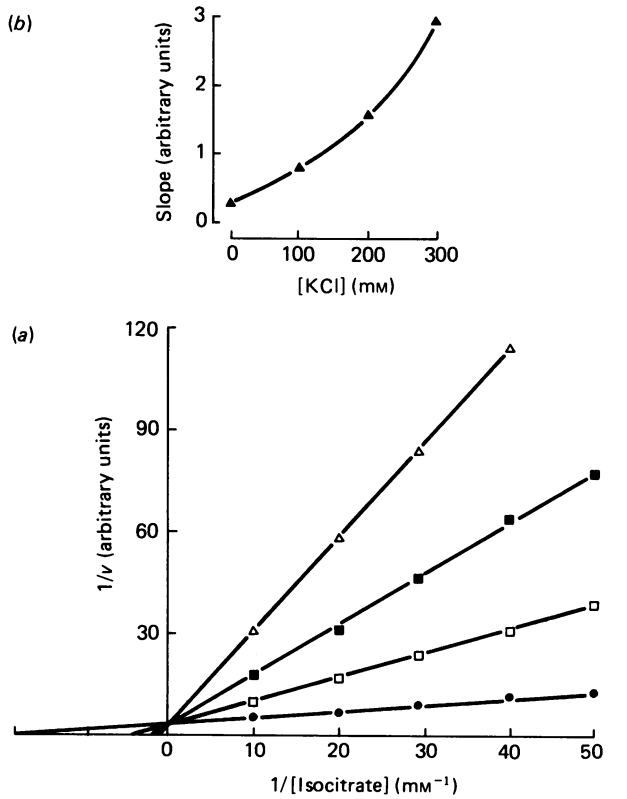

Fig. 7. Inhibition by KCl of the cleavage reaction catalysed by isocitrate lyase

(*a*) Double-reciprocal plots of initial velocity against isocitrate concentration, obtained by the lactate dehydrogenase-coupled assay. Each point represents a single determination. The KCl concentrations were 0 (●), 100 mM (□), 200 mM (■) and 300 mM (△). (*b*) Replot of the slopes in (*a*) against KCl concentration.

the following compounds inhibited isocitrate lyase activity by less than 10%: glutamate, aspartate, citrate, fumarate, malate, fructose 1,6-bisphosphate, fructose 6-phosphate, ATP, ADP, AMP, GTP, $NADP^+$, NAD^+ and NADH. The effects of pyruvate could not be tested, as this compound interfered with both assay methods.

3-Phosphoglycerate gave linear competitive inhibition against isocitrate in the cleavage reaction, with a K_i of 0.8 mM at pH 7.3 and 2-oxoglutarate gave non-competitive inhibition with a K_i of 1.35 mM at pH 7.3 (Table 2). These compounds presumably act as analogues of glyoxylate and succinate respectively. The intracellular content of 2-oxoglutarate in cells growing on acetate [14] corresponds to a concentration of 0.07 mM, so it seems unlikely that this metabolite is a significant effector of isocitrate lyase in intact cells. However, it has been reported that the intracellular concentration of 3-phosphoglycerate is 2.5 mM in cells growing on acetate [12]. This concentration is sufficient to have a significant impact on the activity of isocitrate lyase; it would raise the apparent K_m of this enzyme from 63 μM to 0.26 mM at pH 7.3. It is already known that 3-phosphoglycerate both activates isocitrate dehydrogenase phosphatase and inhibits isocitrate dehydrogenase kinase [12]. Small changes in the intracellular concentration of 3-phosphoglycerate could therefore have a significant impact on the partition of flux between the glyoxylate bypass and the tricarboxylic acid cycle, mediated by effects on both the phosphorylation state of isocitrate dehydrogenase and the activity of isocitrate lyase.

The K_m value of 604 μM for isocitrate lyase reported by LaPorte *et al.* [6] could be misleading, because it was obtained in the presence of 200 mM-chloride ion, a competitive inhibitor. Our data show that, although the K_m in the absence of competing anion is only 63 μM at physiological pH, it must be at least 0.26 mM in intact cells, and probably higher, owing to the presence of 3-phosphoglycerate, sulphate, phosphate and other anions. Walsh & Koshland [5] showed that the flux through isocitrate dehydrogenase in cells growing on acetate is very close to the maximal catalytic capacity of the enzyme. For *E. coli* ML308, the maximal catalytic capacities of isocitrate dehydrogenase and isocitrate lyase are similar (results not shown). The flux through isocitrate lyase during growth on acetate is some 40–50% of that through the dehydrogenase [5,8]. This implies that the K_m of isocitrate lyase in intact cells must be similar to the intracellular concentration of isocitrate, namely 0.57 mM [9]. It can be concluded that, as proposed previously [9,10], changes in the intracellular concentration of isocitrate, such as the doubling caused by addition of pyruvate to cells growing on acetate [9], do indeed affect the flux through isocitrate lyase.

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