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The Purification and Properties of *Escherichia coli* Methylglyoxal Synthase

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1. Methylglyoxal synthase was purified over 1500-fold from glycerol-grown *Escherichia coli* K 12 strain CA 244. The purified enzyme was inactivated by heat or proteolysis, had a molecular weight of approx. 67000, a pH optimum of 7.5 and was specific for dihydroxyacetone phosphate with K_m 0.47mM. 2. The possibility that a Schiff-base intermediate was involved in the reaction mechanism was investigated but not confirmed. 3. The purified enzyme lost activity, especially at low temperature, but could be stabilized by P_i. Two binding sites for P_i may be present on the enzyme. Of other compounds tested only the substrate, dihydroxyacetone phosphate, and bovine serum albumin showed any significant stabilizing effect. 4. Phosphoenolpyruvate, 3-phosphoglycerate, PP_i and P_i were potent inhibitors of the enzyme. Kinetic experiments showed that PP_i was apparently a simple competitive inhibitor, but inhibition by the other compounds was more complex. In the presence of P_i the enzyme behaved co-operatively, with at least three binding sites for dihydroxyacetone phosphate. 5. It is proposed that methylglyoxal synthase and glyceraldehyde 3-phosphate dehydrogenase play important roles in the catabolism of the triose phosphates in *E. coli*. Channelling of dihydroxyacetone phosphate via methylglyoxal would not be linked to ATP formation and could be involved in the uncoupling of catabolism and anabolism.

Although many early studies claimed that methylglyoxal was formed by glycolysing tissues (Toenniessen & Fischer, 1926; Neuberg & Kobel, 1928, 1930), its formation was subsequently considered to be artifactual and its suggested role as a glycolytic intermediate was dismissed by Meyerhof (1948). Meyerhof & Lohmann (1934) had shown that triose phosphates could be converted into methylglyoxal non-enzymically in an acid-catalysed reaction, and much more recently Riddle & Lorenz (1968) showed that a variety of compounds catalysed the non-enzymic formation of methylglyoxal from dihydroxyacetone and DL-glyceraldehyde. Such reactions were thought to account for the many published reports of methylglyoxal formation by glycolysing tissues.

The demonstration of an enzymic reaction for the conversion of dihydroxyacetone phosphate into methylglyoxal (Cooper & Anderson, 1970) has once more raised the possibility that methylglyoxal may play a physiological role in glucose catabolism.

In the present paper we describe the preparation of highly purified methylglyoxal synthase from *Escherichia coli* and report several of its properties, which suggest that methylglyoxal synthase plays a physiological role in the cell. A preliminary account of some of this work has been published (Hopper & Cooper, 1971).

Materials and Methods

Enzyme assays

Both coupled and direct methods were used to assay methylglyoxal synthase. In assay 1 the reaction mixture contained, in 1ml: imidazole buffer, pH7.0 (40μmol), dihydroxyacetone phosphate (0.75μmol), GSH, pH7.0 (1.65μmol), glyoxalase I (EC 4.4.1.5) (Sigma type III; sufficient to catalyse the condensation of 0.8μmol of methylglyoxal/min) and enzyme. The increase in E_{240} was measured at 30°C on a recording spectrophotometer. In this system 1μmol of lactoyl-glutathione has E_{240} 3.4 (Racker, 1957). In assay 2 the reaction mixture at 30°C contained, in 0.5ml: imidazole buffer, pH7.0 (20μmol), dihydroxyacetone phosphate (0.75μmol) and enzyme. The methylglyoxal formed was measured colorimetrically by taking 0.1ml samples into 0.33ml of 2,4-dinitrophenylhydrazine reagent (0.1% 2,4-dinitrophenylhydrazine in 2M-HCl) plus 0.9ml of water. After incubation at 30°C for 15min 1.67ml of 10% (w/v) NaOH was added and the E_{555} measured after a further 15min. A molar extinction coefficient of 4.48×10^4 (Wells, 1966) was used to convert readings into μmol of methylglyoxal. One unit of enzyme activity is defined as the amount of enzyme required for the formation of 1μmol of methylglyoxal or

lactoyl-glutathione/min under the assay conditions. Specific activity is defined as the number of units/mg of protein. Rabbit muscle fructose diphosphate aldolase (EC 4.1.2.13) was measured as described by Rajkumar *et al.* (1966). Malate dehydrogenase (EC 1.1.1.37) was assayed as described by Ochoa (1955).

Determination of protein

Soluble protein was determined spectrophotometrically by the method of Warburg & Christian (1941).

Gel electrophoresis

Polyacrylamide-gel electrophoresis was carried out by the method of Davis (1964) except that the sample [containing 5% (w/v) sucrose] was applied directly to the top of the small-pore gel. The gels were stained for 1 h at room temperature in a solution of 1% (w/v) Naphthalene Black in aq. 7% (v/v) acetic acid, then destained electrolytically with aq. 7% acetic acid as the electrolyte. The stained gels were examined by using a Joyce–Loebl mark IIIc double-beam recording microdensitometer.

Chemicals

Dihydroxyacetone phosphate dimethylacetal (dimonocyclohexylamine salt), phosphoenolpyruvate (sodium salt), GSH, fructose 1,6-diphosphate (tetrasodium salt), DL-glyceraldehyde 3-phosphate diethylacetal (monobarium salt), dihydroxyacetone and glyoxalase I (type III) were purchased from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.; 3-phosphoglyceric acid (sodium salt), pig heart malate dehydrogenase and rabbit muscle fructose diphosphate aldolase were purchased from Boehringer Corp. (London) Ltd., London W.5, U.K.; methylglyoxal was obtained from K & K Laboratories Inc., Plainview, N.Y., U.S.A., and was purified as described previously (Cooper & Anderson, 1970). Nagarse, a crystalline bacterial proteinase, was obtained from Nagase and Co. Ltd., Osaka, Japan. All other reagents were of the highest purity commercially available.

Experimental and Results

Purification of methylglyoxal synthase

The enzyme was purified from glycerol-grown cells of *E. coli* CA 244 obtained from the Microbiological Research Establishment, Microbial Products Section, Porton Down, Wilts., U.K. The buffer used throughout the purification was 50mM-imidazole–HCl buffer, pH 7.0.

Step 1. *Preparation of crude extract.* Frozen cells (100g wet wt.) were thawed in 200ml of buffer and 60ml portions were disrupted at ice temperature in an MSE 100W ultrasonic disintegrator for 4min. The sonicate was centrifuged at 30000*g* for 30min at 2°C and the supernatant solution retained.

Step 2. *Heat treatment.* Portions (50ml) of the crude extract were heated rapidly, with continuous stirring, to 75°C, kept at this temperature for 1min and then quickly cooled in ice-water. The heavy precipitate was removed by centrifuging at 30000*g* for 15min at 2°C and discarded.

Step 3. *Ammonium sulphate precipitation.* Solid $(NH_4)_2SO_4$ (209g/litre) was added to the heat-treated extract at 0°C and stirred for 60min. The precipitate was removed by centrifuging at 30000*g* for 15min at 2°C and discarded. More $(NH_4)_2SO_4$ (111g/litre) was added to the supernatant solution at 0°C, stirred for 60min and the mixture centrifuged as above. The supernatant solution was discarded and the precipitate dissolved in 3ml of buffer containing 1mM-KH_2PO_4 to stabilize the enzyme (see below).

Step 4. *Gel filtration on Bio-Gel P150.* The enzyme solution from the previous step was applied to a column (2.5cm×92cm) of Bio-Gel P150 (100–200 mesh) previously equilibrated against buffer containing 1mM-KH_2PO_4. The column was eluted at room temperature with buffer containing 1mM-KH_2PO_4 at a flow rate of 22ml/h and 2.7ml fractions were collected. The enzyme was eluted between fractions 52 and 72 and the most active fractions were pooled.

Step 5. *Second ammonium sulphate precipitation.*

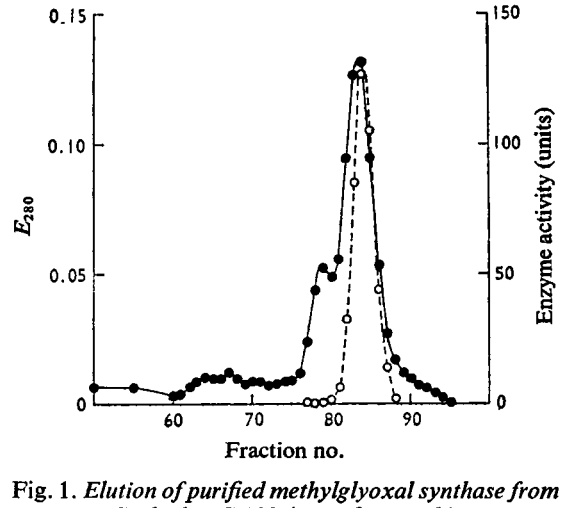


Fig. 1. *Elution of purified methylglyoxal synthase from Sephadex G-100 (superfine grade)*

Details of the procedures used are given in the text; ●, E_{280}; ○, enzyme activity.

Table 1. *Summary of the purification of methylglyoxal synthase*

For experimental details see the text.

Stage of purification		Vol. (ml)	Total protein (mg)	Total units	Specific activity	Recovery (%)	Purification
(1) Crude extract		230	5405	1695	0.32	100	1
(2) Heat treatment		205	496	1440	2.9	85	9
(3) First $(NH_4)_2SO_4$ fractionation		3.5	57	1220	21.4	72	66.6
(4) Pooled fractions from Bio-Gel P150 column		24.2	5.3	776	141.0	46	440
(5) Second $(NH_4)_2SO_4$ fractionation		2.1	3.5	611	174.0	36	543
(6) Peak fractions from Sephadex G-100 column	83	2.1	0.275	86	310.0	5.0	966
	84	2.1	0.288	127	441.0	7.5	1375
	85	2.1	0.200	106	530.0	6.3	1650
	86	2.1	0.088	44	500.0	2.6	1550

Solid $(NH_4)_2SO_4$ (243 g/litre) was added to the pooled fractions and treated as described under Step 3. The precipitate was discarded and more $(NH_4)_2SO_4$ (132 g/litre) was added to the supernatant. The precipitate was dissolved in 2 ml of buffer containing 1 mM-KH_2PO_4 and retained for the next step.

Step 6. Sephadex G-100 gel filtration. The enzyme solution from the previous step was applied to a column (2.5 cm × 89 cm) of Sephadex G-100 (superfine grade) equilibrated against buffer containing 1 mM-KH_2PO_4. The column was eluted at room temperature with buffer containing 1 mM-KH_2PO_4 at a flow rate of 8 ml/h and 2.1 ml fractions were collected. The protein-elution profile is shown in Fig. 1. Fractions containing the purified enzyme were stored at 4°C.

The results from such a purification, which was followed by using assay 1, are shown in Table 1. The highest final specific activities varied from 350 to 530 in different preparations.

Properties of the enzyme

Evidence for its protein nature. Since many compounds can catalyse the formation of methylglyoxal (Bonsignore *et al.*, 1970; Riddle & Lorenz, 1968) it was important to establish the protein nature of methylglyoxal synthase. Its general behaviour during the purification procedures was consistent with that of a high-molecular-weight compound. The purified enzyme was completely inactivated by heating at 100°C for 1 min and when 1 ml of an enzyme solution (specific activity 393; 70 units/ml of 50 mM-imidazole-HCl buffer, pH 7.0, containing 1 mM-KH_2PO_4) was incubated with 10 μl of a solution of a crystalline proteolytic enzyme (Nagarse, 7.0 mg/ml) at 30°C no activity remained when assayed after 35 min. A control without the proteolytic enzyme retained full activity.

Although a protein identified as myoglobin was shown to catalyse methylglyoxal formation from acetoacetate in sheep heart the rate of the reaction was proportional to the square root of the myoglobin concentration (Milligan & Baldwin, 1967). For

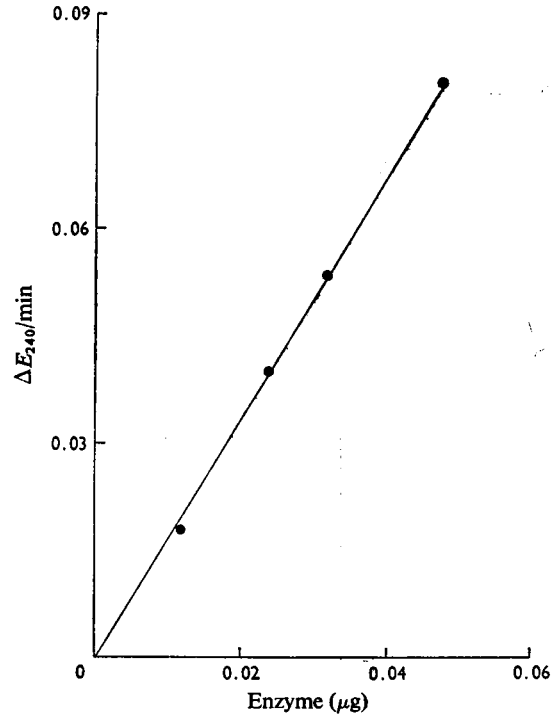


Fig. 2. *Dependence of reaction rate on methylglyoxal synthase concentration*

Dilutions of the enzyme solution (specific activity 530; 50 units/ml) were made in 50 mM-imidazole buffer, pH 7.0, containing 10 mg of bovine serum albumin/ml. Equal volumes (0.01 ml) were taken for assay in the coupled system (assay 1).

methylglyoxal synthase the rate of reaction was directly proportional to the amount of enzyme added, as shown in Fig. 2.

When methylglyoxal synthase was run on Sephadex G-100 or Bio-Gel P150 columns with proteins of known molecular weight its elution was slightly retarded relative to bovine serum albumin and was almost coincident with pig heart malate dehydrogenase. Since both bovine serum albumin and malate dehydrogenase are reported to have molecular weights of approx. 67000 (Loeb & Scheraga, 1956; Murphey *et al.*, 1967) the molecular weight of methylglyoxal synthase would appear to be close to 67000.

Purity of the enzyme. To obtain some indication of the purity of the preparation, fraction 85 from the Sephadex G-100 column (Table 1) was subjected to disc-gel electrophoresis at pH8.9. On staining for protein with Naphthalene Black one major band and two minor bands were seen (Fig. 3). When corresponding sections from an unstained gel were cut out, homogenized in 0.45ml of 50mM-imidazole buffer, pH7.0, and assayed for methylglyoxal synthase by using assay 2, only the major band was active. From the microdensitometer tracing the major band accounted for more than 70% of the total protein. When the electrophoresis was carried out in the presence of 1mM-KH_2PO_4 the mobility of the enzyme band was significantly increased whereas the other two bands were not affected (Fig. 3).

pH–activity curve. When the enzyme activity was measured in various buffers over the range pH 6–9 the pH optimum was found to be close to pH7.5 (Fig. 4). At most pH values the enzyme was more active in imidazole buffer than in either maleate or triethanolamine buffers.

Specificity of the enzyme. Although crude extracts of *E. coli* could form methylglyoxal from dihydroxyacetone phosphate, glyceraldehyde 3-phosphate or fructose 1,6-diphosphate the purified enzyme reacted specifically with dihydroxyacetone phosphate. When DL-glyceraldehyde 3-phosphate was tested (assay 2) methylglyoxal accumulated only when triose phosphate isomerase was included in the assay mixture. Similarly fructose 1,6-diphosphate served as a substrate only when fructose 1,6-diphosphate aldolase was present. No reaction was detected with dihydroxyacetone at concentrations up to 15mM, even with ten times the usual amount of enzyme.

Stabilization of the enzyme. In early experiments methylglyoxal synthase showed a pronounced loss of activity during gel filtration. Since P_i was an allosteric effector of the enzyme (Hopper & Cooper, 1971) it was thought possible that P_i would protect against this inactivation. When 1mM-P_i was incorporated into the elution buffer a dramatic increase in recovery of the enzyme was seen. Accordingly 1mM-P_i was always incorporated into the buffer to stabilize the enzyme.

When the P_i concentration in the enzyme solution was decreased a rapid loss of activity occurred (see Table 2), and Fig. 5(*a*) shows the extent of inactivation at different P_i concentrations. When these results were plotted in a double-reciprocal form (Fig. 5*b*),

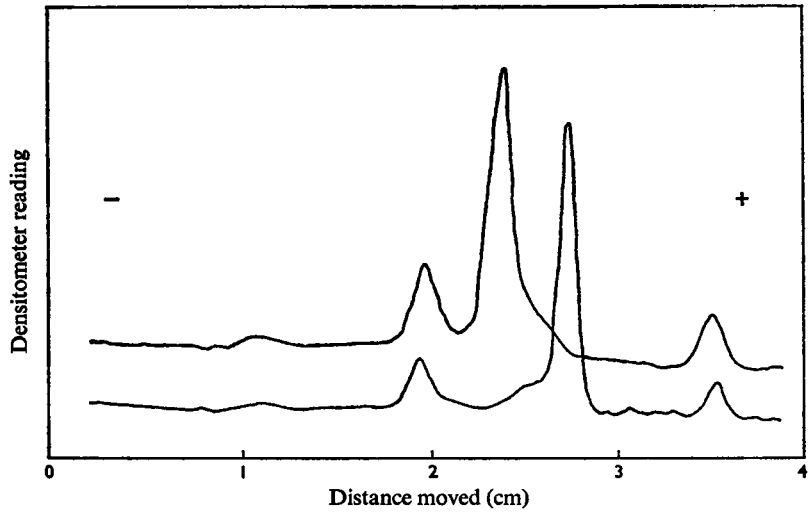


Fig. 3. *Polyacrylamide-gel electrophoresis of highly purified methylglyoxal synthase*

Gels were run in the presence (lower trace) and absence (upper trace) of 1mM-KH_2PO_4. Details of the procedures are given in the text.

a parabolic relationship between stabilization and P_i concentration was observed. However, this could be converted into a linear relationship by plotting the reciprocal of the square of the P_i concentration (Fig. 5*c*). Surprisingly this loss of activity was much more pronounced at 0°C than at room temperature.

Other inhibitors of methylglyoxal synthase (Hopper & Cooper, 1971) did not stabilize the enzyme to any great extent (Table 2). Of several compounds tested only dihydroxyacetone phosphate and bovine serum albumin showed any appreciable stabilizing ability (Table 2).

Treatment of the enzyme with sodium borohydride. Since Bonsignore *et al.* (1970) showed that lysine catalysed methylglyoxal formation from glyceraldehyde, probably via the formation of a Schiff-base intermediate, and Mel'nichenko *et al.* (1969) reported that lysine catalysed methylglyoxal formation from glyceraldehyde 3-phosphate in a similar way, it was possible that the methylglyoxal synthase reaction also involved the formation of a Schiff-base intermediate. To test this possibility methylglyoxal synthase was treated with sodium borohydride in the presence and absence of dihydroxyacetone phosphate. The procedure used was essentially that of Grazi *et al.* (1962) but with 50mM-sodium acetate buffer, pH 5.5, instead of sodium phosphate buffer, pH 6.0, and 2mM-dihydroxyacetone phosphate. Since P_i might interfere with the binding of dihydroxyacetone phosphate to methylglyoxal synthase (Hopper & Cooper, 1971), 1ml of purified enzyme was stabilized with 10mg of bovine serum albumin and dialysed against 100ml of 50mM-acetate buffer, pH 5.5, for 3h, with one change of buffer, to remove P_i. However, no evidence for the formation of a Schiff base was detected, since methylglyoxal synthase treated with sodium borohydride in the presence of dihydroxyacetone phosphate had the same activity as enzyme treated in the absence of dihydroxyacetone phosphate. This was about 50% of the original activity. Under these same conditions commercial fructose diphosphate aldolase treated with sodium borohydride in the presence of dihydroxyacetone phosphate retained only 4% of the activity observed after treatment of the enzyme in the absence of dihydroxyacetone phosphate.

Kinetic studies. Assay 1 was used except where assay 2 is specifically stated. When the activity of the purified enzyme was measured at various concentrations of dihydroxyacetone phosphate and the initial rates were plotted against substrate concentration, a rectangular hyperbola was obtained, as shown in Fig. 6(*a*). In a double-reciprocal plot (Lineweaver & Burk, 1934) these results gave a straight line, the K_m for dihydroxyacetone phosphate being 0.47mM. However, when the experiments were repeated in the

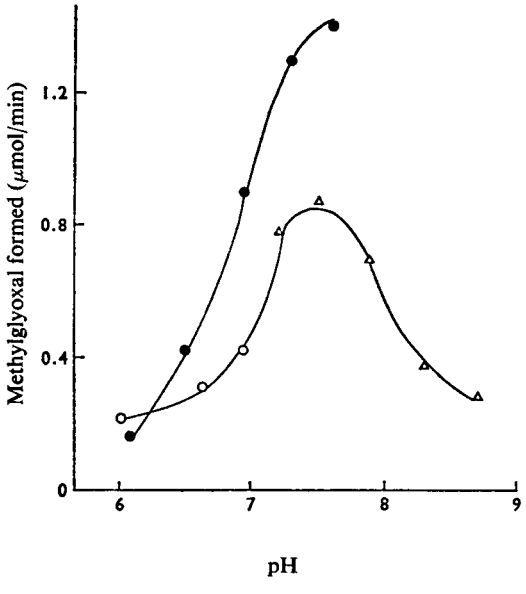


Fig. 4. *pH–activity curve for methylglyoxal synthase*

The enzyme (specific activity 530) was assayed directly (assay 2) at various pH values in the following buffers at 0.16M final concentration; ○, maleate–HCl; ●, imidazole–HCl; △, triethanolamine hydrochloride–NaOH.

Table 2. *Stabilization of methylglyoxal synthase by various compounds*

Purified enzyme (specific activity 441, 60 units/ml) in 50mM-imidazole buffer, pH 7.0, containing 1mM-KH_2PO_4 was diluted 20-fold into 50mM-imidazole buffer, pH 7.0, containing the test compound at 1mM concentration unless stated otherwise. The enzyme was assayed immediately on dilution and after 3.5h at 0°C.

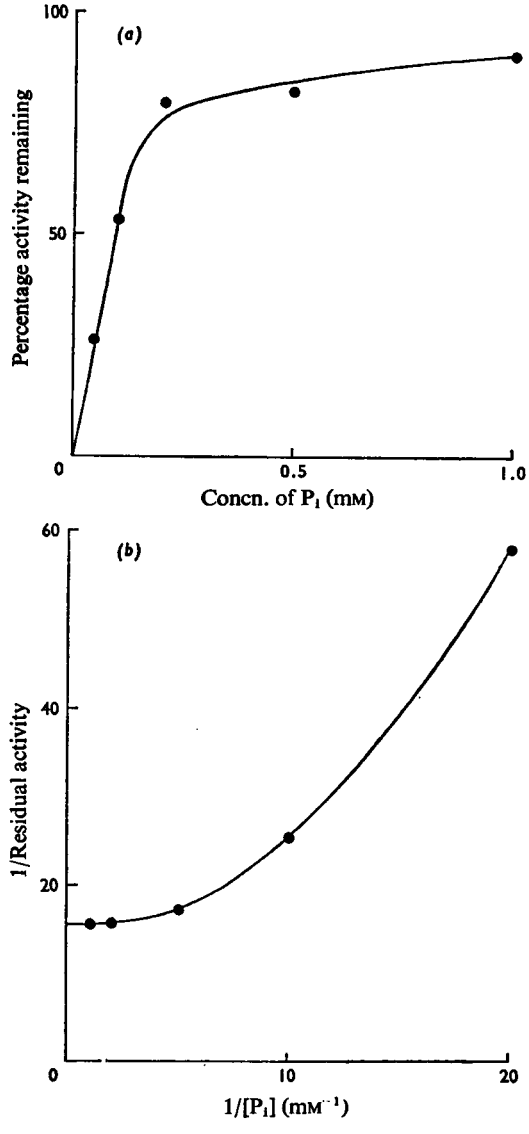
Test compound	Activity remaining (%)
None	9.9
P_i	90.7
PP_i	21.4
Glucose 6-phosphate	23.0
Phosphoenolpyruvate	15.1
Fructose 1,6-diphosphate	28.5
3-Phosphoglycerate	28.1
Dihydroxyacetone phosphate	69.0
GSH (3mM)	10.0
Dithiothreitol (0.5mM)	10.0
Bovine serum albumin (10mg/ml)	65.0
None (temp. 22°C)	40.3

presence of P_i the results obtained were quite different. The relationship between initial rate and substrate concentration was now sigmoidal (Fig. 6*a*) and the double-reciprocal plot gave a parabola rather than a straight line. When the results were plotted by using the modified Hill equation (Atkinson *et al.*, 1965) a straight line with a slope of 2.6 was obtained. This suggested at least three dihydroxyacetone phosphate-binding sites, and when the initial rates were plotted against the cube of the dihydroxyacetone phosphate concentration in a double-reciprocal plot a straight-line relationship was observed (Fig. 6*b*).

Arsenate, an analogue of P_i, was a less effective inhibitor, but the type of inhibition was the same (Fig. 7). Similarly when the P_i concentration was varied at a constant dihydroxyacetone phosphate concentration and the results were plotted by the method of Dixon (1953), a parabolic relationship was again observed (Fig. 8).

The effect of P_i was shown to be independent of the purity of the enzyme and the method of assay. Crude extracts prepared from triose phosphate isomerase-negative mutants (Anderson & Cooper, 1969) (where the isomerization of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate was excluded) showed the same response when assayed either direct (assay 2) or in the coupled assay.

Although DL-glyceraldehyde 3-phosphate was not a substrate for the enzyme its role as a modifier was investigated. When used at 3mM concentration in assay 2 it did not inhibit the reaction nor could it serve as a dihydroxyacetone phosphate analogue in overcoming P_i inhibition.



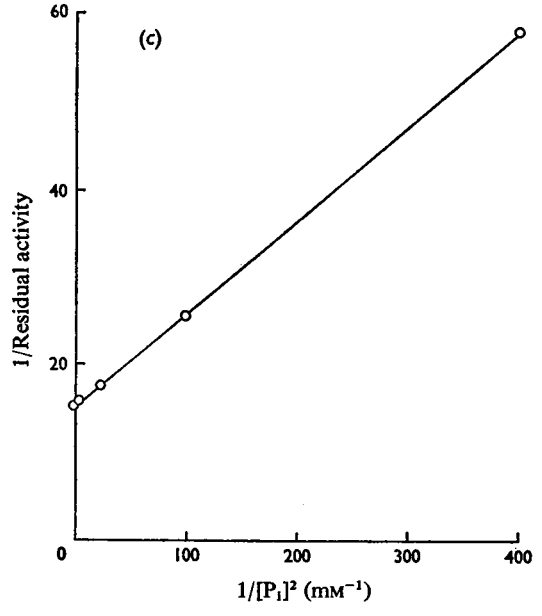


Fig. 5. *Effect of P_i concentration on the stabilization of methylglyoxal synthase*

Enzyme solution (specific activity 530, 50 units/ml) was diluted 20-fold into 50mM-imidazole buffer, pH7.0, containing various concentrations of P_i and kept at 0°C. The dilutions were assayed immediately and after 5h at 0°C. (*a*) Percentage activity remaining versus P_i concentration; (*b*) double-reciprocal plot of residual activity versus P_i concentration; (*c*) double-reciprocal plot of residual activity versus square of P_i concentration.

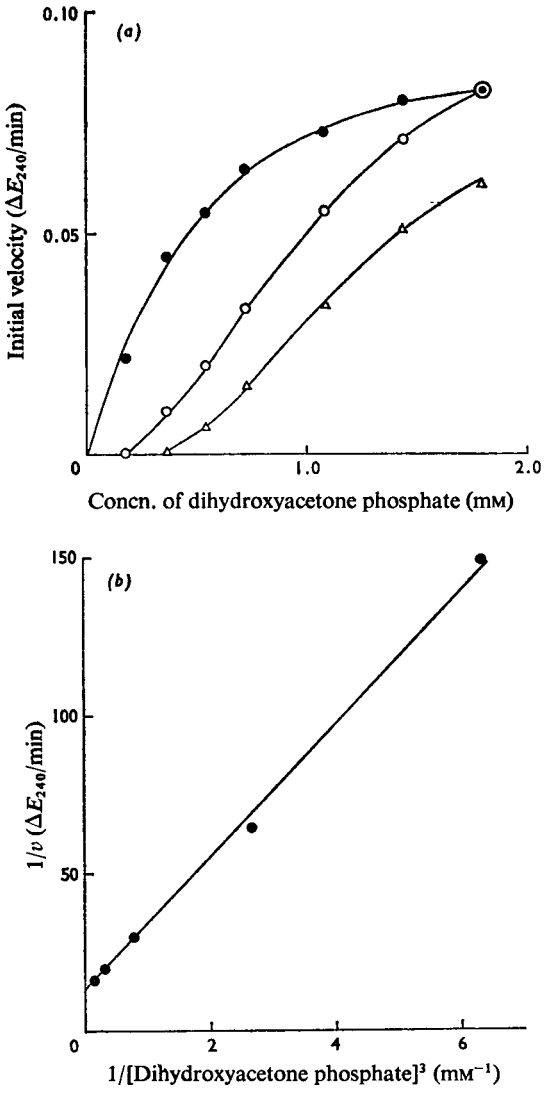


Fig. 6. *Effect of P_i on the kinetics of methylglyoxal synthase*

(*a*) Rate versus substrate concentration for methylglyoxal synthase (specific activity 530) in the presence and absence of P_i; ●, no P_i; ○, 0.2mM-P_i; △, 0.3mM-P_i. (*b*) Double-reciprocal plot of rate against dihydroxyacetone phosphate concentration cubed for reaction with 0.3mM-P_i.

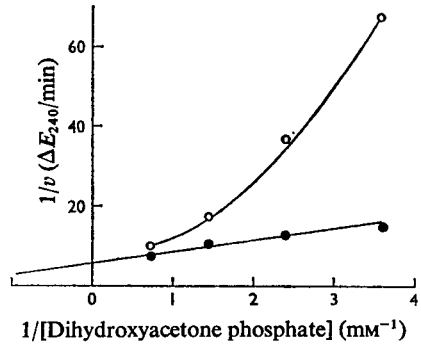


Fig. 7. *Double-reciprocal plot of rate versus substrate concentration for methylglyoxal synthase (specific activity 7.3) in the presence and absence of sodium arsenate*

●, No arsenate; ○, 2mM-sodium arsenate, pH7.0.

Further kinetic studies on the inhibition of methylglyoxal synthase by PP_i, 3-phosphoglycerate and phosphoenolpyruvate have confirmed and extended the results obtained previously with the less-pure enzyme (Hopper & Cooper, 1971). When the concentration of PP_i was varied at a constant dihydroxyacetone phosphate concentration and the results were plotted by the method of Dixon (1953), a linear relationship was observed giving a K_i of 0.095mM (Fig. 8). This response of the enzyme to PP_i was thus quite distinct from its response to P_i. The type of inhibition caused by 3-phosphoglycerate and phosphoenolpyruvate was different again, as shown in Fig. 9.

Discussion

Despite recent reports of the non-enzymic formation of methylglyoxal from triose and triose phosphates (Bonsignore *et al.*, 1970; Mel'nichenko *et al.*, 1969) the results presented in this paper reinforce the view (Cooper & Anderson, 1970) that the formation of methylglyoxal from dihydroxyacetone phosphate by *E. coli* extracts is an enzymic process. The enzyme involved, methylglyoxal synthase, is quite specific and is unable to form methylglyoxal from glyceraldehyde 3-phosphate. Although Wang *et al.* (1964) reported an enzymic formation of methylglyoxal from glyceraldehyde 3-phosphate it is possible that their preparations were contaminated with triose phosphate isomerase and that dihydroxyacetone phosphate was the true substrate. On the other hand the pH optimum they found was quite different from that reported here for methylglyoxal synthase.

The studies on the kinetic properties of the highly purified methylglyoxal synthase and its stabilization by P_i support the suggestion that it is an allosteric enzyme (Hopper & Cooper, 1971). Moreover, under certain conditions methylglyoxal synthase appeared

to be a cold-labile enzyme, a property often found in allosteric enzymes. Although several metabolites were inhibitors of methylglyoxal synthase P_i was perhaps the most interesting. The results of the Hill plot suggested that in the presence of P_i there were at least three dihydroxyacetone phosphate-binding sites on the enzyme, and the parabolic relationship between initial velocity and dihydroxyacetone phosphate concentration observed in the double-reciprocal plot was converted into a linear relationship by plotting the reciprocal of the cube of the dihydroxyacetone phosphate concentration.

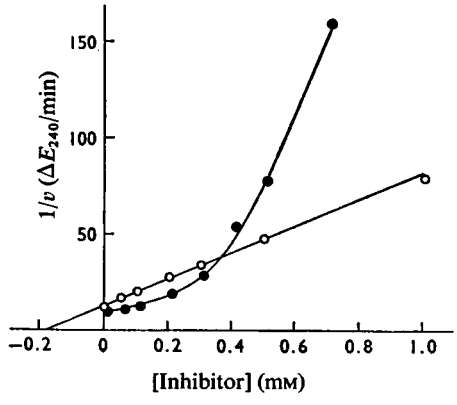


Fig. 8. *Inhibition of methylglyoxal synthase by P_i and PP_i*

The enzyme (specific activity 263) was assayed at a constant dihydroxyacetone phosphate concentration (0.775mM) with various concentrations of inhibitors; ●, P_i; ○, PP_i. The results were plotted by the method of Dixon (1953).

Several experiments suggested that changes in the enzyme structure occurred in the presence of P_i. When the purified enzyme was subjected to disc-gel electrophoresis in the presence of 1mM-P_i its rate of migration was significantly faster than in the absence of P_i, although the migration of the contaminating proteins was not affected. The ability of P_i, but not of the other inhibitors, to prevent the cold-inactivation of the enzyme was another indication that P_i affected the enzyme structure. The stabilization of the enzyme at various concentrations of P_i did not appear to correspond to a simple reversible association between enzyme and P_i, since a double-reciprocal plot of residual activity against P_i concentration showed a non-linear relationship. Since this was converted into a linear form by plotting the reciprocal of the square of the P_i concentration it was possible that there were two P_i-binding sites on the enzyme.

Although the kinetics of inhibition by phosphoenolpyruvate and 3-phosphoglycerate were more normal than those seen for P_i inhibition neither corresponded to simple competitive or non-competitive inhibition. It seems likely from the results that both phosphoenolpyruvate and 3-phosphoglycerate bind to more than one form of the enzyme. On the

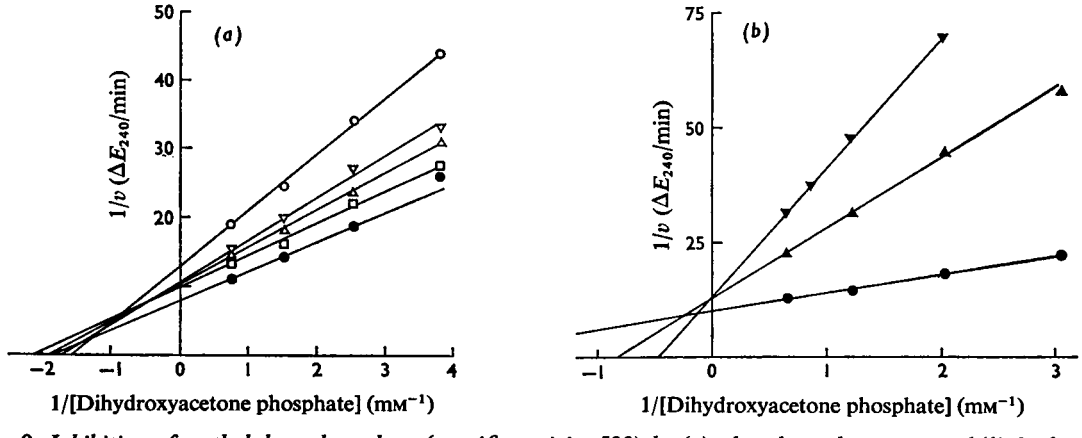


Fig. 9. *Inhibition of methylglyoxal synthase (specific activity 530) by (a) phosphoenolpyruvate and (b) 3-phosphoglycerate*

Initial rates were measured at various dihydroxyacetone phosphate concentrations in the presence and absence of inhibitor and the results plotted in the double-reciprocal form. (*a*) Phosphoenolpyruvate at the following concentrations: ●, no inhibitor; □, 0.1mM; △, 0.2mM; ▽, 0.3mM; ○, 0.5mM. (*b*) 3-Phosphoglycerate at the following concentrations: ●, no inhibitor; ▲, 1mM; ▼, 2mM.

other hand PP_i seemed to be a simple competitive inhibitor.

A possible physiological role for methylglyoxal synthase in providing an alternative catabolic fate for triose phosphates formed during glycolysis can be concluded from that nature of its inhibition by P_i. In crude extracts the specific activity of methylglyoxal synthase was similar to the reported activity of glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) (D'Alessio & Josse, 1971), suggesting that methylglyoxal synthase could make a significant contribution to triose phosphate catabolism. The complementary nature of methylglyoxal synthase and glyceraldehyde 3-phosphate dehydrogenase was also apparent from a study of the role of P_i. Thus methylglyoxal synthase was strongly inhibited by concentrations of P_i that are close to the K_m value for P_i as a substrate for glyceraldehyde 3-phosphate dehydrogenase (Velick & Furfine, 1963). As long as the P_i concentration remains adequate for glyceraldehyde 3-phosphate dehydrogenase activity methylglyoxal synthase would be active only at high dihydroxyacetone phosphate concentrations. However, should the P_i concentration fall sufficiently to decrease the glyceraldehyde 3-phosphate dehydrogenase activity the inhibition of methylglyoxal synthase would be decreased and triose phosphate catabolism could then proceed via methylglyoxal. This switch-over would have a dual effect, since it would make P_i available again for the glyceraldehyde 3-phosphate dehydrogenase reaction and the methylglyoxal could eventually give rise to lactate (Cooper & Anderson, 1970), which would be an energy source under aerobic conditions. Thus the interplay between P_i and dihydroxyacetone phosphate concentrations would divert triose phosphates to either 1,3-diphosphoglycerate or methylglyoxal. The diversion of triose phosphate to pyruvate via methylglyoxal and lactate would not be coupled to ATP synthesis and may thus play a role in the uncoupling of catabolism and anabolism, a phenomenon that has been recognized for many years (Gunsalus & Shuster, 1961).

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