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Characteristics of alanine:glyoxylate aminotransferase from *Saccharomyces cerevisiae*, a regulatory enzyme in the glyoxylate pathway of glycine and serine biosynthesis from tricarboxylic acid-cycle intermediates

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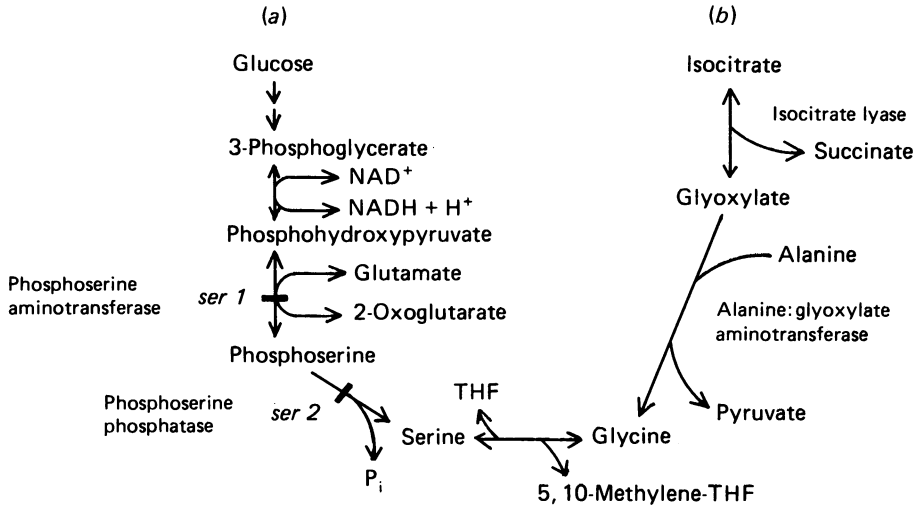
Alanine:glyoxylate aminotransferase (EC 2.6.1.44), which is involved in the glyoxylate pathway of glycine and serine biosynthesis from tricarboxylic acid-cycle intermediates in *Saccharomyces cerevisiae*, was highly purified and characterized. The enzyme had M_r about 80000, with two identical subunits. It was highly specific for L-alanine and glyoxylate and contained pyridoxal 5'-phosphate as cofactor. The apparent K_m values were 2.1mM and 0.7 mM for L-alanine and glyoxylate respectively. The activity was low (10 nmol/min per mg of protein) with glucose as sole carbon source, but was remarkably high with ethanol or acetate as carbon source (930 and 430 nmol/min per mg respectively). The transamination of glyoxylate is mainly catalysed by this enzyme in ethanol-grown cells. When glucose-grown cells were incubated in medium containing ethanol as sole carbon source, the activity markedly increased, and the increase was completely blocked by cycloheximide, suggesting that the enzyme is synthesized *de novo* during the incubation period. Similarity in the amino acid composition was observed, but immunological cross-reactivity was not observed among alanine:glyoxylate aminotransferases from yeast and vertebrate liver.

INTRODUCTION

Two main pathways have been postulated for glycine and serine biosynthesis in *Saccharomyces cerevisiae*, i.e. the phosphoglycerate pathway from glycolytic intermediates and the glyoxylate pathway from tricarboxylic acid-cycle intermediates (Scheme 1) (Deboiso & Stoppani, 1967; Ulane & Ogur, 1972). From experiments using two serine and glycine auxotrophs (*ser 1* and *ser 2*), which were found to be blocked in the phosphoglycerate pathway, it has been suggested that the glyoxylate pathway was not functional when cells grew on glucose, but was the principal pathway for glycine and serine formation when cells grew on acetate (Ulane & Ogur, 1972). In the glyoxylate pathway, glyoxylate is produced from isocitrate by isocitrate lyase (EC 4.1.3.1) and transaminated to glycine by alanine:glyoxylate aminotransferase (EC 2.6.1.44). The glycine generated by transamination serves as both a C_1 donor via glycine synthase (EC 2.1.2.10) and a C_1 recipient for production of serine via serine hydroxymethyltransferase (Jones & Fink, 1983). Deboiso & Stoppani (1967) suggested that the conversion of [1-^{14}C]glyoxylate and glycollate into glycine and serine via transamination prevailed over their



Scheme 1. Phosphoglycerate pathway (*a*) and glyoxylate pathway (*b*) of glycine and serine biosynthesis in *S. cerevisiae*

Details of the pathways are described in the text. Abbreviation: THF, tetrahydrofolate.

incorporation into the tricarboxylic acid cycle and into the glyoxylate cycle (via malate), when yeast cells actively oxidized acetate.

Alanine:glyoxylate aminotransferase is thus at the critical point of glyoxylate metabolism in *S. cerevisiae*. It is, however, not clear whether a specific aminotransferase catalysed the reaction or how the reaction was regulated in *S. cerevisiae* and other lower eukaryotes. The present paper describes the characteristics and regulation by synthesis *de novo* of a highly specific yeast alanine:glyoxylate aminotransferase. Yeast was found to contain only a single form of this enzyme. In contrast, vertebrate liver has been reported to contain two distinct isoenzymes (Noguchi *et al.*, 1978). We examined whether the yeast enzyme and the vertebrate isoenzymes originate from a common ancestral protein; the yeast enzyme and the vertebrate isoenzymes were compared with respect to physical, enzymic and immunological properties.

EXPERIMENTAL

Yeast strain

Commercial baker's yeast (Kyowa Brewery Co., Tokyo, Japan) was used for the purification of the enzyme. *S. cerevisiae* 101D (*ade 2, lys 9*, a), kindly supplied by Dr. M. Fukunaga, (University of Environmental and Occupational Health, Kitakyushu, Japan), was used for studying the regulation of the enzyme activity.

Culture of cells on different carbon sources

Cells were grown at 30 °C aerobically in a complex medium (Ulane & Ogur, 1972) containing, per litre, 10 g of glucose, 6 g of potassium acetate or 20 g of ethanol as carbon source. Cell density was determined at 600 nm. Cells were collected by centrifugation at 1000 *g* for 10 min and washed twice with distilled water.

Incubation of glucose-grown cells in medium containing ethanol as sole carbon source

Cells were grown in the complex medium containing 100 instead of 10 g of glucose/litre for 17 h, collected and washed as above. The cells were suspended (10 g wet wt./litre) in medium containing, per litre, 5 g of KH_2PO_4, 5 g of K_2HPO_4, 1 g of KCl, 0.5 g of $MgSO_4,7H_2O$, 1 g of $(NH_4)_2SO_4$ and 20 g of ethanol, pH 6.7, and incubated at 30 °C aerobically for the indicated time and collected and washed as above.

Preparation of crude extract

Cells were suspended in ice-cold 50 mM-potassium phosphate, pH 7.5, sonicated for 4 min (eight 30 s bursts at 1 min intervals) on ice at 110 W in a UR 200P ultrasonic vibrator (Tomy Seiko Co., Tokyo, Japan) and centrifuged at 20000 *g* for 10 min. The supernatant (crude extract) was used for enzyme assays.

Determination of enzyme activity

Transamination between various L-amino acids and 2-oxo acids were assayed as previously described (Noguchi *et al.*, 1978). The reaction mixture contained, unless specified otherwise, 40 mM L-amino acid, 5 mM 2-oxo acid, 40 μM-pyridoxal 5'-phosphate, 0.1 M-potassium phosphate buffer, pH 8.0, and enzyme preparation in a total volume of 0.4 ml. A unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of product or a decrease in substrate of 1 μmol/min at 37 °C.

Purification of alanine:glyoxylate aminotransferase from *S. cerevisiae*

Freshly frozen commercial baker's yeast (500 g) was broken and thawed in a 1-litre beaker at room temperature and extracted as described by Roschlau & Hess (1972). After addition of 30 ml of toluene, the suspension was cytolysed at 37 °C for 1 h. After addition of 100 ml of 25% (v/v) glycerol the preparation was kept for 1 h at room temperature. The pH was adjusted to 7.5 with 1 M-KOH. The suspension was left for 15 h at room temperature and then centrifuged at 10000 *g* for 30 min at 4 °C. All subsequent procedures were carried out at 0–4 °C, unless specified otherwise. To the supernatant, sodium pyruvate (1 mM) and pyridoxal 5'-phosphate (100 μM) were added. The solution was warmed rapidly to 60 °C and maintained at this temperature for 1 min with constant stirring, and then quickly chilled in an ice-cold bath. The inactive precipitate formed was removed by centrifugation at 10000 *g* for 10 min. The solution was dialysed overnight against 5 mM-potassium phosphate buffer, pH 6.8, containing 1mM-sodium pyruvate, 100 μM-pyridoxal 5'-phosphate and 10% glycerol (buffer A). The inactive precipitate formed was removed by centifugation at 10000 *g* for 10 min. The non-diffusable portion was applied to a column of phosphocellulose (3 cm × 13 cm) previously equilibrated with buffer A. After washing of the column with 500 ml of buffer A, the enzyme was eluted with a 1 litre linear gradient of KCl (0–500 mM) in buffer A; 20 ml fractions were collected. The enzyme was eluted at 270 mM-KCl. Active fractions were pooled and directly applied to a column of hydroxyapatite (2.2 cm × 13.5 cm), previously equilibrated with 5 mM-potassium phosphate buffer, pH 7.5, containing 1 mM-sodium pyruvate, 100 μM-pyridoxal 5'-phosphate and 10% glycerol (5 mM buffer B). After washing of the column with 200 ml of 5 mM buffer B, the enzyme was eluted with a 1 litre linear gradient of phosphate concentration (5–250 mM buffer B); 20 ml fractions were collected. The enzyme was eluted at 180 mM-phosphate. Active fractions were pooled, concentrated to 4.5 ml by Diaflo ultrafiltration with (*a*) G 10 T filter (Ulvac, Tokyo, Japan), and applied to a column of Sephadex G-200 (2.5 cm × 90 cm) previously equilibrated with 50 mM buffer B. The enzyme was eluted with the same buffer at a flow rate of 25 ml/h. The effluent was collected in 3 ml fractions. The active fractions were pooled and concentrated by ultrafiltration. A 0.5 ml sample of the concentrated solution was diluted with the same volume of 10% glycerol and applied to a column of Matrex Green A (2 ml), equilibrated with 25 mM-potassium phosphate buffer, pH 7.5, containing 10% glycerol (buffer C). After washing of the column with buffer C (30 ml), the enzyme was eluted with a linear NaCl gradient (0–1 M) in buffer C (100 ml). The enzyme was eluted at about 250 mM-NaCl. Active fractions were pooled and concentrated by ultrafiltration.

Immunological procedure

An antibody was prepared by the intradermal injection of 50 μg of the purified yeast enzyme in complete Freund's adjuvant into a Japanese White-strain rabbit (2 kg), and 1 month later the rabbit was again immunized

with 50 μg of the enzyme as above. Antibodies could be detected in the animal serum by the Ouchterlony (1949) double-diffusion technique within 1 month of the initial injection. The serum was collected after 10 days of the second immunization and stored at −20 °C.

Amino acid analysis

Amino acid analysis was performed with a HLC-825AA amino acid analyser (Toyo Soda Kogyo, Tokyo, Japan). Protein samples were dialysed against 25 mM-$(NH_4)_2CO_3$, and then hydrolysed in 4.0 M-methanesulphonic acid at 115 °C for 23 h (Simpson *et al.*, 1976).

Other methods

Protein was determined by the method of Bradford (1976). Polyacrylamide-gel disc electrophoresis (Davis, 1964), sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Laemmli, 1970) and sucrose-density-gradient centrifugation for the determination of approximate M_r (Martin & Ames, 1961) were carried out as described in the cited references.

Reagents

Sephadex G-200 and phosphocellulose (P-11) were obtained from Pharmacia and Whatman respectively. Matrex Green A was a product of Amicon. Other materials used were obtained as stated previously (Noguchi *et al.*, 1978).

RESULTS

Purification of alanine:glyoxylate aminotransferase

Table 1 shows the results of the purification of alanine:glyoxylate aminotransferase from *S. cerevisiae*. About 350-fold purification was achieved, with a recovery of about 13%. The enzyme preparation (in 25 mM-potassium phosphate buffer, pH 7.5, containing about 100 mM-NaCl, 100 μM-pyridoxal 5′-phosphate and 10% glycerol) may be stored at −20 °C for at least 3 months without loss of activity. Little or no activity was lost when it was stored at 0–4 °C for 2 weeks.

Criteria of purity

Sodium dodecyl sulphate/polyacrylamide (12.5%)-disc-gel electrophoresis resulted in the detection of a single protein band (Fig. 1). Upon polyacrylamide (7.7%)-disc-gel electrophoresis at pH 8.7 in the absence of sodium dodecyl sulphate, a broad protein band with a mobility of 0.23 (relative to that of Bromophenol Blue marker) was obtained. The gel run in parallel was cut into 2 mm slices and assayed for alanine:glyoxylate aminotransferase activity. The protein band and activity were coincident.

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of purified yeast alanine:glyoxylate aminotransferase

A 6 μg sample of protein and a 12.5%-acrylamide gel were used. The migration was from top to bottom. Gel was stained for protein in 0.5% Coomassie Blue in methanol/acetic acid/water (5:1:5, by vol.) and destained by diffusion in 7% acetic acid.

Catalytic properties

The specificity of the purified enzyme preparation towards various 2-oxo acids was determined with L-alanine (40 mM) as amino donor. The enzyme utilized only glyoxylate. Other 2-oxo acids used (hydroxypyruvate, 2-oxoglutarate, phenylpyruvate and 2-oxo-4-methylthiobutyrate) were all inactive (< 1%).

The specificity of the purified enzyme preparation to various L-amino acids was determined with glyoxylate (5 mM) as amino acceptor. The enzyme utilized only L-alanine. Other L-amino acids used were all inactive (< 2%) (serine, threonine, glutamic acid, glutamine, aspartic acid, asparagine, ornithine, leucine, valine, isoleucine, histidine, phenylalanine, tryptophan and tyrosine).

The relationship between pH and the activity of the purified enzyme was studied (Fig. 2). Buffers used were potassium phosphate (0.1 M) between pH 6.0 and pH 8.0, triethanolamine/HCl (0.1 M) between pH 7.5 and

Table 1. Purification of alanine:glyoxylate aminotransferase from *S. cerevisiae*

Details of the purification procedures are described in the text.

	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)
Crude extract	6354	3240	0.51	1
Heat treatment	1472	2095	1.42	2.8
Phosphocellulose	295	1590	5.40	10.6
Hydroxyapatite	13.3	1224	92.3	181
Sephadex G-200	5.9	616	104.4	205
Matrex Green A	2.4	432	180.2	353

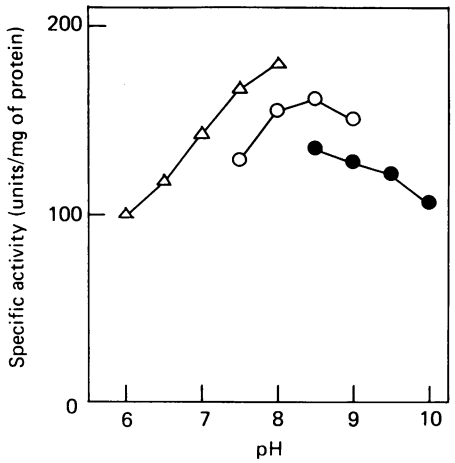


Fig. 2. Effect of pH on the activity of alanine:glyoxylate aminotransferase

Buffers used (0.1 M) were potassium phosphate (△), triethanolamine/HCl (○) and sodium pyrophosphate (●). Details of the assay were described in the text.

pH 9.0, and sodium pyrophosphate (0.1 M) between pH 8.5 and pH 10. The enzyme preparation had a pH optimum near pH 8.5.

The apparent K_m values of the purified enzyme were determined from double-reciprocal plots of initial velocity and substrate concentration. The apparent K_m value for L-alanine with glyoxylate (5 mM) was 2.1 mM, and that for glyoxylate with L-alanine (40 mM) was 0.7 mM.

The reverse transamination reaction of the purified enzyme with glycine (40 mM) and pyruvate (2 mM) as substrates was examined. A negative result was obtained; the enzyme was found to transfer the amino group from L-alanine to glyoxylate.

M_r and isoelectric point

The M_r of the purified enzyme preparation was estimated to be about 80000 by sucrose-density-gradient centrifugation. Sodium dodecyl sulphate/polyacrylamide-disc-gel electrophoresis gave a M_r of about 40000, suggesting that the enzyme consisted of two identical subunits of this M_r.

Isoelectric focusing of the crude extract on a pH 3–10 Pharmalyte gradient gave activity in the range pI 7–9, with three activity peaks with pI 7.4, 7.8 and 8.3.

Amino acid composition

The amino acid composition of the purified yeast alanine:glyoxylate aminotransferase is presented in Table 2. The content of cysteine was not determined. The major amino acid residues were aspartic acid, serine, glutamic acid, glycine, alanine, valine, leucine, and lysine. Tryptophan was not detected. The amino acid compositions of alanine:glyoxylate aminotransferase 2 (AGT 2) from pig liver and alanine:glyoxylate aminotransferase 1 (AGT 1) from rat liver are also presented for comparison. The compositions of the three enzymes, shown in residues/100 residues, were similar to each other, except for some amino acid residues (e.g. glutamic acid, glycine or methionine).

Table 2. Amino acid composition of alanine:glyoxylate aminotransferases

Purified alanine:glyoxylate aminotransferase (100 μg) was hydrolysed in a sealed evacuated ampoule in 4.0 M-methanesulphonic acid for 23 h at 115 °C. The number of amino acid residues per molecule of subunit of the enzyme was calculated on the basis of a subunit M_r of 40000 for yeast enzyme and 50000 for pig enzyme. The data for rat liver alanine:glyoxylate aminotransferase 1 (Noguchi *et al.*, 1978), which is identical with phenylalanine:pyruvate aminotransferase, are from Shih & Chan (1978). Pig liver alanine:glyoxylate aminotransferase 2 was prepared as described (Takada & Noguchi, 1982). Key: A, residues/100 residues; B, residues/subunit; AGT, alanine:glyoxylate aminotransferase; n.d., not determined.

	Yeast AGT		Rat AGT 1		Pig AGT 2	
	A	B	A	B	A	B
Asp	9.49	35	10.90±0.10	40	7.70	34
Thr	5.46	20	5.45±0.07	20	5.97	26
Ser	9.72	36	7.04±0.07	26	6.39	28
Glu	7.66	28	15.11±0.17	55	11.73	52
Pro	1.49	6	n.d.		2.09	9
Gly	7.80	29	13.01±0.16	47	9.46	42
Ala	8.94	33	8.04±0.11	29	7.10	31
Val	7.65	28	7.37±0.21	27	6.57	29
Met	0.77	3	0.47±0.03	2	4.34	19
Ile	5.66	21	4.57±0.07	17	5.91	26
Leu	9.72	36	9.49±0.11	35	9.06	40
Tyr	2.59	10	1.62±0.18	6	2.22	10
Phe	6.37	24	n.d.		6.43	28
His	2.83	10	3.39±0.04	12	3.99	18
Lys	8.02	30	6.16±0.01	23	5.37	24
Trp	0	0	n.d.		0	0
Arg	2.90	11	3.58±0.19	13	5.30	23
		360		352		439

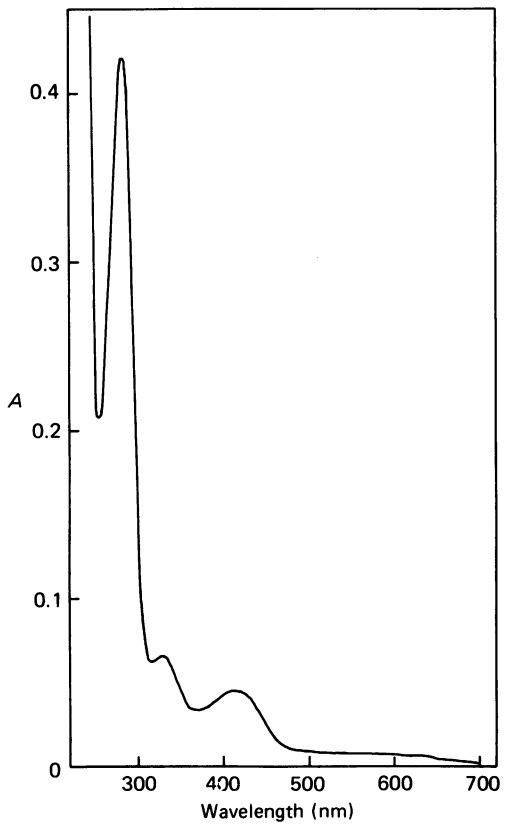


Fig. 3. Absorption spectrum of yeast alanine:glyoxylate aminotransferase

The buffer was 50 mM-potassium phosphate, pH 7.2, containing 0.1 M-NaCl.

Absorption spectrum and cofactor requirement

The absorption spectrum of the purified enzyme showed three maxima (Fig. 3). That at 280 nm is typical of proteins and is caused by aromatic amino acid residues in the peptide chains. The peak at 415 nm is probably due to pyridoxal 5′-phosphate bound to the protein. The peak at 325 nm probably represents the pyridoxamine form (Taylor & Jenkins, 1966). Dialysis (16 h at 4 °C) of the purified enzyme against 20 mM-L-alanine in 50 mM-potassium phosphate buffer, pH 7.5, did not lead to decrease in the activity assayed without added pyridoxal 5′-phosphate (holo-activity), suggesting that the treatment did not result in resolution of the cofactor from the enzyme. The dialysed enzyme showed aborption maxima only at 280 nm and 325 nm, but not at 415 nm. When the purified enzyme was incubated for 1 h at 37 °C in 50 mM-potassium phosphate buffer, pH 7.0, in the presence of 20 mM-L-alanine, the activity fell by 40%. When the enzyme was incubated with 40 mM-L-alanine, followed by dialysis against a series of phosphate buffers, as described by Churchich & Moses (1981), the activity fell by 32%. In the latter two cases, the treated enzymes regained most of the original activity after addition of pyridoxal 5′-phosphate. These results showed that the enzyme contained pyridoxal 5′-phosphate as cofactor.

Inhibition by carbonyl reagents

The activity of the purified enzyme was inhibited by the addition of carbonyl reagents (amino-oxyacetate, hydroxylamine, semicarbazide and isonicotinic acid hydrazide) to the reaction mixture (Table 3). Amino-oxyacetate was the most effective inhibitor and completely inhibited the reaction. This inhibition is probably due to the binding of the inhibitor to the aldehyde group of the coenzyme, pyridoxal 5′-phosphate.

Effect of carbon source on alanine:glyoxylate aminotransferase activity

Table 4 shows the specific activity of alanine:glyoxylate aminotransferase in crude extracts of cells grown on different carbon sources. The activity was very low in the exponential phase when cells grow on glucose, but remarkably high when cells grow on acetate or ethanol as sole carbon source or in the stationary phase in the glucose medium. It has been reported that isocitrate lyase, another enzyme involved in the glyoxylate pathway of glycine and serine formation, increased in activity when glucose was completely replaced by ethanol or acetate in the medium (Polakis & Bartley, 1965; Gosling & Duggan, 1971). These findings suggested that the glyoxylate pathway was not functional when cells grow on glucose, but was functional when only a C_2 compound was available for glycine and serine formation.

Specificity of the glyoxylate transamination in ethanol-grown cells and commercial baker's yeast

The crude extract of the ethanol-grown *S. cerevisiae* and commercial baker's yeast were tested for specificity for L-amino acids with glyoxylate as amino acceptor as

Table 3. Effect of inhibitors on the activity of alanine:glyoxylate aminotransferase

Details of the assay are described in the text. Pyridoxal 5′-phosphate was not added to the reaction mixture.

Addition	Concn. (mM)	Activity (%)
None		100
Semicarbazide	1	66
	2	30
Hydroxylamine	0.25	58
	0.5	29
Isonicotinic acid hydrazide	2	86
	3	65
Amino-oxyacetic acid	0.05	0
	0.1	0

Table 4. Effect of carbon source on the activity of alanine:glyoxylate aminotransferase

S. cerevisiae 101D was grown at 30 °C aerobically in a complex medium (Ulane & Ogur, 1972) containing 10 g of glucose, 6 g of potassium acetate or 17.5 g/ethanol per litre as sole carbon source. The activity was assayed in the crude extract as described in the text.

Carbon source	Growth phase	Activity (munits/mg of protein)
Glucose	Mid-exponential	10
	Stationary	274
Acetate	Mid-exponential	439
	Stationary	74
Ethanol	Mid-exponential	930
	Stationary	411

described above. The extracts utilized only L-alanine. Neither serine:glyoxylate aminotransferase nor glutamate:glyoxylate aminotransferase activity was detected. It is suggested that alanine:glyoxylate aminotransferase catalysed most of the glyoxylate transamination in the cells grown on C_2 compounds.

Induction of alanine:glyoxylate aminotransferase

When cells grown on 10% glucose as sole carbon source for 17 h were transferred to medium containing 2% ethanol as sole carbon source, alanine:glyoxylate aminotransferase activity markedly increased from 180 munits/mg of protein at the beginning to 600 in 4 h, and 720 in 8 h. The increase was completely inhibited by cycloheximide (100 μg/ml), but not at all by chloramphenicol (500 μg/ml). These results suggested that alanine:glyoxylate aminotransferase was synthesized *de novo* during the incubation period.

Immunological properties

Rabbit antiserum against yeast alanine:glyoxylate aminotransferase was raised as described in the Experimental section. The specificity of the antiserum was tested by Ouchterlony double-diffusion analysis; only a single precipitin band was observed with crude extract of ethanol-grown cells and commercial baker's yeast as antigen. Alanine:glyoxylate aminotransferases 1 and 2 from rat and pig liver respectively (Noguchi *et al.*, 1978; Takada & Noguchi, 1982), alanine:glyoxylate aminotransferase from mackerel liver (Noguchi *et al.*, 1984) and those from frog and turtle liver (Y. Takada & T. Noguchi, unpublished work) did not react with the antibody against yeast enzyme on Ouchterlony double-diffusion analysis.

DISCUSSION

In the present paper the characteristics of a highly specific yeast alanine:glyoxylate aminotransferase are documented. Until now alanine:glyoxylate aminotransferase with high substrate specificity has been reported only in higher animals (Noguchi *et al.*, 1978). The alanine:glyoxylate aminotransferase activity in plant leaves has been due to the actions of glutamate:glyoxylate aminotransferase and serine:glyoxylate aminotransferase (Noguchi & Hayashi, 1980; Noguchi & Fujiwara, 1982; Nakamura & Tolbert, 1983). In yeast cells most of the glyoxylate transamination is irriversibly catalysed by a specific alanine:glyoxylate aminotransferase. Spectroscopic evidence and inhibition by carbonyl reagents suggested that the enzyme contained pyridoxal 5′-phosphate as a cofactor. The apparent K_m value for L-alanine (2.1 mM) was relatively low compared with those of alanine:glyoxylate aminotransferases from other sources (Noguchi *et al.*, 1978).

A single protein band was obtained on sodium dodecyl sulphate/polyacrylamide-disc-gel electrophoresis. However, isolectric focusing on a pH 3–10 Pharmalyte gradient resulted in the detection of multiple activity peaks with pI volumes of 7.4, 7.8 and 8.3. The same phenomenon has been observed for avian liver 4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) by Wada *et al.* (1975). They suggested that this phenomenon may be explained by non-enzymic deamidation of a single nascent enzyme. The heterogeneity of the present yeast alanine:glyoxylate aminotransferase might be also explained by this mechanism, or by other post-translational modifications.

The alanine:glyoxylate aminotransferase activity was very low when the yeast cells were grown on glucose as sole carbon source, but became markedly high when the cells were grown on ethanol or acetate as sole carbon source. Incubation of glucose-grown cells in medium containing ethanol as sole carbon source led to a marked increase in the activity. The increase was blocked by cycloheximide, suggesting that the enzyme was synthesized *de novo* during the incubation period. Cycloheximide has been reported to block completely the increase in activity of isocitrate lyase, another regulatory enzyme in the glyoxylate pathway of glycine and serine biosynthesis, during adaptation to acetate utilization (Gosling & Duggan, 1971). It is suggested that the glyoxylate pathway is regulated by synthesis of these enzymes *de novo*.

Alanine:glyoxylate aminotransferase has been reported to be present in the liver and kidney of a wide range of animal species (mammals, birds, reptiles and fish) (Rowsell *et al.*, 1969). The liver enzymes have been reported to catalyse quantitatively the most important transamination of glyoxylate in mammals (Rowsell *et al.*, 1969). Mammalian liver enzymes have been suggested to be involved in gluconeogenesis (Rowsell *et al.*, 1969) and prevention of glyoxylate toxicity (Weinhouse & Friedman, 1951; Noguchi & Takada, 1979; Takada *et al.*, 1984). Two distinct isoenzymes of alanine:glyoxylate aminotransferase, designated AGT 1 and AGT 2 respectively,

have been reported in mammalian liver. AGT 1 has a M_r of about 80000, consists of two identical subunits of M_r about 40000, and has serine:pyruvate aminotransferase activity. AGT 2 has a M_r of about 200000, consists of four identical subunits of M_r about 50000, and is highly specific for L-alanine and glyoxylate (Noguchi *et al.*, 1978; Takada & Noguchi, 1982). Yeast alanine:glyoxylate aminotransferase was similar to AGT 1 with respect to M_r and subunit structure and to AGT 2 with respect to substrate specificity. The amino acid compositions of yeast and vertebrate enzymes were similar, except for some amino acids, but they were not immunologically cross-reactive. Immunological cross-reactivity is observed only between proteins that show at least 60% sequence homology (Prager & Wilson, 1971). Therefore it is not clear whether these enzymes have arisen by divergent evolution from a common ancestral protein. Detailed comparison of amino acid sequences of these protein is required to clarify this point.

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