The Purification and Properties of the Tyrosine-2-Oxoglutarate Transaminase of *Saccharomyces cerevisiae*

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Studies by SentheShanmuganathan (1956, 1960) and SentheShanmuganathan & Elsdén (1958) have established that in the production of fusel oil by *Saccharomyces cerevisiae* transamination is the first step in the conversion of an amino acid into the corresponding alcohol containing one less carbon atom. The formation of tyrosol by washed-cell suspensions and by cell-free extracts of yeast has been studied in detail (SentheShanmuganathan & Elsdén, 1958). The work described in this paper deals with the purification and resolution of the tyrosine-2-oxoglutarate transaminase from *S. cerevisiae* and the subsequent studies of kinetics and substrate specificity with the purified enzyme. A preliminary report of these findings has been published (SentheShanmuganathan, 1958).

MATERIALS AND METHODS

Organism. The organism used throughout this work was *Saccharomyces cerevisiae* (strain 5 of the Departmental collection). The method of growing the cells and the preparation of washed cells have been described by SentheShanmuganathan & Elsdén (1958).

Amino acids. All the amino acids (L. Light and Co. Ltd., Colnbrook, Bucks.) were checked for purity by paper chromatography (Woiwod, 1949). Solutions of the amino acids were adjusted to pH 7.2 with 0.2N-NaOH before use.

Phosphate buffers. The phosphate buffers used in this work were Na_2HPO_4 - KH_2PO_4 mixtures.

2-Oxo acids. 2-Oxoglutarate was prepared by the method of Clutterbuck (1927). Oxaloacetic acid was a gift from Professor Sir Hans Krebs, F.R.S.; the sodium salt was prepared as described by Elsdén & Ormerod (1956). *p*-Hydroxyphenyl pyruvate was prepared as described by SentheShanmuganathan & Elsdén (1958).

Pyridoxal phosphate (synthetic). This was the gift of Dr Morrison of Roche Products Ltd., Welwyn Garden City, Herts.

Preparation of extracts to test transaminase activity. Dialysed cell-free extracts (termed 'Hughes extracts') of *S. cerevisiae* were used in all the experiments. The mass of broken cells, obtained by crushing the yeast in a Hughes press (Hughes, 1951), was incubated with 0.1M-phosphate buffer, pH 7.2 (12 ml./g. dry wt. of cells), for 1 hr. at 37°. The mixture was then centrifuged at 19 600 *g* for 20 min. at -2°, the supernatant was separated and the cell debris

suspended in an equal volume of the same buffer. The pH values of extracts (i.e. the supernatants) so obtained varied between 6.5 and 6.7 and were adjusted to pH 7.2 with 0.2N-NaOH. The extracts contained a high concentration of free glutamate and this was eliminated by dialysing against 0.02M-KCl at 1-2° for 16-18 hr. with continuous stirring (Bigger-Gehring, 1955). The contents of the dialysing bag did not contain any precipitate. Both the dialysed extract and the suspension of cell debris were tested for transaminase activity between 2-oxoglutarate and either tyrosine or leucine. The whole of the transaminase activity was found in the cell-free extract.

Estimation of protein. This was by the turbidimetric method of Bücher (1947) adapted by Kornberg (1950) for use with the spectrophotometer. The details of the procedure used in this Department are as follows. To the sample containing no more than 0.8 mg. of protein in 2.6 ml. is added 0.3 ml. of 0.6M-(NH_4)₂SO₄ and *E* at 340 m μ is measured with the Unicam SP. 500 spectrophotometer and a cuvette of 1 cm. light-path. Trichloroacetic acid (0.1 ml. of 50%, w/v) is then added, the whole mixed and *E* is measured again after 2 min. With most proteins, including those in microbial extracts, the increase in extinction is directly proportional to the amount of protein present; this, however, does not hold for serum proteins (Dr J. L. Peel, unpublished work). The slope of the calibration curve varied somewhat with the protein used as standard, but approximated to an increase in extinction of 1.0/mg. of protein. Since the choice of the protein standard is arbitrary this value was, for convenience, used for all determinations.

Estimation of glutamic acid. Glutamic acid was estimated by the specific decarboxylase method of Gale (1945). The formation of glutamate was also followed by paper-partition chromatography with propanol-water (80:20). This solvent was chosen because it gave a good separation of glutamate from glutathione (Hanes, Hird & Isherwood, 1952). After the chromatogram had been developed, glutamic acid was detected by spraying with a solution of ninhydrin (Woiwod, 1949).

Assay of transaminase activity. Incubations were made in duplicate in 15 ml. test tubes at 37° unless stated to the contrary. Except where indicated differently, the complete system contained, in a total volume of 3 ml.: pyridoxal phosphate (0.04 μ mole), enzyme solution (0.1 ml.), 2-oxoglutarate (10 μ moles when tyrosine was the amino acid and 20 μ moles in all other cases) and glutathione (1 μ mole) in 67 mM-phosphate buffer; during the purification procedure, the pH value was 7.2 and in studies with the purified enzyme the pH value was 7.8. The mixture was incubated for 10 min. before the addition of L-tyrosine (5 μ moles) or L-leucine (10 μ moles). Two control systems

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were used, one without the amino acid and the other without 2-oxoglutarate. At the end of the incubation period, 0.2 ml. of 40% (w/v) trichloroacetic acid was added and, after heating in a boiling-water bath for 3 min., the denatured protein was removed by centrifuging. Glutamate or *p*-hydroxyphenyl pyruvate was estimated in the supernatant. No glutamate or *p*-hydroxyphenyl pyruvate was formed in the control systems. One unit of enzyme activity is defined as that amount of enzyme which catalyses the formation of 1 μ mole of *p*-hydroxyphenyl pyruvate or glutamate in 30 min. at 37° under the standard conditions described above. The specific activity is the number of units of activity/mg. of protein and the purification factor is the ratio of the specific activity of the purified fraction to that of the initial material.

Estimation of 2-oxo acids. 2-Oxoglutarate was estimated by the method of Krebs (1950). *p*-Hydroxyphenyl pyruvate was estimated by the method of Briggs (1922) as modified by Canellakis & Cohen (1956). The extinction of the coloured solution obtained by mixing the *p*-hydroxyphenyl pyruvate with a mixture of ammonium phosphomolybdate and KH_2PO_4 was determined, after 1 hr., with the EEL colorimeter (Evans Electroselenium Ltd., Harlow, Essex) and a red filter. With this 2-oxo acid, a linear relationship was obtained between colour intensity and concentration up to 0.55 μ mole/ml. (Fig. 1).

RESULTS

Thiol groups and activity

In the early experiments there was a rapid loss of transaminase activity during purification of the enzyme. Activity was restored by the addition of glutathione or cysteine, indicating that SH groups were probably important for optimum activity of the transaminase. Because of these results, *p*-chloromercuribenzoate, a reagent known to combine with SH groups, was tested for its effect on transaminase activity in 'Hughes extracts'. 2.83 μM -*p*-Chloromercuribenzoate inhibited trans-

aminase activity by 56%, but increasing the concentration to as much as 0.565 mM did not cause any appreciable increase in the degree of inhibition (Table 1). In these experiments the *p*-chloromercuribenzoate was incubated together with the enzyme and pyridoxal phosphate for 10 min. before the addition of the tyrosine. Inhibition may have been only partial because of protection of some of the SH groups by the cofactor. However, if the cofactor was added after *p*-chloromercuribenzoate had been incubated with the enzyme for 10 min., the results were essentially the same as before. Since these experiments were done with the crude cell-free extract it was possible that two or more transaminases for tyrosine were present and that SH groups were essential for the optimum activity of only one of the enzymes. Alternatively, if only one transaminase is present in the crude extract, it is feasible that only some of the SH groups are required for maximal activity. It is well known that in some enzymes, e.g. adenosine triphosphatase (Singer & Barron, 1944), all the thiol groups of the native protein are essential for activity, whereas in other enzymes, e.g. urease (Hellerman, Chinard & Deitz, 1943), only some of them are essential. Reactivation of transaminase inhibited by *p*-chloromercuribenzoate was achieved by the addition of glutathione (0.01 M) or cysteine (0.01 M) to the reaction mixture (Table 2). In subsequent experiments glutathione was always included in the test system for transaminase activity.

Purification procedure for tyrosine transaminase

The general properties of the transaminase with regard to the stability to acid and heat and to adsorption on to calcium phosphate gel and subsequent elution were established in small-scale experiments. Table 3 summarizes the steps and

Fig. 1. Calibration curve (EEL spectrophotometer readings) for the estimation of *p*-hydroxyphenyl pyruvate. Estimated by the method of Canellakis & Cohen (1956).

Table 1. Effect of *p*-chloromercuribenzoate on tyrosine-2-oxoglutarate-transaminase activity

The system (3 ml.) containing the enzyme (0.1 ml. of Hughes extract), pyridoxal phosphate (0.04 μ mole), 2-oxoglutarate (10 μ moles) and *p*-chloromercuribenzoate was incubated for 10 min. before the addition of L-tyrosine (5 μ moles). *p*-Hydroxyphenyl pyruvate formed in 30 min. at 37° was estimated by the method of Canellakis & Cohen (1956). (Unit of activity is defined in the Materials and Methods section.)

Concn. of <i>p</i> -chloromercuri- benzoate (μM)	Total transaminase activity (units/ml.)	Inhibition (%)
0	22.8	—
2.83	10.2	56
5.66	10.1	56
8.49	10.2	56
11.30	10.1	56
565	10.2	56

Table 2. *Reactivation of the tyrosine-2-oxoglutarate transaminase inhibited by p-chloromercuribenzoate*

The system (3 ml.), containing the enzyme solution (0.1 ml. of Hughes extract), pyridoxal phosphate (0.04 μ -mole), 2-oxoglutarate (10 μ moles) and *p*-chloromercuribenzoate (as given), was incubated for 10 min.; then either glutathione or cysteine (0.01 M) was added and the mixture incubated for a further period of 10 min. before addition of L-tyrosine (5 μ moles). *p*-Hydroxyphenyl pyruvate formed in 30 min. at 37° was estimated by the method of Canellakis & Cohen (1956).

Concn. of <i>p</i> -chloromercuri- benzoate (μ M)	Total transaminase activity (units/ml.)		
	No addition	Glutathione (0.01 M)	Cysteine (0.01 M)
0	22.8	31.2	31.2
2.83	10.2	31.2	31.0
5.66	10.1	30.8	30.8

yields of a typical preparation. In order to obtain sufficient material and because freshly grown yeast was required, a series of extracts was prepared over a period of 2 weeks. Each extract was dialysed against mM-phosphate buffer, pH 7.2, for 12–16 hr. at 1–2° and then stored at –20°. All the preparations were subsequently mixed to provide the initial preparation. Acid precipitation was the first step. Acetic acid (2N) was added rapidly until the pH value was 4.1; the preparation was then centrifuged at 19 600 *g* for 20 min. at –2° and the precipitate discarded (yield of enzyme: 95–97% with a sixfold purification). The supernatant was adjusted to pH 5.5 with 0.2N-NaOH and the temperature raised rapidly (4 min.) to 60°. The preparation was kept at this temperature for 6 min. and cooled and the inactive precipitate was removed by centrifuging. This treatment gave a twofold purification.

Table 3. *Purification of tyrosine transaminase*

Step	Volume (ml.)	Protein (mg.)	Total activity (units)	Specific activity	Purifi- cation factor	Yield (%)
(i) Dialysed crude cell-free extract	380	10 640	5 152	0.48	1	100
(ii) Acid treatment: brought to pH 4, ppt. discarded	360	1 400	5 010	3.5	7	97
(iii) Heat treatment. Supernatant from (ii) at 60° for 6 min. at pH 5.5, ppt. discarded	350	600	4 200	7.0	14	81
(iv) Ca ₃ (PO ₄) ₂ gel treatment:						
(1) 0.66 mg. } (dry wt. of	40	140	900	6.4	13	17
(2) 0.5 mg. } gel/mg. of protein) {	40	105	2 140	20	41	41
(3) 0.5 mg. }	30	136	1 010	7.4	15	19
Gel from 1 and 3 combined, eluted and eluate treated						
(1) 0.66 mg. } (dry wt. of	—	—	—	—	—	—
(2) 0.5 mg. } gel/mg. of protein) {	40	60	1 068	17	35	20
(3) 0.5 mg. }	—	—	—	—	—	—
(v) (NH ₄) ₂ SO ₄ fractionation of combined (iv) two eluates						
Unfractionated	100	165	3 208	19	39	62
0–50%	20	25	132	5.2	11	2
50–65%	30	80	2 329	29	60	45
65–85%	20	37	642	17	35	12
(vi) (NH ₄) ₂ SO ₄ fractionation of (v) 50–65%						
Unfractionated	40	80	2 344	29.3	61	45
0–50%	—	0	—	—	—	—
50–58%	15	40	1 652	41.3	86	32
58–65%	15	20	680	34	70	13
65–75%	5	6	48	8	—	—
(vii) (NH ₄) ₂ SO ₄ fractionation of 50–58% and 58–65% from (vi)	40	60	2 344	40	83	45
0–50%	—	0	—	—	—	—
50–54%	15	20	1 071	53	110	20
54–60%	15	14	688	49	100	13
60–65%	15	16	375	22	46	7

The supernatant resulting from the heat-treatment was brought to pH 6.0 and stirred with calcium phosphate gel (final concentration: 0.66 mg. of gel/mg. of protein). The gel (P1), which was collected by centrifuging, contained only 17% of the initial activity. A further 0.5 mg. of gel/mg. of protein was added and the resulting precipitate (P2) contained 41% of the activity. A third treatment with 0.5 mg. of gel/mg. of protein gave precipitate P3, which contained a further 19% of the activity. The enzyme was eluted from P2 by stirring it with 0.1M-phosphate buffer, pH 7.2, for 1 min. The gel was centrifuged off and the eluate stored at -20° until required. Meanwhile, P1 and P3 were pooled, the enzyme was eluted with phosphate buffer as described above and the eluate dialysed against mM-phosphate buffer, pH 7.2, for 12–16 hr. The dialysed eluate so obtained was then subjected to a further gel treatment, the eluate from which contained 20% of the original activity. This was mixed with the eluate from P2.

The combined eluates were brought to 50% saturation with $(\text{NH}_4)_2\text{SO}_4$ and after 5 min. the precipitate was removed by centrifuging (19 600 g for 10 min. at -2°). The supernatant was brought to pH 7.2 (0.2N-NaOH) and the $(\text{NH}_4)_2\text{SO}_4$ concn. to 65% saturation; the precipitate was removed by centrifuging. The supernatant was again brought to pH 7.2 and the $(\text{NH}_4)_2\text{SO}_4$ concn. to 85% and the precipitate was collected. Each precipitate was dissolved in 0.1M-phosphate buffer, pH 7.2, and insoluble material was removed. The bulk of the enzyme (45%) was in the 50–65% fraction, with a 60-fold purification, and this fraction was re-fractionated between the limits 0–50, 50–58, 58–65 and 65–75% (w/v) $(\text{NH}_4)_2\text{SO}_4$. Activity was mainly in the 50–58% and 58–65% fractions; these two fractions were combined and re-fractionated. The 50–54% and 54–60% fractions contained most of the activity (20%, purification 110 times, and 13%, purification 100 times, respectively). Dialysis of the 54–60% fraction produced no further change and the material from this fraction was used in the subsequent studies of the properties of the tyrosine-2-oxoglutarate transaminase. This preparation had no effect on the four reactants (L-tyrosine, 2-oxoglutarate, *p*-hydroxyphenyl

pyruvate and L-glutamic acid) when incubated with any one of them alone.

Properties of the partially purified tyrosine transaminase

Specificity. The specificity of the purified enzyme preparation was examined by incubating it with various amino acids, 2-oxoglutarate, glutathione and buffer at pH 7.8. At the end of the incubation period, the mixture was held at 100° for 5 min. to inactivate the enzyme and was then centrifuged. The formation of glutamic acid, detected by paper chromatography, was taken as an indication of enzymic activity. For comparative purposes equal volumes of the various supernatants were placed on the chromatograms. Of the ten amino acids tested for their ability to transaminate with 2-oxoglutarate, only tyrosine gave rise to a large distinct spot corresponding to glutamic acid. Leucine, norleucine, methionine, tryptophan and phenylalanine formed only very small amounts of glutamic acid (faint spots) and there was no detectable glutamic acid formed from isoleucine,

Fig. 2. Progress curve for the formation of a *p*-hydroxyphenyl pyruvate from tyrosine. Experimental conditions are described in the text. The experimental system was contained in 0.1M phosphate buffer, pH 7.8. Temp., 37° . Amount of enzyme protein used was 6.67 μg. The keto acid formed was measured at 10 min. intervals.

Table 4. Activities of leucine- and tyrosine-transaminase systems

Preparation 1 was a dialysed crude extract and preparation 2 was obtained by adjusting preparation 1 to pH 4.0 and discarding the precipitate. Transaminase activity was measured as described in the text.

Preparation	Total protein (mg.)	Leucine transaminase		Tyrosine transaminase		Specific activity (tyrosine) Specific activity (leucine)
		Total units in extract	Specific activity (units/mg.)	Total units in extract	Specific activity (units/mg.)	
1	2200	2400	1.1	1310	0.59	0.54
2	320	630	1.9	1230	4.0	2.11

valine, norvaline and aspartic acid. The dialysed crude extract catalysed the transamination of 2-oxoglutarate with each of the ten amino acids tested and glutamic acid was formed in good yield (SentheShanmuganathan, 1960); it is clear that the final preparation had been substantially freed from these other transaminases. In the dialysed crude Hughes extract, the activity for leucine was nearly twice that for tyrosine, as judged by the rate of glutamic acid formation. After acid precipitation at pH 4, only 26 % of the leucine activity remained in the supernatant; the activity for tyrosine remained unchanged (Table 4). Hence it would appear that transamination involving leucine is not catalysed by the same enzyme as that concerned with tyrosine. The ability of the purified enzyme to transfer amino groups from L-tyrosine to pyruvate or oxaloacetate was also tested, but neither of these keto acids could replace 2-oxoglutarate. The amount of *p*-hydroxyphenyl pyruvate formed by the complete system at pH 7.8 increased linearly with time up to 30 min. (Fig. 2), and in all further work the incubation period was 30 min. or less.

Effect of pH on activity. With the standard incubation system, but with different buffers, the effect of pH on activity was determined. The optimum pH was between pH 7.8 and 8.0 at 37° (Fig. 3). The activity fell sharply on the acid side of the optimum pH but decreased only a small amount on the alkaline side (up to pH 10).

Effect of enzyme concentration. The rate of formation of *p*-hydroxyphenyl pyruvate as a function of the amount of enzyme preparation added to the

system was linear up to about 4.5 μ g. of protein/ml. of reaction mixture (Fig. 4). With higher concentrations of the enzyme preparation the rate declined, which may have been due to inhibition by the *p*-hydroxyphenyl pyruvate produced in the reaction (cf. Canellakis & Cohen, 1956).

Effect of pyridoxal phosphate. The purified enzyme was completely resolved and was inactive unless pyridoxal phosphate was added to the system. The enzyme was saturated at 2.8 μ M-pyridoxal phosphate (Fig. 5).

Effect of temperature. The rate of transamination increased with temperature, and there was a linear relationship between 25° and 37° (Fig. 6). The temperature coefficient (Q_{10}) over the range 27–37° was 1.87.

Michaelis constants for tyrosine, 2-oxoglutarate and coenzyme. In order to determine the Michaelis constants (K_m) for any one of the reactants tyrosine, 2-oxoglutarate and pyridoxal phosphate, sufficient of the other two compounds must be present to ensure that the enzyme system is saturated and that the only material influencing the rate of the reaction is the substance under test (cf. Alberty, 1953). Precautions were taken to ensure that these conditions applied, and the observed rates of reaction were plotted graphically according to the method of Lineweaver & Burk (1934). The K_m values obtained from the three experiments in which the concentration of tyrosine,

Fig. 3. Effect of pH on the activity of purified tyrosine transaminase. Experimental conditions are described in the text. Phosphate buffers (0.1M) were used up to pH 7.5; above pH 7.5 pyrophosphate buffers (0.1M) were used. Temp., 37°. The amount of *p*-hydroxyphenyl pyruvate formed in 30 min. was determined. The amount of enzyme protein used was 6.67 μ g.

Fig. 4. Effect of enzyme concentration on the formation of *p*-hydroxyphenyl pyruvate from tyrosine. Experimental conditions are described in the text. The purified enzyme preparation used contained 66.7 μ g. of protein/ml., in 0.1M-phosphate buffer, pH 7.8. Temp., 37°; incubation period 30 min. The activity was measured in the presence of different amounts of the enzyme.

2-oxoglutarate and pyridoxal phosphate were varied one at a time are shown in Table 5, and, for comparison, those obtained by Canellakis & Cohen (1956) for the analogous enzyme from dog liver are also included.

Energy of activation and change in heat content of enzyme-substrate complex. With different concentrations of L-tyrosine and saturating concentrations

Fig. 5. Effect of concentration of pyridoxal phosphate on the formation of *p*-hydroxyphenyl pyruvate from tyrosine. Experimental conditions are given in the text. Pyridoxal phosphate was added as required. The purified enzyme (equivalent to 6.67 μg. of protein) and the substrates were present in 0.1M-phosphate buffer, pH 7.8. *p*-Hydroxyphenyl pyruvate formed in 30 min. at 37° was estimated.

Fig. 6. Effect of temperature on the formation of *p*-hydroxyphenyl pyruvate from tyrosine. Experimental details are given in the text. The system was contained in 0.1M-phosphate buffer, pH 7.8. The initial rate of formation of *p*-hydroxyphenyl pyruvate at each temperature was measured at the end of 10 min. incubation period.

of 2-oxoglutarate and pyridoxal phosphate, the maximum velocities of reaction at different temperatures were calculated from the rates of reaction according to the method of Lineweaver & Burk (1934). There was a linear relationship between $\log(V_{\max})$ and the reciprocal of the absolute temperature (Fig. 7), and since the slope of the graph is $-Ea/R$ (Gibson, 1953), Ea , the energy of activation, is 15.2 kg.cal. for the temperature range 25–46°.

If the Michaelis-Menten theory is assumed to apply, then since K_m is the reciprocal of an equilibrium constant it can be deduced from the van 't Hoff isochore that

$$\frac{d(\log K_m)}{d(1/T)} = \frac{\Delta H_m}{R},$$

Table 5. K_m values for tyrosine, 2-oxoglutarate and pyridoxal phosphate

The complete system contained in a total volume of 3.0 ml.: pyridoxal phosphate (48 μM), L-tyrosine (33 mM) and 2-oxoglutarate (50 mM) in 0.1M-phosphate buffer, pH 7.8; Temp., 37°.

Substrate	K_m	
	Yeast enzyme	Dog-liver enzyme
Tyrosine	1.66 mM	0.71 mM
2-Oxoglutarate	2.5 mM	0.16 mM
Pyridoxal phosphate	2.38 μM	1.0 μM

Fig. 7. Determination of energy of activation of enzyme-substrate complex. Maximum reaction velocity ($V_{\max}$) was determined at different temperatures. At each temperature the rate of reaction for different tyrosine concentrations was measured and $V_{\max}$ determined by the method of Lineweaver & Burk (1934). Slope of graph = $-Ea/R$. The experimental system contained, in a total volume of 3.0 ml. in 0.1M-phosphate buffer, pH 7.8: pyridoxal phosphate (48 μM), 2-oxoglutarate (50 mM), enzyme solution (0.1 ml., 6.67 μg. of protein). Incubation period was 10 min.

Fig. 8. Determination of change in heat-content in the formation of enzyme-substrate complex. Michaelis constant (K_m) for tyrosine was determined at different temperatures. At each temperature the rate of reaction for different tyrosine concentrations was measured and then K_m was determined by the method of Lineweaver & Burk (1934). The concentrations of the various substrates added were as described under Fig. 7.

where ΔH_m is the change in the heat content accompanying the formation of the enzyme-substrate complex. Experiment showed a linear relationship between $\log K_m$ and the reciprocal of the absolute temperature (Fig. 8). The slope equalled $(30/21) \times 10^3$ and corresponds to $\Delta H_m/R$, whence ΔH_m is found to be 6.5 kg.cal. for the temperature range 25–46°.

DISCUSSION

Saccharomyces cerevisiae can bring about transamination reactions involving several amino acids, and it seems likely from the work described here that there is more than one transaminase responsible for these reactions. The preparation of the tyrosine enzyme obtained, although not pure, showed little transaminase activity for amino acids other than tyrosine, whereas crude cell-free extracts contain a leucine transaminase apparently more active than the tyrosine enzyme. Like other transaminases, the tyrosine enzyme was activated by pyridoxal phosphate, and the enzyme was saturated when the coenzyme concentration was $2.8 \mu\text{M}$. This value is close to that ($4 \mu\text{M}$) obtained by Canellakis & Cohen (1956) for the tyrosine transaminase of dog liver. No evidence has been obtained that the purified tyrosine transaminase of yeast has any properties which are markedly different from those of other purified transaminases (cf. O'Kane & Gunsalus, 1947).

SUMMARY

1. Tyrosine-2-oxoglutarate transaminase has been purified more than 100-fold with an overall

yield of 33% from a dialysed crude extract of *Saccharomyces cerevisiae*.

2. The purified enzyme required pyridoxal phosphate for activity, and glutathione increased transaminase activity by 50%.

3. Neither pyruvate nor oxaloacetate replaced 2-oxoglutarate and only a very slight activity was observed when tryptophan, methionine, phenylalanine, leucine or norleucine replaced tyrosine, suggesting that the enzyme is highly specific.

4. At pH 7.8 and 37° the Michaelis constants for tyrosine, 2-oxoglutarate and pyridoxal phosphate are 1.66 mM, 2.5 mM and $2.38 \mu\text{M}$ respectively.

5. The temperature coefficient over the range 27–37° is 1.87 and the energy of activation determined over the range 25–46° is 15.2 kg.cal. The change in heat content accompanying the formation of the enzyme-substrate complex is 6.5 kg.cal. over the temperature range 25–46°.

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