ISOLATION OF A TYROSINE-ACTIVATING ENZYME FROM BAKER'S YEAST*, **, ***

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INTRODUCTION

The discovery by HOAGLAND *et al.*[1,2] of amino acid activating enzymes in the particle-free supernatant of pancreas homogenate stimulated various authors[3-7] to further research on this subject.

The enzymes carry out an amino acid-dependent exchange of radioactive ^{32}PP into ATP according to

$$ATP + {}^{32}PP \rightleftharpoons AT^{32}P + PP$$

as well as the formation of aminoacylhydroxamate in the overall reaction:

$$ATP + \text{amino acid} + NH_2OH \longrightarrow \text{aminoacylhydroxamate} + AMP + PP.$$

They have been found in animal tissue[1-4] as well as in plants[5] and micro-organisms[6,7]. A tryptophan-[3] and methionine-[7]activating enzyme have been highly purified and were found to be rather specific for the amino acid substrate.

In an attempt to obtain additional information on this type of activation reaction we undertook the isolation and purification of such an enzyme from baker's yeast. Following the hydroxamate formation as a rather easy method for determining the enzymic activity, we could obtain a reasonably pure tyrosine-activating enzyme preparation from extracts of ether–CO_2 frozen baker's yeast. In this paper a detailed description is presented of the purification procedure and characterization of this enzyme.

MATERIALS AND METHODS

The ammonium sulfate used in the preparation of the enzyme was the analytical grade Analar, purchased from the British Drug Houses.

Amino acids were obtained from Hofmann La Roche, Basle, Switzerland.

Tyrosine hydroxamic acid was prepared from methyl tyrosinate and salt-free hydroxylamine in methanol solution, according to the method of SAFIR AND WILLIAMS[9]. It was used as colorimetric standard for tyrosine as well as for the other amino acids.

Hydroxamates were determined as ferric salts after addition of an acidified solution of $FeCl_3$, according to the method of LIPMANN AND TUTTLE[10]. The color of the ferric tyrosine acyl hydroxamate proved to be rather dependent on the final acidity of the reaction mixture. Therefore,

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** The following abbreviations will be used: ATP, adenosine triphosphate; AMP, adenosine monophosphate; PP, inorganic pyrophosphate; P_i, orthophosphate; PPase, inorganic pyrophosphatase; TRIS, tri(hydroxymethyl)aminomethane; PCMB, *p*-chloromercuribenzoate.

*** A preliminary note on this subject has been published[8].

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the measurements were rigorously standardized and carried out under similar conditions as used in the determination of the enzymically formed hydroxamate.

Salt-free hydroxylamine was prepared according to BEINERT *et al.*[11]. The concentration was determined colorimetrically[12].

Crystalline pyrophosphatase was a gift from Dr. KUNITZ.

Crystalline ATP and other nucleotides were purchased from Pabst Laboratories. ^{32}P was obtained from Philips Roxane, Weesp, The Netherlands. ^{32}PP was prepared by pyrolysis of K_2HPO_4 and $Na_2H^{32}PO_4$. It contained 1–2% orthophosphate. Phosphate determinations were made by the method of FISKE AND SUBBAROW[13]. PP was determined as P_i after acid or enzymic hydrolysis. Radioactivity was determined by wet-counting samples on steel planchets.

Protein was determined turbidimetrically with trichloroacetic acid using bovine serum albumine (Povite Co., Amsterdam) as a standard[14].

The hydroxamic acid assay was similar to that used by DAVIE, KONINGSBERGER AND LIPMANN[3]. Routinely the enzyme was incubated, with and without amino acid substrate, at 30° C, pH 7.15, in a reaction mixture containing 15 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles amino acid (tyrosine was given as a suspension titrated to pH ± 7.5; the final reaction mixture contained at least 6 μmoles dissolved tyrosine/ml), 1000 μmoles salt-free NH_2OH, and 200 μmoles TRIS buffer/ml, 10 μg crystalline pyrophosphatase/ml was added until it was proved that even the most highly purified enzyme preparations still contained enough PPase activity to prevent any product inhibition[3] by the PP formed during the course of the reaction.

One-ml samples were removed at appropriate intervals and added to 2.5 ml of a solution that contained 6% $FeCl_3$, 5% trichloroacetic acid, and 0.66*N* HCl, for the determination of the hydroxamic acid.

The enzymic unit was chosen according to DAVIE, KONINGSBERGER AND LIPMANN[3] as the amount of enzyme which forms 1 μmole hydroxamate/h in the standard assay. Specific activity is given as μmoles hydroxamate formed/mg protein/h.

Fig. 1 demonstrates the proportionality between enzyme concentration and hydroxamate formation.

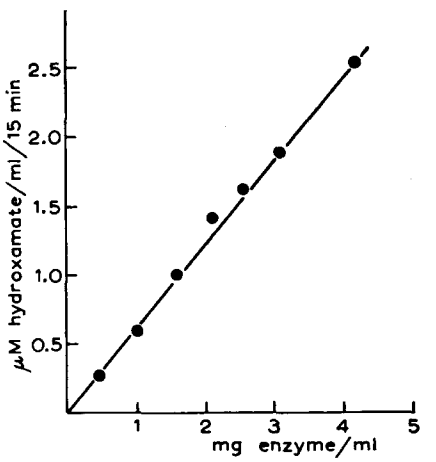

Fig. 1. Effect of enzyme concentration on hydroxamate formation. Assay contains: 15 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles L-tyrosine, 1000 μmoles NH_2OH, 200 μmoles TRIS buffer, 10 μg pyrophosphatase, and 0.52–4.2 mg enzyme, per 1.0 ml. Incubation at 30°, pH 7.8, for 15 min.

The procedure for the ATP-pyrophosphate exchange was essentially the same as described in ref. [3]. Treatment with ion-exchange resin of the enzyme proved to be unnecessary. Any residual amino acids did not influence the measurements. The enzyme preparations were incubated with 15 μmoles ATP, 3 μmoles $MgCl_2$, and 1 μmole NaF to repress the PPase activity for at least 95%, 2 μmoles ^{32}PP containing 1000 c.p.m., and 100 μmoles phosphate buffer, pH 7.15, per ml at 30° C. Appropriate samples of the reaction mixture were added to the same volume of 12% trichloroacetic acid. The PP and ATP were separated by adsorption to charcoal according to the method of CRANE AND LIPMANN[15]. The results were calculated by the equations derived by DUFFIELD AND CALVIN[16].

The rate of exchange, *R*, in μmoles/min is given by

$$R = \frac{[ATP]\,[PP]}{[ATP] + [PP]} \cdot \frac{2.3}{t} \cdot \log \frac{100}{100 - \%\ \text{exchange}}$$

[ATP] and [PP] are the total concentrations of the reactants in μmoles/ml; *t* is time in min; % exchange is:

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$$\% \text{ exchange} = \frac{AT^{32}P_t}{15/17\ (AT^{32}P_t + {}^{32}PP_t)} \times 100$$

$AT^{32}P_t$ and ${}^{32}PP_t$ refer to total radioactivity in ATP and PP at time t applying only when, as in the present experiments, different amounts of ATP and ^{32}PP are added. In this case 15/17 of the added ^{32}PP is shifted into ATP after equilibration.

Preparation of the enzyme

(1) *Crude extract*

4000 g of freshly-obtained baker's yeast are crumbled and frozen for 3–4 h in ether–CO_2. After removal of the residual ether and CO_2, the yeast is thawed out and extracted overnight by stirring after the addition of 11.2 g KCl/1000 g yeast. All further steps in the purification are carried out at 0–4° C.

The crude homogenate is centrifuged for 20 min at 5000 × *g* in a PR International Centrifuge. The supernatant (1200–1500 ml) is passed through several layers of cheese-cloth giving *crude 0.15 KCl extract-Sup I.*

(2) *Dialysis*

Sup I is dialysed in Nojax-Viscora bags against 10 l distilled water for 4 h and against a fresh 10 l batch for another 18 h. The content of the bags is centrifuged for 20 min at 5000 × *g*, giving 1500–1800 ml *dialysed-Sup II.* The precipitate is discarded.

(3) *Ammonium sulfate fractionation*

0.3135 g ammonium sulfate (analytical grade) per ml Sup II is added to give 0.5 saturation. The solution is centrifuged for 20 min at 5000 × *g*. The precipitate is discarded. The supernatant is brought to 0.6 saturation by addition of 0.065 g ammonium sulfate per ml supernatant. The precipitate is collected by centrifugation for 25 min at 5000 × *g* and dissolved in 130 ml ice-cold distilled water to give *Am_2SO_4 I-Sup III.*

(4) *First acid ammonium sulfate fractionation*

0.12 g ammonium sulfate per ml Sup III is added. The pH of the resulting solution is slowly lowered by addition of 0.2 *M* acetic acid to pH 4.5, using an Electrofact pH-meter (previously calibrated with buffer at room temperature). More $AmSO_4$ is added by addition of 0.133 ml saturated ammonium sulfate solution per ml original Sup III. (The Am_2SO_4 soln. was saturated at room temperature.) The mixture is centrifuged for 10 min at 11,000 × *g* in a PR International Centrifuge (high speed–high capacity attachment). The precipitate is discarded, and the supernatant further saturated with ammonium sulfate by addition of 0.2 ml ammonium sulfate solution per ml original Sup III. Centrifugation follows for 10 min at 11,000 × *g*. The precipitate is dissolved very quickly in 30 ml ice-cold distilled water and immediately centrifuged for 2 min at 7000 × *g*. The supernatant is brought to pH 6.5–7.0 by addition of a few drops of 1 *M* Na_2CO_3 solution, giving *Am_2SO_4 II (pH 4.5)-Sup IV.* If larger preparations are made Sup IV may be kept frozen in the presence of the residual $(NH_4)_2SO_4$, which stabilizes the enzyme. During four days[1] storage at — 15° C, 20%–30% loss in activity was observed.

(5) *Adsorption of impurities on $Ca_3(PO_4)_2$-gel*

The protein concentration of Sup IV is adjusted to 20 mg/ml by addition of distilled water. The solution is mixed with one fourth of its volume of cold $Ca_3(PO_4)_2$-gel prepared according to the method of KUNITZ[17]. The mixture is centrifuged for 10 min at 11,000 × *g*. The precipitate is discarded. The supernatant is brought to 0.6 saturation by addition of 0.4 g solid ammonium sulfate per ml supernatant. The precipitate that is collected by centrifugation for 10 min at 11,000 × *g* is dissolved in 25 ml distilled water, and a second treatment with $Ca_3(PO_4)_2$-gel is given in the same way. The final precipitate is dissolved in 20 ml distilled water and rapidly dialysed for 4 h against 10 × its final volume 0.05 *M* KCl – 0.01 *M* phosphate buffer, pH 6.8, (*ca.* 200–250 ml), giving *$Ca_3(PO_4)_2$–Am_2SO_4 III-Sup V.*

(6) *Second acid ammonium sulfate fractionation*

Sup V is subjected to a second acid ammonium sulfate fractionation, which is made in essentially the same way as described above. 0.12 g ammonium sulfate is added per ml Sup V, and the pH is slowly adjusted by addition of 0.2 *M* acetic acid to pH = 4.5. 0.3 ml saturated $(NH_4)_2SO_4$ solution per ml original Sup V is added, and the resulting mixture centrifuged for 10 min at 11,000 × *g*. The supernatant is further saturated by addition of 0.5 ml/ml original Sup V. The precipitate collected by centrifugation at 11,000 × *g* is dissolved in 5 ml water and adjusted to pH 6.5–7.0. This preparation, *Am_2SO_4 IV (pH 4.5)-Sup VI*, is frozen for overnight storage.

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By addition of more $(NH_4)_2SO_4$ to the supernatant a small third precipitate can be collected. The first precipitate and this one can be refractionated if immediately dissolved and brought to pH 6.5–7.0.

(7) *Final ammonium sulfate fractionation*

To obtain a somewhat higher specific activity a final ammonium sulfate fractionation can be made. Therefore, Sup VI is thawed and rapidly dialysed for 4 h against 40 ml 0.05 *M* KCl – 0.01 *M* phosphate buffer, pH 6.8. The solution is brought to 0.45 saturation by addition of 0.27 g $(NH_4)_2SO_4$/ml and centrifuged for 10 min at 11,000 × *g*. Addition of 0.10 g $(NH_4)_2SO_4$/ml brings the supernatant to 0.6 saturation. The precipitate collected by centrifugation for 10 min at 11,000 × *g* is dissolved in 4 ml distilled water giving Am_2SO_4 VII-Sup VII. This preparation may be stored at —15° C for about 10 days, losing 30% of its activity during storage if repeatedly thawed and frozen. Table I shows a typical purification and recovery chart starting with 4000 g of baker's yeast.

TABLE I

A TYPICAL PURIFICATION CHART FOR 4000 g OF BAKER'S YEAST

Fraction	*Protein (mg)*	*μmoles hydroxamate/mg/h*	*Total units*	*Recovery (%)*
Crude 0.15 KCl-Sup I	45,000	± 0.08	3600	—
Dialysed-Sup II	22,000	0.11	2400	75
Am_2SO_4I-Sup III	4,500	0.5	2250	70
Am_2SO_4 II (pH 4.5)-Sup IV	900	1.7	1500	43
$Ca_3(PO_4)_2$–Am_2SO_4 III-Sup V	500	2.2	1100	30
Am_2SO_4 IV (pH 4.5)-Sup VI	150	3.7	550	15
Am_2SO_4 V-Sup VII	110	4.5	500	14

Purity of the enzyme

Ultracentrifuge and electrophoretic analysis

We have already reported[8] that the combined results of electrophoresis and ultracentrifuge studies suggested that the enzyme preparation at the last stage of the isolation procedure is about 70% pure. At that stage the enzyme has then been purified about 50-fold with respect to the first particle-free supernatant. The ultracentrifuge analyses were made with a Spinco analytical ultracentrifuge in a 0.10 *M* KCl–0.01 *M* phosphate buffer solution, pH 6.8. In Fig. 2 the results of a representative experiment are shown. It demonstrates the presence of four components, the major one consisting of about 70% of the total protein.

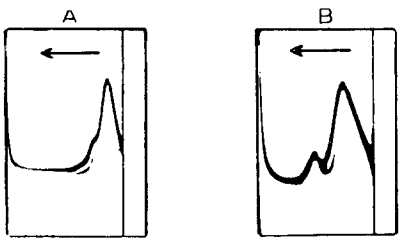

Fig. 2. Ultracentrifuge pattern for a 70-fold purified enzyme preparation (see text) in 0.10 *M* KCl – 0.01 *M* phosphate buffer (pH 6.8) solution at 56,100 r.p.m. and about 18° C. (A) After 27 min; the bar angle is 50°; (B) after 46 min; the bar angle is 50°.

Electrophoretic analysis of the same preparation under identical conditions showed a very similar pattern with a major component comprising 70% of the total protein in addition to a few minor components. Furthermore, it could be proved by a paper-electrophoresis experiment, as drawn schematically in Fig. 3, that the enzymic activity is associated with the major component.

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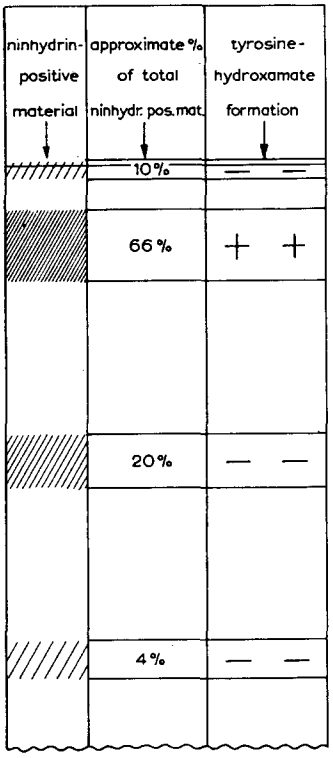

Fig. 3. Schematic outline of a paper electrophoresis experiment indicating that the enzymic activity is associated with the major component. A 50-fold purified preparation (see text) in 0.01 *M* phosphate buffer (pH 6.8) solution was subjected to electrophoresis on Whatman No. 3 filter paper for 17 h at 120 V.

TABLE II

AMINO ACID ACTIVATION BY BAKER'S YEAST AS MEASURED BY HYDROXAMATE FORMATION AND PYROPHOSPHATE EXCHANGE

Substrate	*Crude preparation of Sup I*, ***	*Dialysed- -Sup II**	*Am_2SO_4 V- -Sup VII**	*Am_2SO_4 IV- -Sup VI****
Tyrosine	0.07	0.11	4.2	6
Methionine	0.020	0.037	0.0	0.0
Phenylalanine	0.020	0.030	0.0	0.0
Tryptophan	0.022	0.0	0.0	0.0
Glutamine	0.14	0.40	0.0	0.0
Glutamic acid	0.16	0.40	0.0	0.0

Amino acids not mentioned in the table had no measurable influence on the hydroxamate formation.

* Specific activity in micromoles hydroxamate/mg protein/h.

** 0.15 KCl extract – Sup I was subjected to a rough ammonium sulfate fractionation. The crude preparation used contained the protein collected between 0.3–0.7 saturation, which accounts for at least 95 % of all protein present.

*** Radioactive exchange in μmoles/mg/h. See also text of section on exchange.

Specificity

Another indication of the reasonable purification of the enzyme can be found in Table II, which compares the activities of the crude extract and dialysate towards various amino acids with those of the final enzyme preparation. As can be seen the enzyme appears to be completely substrate-specific.

Hydroxamate formation

The influence of the experimental conditions on the hydroxamate formation is shown in Figs. 4–10.

The influence of pH (Fig. 4) is in rather good agreement with the observations of DAVIE, KONINGSBERGER AND LIPMANN[3] on tyrosine activation by crude beef pancreas extracts.

The enzyme shows a high affinity for tyrosine (Fig. 5). The saturation concentration is about 2.0 μmoles tyrosine/ml under the conditions of this experiment.

The affinity for ATP is considerably less (Fig. 6). Saturation is reached at 10 μmoles ATP/ml.

Hydroxamate is formed only in the presence of rather high concentrations of NH_2OH (Fig. 7). This observation is in good agreement with those of HOAGLAND *et al.*[1,2], NOVELLI *et al.*[6] and LIPMANN *et al.*[3]. The primary reaction product of the interaction of the enzyme, tyrosine, and ATP, either is not accessible to hydroxylamine or reacts only sluggishly with it. To avoid neutralization of higher concentrations of

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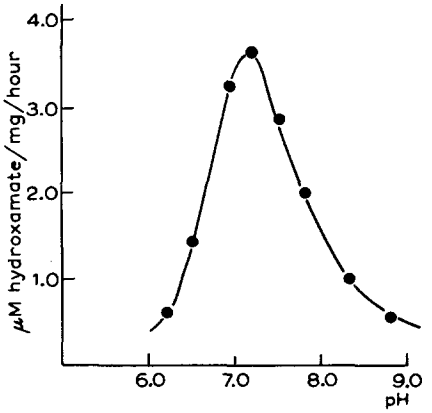

Fig. 4. pH curve for tyrosine–hydroxamate formation. Assayed under standard conditions described under METHODS.

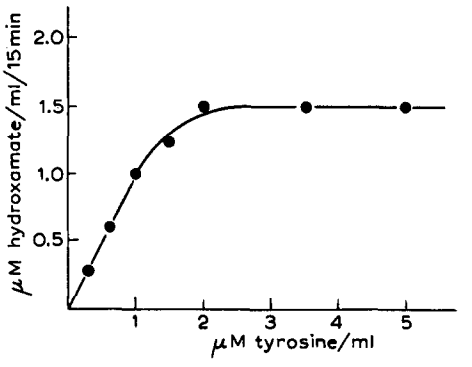

Fig. 5. Effect of tyrosine concentration on hydroxamate formation. Assayed under standard conditions described under METHODS.

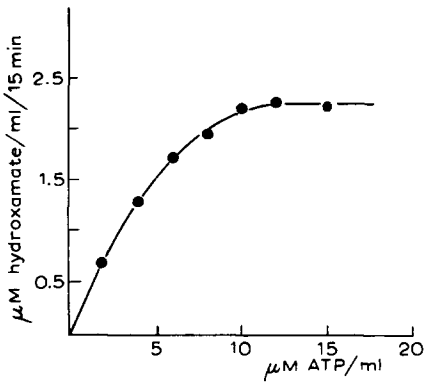

Fig. 6. Effect of ATP concentration on hydroxamate formation. Assayed under standard conditions described under METHODS.

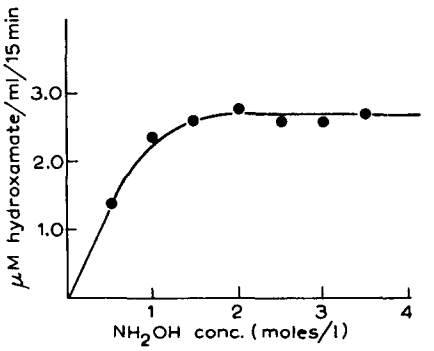

Fig. 7. Effect of NH_2OH concentration on hydroxamate formation. Assayed under standard conditions described under METHODS.

base in color tests we studied the reaction routinely in 1 *M* NH_2OH. Maximal activities are, therefore, somewhat higher than those reported in the routine analysis.

In a study on the balance between hydroxamate- and pyrophosphate formation as indicated by various authors[1,3,6] it proved to be impossible to obtain any accumulation of pyrophosphate owing to the presence of small amounts of pyrophosphatase in our final enzyme preparations. These small amounts were still found to be able to split about 25 μmoles pyrophosphate/mg/h, which according to KUNITZ[17] means a pyrophosphatase activity of about 0.025 units. This amount is considerably less than that present in original yeast extracts[17] (0.28 units), but clearly enough to account for the absence of any pyrophosphate accumulation. The observation[3] of product inhibition of the hydroxamate formation by PP could, therefore, only be confirmed if under routine conditions considerable amounts of pyrophosphate (15 μmoles/ml) and 1 μmole NaF/ml were added to repress the PPase activity to less than 5% of the original value. A complete linearity with time is observed with or without added pyrophosphatase (Fig. 8).

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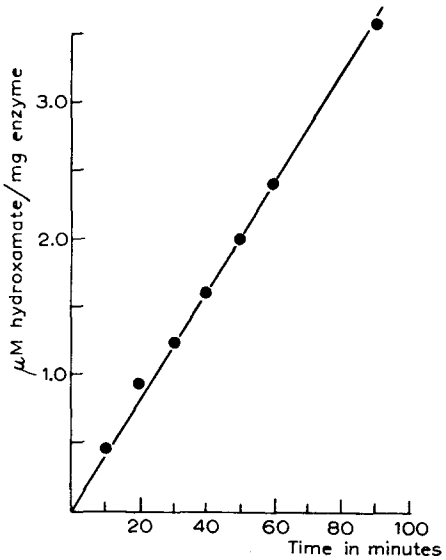

Fig. 8. Hydroxamate formation in presence of crystalline pyrophosphatase. Assay contains: 15 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles L-tyrosine, 1000 μmoles NH_2OH, 200 μmoles TRIS buffer, 10 μg pyrophosphatase and 1 mg enzyme/ml. Incubation at 30° C, pH 7.8.

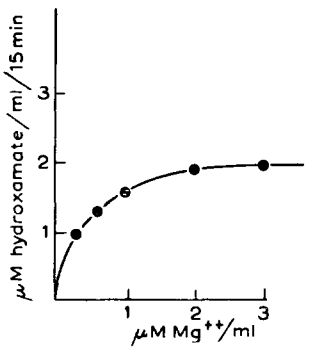

Fig. 9. Effect of Mg^{++} concentration on hydroxamate formation. Assayed under standard conditions described under METHODS.

TABLE III

COMPARISON OF HYDROXAMATE FORMATION AND PYROPHOSPHATE FORMATION, MEASURED AS INORGANIC PHOSPHATE/2

Assay contains: 15 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles L-tyrosine, 1000 μmoles NH_2OH, 200 μmoles TRIS buffer, and 1.5 mg enzyme, per ml. No crystalline pyrophosphatase was added. Incubation at 30° C, pH 7.2.

Time	*Hydroxamate μmoles/ml*	*P_i/2 μmoles/ml*
10	1.0	1.1
20	1.9	1.9
30	2.9	2.7

The hydroxamate formed was identified as tyrosine hydroxamate by paper chromatography using a butanol–acetic acid–water system.

However, if pyrophosphate, produced by the enzymic reaction, is determined as inorganic phosphate a good equivalence is found. This is shown in Table III.

The reaction was found to be absolutely dependent on the presence of magnesium ions (Fig. 9). Pyrophosphatase also needs Mg^{++}-ions. However, for optimal conditions only 1 μmole/ml is needed, while saturation in the hydroxamate test is reached at 3 μmoles/ml. SO_4^{--}-ions were found to have a slight inhibiting effect on the enzymic activity.

Although the addition of sulfhydryl compounds was not found to have a stimulatory effect on the most highly purified preparations, a strong inhibition of the enzymic reaction by *p*-chloromercuribenzoate is observed (Fig. 10). The inhibition can be completely reversed by addition of 2 μmoles glutathione/ml. *p*-Chloromercuribenzoate has no effect on pyrophosphatase.

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ATP-pyrophosphate exchange

The amino acid-dependent exchange reaction between radioactive pyrophosphate and ATP forms an alternative method for determining the enzymic activity[1–3,6]. Moreover, the comparison of the hydroxamate formation with the exchange reaction opens the possibility for a better understanding of the enzymic reaction itself as hydroxylamine is clearly only a substitute of the natural cellular acceptor of the product of interaction of the enzyme, tyrosine, and ATP.

The experimental conditions were only slightly altered in comparison with those used in ref. [3]. To repress any residual pyrophosphatase activity 1 μmole NaF/ml was added. As far as could be judged the influence of F^--ion is limited to pyrophosphatase,

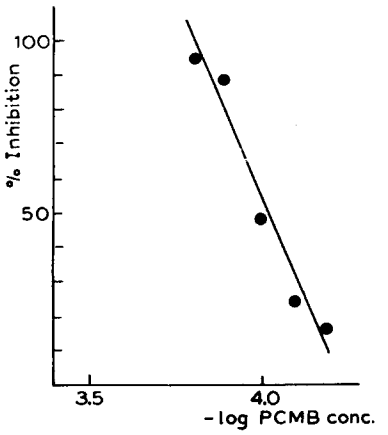

Fig. 10. Effect of PCMB on hydroxamate formation. Assay contains 15 μmoles ATP, 10 μmoles $MgCl_2$, 10 μmoles L-tyrosine, 200 μmoles TRIS buffer, and 2.6 mg enzyme/ml. Preincubated with various concentrations of PCMB in μmoles/ml for 5 min at 30° C. Reaction started by the addition of 1000 μmoles NH_2OH.

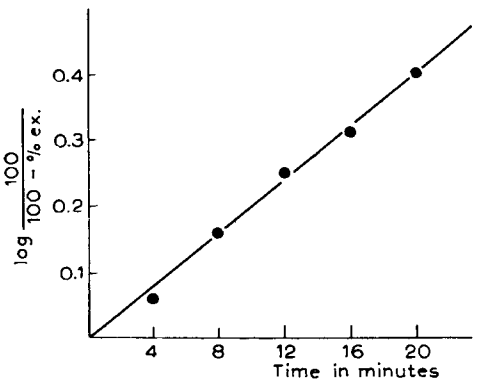

Fig. 11. Time curve for ^{32}PP exchange. Assayed under standard conditions described under METHODS.

only. The hydroxamate reaction is not measurably inhibited. The TRIS buffer proved to inhibit the exchange reaction to a small extent and, therefore, was replaced by a 0.1 *M* phosphate buffer, pH 7.15. The experiments on the ATP-pyrophosphate exchange were generally performed with preparations as far purified as step 6. Accordingly, these preparations showed a hydroxamate activity of about 80% of those reported for our most highly purified preparations. In Fig. 11 a typical time curve for the reaction is given.

The dependence of the exchange reaction on addition of tyrosine can be seen in Table IV. The effects were realized without any ion-exchange treatment of the enzyme for removal of residual amino acids.

In Table II, column 4, it can be observed, that the enzyme is substrate-specific.

A comparison of the rates of hydroxamate formation and ^{32}PP exchange as measured under experimental conditions seems not to confirm the conclusion of the limited character of the hydroxamate reaction as a measure for the enzymic activity. NOVELLI *et al.*[6], *e.g.*, found a rather large discrepancy between the exchange reaction and the hydroxamate formation, but DAVIE, KONINGSBERGER AND LIPMANN[3] observed

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TABLE IV

EFFECT OF TYROSINE CONCENTRATION ON THE EXCHANGE REACTION

L-tyrosine added μmole/ml	*Rate of exchange μmoles/mg/h*
0	0
0.01	0.8
0.05	2.7
0.1	4
0.4	6
0.7	6
1.0	6
2.5	6
5.0	6
10.0	6

Assay contains: 15 μmoles ATP, 2 μmoles PP, 3 μmoles $MgCl_2$, 1 μmole NaF, 100 μmoles 0.1 *M* phosphate buffer (pH 7.2), 1.0 mg enzyme, and varying amounts of L-tyrosine per ml.

the opposite. Here, also, if maximal values of the color test are counted (somewhat higher than those reported in routine analysis), a reasonable equivalence seems to exist. A rate of hydroxamate formation (5.4 μmoles/mg/h) can be reached, while for preparations of purification stage 7 an exchange rate is found of 7.5 μmoles/mg/h (see also Table II, columns 3 and 4).

In order to check our experimental conditions for the ATP-pyrophosphate exchange we prepared a tryptophan-activating enzyme from beef pancreas by the procedure described by DAVIE, KONINGSBERGER AND LIPMANN[3]. This enzyme showed a completely equivalent exchange rate under both their and our conditions.

DISCUSSION

The discovery by HOAGLAND *et al.*[1,2] of the ATP-linked carboxyl activation of amino acids by way of pyrophosphate elimination by enzymes of the supernatant of rat-liver homogenate confirmed an earlier suggestion[18,19] that a type of reaction analogous to the ATP-coupled acetate- and fatty acid activation might be involved in the activation of amino acids for protein synthesis.

Since only two amino acid-activating enzymes[3,7] had been purified so far, it did not seem certain yet whether a specific enzyme existed for each amino acid. The purification of such an enzyme from baker's yeast, specifically activating tyrosine, which we have described in this paper, supplies new evidence for the general theory that for each amino acid a specific enzyme is available that can be isolated and purified.

By comparing the rate of hydroxamate formation as well as the rate of ATP–pyrophosphate exchange found for this tyrosine-activating enzyme with those observed for the other isolated enzymes[3,7], the following remarks can be made.

The observed rates are very small with respect to those found by DAVIE, KONINGSBERGER AND LIPMANN[3] for a tryptophan-activating enzyme, isolated from beef pancreas, but they are of the same order as those observed by BERG[7] for a 50-fold purified methionine-activating enzyme from brewer's yeast. With respect to the statement about the purity of the enzyme, which we suppose to be about 70%, it

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can be said that this proposal is based only on ultracentrifugal and electrophoretic observations. It is, of course, possible that we have overestimated the purity of our enzyme but the ultracentrifugal and electrophoretic evidence points definitely to a purity of 70% in our best preparations. Moreover, a rough calculation can show that enough of the enzyme is present in yeast to account for all the tyrosine built into proteins during the growth period of this organism. In normal growth the amount of cellular material is doubled in 5 to 8 h. Since yeast protein contains, on the average, 5% tyrosine this means that for the synthesis of 100 mg protein about 4 μmoles tyrosine/h are needed. According to our kinetic results this quantity can be reached if less than 0.5% of the cell protein is formed by the tyrosine-activating enzyme. As 4000 g of commercial baker's yeast contain about 500 g protein this should contain less than 2.5 g of the enzyme. According to our purification Table I, 45 g yeast protein (Sup I) contain 0.55 g of the pure enzyme. It is not unreasonable to assume that a more effective destruction of the yeast would free 4 or 5 times as much of the enzyme.

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SUMMARY

The extracts of ether–CO_2-frozen baker's yeast contain enzymes that catalyze the ATP-linked amino acid activation by way of pyrophosphate elimination. From the extract a tyrosine-activating enzyme could be isolated, which, judging from ultracentrifugation and electrophoretic data, was about 70% pure at the final stage of the isolation procedure. The enzymic activity is measured by tyrosine-specific hydroxamate formation as well as by tyrosine-linked ATP-pyrophosphate exchange. The enzyme proved to be substrate-specific.

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