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BIOCHEMISTRY

THE ISOLATION OF A TYROSINE-ACTIVATING ENZYME FROM BAKER'S YEAST.

A PRELIMINARY NOTE [1], [2]

BY

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In the recent literature, considerable attention is being paid to amino acid-activating enzymes of the type described for the first time by HOAGLAND [1, 2] about two years ago. These enzymes catalyze an amino acid-dependent exchange reaction resulting in the incorporation of radioactive PP into ATP according to:

$$ATP + PP^{32} \underset{+\text{ amino acid}}{\leftrightarrows} ATP^{32} + PP$$

as well as the formation of amino hydroxamic acids according to:

$$ATP + \text{amino acid} + \text{hydroxylamine} \rightarrow \text{amino hydroxamic acid} + AMP + PP$$

Hydroxylamine should not be considered as a "natural acceptor" of the activated amino acid; it is used in very high concentrations as a trap, providing a rather easy determination of enzymatic activity.

The presence of amino acid-activating enzymes has been demonstrated in animal tissues [1, 2, 3] as well as in micro-organisms [4, 5, 6]. A tryptophan [3]—and a methionine [6]—activating enzyme have been highly purified; their interaction with the amino acid-substrate seems to be rather specific. Therefore, a study on the activation of other amino acids may be helpful in any attempt to get more information about the possible relation of this type of activation reactions with the biosynthesis of proteins.

Following the hydroxamate formation as an easily determinable measure of the enzymatic activity, we have obtained a reasonably pure tyrosine-activating enzyme preparation from extracts of ether-CO_2 frozen baker's yeast.

Freshly obtained baker's yeast is frozen for 3–4 hours in ether-CO_2 and extracted in the cold after the addition of 11.2 gr KCl per 1000 gr yeast. Subsequently, this extract is subjected to the following steps for purification: dialysis, ammonium

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[2] The following abbreviations will be used: ATP adenosine triphosphate; AMP adenosine monophosphate; PP inorganic pyrophosphate.

sulfate fractionation at pH 5.3 and pH 4.5, adsorption of impurities on Ca-phosphate gel, short dialysis and precipitation of impurities with protamine sulfate followed by ammonium sulfate fractionation at neutral pH. All ammonium sulfate fractions were collected at about 0.5–0.6 saturation.

The combined results of electrophoresis- and ultracentrifuge experiments with an enzyme preparation that had been purified 50-fold with respect to the first particle-free supernatant indicated that the enzyme was about 70 % pure at that stage of the isolation procedure. This is illustrated in figs. 1 and 2. The ultracentrifuge pattern of this preparation shows the presence of four components, the major one consisting of about 70 % of the total protein (figs. 1A, 1B).

A very similar pattern was obtained with this preparation in the same buffer solution in a LEIS Micro-Electrophoresis apparatus. In addition, it could be proved that the enzymatic activity is associated with the major component by a paper electrophoresis experiment as drawn schematically in fig. 2.

A full account of the isolation procedure and a description of the properties of the enzyme will be published later.

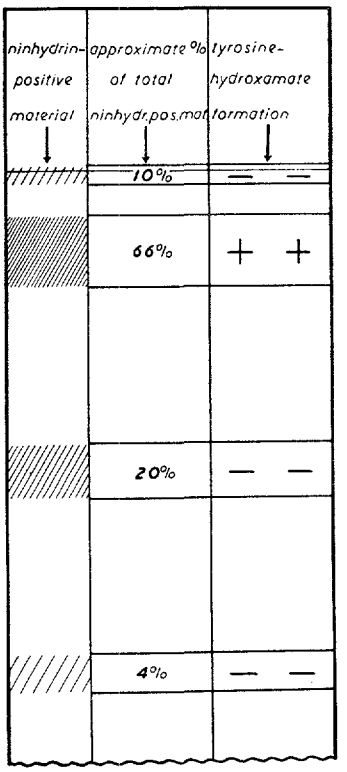

Fig. 2. Schematic outline of a paper electrophoresis experiment indicating that the enzymatic activity is associated with the major component. A 50-fold purified preparation (see text) in 0.01 m phosphate buffer (pH 6.8) solution was subjected to electrophoresis on Whatman no. 3 filter paper for 17 hours at 120 Volt.

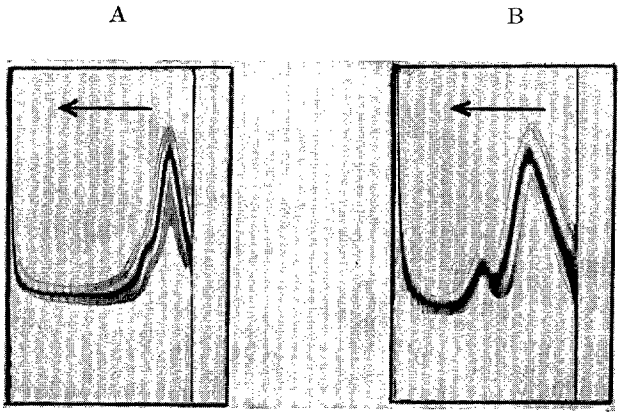

Fig. 1. Ultracentrifuge patterns for a 50-fold purified enzyme preparation (see text) in a 0.10 m KCl — 0.01 m phosphate buffer (pH 6.8) solution at 56.100 r.p.m. and about 18°. *A*, After 27 min.; bar-angle 50°. *B*. After 46 min.; bar-angle 50°.

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