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The Preparation and Purification of Protyrosinase

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The presence of the inactive form of the enzyme tyrosinase in the egg of the grasshopper (Melanoplus differentialis) is of considerable interest since it has been found possible to isolate and activate it by various physico-chemical means (Bodine and Carlson, 1940). The reproductibility of these results depends to a great degree upon the purity of the sample of proenzyme obtained. Extensive purification of the enzyme has been carried out and the general physico-chemical properties of various preparations are noted below.

The procedures used in obtaining different preparations of protyrosinase are as follows:

Grind eggs in cold in 0.9% NaCl—10 cc. NaCl per gm. eggs. Centrifuge 5 minutes at high speed—Remove A and C layers. (*Sample B* removed here)

Centrifuge 5 minutes at high speed—Remove A and C layers. Add 1 gm. of KH_2PO_4 to each 500 cc. of supernatant and allow to stand at 0° C. at least 2 hours. Centrifuge 10 minutes at high speed—discard precipitate.

(*Sample* B_1 of supernatant)

Add 12 gms. $(NH_4)_2SO_4$ for each 88 cc. of supernatant—allow to stand at 0° C. for at least 2 hours. Centrifuge 10 minutes at high speed—discard precipitate.

(*Sample B*-12 of supernatant dialized against 3 changes of 0.9% NaCl over a 24 to 48 hour period) Add 6.6 gms. $(NH_4)_1SO_4$ for each 84 cc. of supernatant—Allow to stand at least 2 hours at 0°C. Centrifuge 10 minutes at high speed.

Precipitate

Take up precipitate in volume of NaCl one-half that of total volume in preceding step. Allow to stand at least one hour at 0°C. Centrifuge 10 minutes at high speed—discard precipitate.

(*Sample B-18* of supernatant dialized against 3 changes of NaCl over a 36 to 48 hour period).

Reprecipitate with 18 gms. $(NH_4)_2SO_4$ for each 82 cc. of supernatant. Allow to stand at least 2 hours at 0° C. Centrifuge at high

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speed for 10 minutes. Discard supernatant. Precipitate taken up in same volume of NaCl as before precipitation.

Sameple B-18a dialized against 3 changes of NaCl over a period of 36 to 48 hours.

Supernatant

Add 7.5 gms. $(NH_4)_1SO_4$ for each 75 cc. of supernatant. Allow to stand for at least 2 hours at 0° C. Centrifuge 10 minutes at high speed. Discard supernatant. Precipitate taken up in NaCl (one-half volume of former step) and allowed to stand. Centrifuge 10 minutes at high speed. Discard precipitate. Supernatant dialized against 3 changes of NaCl over a period of 36 to 48 hours. (Sample B-26).

Other Procedures

Bentonite—Adsorbs protyrosinase partially activating it. Have not yet been able to remove it.

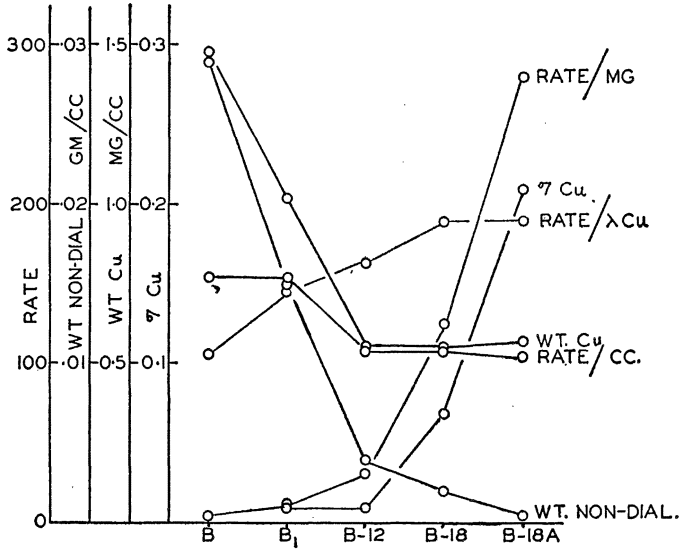

Figure 1—Shows graphically a summary of the data obtained from analyses of 5 different proenzyme preparations. Data represent averages of 5 separate determinations for each preparation. Rate equals the reciprocal of the time necessary for the oxidation of the substrate, tyramine HCl to attain half completion (for further details as to methods, etc., see Bodine and Carlson (1940). Ordinates, show different properties of preparations; abscissa, letters designating preparations. Nitrogen content of B-18a = 127.5 mg/cc or 14.2%.

Alumina—Does not adsorb the protyrosinase nor does it increase the rate per mg. i.e., remove impurities.

Aerosol or Sodium Oleate—Addition of these to activate causes slight precipitate to form. After centrifuging, all activity is in the supernatant. Dialysis against NaCl will not remove oleate or aerosol so it was impossible to reverse activation.

Data for 5 different preparations are summarized graphically in figure 1. Beginning with the rather crude preparation (B), a marked increase in the general activities of the enzyme is noted as the extraneous materials are gotten rid of. The final preparation of protyrosinase (B-18a) had 0.21% of copper. Kubowitz (1937) found 0.18% copper in his preparation of tyrosinase; Keilin and Mann (1938) 0.30%, and Nelson and Dawson (1944) 0.21%. An analysis of the nitrogen content of B-18a gave an average of 14.2%. The ultraviolet absorption spectrum of sample B-18a shows marked breaks at 310 and 240 μm (Bodine and Carlson, 1954).

In summary, methods are given for the purification of protyrosinase as well as the general physico-chemical properties of the samples so produced.

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