Studies on Liver Arginase. II. The Separation of a Single Protein with Arginase Activity

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An extensive study of the properties of horse liver arginase revealed that although it is extremely sensitive to pH values lower than 5.0, yet it can withstand unusually high temperatures in the neutral range. It is neither precipitated nor its activity impaired by such treatment. Manganese-treated crude extracts at pH 7.2—7.7, can be heated directly on the flame to a temperature of 80—90° C. A lot of inert proteins closely associated with the enzyme can thus be removed. Moreover, the enzyme activity is unaffected by lead ion in any concentration. The amount of lead ion that can be added to the enzyme extract without causing its precipitation depends upon the concentration of the enzyme protein and previous treatments; such as, addition of manganese and heating. The enzyme is more easily precipitated with lead if previously heated. Unheated liver extracts can be treated with considerably high concentrations of lead (about 8 mg Pb^{++}/ml) without any change in the enzyme which remains in solution. This fact was first mentioned and utilized for the partial purification of arginase by Safwat Mohamed and Greenberg [1]. Their procedure was utilized later by Thompson [2, 3] with some additional steps. The use of the combination of Mn^{++} and phosphate ions as a means of purification of arginase, reported by Thompson, has been tried by the author and was found to entail a considerable loss to the enzyme. The disappearance of the color in Thompson's final extract (K)[3] is due more to dilution than to removal of the coloring matter. Still more loss is to be encountered if the final dilute extract (K) is to be concentrated through the use of acetone or ammonium sulfate.

In the following a brief account is given of the procedure that yields arginase of the highest purity and activity.

a) Three hundred g acetone-dried horse liver, extracted with 3 l distilled water containing 2 g $MnSO_4 \cdot 4H_2O$. Adjust pH to 7.5. Stir continuously for 8 h at room temperature. Centrifuge and discard residue.

b) Supernatent (I) at 3° C is mixed with 0.7 volumes cold acetone with stirring. Let stand at 3° C for 8 h. Centrifuge in cold room. Discard residue.

c) Supernatent, at 3° C, mixed with 0.5 volumes cold acetone with stirring and left at 3° C for 8 h. Centrifuge in cold room and discard supernatent.

d) Precipitate is taken up in distilled water. Extract (II); add 0.5 g $MnSO_4$, adjust pH to 7.5. Heat to 80° C and cool rapidly. Centrifuge and discard precipitate.

e) To supernatent (III) add $Pb(CH_3COO)_2 \cdot 3H_2O$ to give 2.0 mg Pb++/ml. Mix thoroughly and adjust pH to 7.5. Let stand at room temperature for 2 h Centrifuge.

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either of these pairs forming double molecules around the centres of symmetry in (000) and (0 $\frac{1}{2}$ $\frac{1}{2}$) respectively.

This structure is in agreement with interatomic distances, previously reported, with a Patterson synthesis of the 0*kl* data and can account satisfactorily for the relative intensities of the reflexions.

Further data and a more detailed description of this structure will be given in another paper.

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Table 1.

Extract	Volume ml	AU/ml	Total AU	mg N/ml	AU/mg N
I	2800	90	252000	4.0	22
II	650	380	251000	2.0	190
III	650	350	228000	0.6	580
IV	650	250	162000	0.4	600
IV+Mn	650	450	292000	0.4	1100
V	50	2100	110000	3.0	700
V+Mn	50	5000	250000	3.0	1650
VI	180	900	160000	1.2	750
VII	70	2000	140000	2.6	780

AU signifies arginase units, a term adopted by Safwat Mohamed and Greenberg [1].

By AU/ml is meant arginase units per ml of extract. AU/mg N = arginase units per mg N in 1 ml of extract.

f) Supernatent (IV) is mixed at 3° C with 0.7 volumes cold acetone. Let stand for 8 h and centrifuge in cold room.

g) Supernatent + 0.5 volumes cold acetone and left at 3° C for 8 h Centrifuge and the greenish blue precipitate is taken up in distilled water (V).

h) The precipitate formed in (e) contains considerable arginase. Take up in phosphate buffer, pH 7.5. Let stand with shaking overnight and centrifuge. Extract (VI) is mixed at 3° C with 1.2 volumes cold acetone, centrifuge after 8 h. Precipitate was taken up in phosphate buffer, pH 7.0 (VII).

Extract (V) contains very active arginase which is strongly activated by Mn^{++}. Electrophoresis at pH 7 and pH 6.0 showed the presence of a single homogeneous protein. The protein was precipitated several times with acetone in the cold (1.2 volumes). A greenish blue protein was always recovered. It dries into a white powder with bluish tinge.

Electrophoresis of (VII) showed the presence of three peaks with arginase forming 85—90 % of the total protein present.

From Table 1 it is evident that part of the arginase is split up, under the effect of heat and Pb^{++} ions, into smaller fragments still very active and can be much further activated by Mn^{++} ions. This fraction (V) was separated in a pure, yet not crystalline, form. In fraction (VI) the remainder of the enzyme, probably intact, could be separated along with two other proteins; arginase forming 85—90 %.

In a later publication a more detailed report will be presented.

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On the Reducing Sugars in Sera from Pregnant and Lactating Women

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The statement by Lövgren [1] that sera from pregnant and lactating women contain lactose only and no glucose as determined by his osazone formation method seemed to the present authors so surprising that it was desirable to check its validity.

It is well known that the microscopical identification of osazone crystals can be very difficult. This is especially the case if more than one osazone-forming sugar are simultaneously present or if the mother liquor is contaminated with other crystal-