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Studies on Liver Arginase

I. On its Crystallization by Bach

M. SAFWAT MOHAMED*

Biokemiska Institutet, Stockholm Högskola, Stockholm, Sweden

The procedure of purification of liver arginase published by Bach[1], in which he claims the crystallization of the enzyme, was repeated several times in this laboratory. The purpose of this communication is to report that the findings of Bach in regards to the purity of the preparation, the assumed crystalline structure, and the role of manganese are completely in error.

The purification method is relatively simple, though it requires the consumption of large quantities of ammonium sulfate for the repeated dialysis which is somewhat tedious.

EXAMINATION OF THE FINAL PRECIPITATE

The final product containing the round structures (assumed hexagonal crystals) contains considerable amounts of amorphous proteins. Thus, assigning arginase activity to a particular constituent in the mixture is impossible. Tests for the presence of catalase, phosphatase and esterase were carried out showing only the presence of the latter in rather large concentration.

The heterogeneous nature of the preparation was further ascertained by means of electrophoresis **. This was carried out in phosphate buffers of ionic strength 0.1 at pH values of 7.0 and 5.9. The solution containing 0.8 per cent protein was first dialyzed against running distilled water for 48 hours to remove the ammonium sulfate, followed by dialysis for 24 hours against the buffer which was to be used in the electrode compartments of the Tiselius aparatus.

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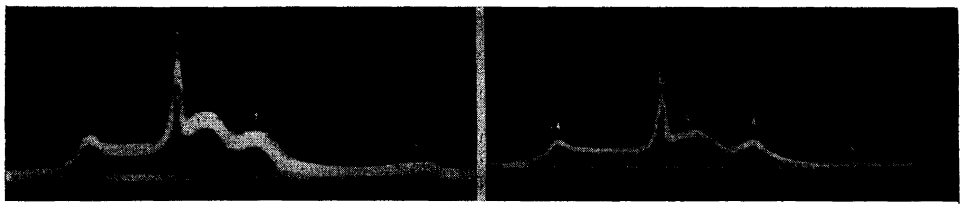
** Thanks are due to Dr. E. Hultin of this institute for running the tests and for his continuous help and cooperation.



a *b*

Fig. 1. Electrophoresis of Bach's purified arginase. a: pH 7.0 (ascending limb). b: pH 5.9 (ascending limb).

The diagrams reproduced (Fig. 1) show the presence of five proteins in the preparation. They all move towards the anode at both pH 7.0 and 5.9 except the slowest component a which moves towards the cathode at the latter pH. Table 1 gives the mobilities of the components at the two pH values.

Table 1. Calculated mobilities of the components present in Bach's purified arginase.

Boundary	Mobility ($\times 10^5$ cm^2 volt^{-1} sec^{-1})	
	pH 7.0	pH 5.9
a	0.35	— 0.66
b	0.92	0.25
c	1.45	0.46
d	1.75	0.80
e	2.95	2.00

Electrophoresis of arginase partially purified according to Mohamed and Greenberg [2] showed the presence of the three components having mobilities corresponding to boundaries b, c, and e (Fig. 2).

Fig. 2. Electrophoresis of purified arginase (Mohamed and Greenberg). pH 7.0 (descending limb).

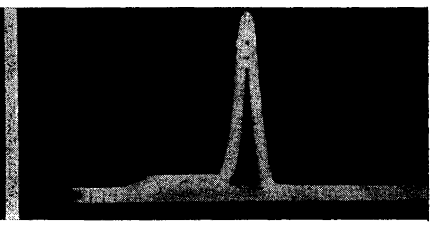


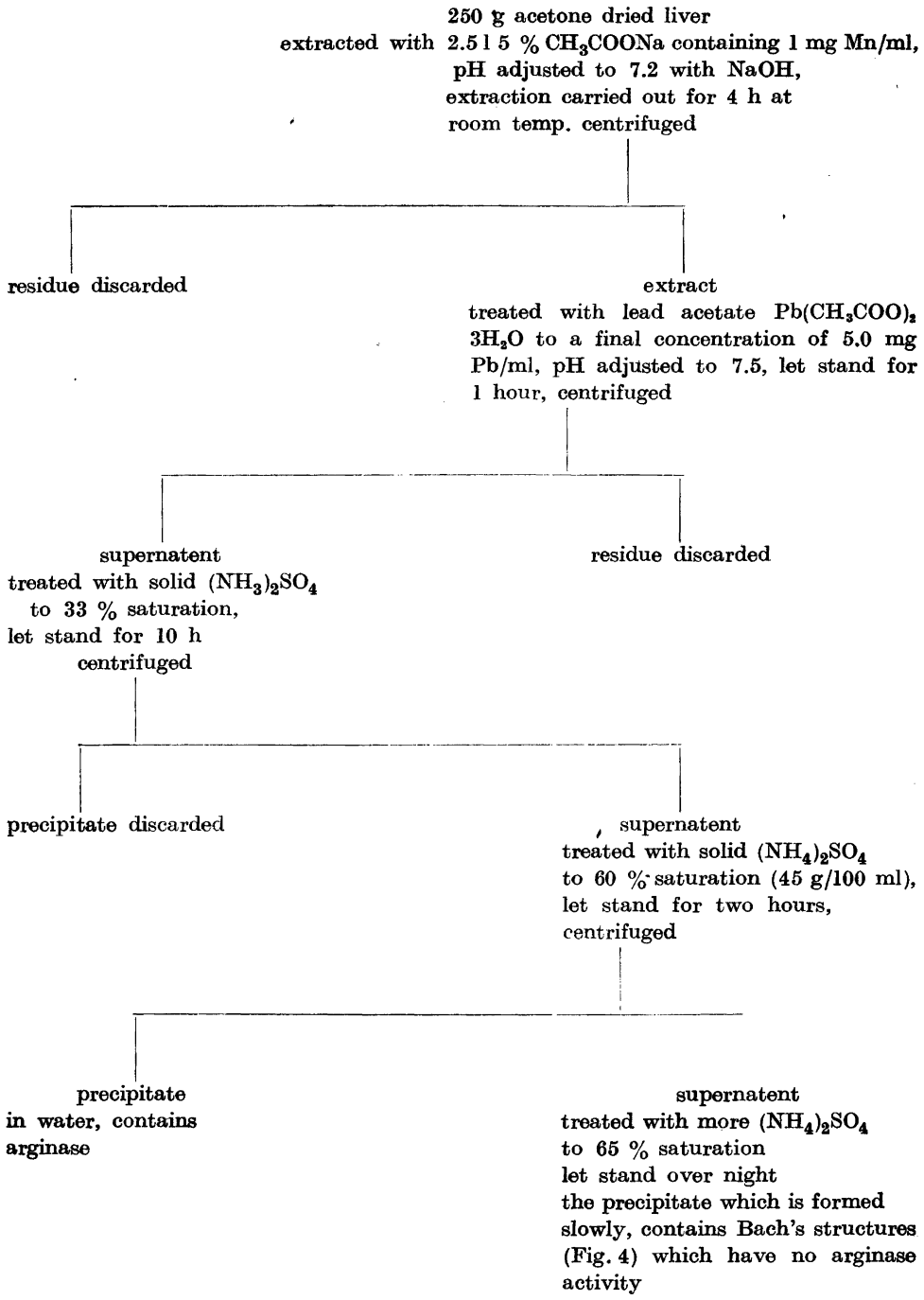
Fig. 3. Electrophoresis of crystalline liver esterase. pH 7.0 (ascending limb).

Fig. 4. Round structures of Bach.

The crystallization of a protein from liver possessing esterase activity was achieved [3]. Electrophoresis experiments were carried out at pH 7.0 (Fig. 3), and pH 5.8 showing the presence of a single homogeneous component with a mobility of 2.96 ($x10^5 cm^2 volt^{-1} sec^{-1}$) at pH 7.0 and 1.98 at pH 5.8. These values correspond very closely to those of component e in Bach's preparation.

A close study under the microscope of the round structures, claimed to be arginase crystals, revealed that each is composed of a network of filaments with part of the mother liquor and amorphous proteins entrapped inside along with one or more air bubbles. The presence of air bubbles could be observed by putting a slide with a part of the suspension in a vacuum desiccator. The bubbles could clearly be seen to expand in volume, then collapse on exposure to the air. The whole structure is then partly or completely destroyed. This also occurs when the preparation is left to dry on the slide giving the illusion af resolution.

The next step was to find out if these structures actually constituted the enzyme arginase. A procedure was finally adopted by which they were separated in a fraction which proved to be completely devoid of arginase activity. The enzyme was separated in a different fraction. The procedure is outlined as follows:


250 g acetone dried liver
extracted with 2.5 l 5 % CH_3COONa containing 1 mg Mn/ml, pH adjusted to 7.2 with NaOH, extraction carried out for 4 h at room temp. centrifuged
residue discarded
extract
treated with lead acetate $Pb(CH_3COO)_2$ $3H_2O$ to a final concentration of 5.0 mg Pb/ml, pH adjusted to 7.5, let stand for 1 hour, centrifuged
supernatent
treated with solid $(NH_3)_2SO_4$ to 33 % saturation, let stand for 10 h centrifuged
residue discarded
precipitate discarded
supernatent
treated with solid $(NH_4)_2SO_4$ to 60 % saturation (45 g/100 ml), let stand for two hours, centrifuged
precipitate
in water, contains arginase
supernatent
treated with more $(NH_4)_2SO_4$ to 65 % saturation
let stand over night
the precipitate which is formed slowly, contains Bach's structures (Fig. 4) which have no arginase activity

Moreover, if the crude liver extract is incubated with trypsin in presence of Mn ions before employing Bach's method the round structures fail to appear in the final product yet it still possesses powerful arginase activity.

ACTIVATION BY MANGANESE

Contrary to Bach's observation it was clearly found that manganous ions powerfully activate the enzyme in all stages of the purification. The purest stage in Bach's method (47 per cent saturated ammonium sulfate precipitate) is strongly activated by both cobalt and manganese salts. Incubation of the extract with 2.5×10^{-3} *M* of either Mn^{++} or Co^{++} for one hour at 40° C and pH 7.0 resulted in 2—3 fold activation.

SUMMARY

Electrophoretic studies of arginase purified according to Bach showed the heterogeneous nature of the preparation.

The round structures are neither crystalline nor do they contain arginase activity.

Manganese and cobalt salts are powerful activators of the enzyme in all stages of purification so far attained.

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2. Mohamed, M. S., and Greenberg, D. M. *Arch. Biochem.* **8** (1945) 349.
3. Mohamed, M. S. *Acta Chem. Scand.* **2** (1948) 90.

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