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Studies on Liver Arginase

IV. Evidence for the Presence of Two Enzymes with Arginase Activity

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The conflicting reports in the literature and the peculiar and unexplained properties ascribed to arginase have been reviewed and studied by the author. It is the aim of this communication to put some end to these conflictions by proving that liver extracts contain two enzymes with arginase activity. These two enzymes have quite different and rather interesting properties.

If one studies the pH-activity curves of arginase preparations [1–3], one is confronted with a sharp optimum at pH 9.8—10. In the less alkaline region, however, the activity extends, although at a lower level, but nevertheless, it forms a distinct and persistent bulge between pH 7.0 and 8.0. The curve can actually be resolved into two separate curves partially superimposed (Fig. 1). One curve would have its maximum at around pH 7.4—7.6 (curve I) and the second at pH 9.9—10 (curve II). If one measures the activity at pH 8.0 or 9.0 it would show a combination of activities of the two enzymes; thus giving no true picture of either enzyme at all. This will explain many peculiarities such as, the variation of the Michaelis-Menten constant (K_s) with the change of pH of the determination, as was reported by Greenberg and Mohamed [4].

When a liver extract is dialyzed against water, and the pH activity relationship of the dialyzed extract is plotted, a curve very much similar to curve II alone is obtained. The characteristic bulge (pH 7.0—8.0) is completely absent. This is taken to mean that the enzyme represented by curve I (may be called arginase *a*) must be inhibited by dialysis while the enzyme (arginase *b*) represented by curve II remains practically unchanged. Experiments on prolonged dialysis reported by Mohamed [5] showed that arginase activity,

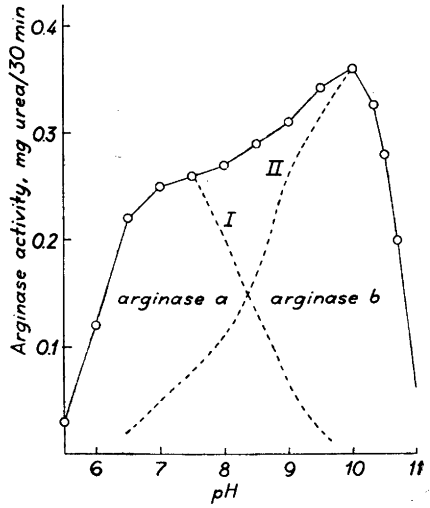

Fig. 1. pH-activity curve of liver arginase resolved into two separate curves.

measured at pH 9.9 remained practically the same throughout a dialysis period of 360 hours at room temperature.

A further means of differentiating between the two enzymes is by making use of the metal activators, namely, Mn^{++}, Co^{++}, and Ni^{++} ions. Manganous ions definitely activate arginase *b* alone, while Co^{++} and Ni^{++} ions activate arginase *a* only. Water dialyzed extracts tested at pH 9.9 did not respond when incubated with Co^{++} or Ni^{++} ions, but were distinctly activated by Mn^{++} ions [5]. In fact, Mn^{++} ions may have a definite inhibitory action on arginase *a*.

This difference in specificity of these metal ions in activating either one of the two enzymes clears up a great deal of the controversy in the literature concerning the part these ions play. It is clear when dealing with crude or partially purified extracts (containing the two enzymes), how important it is to select the right pH for measurements of the activity in the presence of the different ions in order to get the right information.

The effect of borate ion on arginase activity [4, 5] clearly adds more evidence to the presence of two enzymes. The pH-activity curve of borate extracted arginase has a shape very similar to a water dialysed extract, *i. e.*, arginase *a* is completly absent in both. The activity at pH 7.0 is very low amounting only to the activity of arginase *b* at that pH. The inhibiting effect of borate cannot be released by Co^{++} ions [5]. Only when the borate ions are removed does arginase *a* activity reappear and then it can be activated by cobalt or nickel.

When a liver extract is heated at 65—80° C, cooled and then treated with lead ions (2 mg/ml) a precipitate is obtained leaving a supernatent with bluish color. Both precipitate and supernatent contain strong arginase activity.

The above procedure was utilized in separating a pure arginase (electrophoretically) from the supernatent [6]. It represents arginase *b*, and thus far, it has been shown to be strongly activated by Mn^{++} but not by Co^{++} ions and is not affected by dialysis. The precipitate obtained by treatment with lead contains arginase *a*, although not in a pure state. Work is in progress to purify it and to further elucidate the properties of both enzymes.

The purified arginase *b* was erronuously reported as resulting from splitting of a large molecule of arginase under the effect of heat and lead ions. However, after studying its properties and reviewing the forementioned points of evidence the only conclusion reached is that there are two arginases with the following properties:

Arginase *a*	Arginase *b*
1. pH optimum: 7.4—7.6.	1. pH optimum 9.9—10.
2. Activated by Co^{++} and Ni^{++} ions and inhibited by Mn^{++} ions.	2. Activated by Mn^{++} ions. Unaffected by Co^{++} or Ni^{++} ions.
3. Inactivated by dialysis; activity restored by Co^{++} and Ni^{++} ions.	3. Dialysis has little effect.
4. Reversibly inhibited by borate, inhibition not released by Co^{++} ions.	4. Borate has no effect.
5. Easily precipitated by heat and Pb^{++} ions.	5. Remains in solution when heated and mixed with Pb^{++} ions.

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