# THE REGULATION OF RAT-LIVER XANTHINE OXIDASE: ACTIVATION BY PROTEOLYTIC ENZYMES

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## 1. Introduction

We have reported that the xanthine oxidase (xanthine-oxygen oxidoreductase, ED 1.2.3.2) of freshly-prepared rat liver supernatant oxidizes xanthine with oxygen, and more rapidly with $NAD^+$ or methylene blue (MB) as electron acceptors. The enzyme was "activated" upon storage at −20° for some hours, i.e., the rate of the reaction with oxygen reached that observed in the presence of $NAD^+$. The activity in the presence of $NAD^+$ seemed unchanged, as determined by the rate of uric acid formation, but no NADH was formed by the "activated" supernatant during the oxidation of xanthine, thus indicating that oxygen and not $NAD^+$ was the acceptor. No changes were observed in the activity measured in the presence of MB [1]. We report now that an apparently similar activation is brought about by treatment of rat liver supernatant with proteolytic enzymes.

## 2. Experimental

Rat liver supernatant was prepared in 0.1 M tris-HCl buffer, pH 8.1 as described previously [1], and samples were incubated at 37° for 30 min in the same buffer with protease, trypsin, chymotrypsin and pronase. A portion of the liver was homogenized in 0.15 M HCl, and was incubated in 0.1 M sodium phosphate buffer, pH 6.0, with papain. The effect of pepsin could not be tested, since incubation at pH 2.5 (adjusted with HCl) with or without pepsin destroyed the enzyme activity. A control sample was kept in ice, and another one was incubated without additions. The samples were then dialyzed against 0.1 M tris buffer, pH 8.1 and their xanthine oxidase activity was assayed essentially according to Rowe and Wyngaarden [2], by following spectrophotometrically at 292 mμ the formation of uric acid, with oxygen (air), with or without addition of $NAD^+$ or MB [1]. The formation of NADH was determined from the changes of absorbance at 340 mμ.

## 3. Results and comments

The results are summarized in table 1. The incubation of the supernatant alone brought about a partial activation, i.e., a moderate enhancement of the activity with oxygen and some reduction of the formation of NADH. When supernatant was incubated with proteolytic enzymes the xanthine oxidase activity with oxygen reached that observed with $NAD^+$ and the formation of NADH was abolished, whereas the activity with MB did not undergo significant changes. It was checked that supernatants, either before or after the incubation, did not oxidize NADH. The effect of trypsin was not seen, if soya-bean trypsin inhibitor was added. No further changes were observed if the samples treated with proteolytic enzyme were kept at −20°, thus indicating that the "activation" was complete.

It has been reported that several forms of xanthine oxidase are obtained by proteolysis of the enzyme prepared from milk [3]. The changes of the rat liver enzyme caused by proteolysis are apparently similar to the "activation" observed after storage of the supernatant at −20°. These results and those reported already [1] allow to conclude that in rat liver super-

Table 1
Activation of xanthine oxidase of rat liver supernatant after incubation with proteolytic enzymes.

| Treatment | Xanthine oxidase activity | | | |
|---|---|---|---|---|
| | $O_2$ mμmoles of uric acid formed/min/100 mg of liver | NAD mμmoles of uric acid formed/min/100 mg of liver | NAD mμmoles of NADH formed/min/100 mg of liver | MB mμmoles of uric acid formed/min/100 mg of liver |
| None | 5.7 | 29.4 | 24.2 | 49.1 |
| Incubation at 37° for 30 min with | | | | |
| No additions | 11.4 | 29.4 | 15.8 | 48.3 |
| Protease (100 μg/ml) | 17.2 | 26.2 | 11.0 | 42.5 |
| Protease (500 μg/ml) | 32.7 | 31.1 | 0 | 41.7 |
| Trypsin (100 μg/ml) | 31.1 | 31.1 | 0 | 48.3 |
| Trypsin (100 μg/ml) plus trypsin Inhibitor (200 μg/ml) | 12.2 | 28.6 | 17.0 | 47.4 |
| Chymotrypsin (100 μg/ml) | 31.1 | 31.1 | 0 | 47.4 |
| Papain (100 μg/ml) | 27.0 | 27.0 | 0 | 48.3 |

The enzyme assays were performed at 25° in a mixture containing, in 3 ml, 0.1 M tris-HCl buffer, pH 8.1, 60 μM xanthine, 0.67 mM $NAD^+$ or 0.033 mM MB (when present), and 0.2 ml of rat liver supernatant.

natant xanthine oxidase exists in two forms: an oxidase, capable of reacting with oxygen, and a dehydrogenase, for which $NAD^+$ is an acceptor, whereas no differences exist in the reactivity of both forms with MB. The dehydrogenase can be activated into the oxidase upon storage at −20° or upon treatment with proteolytic enzymes, and possibly by other treatments. Experiments are in progress to clarify the nature of the change(s) resulting in the "activation" and the possible physiological significance of the two forms and of their interconversion.

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## References

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