# ISOENZYMICITY IN MOUSE LIVER GUANINE DEAMINASE DEMONSTRABLE UNDER SUBSTRATE STRESS

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SUMMARY : Two isoenzymes of guanine deaminase could be demonstrated in the liver of mice subjected to guanine stress while the saline-treated controls showed only one. The one appearing under stress was a regulatory protein showing a sigmoidal substrate saturation curve, but was not influenced by GTP, allantoin or $Mg^{2+}$

Guanine daminase activity in the liver and brain of mouse and rat was mostly localised in the 15,000 x g supernatant (1). We have reported earlier that of the four tissues studied mouse liver contained the least enzyme activity (2). Also the isoenzymicity demonstrable in the other tissues was lacking in the mouse liver (1). Partially purified enzyme from mouse liver was shown to be unaffected by GTP, allantoin or $Mg^{2+}$ unlike the enzyme from the other tissues (3). We have also demonstrated that guanine deaminase activity was inducible in brain and liver of rat and mouse under substrate stress (2). In the present communication we report the observation, in the guanine-stressed mice, of an isoenzyme of guanine deaminase different from and in addition to the one present in the untreated animals. This inducible isoenzyme showed a sigmoidal response to increasing substrate concentration, but was not affected by GTP, allantoin or $Mg^{2+}$.

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MATERIALS AND METHODS : Adult albino male mice were divided into three groups of 24 animals each. The first group was injected with normal saline and the second with guanine throughout the experimental period. The third group received guanine during the first 5 days followed by a mixture of guanine and ethionine thereafter. Guanine and ethionine were suspensions in normal saline and the dosage 120 and 70 mg per kg. body weight per day respectively. The injections were intraperitoneal and were repeated every 24 h. In experiments reported earlier (2), the mice were sacrificed after one injection while in the present experiments they were sacrificed in batches of 8 from each group after receiving 5, 10 and 15 injections. The livers were extracted and homogenized in 0.25 M sucrose solution.

The enzyme assays and the kinetic studies were conducted as described earlier (1, 3). The influence of GTP (at 50 μM), allantoin or $Mg^{2+}$ (both at 1 mM) was tested by including them in the assay system.

The homogenate was centrifuged for 20 min. at 15,000 x g and the supernatant obtained served as the starting material for the partial purification of the enzyme. Briefly, the enzyme activity precipitating between 35-55 % ammonium sulphate saturation was dialysed against water for 6 h and the contents of the dialysis bag were centrifuged. The supernatant was adjusted to 20 mM phosphate, pH 7.0 and loaded on a DEAE-cellulose column (1 x 10 cm) equilibrated with 20 mM phosphate, pH 7.0. The column was washed with the same buffer and eluted stepwise with 65 ml each of 50 mM, 85 mM, 125 mM and 200 mM phosphate buffer, pH 7.0. Five ml fractions were collected and analysed for enzyme activity and protein content.

The protein content in the DEAE-cellulose column eluates was determined spectrophotometrically (4) and in all other fractions by the method of Lowry *et al* (5).

RESULTS AND DISCUSSION

We have reported earlier that guanine deaminase activity in mouse liver has increased by 65 % on a single injection of guanine but decreased slightly to 45-50 % on prolonging the administration for 4 days (2). In the present experiment we found slightly higher induction rates, 77 % after 5 days of stress and 53 % and 50 % after 10 and 15 days respectively. Ethionine-treated animals showed control level activity.

In the present experiment a partial purification of the enzyme from the stressed animals was attempted in order to observe whether the kinetic properties of the enzyme synthesized under substrate stress altered from the normal pattern of the enzyme in untreated animals (1). To afford a comparison, the enzyme activity from saline- and ethionine- treated animals was also partially purified under the same conditions. Guanine deaminase activity from saline-treated animals loaded on a DEAE-cellulose column could be eluted with 125 mM phosphate (A enzyme) with no more of

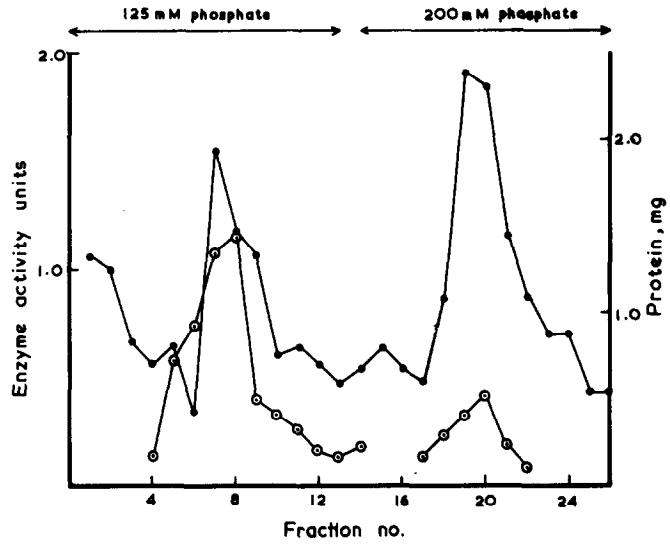


Fig. 1 : Elution pattern of liver supernatant guanine deaminase activity (O) and protein (●) of guanine-stressed mice from DEAE-cellulose column.

activity getting eluted on increasing the molarity of the buffer to 200 mM. That agrees with the earlier reported chromatographic behaviour of the enzyme from the untreated animals (1). But when the preparation from the guanine-treated animals was eluted from the DEAE-cellulose column, there was an additional peak of enzyme activity (B enzyme) appearing in the eluates of 200 mM phosphate buffer. This peak accounted for 14 % of the enzymic activity loaded on the column from animals stressed for 5 days while it increased to 19 % and 32 % for preparations from animals stressed for 10 and 15 days respectively. A typical elution pattern of the enzyme from the stressed animals was shown in Fig. 1. The enzyme preparation from ethionine-treated animals also exhibited a very small but definite shoulder of activity in 200 mM phosphate eluates in spite of the fact that the analysis of the homogenates showed no increase in enzyme activity.

The A enzyme from all three groups of animals showed classical Michaelis-Menten response to increasing substrate concentration as reported

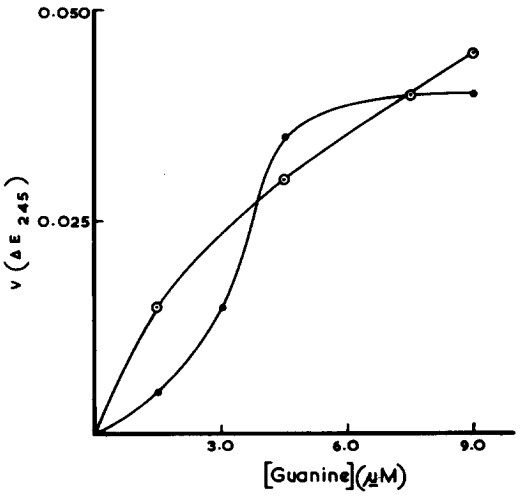


Fig. 2 : Reaction rate-substrate concentration relationship for liver guanine deaminase isoenzymes A (O) and B (●) from guanine-stressed mice.

for the enzyme in untreated animals (1), while the B enzyme of guanine-treated animals showed a sigmoidal response (Fig. 2). But unlike the guanine deaminase isoenzymes from rat liver and brain or mouse brain, both the isoenzymes in the guanine-treated animals and the A enzyme in saline- or ethionine-treated animals were unaffected by GTP, allantoin or $Mg^{2+}$.

Obviously mouse liver also has two molecular species of guanine deaminase like mouse brain and rat brain and liver. Only the A enzyme is demonstrable under normal conditions while the B enzyme is in very low concentrations. Substrate stress reveals this isoenzymicity clearly by elevating the concentration of the B isoenzyme to demonstrable levels. It is significant that the concentration of the regulatory isoenzyme is enhanced under substrate stress.

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