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An enzymatic model for some effects of methoxyethylmercury acetate on rat liver glutamate dehydrogenase

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ORGANIC mercury compounds may stimulate or inhibit glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating), EC 1.4.1.3.).[1, 2] They may likewise modify the effect of allosteric ligands[3] on the enzyme.[4] An investigation of the effects of methoxyethylmercury acetate* on crude rat liver glutamate dehydrogenase has been included in a more comprehensive study on the effect of mercury compounds on animal tissues. Below will be described the *in vitro* results, while the *in vivo* results will be published elsewhere. The enzymatic model for glutamate dehydrogenase proposed by di Prisco and Strecker[5] has been adopted.

MATERIALS AND METHODS

Ultrasonically-treated rat liver mitochondrial suspensions, passed through a Sephadex G 25 column equilibrated with 0·05 M phosphate buffer, were used as the enzyme. This enzyme could be stored at 4° for several days without loss of activity. Protein content was estimated using the biuret method. Enzymatic activity was measured as change in extinction at 340 mμ during 60 sec, after a short period of equilibration. The reaction mixtures appear from the Figures. The reactions were started by adding MeEHg treated, or nontreated, enzyme to the incubation mixture. The treatment consisted of preincubation, with different amounts of MeEHg, for 10 min at room temperature. Control series, without addition of mercury, were run simultaneously. The readings were done on a Zeiss selfrecording spectrophotometer RPQ 20, at 25°.

To evaluate the effect of MeEHg, the activity of glutamate dehydrogenase was tested as indicated at pH 7·5 and 8·5. The activity of the MeEHg treated enzyme with the known allosteric ligands NAD,[6–8] ADP,[6] and L-leucine,[9] was also investigated.

RESULTS

The NAD induced stimulation of glutamate dehydrogenase in rat liver mitochondria is reversed by MeEHg treatment (Fig. 1). With high NAD concentrations, larger doses of MeEHg cause an inhibitory reaction. This effect is found at pH 7·5 and 8·5 (Fig. 2). At pH 7·5 there is a slight inhibitory effect even at low NAD concentrations, whereas an activation of the enzyme is found at pH 8·5 (Fig. 3).

The allosteric stimulation of the enzyme by L-leucine and ADP is more pronounced at pH 8·5 than 7·5, but this effect is reversed by addition of MeEHg, independent of pH. As to the NAD stimulation, larger amounts of MeEHg have an inhibitory effect. Very large concentrations destroy the stimulated and unstimulated enzymes at the same rate, probably by denaturation (Figs. 3 and 4).

The inhibitory effect of diethylstilboestrol, also believed to be an allosteric effect, is likewise reversed by addition of MeEHg. The results change with pH and NAD concentration as for allosteric activators (Table 1).

DISCUSSION

The effect of organic mercury compounds on the activity of glutamate dehydrogenase is not clear. Bitensky *et al.*[4] proposed that methyl mercury might have a stabilizing effect on the equilibrium between two monomers with different enzymatic activity. The allosteric ligands, which were supposed to disturb this equilibrium, would then be inhibited. The authors did not, however, present

* The seed dressing agent methoxyethylmercury acetate (MeEHg) was a gift from A/B Casco, Stockholm, Sweden.

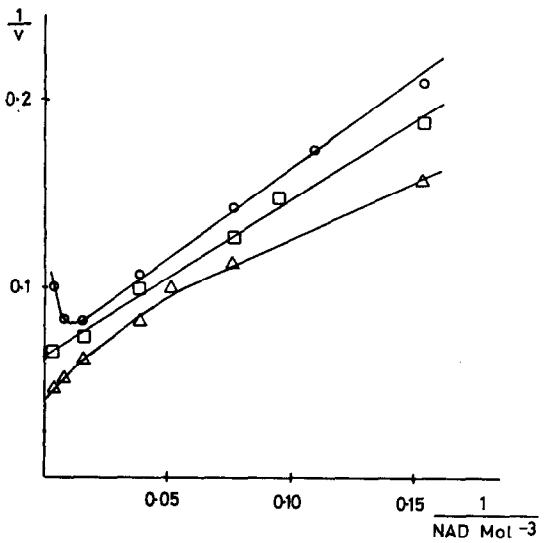

FIG. 1. Reciprocal plot of the glutamate dehydrogenase activity of a mitochondrial suspension from rat liver (△), and of the same suspension pretreated as described in the text with 7·5 μg (□) and 10 μg (○) methoxyethyl-mercury acetate.

The reaction mixture contained 0·13 M L-glutamate, 0·13 M niacinamid, 0·0004 M KCN, and NAD as indicated, in 0·25 M phosphate buffer pH 7·5. The reaction was started by adding enzyme suspension corresponding to 1 mg of protein. $v = \Delta E_{340m\mu} \times 10^2$/min was taken as the enzymatic activity.

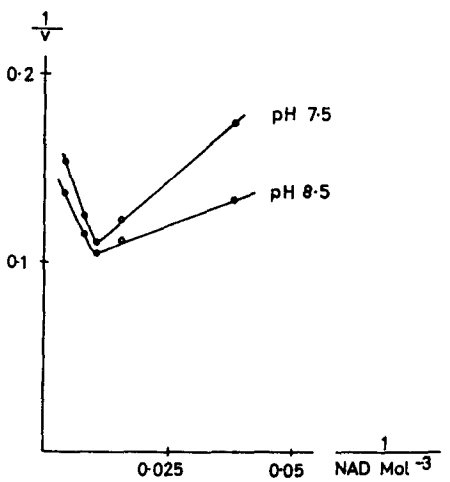

FIG. 2. Reciprocal plot as shown in Fig. 1 with similar conditions. The mitochondrial suspension was preincubated with 10 μg methoxyethylmercury acetate. The reaction mixture was as indicated in Fig. 1, except 0·05 M phosphate buffers pH 7·5 and 8·5 were used.

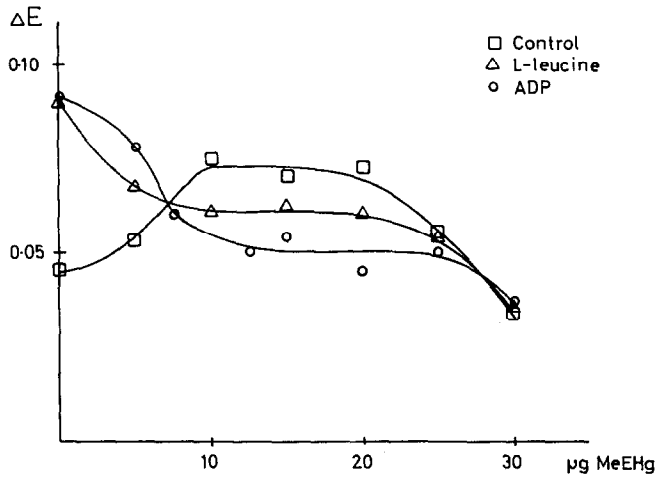

FIG. 3. The effect of methoxyethylmercury acetate pretreatment on the normal and on the "allosteric activated" crude glutamate dehydrogenase from rat liver at pH 8·5.

The reaction mixture contained 0·13 M L-glutamate, 0·03 M niacinamid, 0·0004 M KCN and 0·004 M NAD in 0·05 M phosphate buffer pH 8·5. 0·003 M ADP and 0·01 M L-leucine were added in the reaction mixture as activators. The reaction was started by adding enzyme suspension corresponding to 1 mg of protein, and $\Delta E_{340\ m\mu}$/min was taken as the enzymatic activity.

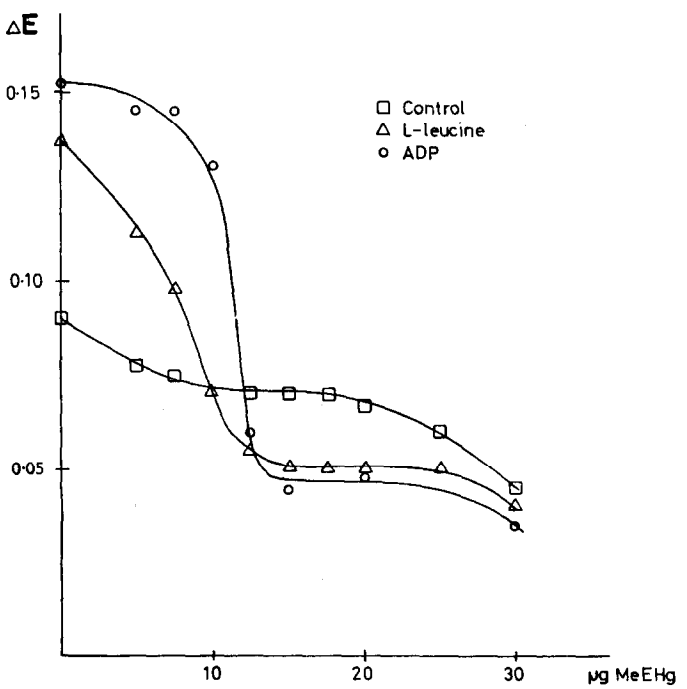

FIG. 4. The effect of methoxyethylmercury acetate pretreatment on the normal and on the "allosteric activated" crude glutamate dehydrogenase from rat liver at pH 7·5.

Except for a phosphate buffer pH 7·5, 0·05 M, the assay conditions were identical to Fig. 3.

evidence that the mercury compound did not react with the allosteric site. Nor could they explain the somewhat unexpected inhibitory effect caused by higher methyl mercury concentrations in connection with ADP.

TABLE 1. VARIATION OF THE DIETHYLSTILBOESTROL-INDUCED INHIBITION OF CRUDE RAT LIVER GLUTAMATE DEHYDROGENASE WITH NAD CONCENTRATION, pH VARIATION AND METHOXYETHYL-MERCURY TREATMENT *in vitro*

	pH 7·5		pH 8·5	
	NAD 4 mM	NAD 8 mM	NAD 4 mM	NAD 8 mM
Control enzyme	100	100	100	100
Methoxyethylmercuryenzyme*	82	78	97	84
Control enzyme + inhibitor†	51	93	26	60
Methoxyethylmercuryenzyme* + inhibitor†	63	78	55	70

Values are given as per cent of control activity. The reaction conditions were as described in the Figures, as was the preincubation with methoxyethylmercury acetate.

* Preincubated with 7·5 μg methoxyethylmercury acetate.

† Diethylstilboestrol, $2{\cdot}5 \times 10^{-5}$ M final concentration in the reaction mixture.

To explain the unstability of the enzyme and the differing responses to allosteric ligands, di Prisco and Strecker[5] proposed a slightly different model of monomers:

$$\underset{\text{Polymer}}{\overset{\text{I}}{\leftrightharpoons}}\ \underset{\text{GDHs}}{\overset{\text{II}}{\leftrightharpoons}}\ \underset{\text{GDHu}}{\overset{\text{III}}{\leftrightharpoons}}\ \underset{\text{GDHi,}}{}$$

where GDHs indicates a stable monomer insensitive to the effect of allosteric ligands and GDHu an equally active but unstable form, sensitive to allosteric ligands and tending to change into the reversible inactive form GDHi.

It seems likely that both allosteric ligands and organic mercury compounds act by changing equilibrium III, corresponding to equilibrium $x \leftrightharpoons y$, as proposed by Bitensky *et al.*[4] Thus an activating effect would point to GDHu as the predominating monomer, and a tendency to generate GDHi would simulate an inhibitory effect. The less pronounced effects of the allosteric ligands at low pH levels could then be explained by the predomination of GDHs at this acidity. The stimulating effect of MeEHg at pH 8·5 and the lack of stimulation at pH 7·5 would then be easily explainable.

The other effects of MeEHg, alone and together with allosteric ligands, do not seem to be dependent on pH level. An explanation seems to be that MeEHg acts by changing the overall equilibrium of the monomers towards GDHs, while the allosteric activators act by stabilizing GDHu. Then the reversal by MeEHg of an inhibitory effect would be quite plausible. The high proportion of GDHs, stable to allosteric effects, on administration of MeEHg, would explain the reversal of the allosteric activating effect. The allosteric activators still combine with the enzyme as shown by the joint effect of MeEHg and these activators. The inhibitory effect of MeEHg in connection with ADP, L-leucine, or activating amounts of NAD, indicates a specific effect of the activators on the monomer GDHs, although no change in the reaction rate with the activators alone is demonstrable.

The slight inhibitory effect of MeEHg at pH 7·5 cannot be explained from these considerations, but the inverse plots suggest competitive inhibition on the coenzyme site with regard to NAD.

The monomer equilibrium of glutamate dehydrogenase is influenced by certain anions.[5] The possibility that the effects of MeEHg be related to the acetate ion must not be overlooked, even if it hardly seems likely. According to Bitensky *et al.*[4] the effects of *p*-chloromercuric benzoate treated enzyme, passed through a Sephadex G 25 column, are similar to those caused by MeEHg. They also found the effects of methyl mercury to be reversed by mercaptoethanol. We observed a reversal of the allosteric effect by MeEHg in MeEHg intoxicated rats where the possibility of anionic influence can be ruled out (unpublished observation).

The present investigation shows that combination of MeEHg with the glutamate dehydrogenase protein from rat liver mitochondria gives rise to different patterns with different effects, depending on the condition of the protein at the moment. The findings may not be applicable to the symptoms

of mercury intoxication in man. On the other hand, the unexplained different liability of equally exposed workers to develop symptoms of mercury intoxication may well be caused by similar mechanisms. In fact, it has been suggested that the explanation for this must be sought in the organ status at the moment. The basic position of allosteric mechanisms in the overall regulation of intracellular metabolism must likewise be borne in mind.

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