Selective Desensitization of the Allosteric Glutamic Dehydrogenase from Blastocladiella by Hg^{++}

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Received October 11, 1968

An integral part of the allosteric concept of Monod, Wyman, and Changeux (1) is that enzymes which are allosteric can lose their regulatory properties without attendant loss of catalytic activity. This phenomenon was given the phenotypic term of *desensitization*. Recently, we have been able to show that three distinct categories of small molecules regulate the overall activity of an NAD-specific glutamic dehydrogenase from *Blastocladiella emersonii* (2, 3, 4). One class, the purine and pyridine nucleotides (AMP, ATP, and NAD^+), interact with the enzyme in a way that permitted us to classify them as 'allosteric' ligands (2). The second class which are mostly metabolites (e.g., citrate, succinate, fructose 1,6-diphosphate, fumarate), and the nonmetabolite EDTA completely inhibit the oxidative deamination of glutamate but *apparently* do not affect the reductive amination of α-ketoglutarate. This unidirectional inhibition mechanism was formulated into a metabolic pattern of control (3). The third class of effectors are cations, Ca^{++} and Mn^{++} specifically, which are strong activators of the reductive amination reaction and inhibitors of the oxidative deamination of glutamate (4). In every case, we emphasized the importance of pH in the manifestation of these modifying influences. At pH 6, there is a complete 'desensitization' of the enzyme to all its effectors.

This communication shows that the enzyme can be selectively desensitized by Hg^{++} against AMP and NAD^+ activation, and ATP inhibition without altering the effects mediated by Ca^{++} and the metabolites.

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EXPERIMENTAL SECTION

A highly purified enzyme preparation with a specific activity 330 times greater than that of the crude extract was used throughout these studies. The procedure for the isolation of the enzyme has been reported (3). Further purification of the enzyme was achieved by passage through a column of sephadex G-200. Reaction rates were measured by means of a Gilford model 2000 recording spectrophotometer in 3 ml cuvettes (10 mm light path) and expressed as $A_{340\ m\mu}\ min^{-1}$. All reactions and incubations were carried out at 25°C.

RESULTS

Glutamic dehydrogenase from *Blastocladiella* can be rapidly desensitized to AMP activation and ATP inhibition when treated with $HgCl_2$ at pH 7. The kinetics of desensitization depend on the concentration of enzyme and on pH. Reproducible systems were obtained by the use of 25-50 µg enzyme in 50 mM Tris-chloride, pH 7, containing Hg^{++} at 6.67×10^{-5} M concentration in a total volume of 0.1 ml. Lower or higher levels of Hg^{++} can lead to partial desensitization or complete inactivation of the enzyme in a very short period. The desensitized enzyme is stable at 0°C for approximately 1 hr.

A previous report (2) had indicated that the nucleotides (AMP, ATP, and NAD^+) are cooperative interacting ligands. Both AMP and ATP were postulated to act at the same allosteric site. The results recorded in Fig. 1 indicate that there is a simultaneous desensitization by Hg^{++} of the enzyme to AMP activation and ATP inhibition (curves, A and C). The process is almost complete within 2 min. This can only mean that the two sites are identical or that they overlap. The homotropic interaction of NAD^+ was also lost (Fig. 2, curve C), implying a common nucleotide regulatory site for all three ligands.

Selective Desensitization. Interestingly, the enzyme is not desensitized to the inhibitory effects of citrate, EDTA and the other metabolites during the period of desensitization to nucleotides (Fig. 1, curves E and F). Only the plots for citrate and EDTA have been included since they were the most

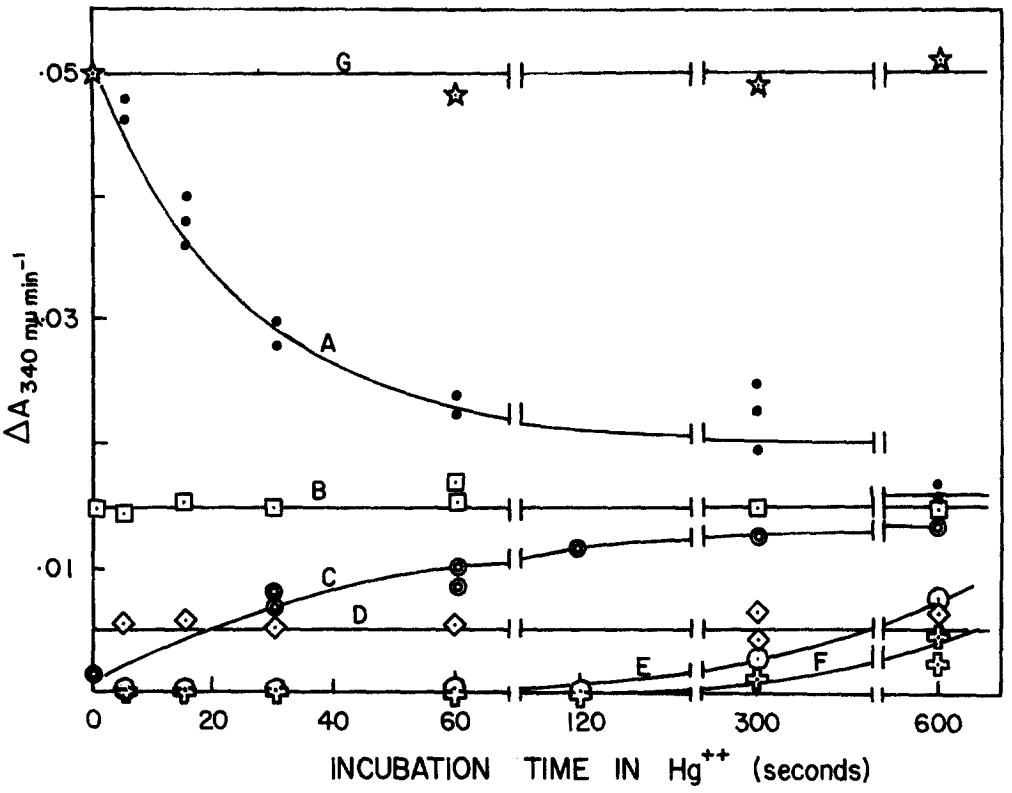

Fig. 1. *Response of B. emersonii glutamic dehydrogenase to various effectors during desensitization in the oxidative deamination of glutamate.* The letters A-G represent the following: A, 1 mM AMP; B, No effector; C, 1 mM ATP; D, 10 mM Ca^{++}; E, 3.33 mM citrate; F, 3.33 mM EDTA; and G, Non-desensitized enzyme. The assay system is as outlined in Table I with 1 mM AMP added.

potent inhibitors that affected the enzyme unidirectionally (3). Ca^{++} which inhibits the oxidative deamination of glutamate and activates the reductive amination of α-ketoglutarate, still showed the same response on the desensitized enzyme (Fig. 1, curve D).

When the enzyme is allowed to interact with Hg^{++} for a longer period (15 min), there is a near complete desensitization of the enzyme to all its various effectors (Table I(a) and (b)). As seen in Fig. 2 (curve E), 0.066 mM EDTA completely inhibits the non-desensitized enzyme. The desensitized enzyme is resistant to as high as 3.33 mM EDTA (curve D).

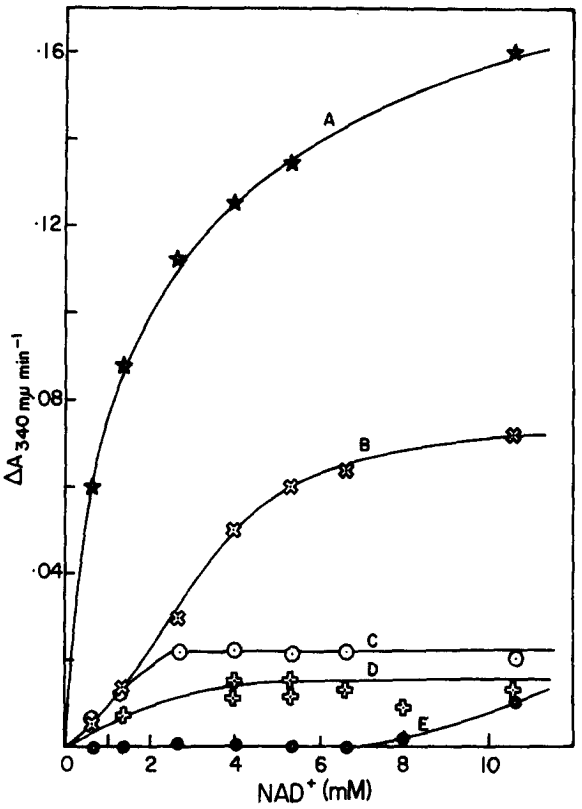

Fig. 2. *Effect of NAD^+ concentration on the reaction rates of desensitized, and non-desensitized glutamic dehydrogenase from B. emersonii.* The letters A-E represent the following: A, Non-desensitized enzyme assayed with AMP; B, Non-desensitized enzyme assayed without AMP; C, Desensitized enzyme assayed with and without AMP; D, Desensitized enzyme assayed with 3.33 mM EDTA; and E, Non-desensitized enzyme assayed with 0.066 mM EDTA.

We had to confirm that all the desensitization kinetics are reflected in the reverse direction as well. Since the metabolites affect the enzyme in a unidirectional manner, only Ca^{++} and AMP effects were considered. From Fig. 3, we deduced that Ca^{++} activation was unaffected during the initial period of desensitization to AMP. Desensitization to Ca^{++} occurred after a longer period of incubation (at least 5 min).

Protection by Ligands. The results of Table 2(a) and (b) show that the substrates and effectors, individually,

Table I. *The effect of various ligands on the activity of desensitized glutamic dehydrogenase from B. emersonii*

25 µl enzyme protein was incubated with 6.7 X 10^{-5} $HgCl_2$ in 50 mM Tris-chloride, pH 7 in a total volume of 0.1 ml for 15 min. The desensitized enzyme was assayed as follows:

(a) during the oxidative deamination of glutamate, in 100 mM Tris-chloride, pH 9, containing 10 mM glutamate; 4 mM NAD^+; effector at the specified concentration.

(b) during the reductive amination of α-ketoglutarate, in 133 mM Tris-chloride, pH 7, containing 500 mM NH_4^+; 0.166 mM NADH; 1.66 mM α-ketoglutarate; effector at the specified concentration.

	(a) *Oxidative Deamination Reaction*		(b) *Reductive Amination Reaction*
Effector	Conc^n (mM)	v_e/v_o	v_e/v_o
None	-	1.02	0.94
AMP	1	1.11	1.05
ATP	1	0.92	1.05
Ca^{++}	10	0.76	1.07
Citrate	3.33	0.70	1.16
EDTA	3.33	0.65	1.03

v_o = reaction rate of non-desensitized enzyme in the absence of effector.

v_e = reaction rate of desensitized enzyme in the presence of effector.

Table 2. *The effect of various effectors and substrates as protective ligands in the desensitization of B. emersonii glutamic dehydrogenase.*

Procedure for desensitization has been outlined in the legend to Table I. Ligands, for protection, were added to the enzyme together with Hg^{++}. The basic assay systems used for the oxidative deamination and reductive amination reactions are as recorded in Table I. Where specified, Ca^{++} and AMP are used as activators of the reactions at 10 mM and 1 mM respectively.

(a) *The Oxidative Deamination of Glutamate*

Protective Ligand(s)	Conc^n (mM)	Reaction Rate*		
		No Activator	plus Ca^{++}	plus AMP
None	-	0.038	-	0.040
NAD^+	4	-	-	0.058
AMP	1	-	-	0.052
ATP	1	-	-	0.044
NADH	0.1	-	-	0.046
Glutamate	10	-	-	0.052
Glutamate + AMP	(10 + 1)	-	-	0.058
α-ketoglutarate	1	-	-	0.060
NAD^+ + AMP	(4 + 1)	-	-	0.110

Table 2. Cont'd

(b) *The Reductive Amination of α-Ketoglutarate*

Protective Ligand(s)	Conc^{n} (mM)	Reaction Rate		
		No Activator	plus Ca^{++}	plus AMP
None	-	0.088	0.090	0.090
Ca^{++}	10	0.100	0.248	0.320
AMP	1	0.120	0.112	0.144
ATP	1	0.120	0.115	0.120
Citrate	3.33	0.096	0.090	0.104

*Reaction rate = $\Delta A_{340\ m\mu}\ min^{-1}$.

provided only partial protection against desensitization. AMP and NAD^{+} proved to be the most effective combination of ligands affording greater than 80% protection. Separately, they were only 30-35% effective. The substrates, glutamate, α-ketoglutarate, and metabolic inhibitors (not recorded) were ineffective. On the other hand, Ca^{++} did serve as an excellent stabilizing ligand (Table 2(b)).

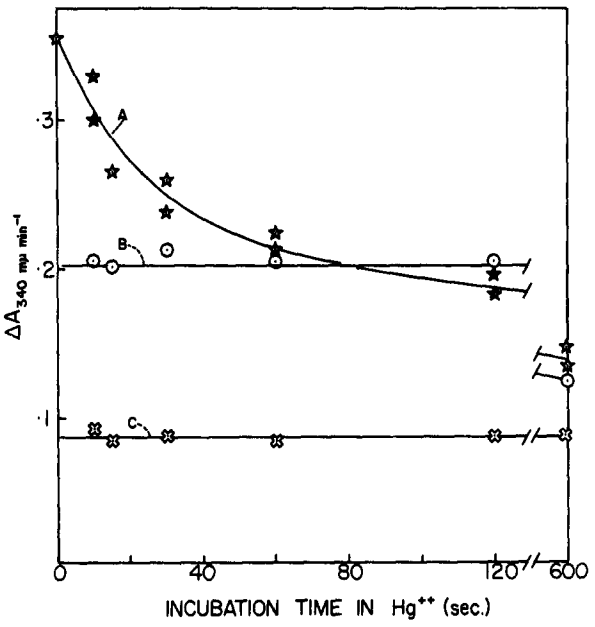

Fig. 3. *Response of B. emersonii glutamic dehydrogenase to various effectors during desensitization in the reductive amination of α-ketoglutarate.* The letters A, B and C represent the following additions to the assay system: A, 1 mM AMP; B, 10 mM Ca^{++}; C, No addition. The assay system as outlined in Table I.

DISCUSSION

The results reported here and elsewhere (2, 3, 4) indicate that the glutamic dehydrogenase from *B. emersonii* is an allosteric enzyme that is controlled by a diversity of ligands. Under carefully controlled conditions, primary and secondary desensitization processes could be elicited. The primary process is confined to the loss of nucleotide activating activity. The secondary process involves the disappearance of all regulatory effects by cations and metabolites. The latter is comparable to the loss of regulatory property at pH 6. The main difference is that whereas the pH effect is reversible, Hg^{++} desensitization has, so far, been found to be irreversible.

The importance of Ca^{++} as an activator of the enzyme was recently emphasized (4). These findings suggest that its mode of action is regulatory.

In many ways, the properties displayed by this glutamic dehydrogenase resemble those of glutamine synthetase from *Escherichia coli* (5, 6, 7) which exists in taut and relaxed forms. The taut form is resistant to organic mercurial inactivation, but the relaxed form is not. The possibility remains that during desensitization of the glutamic dehydrogenase by Hg^{++}, there is a similar transition from relaxed to taut form. If this is true, it provides added support for the allosteric model we have presented elsewhere (2).

ACKNOWLEDGMENT

This work was supported by a grant from the National Research Council of Canada.

REFERENCES

1. Monod, J., Wyman, J., and Changeux, J.P., J. Mol. Biol., 12, 88 (1965).
2. LéJohn, H.B., and Jackson, S., J. Biol. Chem., 243, 3447, (1968).
3. LéJohn, H.B., J. Biol. Chem., 243, 5126 (1968).
4. LéJohn, H.B., Biochem. Biophys. Res. Communs., 32, 278 (1968).
5. Shapiro, B.M., and Stadtman, E.R., J. Biol. Chem., 242, 5069 (1967).
6. Shapiro, B.M., and Ginsberg, A., Biochemistry., 7, 2153 (1968).
7. Valentine, R.C., Shapiro, B.M., and Stadtman, E.R., Biochemistry., 7, 2143 (1968).