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REGULATION OF GLUTAMATE DEHYDROGENASE BY HISTIDINE

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SUMMARY

Histidine and its analogs were demonstrated to activate crystalline beef liver glutamate dehydrogenase (L-glutamate: NAD^+ oxidoreductase (deaminating), EC 1.4.1.2). Activation effects were similar to those of other amino acids such as leucine or norvaline, which have been shown previously by others to exert their activating effect with respect to high concentration and in which kinetic studies also revealed modified values for both K_m and v_{max}.

Unlike ADP, histidine did not alter the fluorescence intensity caused by enzyme–coenzyme binding but increased the sedimentation coefficient of the enzyme slightly in either the native or disaggregated state by NADH.

INTRODUCTION

As far as metabolic control is concerned, both sides of controls are always to be considered, inhibition and enhancement. Allosteric regulators of glutamate dehydrogenase (L-glutamate: NAD^+ oxidoreductase (deaminating), EC 1.4.1.2) are thus classified into inhibitors and activators[1–9] and another category of control mechanisms which involves desensitization against modifiers has been introduced[10–14].

As a regulator of physiological origin, bilirubin inhibition on glutamate dehydrogenase has been described in the previous report[15]. In the present report, as a heterocyclic nitrogen compound of physiological origin, histidine and its analogs, as well as histidine dipeptides, are discussed as possible activators.

Although, some amino acids such as leucine, valine or norvaline[2,8,9] have been known to activate the enzyme together with ADP[1–7], the biological significance of those activation mechanisms is still unknown.

EXPERIMENTAL

Crystalline beef liver glutamate dehydrogenase was obtained from Boehringer and Sons, in 50% glycerol with a specific activity of about 3 units/mg. The enzyme assay was performed in 3.0 ml of 0.05 M potassium phosphate buffer (pH 7.6), 17 mM sodium glutamate and 1 mM NAD^+ with 0.015 unit of crystalline glutamate de-

hydrogenase at 25°. The increased absorbance by NADH at 340 nm during the initial 1 min was measured on a Hitachi EPS-2 spectrophotometer.

Fluorescence measurements were carried out on an Hitachi spectrofluorimeter Model MPF-2A.

Ultracentrifugal patterns were taken by using an Hitachi analytical ultracentrifuge Model UCA-1A, with schlieren optics, in either standard or wedge-type cells of 12 mm at 55 430 rev./min at 20°.

NAD^+ was purchased from General Biochemicals, NADH from Daiichi Pure Chemicals Corp., *N*-acetyl-L-histidine and glycyl-L-histidine from International Chemical and Nuclear Corp., L-histidine from Rikagaku Yakuhin Corp., L-carnosine from Nakarai Chemicals, other histidine derivatives and dipeptides were kindly offered from Prof. Tsunoo's laboratory.

RESULTS

In the present study, histidine showed an activating effect in an almost identical manner to valine or ADP (Fig. 1).

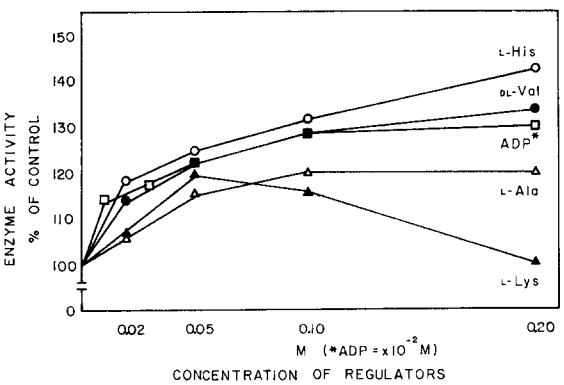

Fig. 1. Effect of various amino acids on glutamate dehydrogenase. The reaction mixture contained 0.015 unit of glutamate dehydrogenase, 1 mM NAD^+ and 17 mM sodium glutamate in 3.0 ml of 0.05 M phosphate buffer (pH 7.6). All amino acids were neutralized if necessary and added to the reaction mixture as indicated above.

Lysine had a similar effect at $5 \cdot 10^{-2}$ M as these effectors, although it was no longer an activator at higher concentrations. Alanine was less effective. A portion of enhanced active material added to the original enzyme activity, in the absence of effectors, could be due to the enzyme affinity for those effectors as substrates. In fact, alanine and other monocarboxylic amino acids were reported to be substrates for glutamate dehydrogenase at as low a concentration as $1 \cdot 10^{-4}$–$1 \cdot 10^{-6}$ M, with an enzyme concentration as high as 2.5 times that required for an enzyme assay with glutamate as substrate[7] and norvaline was effective as a substrate under similar conditions as glutamate but at different pH optima[16]. Under the same experimental conditions with glutamate as substrate, however, those amino acids tested in the present study did not show any enzymatic reaction, which meant a mechanism other than that involving substrates might cause activation of the enzyme when those

TABLE I

ACTIVATION OF GLUTAMATE DEHYDROGENASE ACTIVITY BY HISTIDINE DERIVATIVES

The concentration of the histidine derivatives was $5 \cdot 10^{-2}$ M. The enzyme assay was conducted as in Fig. 1.

Compound added	*Increased rate (% of control)*
None	100.0
L-Histidine	125.5
N-Acetylhistidine	102.2
1-*N*-Methylhistidine	119.6
2-*C*-Methylhistidine	126.0
3-*C*-Methylhistidine	121.6
Glycylhistidine	123.5
L-Anserine	123.5
L-Carnosine	126.5
Homocarnosine	117.2
Ophidine	116.5

effectors were added to the reaction mixture for the glutamate dehydrogenase assay. At $5 \cdot 10^{-2}$ M, histidine derivatives and dipeptides also enhanced glutamate dehydrogenase activity in almost the same way as histidine except for *N*-acetylhistidine (Table I).

Activating effects by these histidine derivatives and dipeptides were concentration dependent, as shown for histidine in Fig. 1 although the data were not presented at different concentrations. For further investigations of the activation mechanism by histidine and its analogs, kinetic studies were shown in Figs. 2 and 3 with histidine as an effector. When histidine was present in the reaction mixture, the K_m was increased from $1.8 \cdot 10^{-4}$ to $3 \cdot 10^{-4}$ M with substrate (Fig. 2) and from $2.6 \cdot 10^{-4}$ to $3.3 \cdot 10^{-4}$ M with NAD^+ (Fig. 3). However, binding sites were non-competitive with substrate or coenzyme, as previously shown to be the case in the activation of the enzyme by valine or leucine[8]. In order to compare the activation

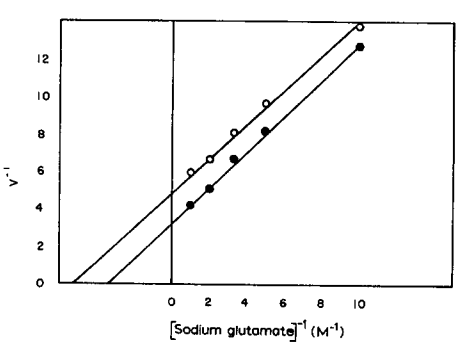

Fig. 2. Lineweaver–Burk plots of the substrate saturation curve. The experimental condition was the same as in Fig. 1 except for the substrate concentrations. ○, control; ●, experimental. $5 \cdot 10^{-2}$ M histidine was present in the experimental assay.

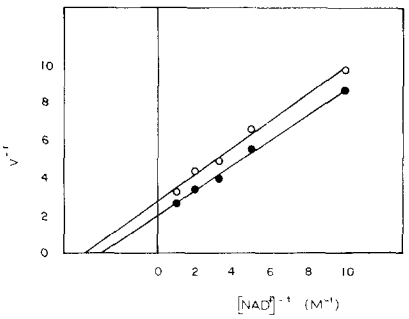

Fig. 3. Lineweaver-Burk plots of the coenzyme saturation curve. The experimental condition was the same as in Fig. 1, except for the coenzyme concentrations. Other notes were indicated in Fig. 2.

mechanism of histidine with ADP, a fluorimetric assay of the enzyme–NADH binding was conducted as shown in Fig. 4.

With ADP, the fluorescence intensity caused by enzyme–NADH binding was decreased, as demonstrated by TOMKINS *et al.*[17], whereas, GTP[18,19] and diethylstilbesterol[17], which inhibit the enzyme activity, enhanced the fluorescence intensity thereby increasing the NADH binding. ADP extended its maximal effect on decreasing the fluorescence intensity produced by the enzyme–NADH complex at $5 \cdot 10^{-5}$ M and on further addition no further decrease was observed, as has been pointed out by others[17]. On the other hand, histidine did not change the fluorescence

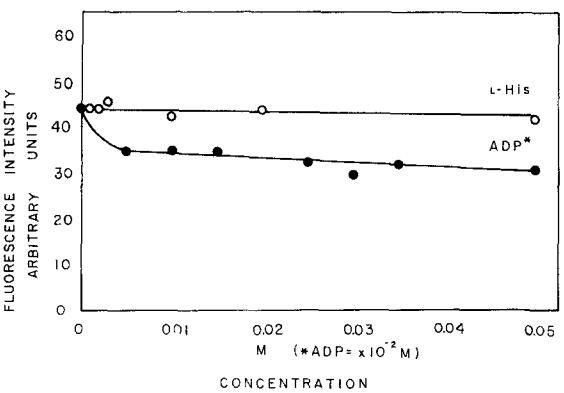

Fig. 4. Effects of modifiers on enzyme–coenzyme binding. A fluorescence assay was performed in 0.3 ml of 0.05 M phosphate buffer (pH 7.6) with 0.1 mg of glutamate dehydrogenase and 15 μM NAD^+. Emission and excitation spectra were at 460 and 340 nm, respectively.

intensity as shown in Fig. 4, and similar concentrations of histidine were lowered to the same degree as ADP. The numbers of NADH binding sites were analyzed by means of a plot according to SCATCHARD *et al.*[20], where, $\bar{\nu}$ represents the numbers of bound NADH per mole of enzyme, and c is the concentration of free NADH. The intercept of the slope with the abscissa indicates the numbers of NADH binding sites and the dissociation constant K_D is calculated from the equation; slope $= -K_D$ as $5.6 \cdot 10^{-5}$ M (Fig. 5). Those NADH binding sites may not necessarily be involved as

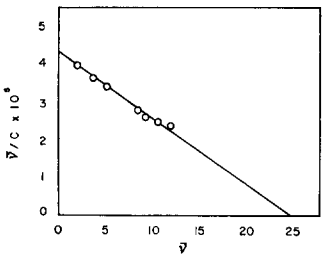

Fig. 5. Scatchard plot of enzyme–coenzyme binding. Moles of bound NADH per mole of enzyme, $\bar{\nu}$, were calculated from titration curves of enzyme and NADH and plotted on the abscissa. c indicated the concentration of free NADH and $\bar{\nu}/c$ was plotted on the ordinate. Otherwise, see the text.

catalytic sites, a fact which was demonstrated in the experiment with the enzyme with a high concentration of urea. Enzyme activities were abolished at higher concentration of urea than 3 M, whereas, the binding capacity with NADH with the enzyme still remained in such a highly dissociated state. From the result in Fig. 6, at least 20–30% of the fluorescence intensity caused by the enzyme–NADH binding remained

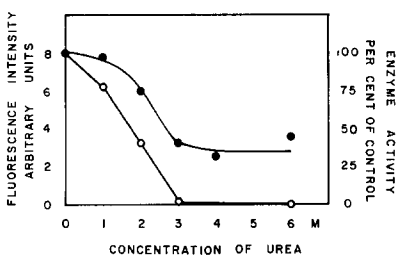

Fig. 6. Effect of urea on enzyme–coenzyme binding. A fluorescence assay was conducted as indicated in Fig. 4 and plotted on the ordinate on the left as ●. Enzyme activity was assayed as indicated in Fig. 1 and plotted on the ordinate on the right as ○.

unaffected. Presumably, 1 catalytically active unit of molecular weight of about 300 000 (refs. 21, 22) has a capacity to bind 24 moles of NADH (*cf.* ref. 19). This has been shown to consist of 6 identical peptide chains[23] of molecular weight of about 50 000, each unit of which has 4 sites for NADH binding and 1 of which is still capable

TABLE II

SEDIMENTATION COEFFICIENT OF GLUTAMATE DEHYDROGENASE WITH VARIOUS EFFECTORS

Analytical condition was indicated in the text. Protein concentration was 2.5 mg/ml.

Effector added	*Concentration (M)*	$s_{w,20}$
None	—	11.9
Histidine	$5 \cdot 10^{-2}$	13.0
ADP	$5 \cdot 10^{-4}$	11.9
NADH	$5 \cdot 10^{-4}$	6.9
Histidine + NADH	$5 \cdot 10^{-2}$ + $5 \cdot 10^{-4}$	7.9

of binding with NADH, regardless of the catalytic activity. FRIEDEN[1] demonstrated that ADP aggregated glutamate dehydrogenase, which was partially dissociated by NADH by increasing the sedimentation coefficient, whereas, ATP accelerated the enzyme dissociation which was induced by NADH. Leucine was also reported to stimulate glutamate dehydrogenase to promote aggregation together with ADP by TOMKINS *et al.*[7]. FAHIEN *et al.*[24] observed a slight decrease in the sedimentation coefficient of glutamate dehydrogenase by the addition of NADH.

In Table II, the sedimentation coefficient of glutamate dehydrogenase is summarized under various conditions. From the result in Table II, when glutamate dehydrogenase is intact, ADP had no effect on the enzyme, whereas, histidine increased the sedimentation coefficient slightly. A similar effect of histidine was also observed in the disaggregated enzyme by NADH, an observation which might or might not be interpreted as a distinct activation mechanism apart from that of ADP.

DISCUSSION

Leucine and DL-norvaline were reported to have an activating effect on glutamate dehydrogenase by YIELDING AND TOMKINS[2] and protecting effects on glutamate dehydrogenase denaturation together with ADP[9]. Among purine nucleotides, ADP has been shown to activate enzyme activity by aggregating the enzyme molecule[1]. Further studies concerning the precise kinetic analysis were carried out by KUN AND ACHMATOWICZ[8] in the presence of substrate analogs, showing that these effects were substrate- as well as coenzyme-dependent.

Among modifiers of glutamate dehydrogenase activity, amino acids[8,9] required an extraordinarily high concentration to exert their activating effect on glutamate dehydrogenase as was shown with histidine and its derivatives (Table I) in the present experiment. However, this seems to be one of the characteristic features of allosteric regulation which extends beyond the stoichiometric dimension.

Kinetic studies have revealed that both K_m and v_{max} were altered by the addition of histidine in the enzyme reaction (Figs. 2 and 3), as was shown by others[8] in case of leucine and valine. On the contrary ADP modified only v_{max} when added to the reaction mixture[9]. ADP decreased the fluorescence intensity of glutamate dehydrogenase in the presence of high NADH to about 30% of the original value (Fig. 4) in accord with the results by FRIEDEN[5] and by TOMKINS *et al.*[17]. However, histidine did not change the fluorescence intensity, which means that unlike ADP, histidine enhances the enzyme activity without affecting the enzyme–coenzyme binding.

A slight enhancement of the sedimentation coefficient of glutamate dehydrogenase by the addition of ADP and a marked aggregation effect of ADP on the enzyme aggregation was demonstrated by CHEN *et al.*[25], with untreated isocitrate dehydrogenase, where, in the presence of ADP, sedimentation constants shifted from 10.3 to 18.6 and 25.7 S depending on the concentration of ADP added.

Taking account of the fact that the sedimentation velocity was modified in the presence of salt such as NaCl, as was shown by BITENSKY *et al.*[9], and that glutamate dehydrogenase activity was increased by the addition of various electrolytes including NaCl or potassium phosphate at high pH (ref. 26), under the present experimental conditions, the salt effect could be ruled out. Therefore, the changes in the sedimentation velocity may be caused by other physical factors than the salt effect, probably

due to the partial aggregation of the enzyme induced by the presence of excess of histidine but not by ADP in the present study.

It is interesting to know that physiological substances of histidine derivatives and its dipeptides showed a similar effect on glutamate dehydrogenase activity to that of histidine (Table I). However, acetylhistidine which is rarely found in mammalians[27] did not show the same histidine effect of glutamate dehydrogenase from mammalian sources.

Further verifications about whether the modification of the catalytic units of the enzyme or some physicochemical changes of the catalytic center of the enzyme take place are expected to be proved by direct visualization of the enzyme molecules.

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