# ALLOSTERIC NATURE OF A GLUTAMINASE ISOZYME IN RAT LIVER

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Sayre and Roberts(1958) purified glutaminase from dog kidney and reported on the mode of its activation by phosphate. More recently, Kvamme et al. (1965) showed that the activation of glutaminase by phosphate involves a conformational change of the enzyme protein of pig kidney. The authors(1966) previously reported that there are two types of glutaminase in both rat kidney and liver. One isozyme requires phosphate to show activity (phosphate dependent, PD), while the other does not require phosphate either as a cofactor or activator (phosphate independent, PI), but is activated by maleate or N-acetyl glutamate. The latter enzyme is polymerized in the presence of a high concentration of the substrate and the activators facilitate the polymerization of the enzyme caused by the substrate. The present paper describes the nature of the phosphate independent glutaminase as an allosteric enzyme at the molecular level.

## MATERIALS AND METHODS

Assay of Glutaminase Activity: Enzyme activity was assayed by determination of the ammonia liberated during incubation, as described previously(N.Katunuma et al.,1966). Specific activity of the PI is defined as m moles of liberated ammonia per mg protein per hour under the assay conditions (N.Katunuma et al., 1966).

Purification of Rat Liver Glutaminase: PI in the liver is much more labile than that in the kidney. The enzyme was purified as reported previously (N.Katunuma et al.,1968). A final purification of 250 fold was achieved. Only glutamine acted as substrate for this enzyme while no other compounds tested, such as asparagine, leucineamide, glycineamide, glucosamine, tyramine, tryptamine and serotonine served as substrates. The enzyme was free from glutamine transaminase and PD activities.

Method of Sucrose Density Gradient Centrifugation: Sucrose density gradient of linear from 5 to 15 % in $5x10^{-2}$M Tris buffer (pH 8.6) in 5 ml tube was prepared and then 0.5 ml (2.5 mg of protein) of the enzyme having specific activity 0.12 m moles per mg per hour was applied on it. The centrifugation was performed at 40,000 rpm for 300 or 600 min. at 5°C in swinging bucket of the HITACHI preparative ultracentrifuge (Model 5Sp-2). The distribution of the enzyme activity was assayed for 8 fractions. Lactic dehydrogenase (LDH) was used as a marker for calculation of the sedimentation constant. LDH activity was assayed by the method of F.Kubowity and P.Ott (1943).

## RESULTS AND DISCUSSION

Liver PI was always assayed in the presence of $10^{-3}$M phosphate, because the enzyme was extremely labile even during the incubation in the absence of phosphate. But more high concentration of phosphate above $5x10^{-3}$M inhibited the PI activity strongly. The effect of phosphate on the PI is entirely different from that on PD which requires $10^{-1}$M phosphate to show the activity. In the presence of a suitable concentration of phosphate ($10^{-3}$M), various compounds, as for example, maleate, N-acetyl glutamate and tricarboxylic acids, had additive activating effects. As shown in Fig. 1, liver PI displayed a typical sigmoidal velocity curve with respect to the substrate (glutamine) concentration. In the presence of an activator (maleate), the sigmoidal curve was converted to a normal curve. The activator caused a decrease in the Km value of the PI for glutamine. When the kinetic data ob-

tained without activator were plotted by the Lineweaver-Burk equation a straight line was not obtained. These kinetics suggest that the enzyme is

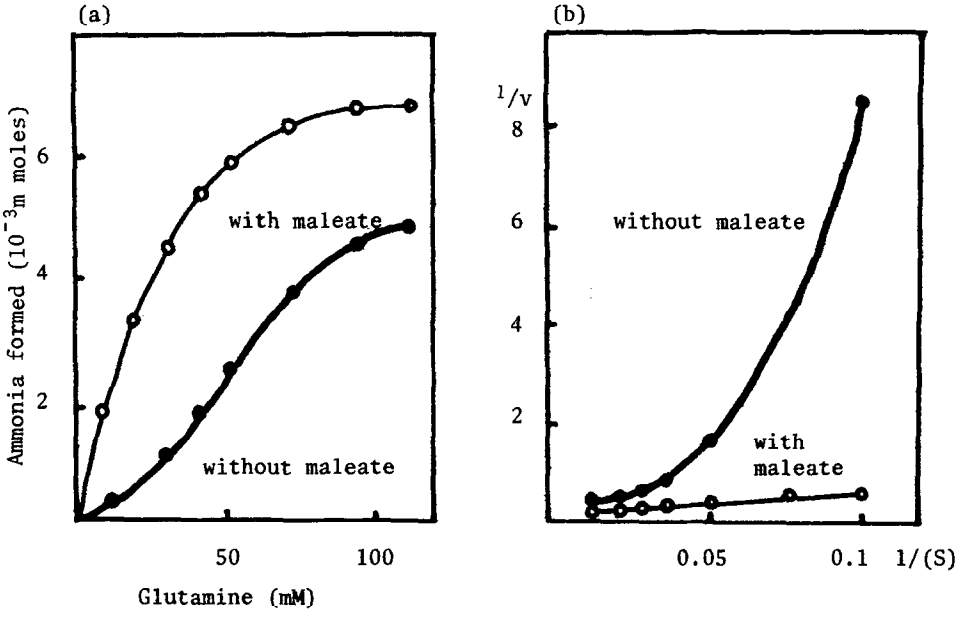


Fig.1 Kinetics of Rat Liver PI

Reaction mixtures contained $5x10^{-2}$ M Tris buffer(pH 8.6), $10^{-3}$M phosphate, 1mg/2ml of the enzyme (Sp.Act. 0.12 m moles/mg/hour), with or without $2.5x10^{-2}$M maleate, and indicated amount of glutamine. And then the reaction mixtures were incubated at 37°C for 15 min. The reactions were terminated by determination of the ammonia liberated during the incubation. Fig.1(a) shows the relationship between the enzyme activities and the substrate concentration with or without maleate ($2.5x10^{-2}$M). The expression by the Lineweaver-Burk equation is shown in Fig.1 (b).

polymerized in the presence of a high concentration of substrate and that the activator facilitates the polymerization of the enzyme molecules induced by the substrate. To demonstrate the allosteric polymerization more directly, sucrose density gradient centrifugation was carried out. Lactic dehydrogenase was used as a marker for calculation of the sedimentation constant. The results in Fig.2(a) show that the sedimentation constant of the enzyme increases in the presence of a high concentration of glutamine($10^{-1}$M). This provides further evidence that the enzyme is polymerized in the presence of the substrate. On the other hand, the addition of maleate alone to the system does not affect the sedimentation constant, as in Fig.2(b). Even when the glutamine concent-

ration is too low($2.5\times10^{-2}$M) to polymerize the enzyme(left side figure of c),

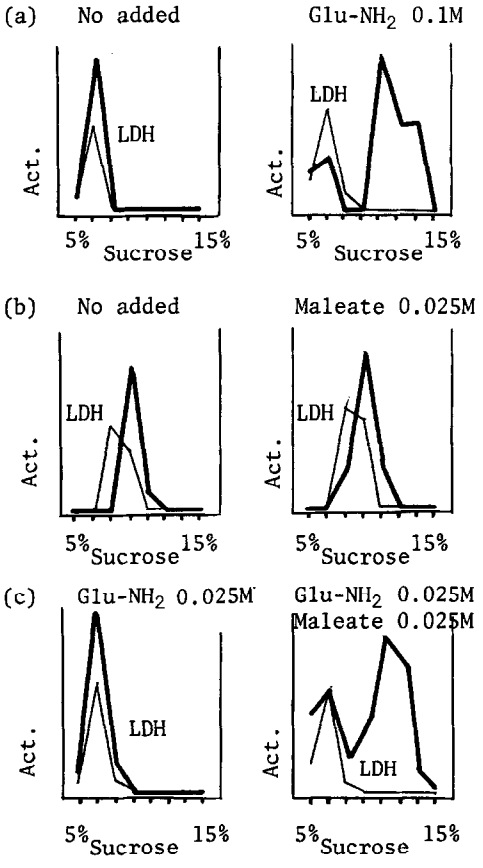


Fig.2 Sucrose Density Gradient Pattern of the PI

Sucrose density gradient conditions were described in the Text.

——: Bold line showes the glutaminase activity.
——: Fine line showes LDH activity as a marker.
Conditions of the centrifugation
(a) Left; no addition
Right; in the presence of $10^{-1}$M glutamine.
(b) Left; no addition
Right; in the presence of $2.5\times10^{-2}$M maleate.
(c) Left; in the presence of $2.5\times10^{-2}$M glutamine.
Right; in the presence of $2.5\times10^{-2}$M glutamine and $2.5\times10^{-2}$M maleate.
The centrifugation was performed for 300 min. on (a) and (c),and for 600 min. on (b).

the simultaneuous presence of maleate or N-acetyl glutamate causes the polymerization of the molecules just as in the presence of a high glutamine concentration ($10^{-1}$M). These results indicate that the enzyme is polymerized by a high concentration of the substrate and activators facilitate the polymerization of the enzyme molecules caused by the substrate.

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