GLUTAMINASE ISOZYMES IN RAT KIDNEY

NOBUHIKO KATUNUMA, IKUKO TOMINO AND HOZUMI NISHINO
Department of Enzyme Chemistry, Institute for Enzyme Research, School of Medicine, Tokushima University, Tokushima, Japan

Received January 3, 1966

In 1958 Sayer and Roberts reported on the mode of activation of glutaminase by phosphate using a dog kidney preparation. More recently Kvamme et al.(1965) showed that the activation by phosphate involves a conformational change of the enzyme protein of pig kidney. The authors found that there are two types of glutaminase in rat kidney. The two are completely different in their properties and only one has been reported previously. This discovery of the second isozyme may be due to our use of rat kidney which contains much of the new enzyme, while dog or pig kidney contain only a little. The present paper gives a comparison of the properties of the two glutaminase isozymes. One isozyme requires phosphate for activity(phosphate dependent) while the other does not require phosphate either as a co-factor or activator(phosphate independent), but is activated by carbonate and maleate ions. Differences in the properties of the two glutaminase isozymes are reported and their metabolic roles in relation to ammonium excretion in the kidney are discussed briefly.

MATERIALS AND METHODS

The kidneys of male rats were used throughout. Enzyme activity was assayed by determination of the ammonia liberated during incubation. The reaction mixture contained L-glutamine

30-40 μmoles, activator 50-300μmoles, enzyme and 0.05M Tris-HCl buffer(pH 7.2 or 8.6) in a total volume of 2 ml. The reaction was carried out for 30 minutes at 37°C, and terminated by the addition of 2 ml of 10 % TCA. After centrifugation, 1 ml of the supernatant was introduced into a Selligson-Shibata ammonium diffusion apparatus. After 20 minutes diffusion ammonia was determined by Nesslerization, and the optical density at 4200Å was measured in a spectrophotometer.

A calcium phosphate gel column was prepared by mixing 3 parts of calcium phosphate and one part of Whatman's cellulose powder. The mixture was suspended in 0.002M potassium phosphate buffer, pH 7.5, and poured into a column(5 X 10-15cm).

RESULTS AND DISCUSSION

1) Separation of Glutaminase Isozymes

The ratios of the activities with and without added phosphate differed with the kidneys of different species. Thus these ratios were 4-6 with rat kidney, 50 with pig kidney, 8-10 with beef kidney, 8-10 with human kidney and 0.5-1.0 with rat liver. This suggested that glutaminase might be a mixture of phosphate independent and dependent enzymes. The actual presence of two forms of glutaminase in rat kidney was demonstrated by their separation by calcium phosphate gel column chromatography. A rat kidney homogenate was prepared in 0.02 M Tris-HCl buffer, pH 8.6, and sonicated at 10 KC for 5 minutes. The sonicate was fractionated with ammonium sulfate. The fraction precipitating at 15-26 g% ammonium sulfate was dissolved in 0.002 M potassium phosphate buffer, pH 7.5, and eluted from a Sephadex G-50 column with the same buffer to remove ammonium sulfate. This eluate was then placed on a calcium phosphate gel column. The column was washed with 0.002 M phosphate buffer, pH 7.5

and then successively eluted with 0.1 M phosphate buffer, pH 8.0 and with 0.4 M trishydroxymethyl aminomethane solution. The enzyme in the former eluate was designated as the phosphate requiring glutaminase, since it did not show any activity without phosphate. The latter fraction contained the phosphate insensitive enzyme, the activity of which was not affected by adding phosphate. The phosphate insensitive enzyme was activated by carbonate and maleate.

The phosphate independent enzyme was readily prepared by taking advantage of its heat stability(see section 2)-b). Thus a rat kidney homogenate sonicate in 0.02 M Tris-HCl buffer (pH 7.2) was heated at 50°C for 30 seconds, centrifuged and the resulting supernatant was fractionated with ammonium sulfate. The fraction precipitating with 16-28 g% ammonium sulfate was passed through a Sephadex G-50 culumn before use. The phosphate independent enzyme prepared by the heat treatment is the same as that eluted with 0.4 M trishydroxymethyl aminomethane from the calcium phosphate gel column and also it is not identical with glutamine ketoacid transaminase of γ-glutamyl transferase.

2) Differences in the Properties of the Glutaminase Isozymes.

a) Effect of Phosphate on the Isozymes. As shown in Table I the effect of added phosphate differed with the glutaminase eluted with 0.1 M phosphate buffer and that eluted with 0.4 M trishydroxymethyl aminomethane solution.

b) Stabilities of the Isozymes. The phosphate dependent isozyme is very unstable and loses its activity completely when heated at 50-55°C for one minute in pH 8.6, 0.02 M Tris buffer or on adding PCMB at the final concentration of 0.1 mM, while the phosphate independent isozyme is unaffected by these treatments. The inactivation of the former isozyme was found

Table I

Effect of Phosphate on the Isozymes

phosphate added μmoles	phosphate dependent μmoles NH_3 formed	phosphate independent μmoles NH_3 formed
0	0	0.64
25	0.6	-
50	1.4	0.68
100	2.0	-
150	2.4	0.60
200	2.4	-

to be completely prevented by the presence of more than 150 μmole/ml of phosphate. It was shown that the phosphate independent isozyme was also inactivated by heat treatment at over 70°C for one minute under the conditions described above.

c) Optimum pH. These isozymes have quite different optimal pH values. When reaction velocity is plotted against the pH a sharp curve with a maximum at pH 8.2-8.7 is obtained with the phephate dependent isozyme, whereas with the phosphate independent glutaminase there is a gentle slope with maximum at pH 7.2-7.6.

d) Michaelis Constants for Glutamine. These isozymes have very different Michaelis constants for glutamine. Thus, the constants of the phosphate dependent and independent isozymes were estimated to be 4×10^{-2}M and 4×10^{-3}M, respectively, by the method of Lineweaver and Burk. The Michaelis constant of the phosphate dependent isozyme was the same with 20 μmoles /ml and with 150 μmoles/ml of added phosphate.

e) Effects of Various Compounds on Activities of Glutaminase Isozymes. As shown in Table II, carbonate and maleate ions were found to activate the phosphate independent isozyme but the phosphate dependent isozyme was not affected by these compounds in the absence of added phosphate. Fumarate and succinate, which

are the trans-isomer and the saturated form of maleate, respectively had no effect. Phosphate, arsenate and sulfate which are effective co-factors of the phosphate dependent isozyme, and also the other eight compounds shown in Table II, had no effect upon the activity of the phosphate independent enzyme.

Table II

Influences of Various Compounds on Glutaminase Activity.

compounds added	phosphate independent (% activity)	phosphate dependent (% activity)
None	(100)*	0
K_2HPO_4	100	(100)**
$NaHCO_3$	150	0-10
Urea	100	
Na_2HAsO_4	100	20-30
Maleate	400	0-12
Fumarate	100	0-11
Succinate	100	0- 8
cis-Aconitate	80	
Citrate	100	0-12
Malic acid	100	
Diamox	100	

* activity without any addition.
** activity with added phosphate.

f) Mode of Activation of the Phosphate Independent Isozyme by Carbonate and Maleate. The phosphate independent isozyme is strongly activated by maleate and weakly by carbonate as shown in Fig. 1. As described in section d), increases in the maximum velocity were observed on addition of these activators. These activations, and also the original enzyme activity were unaffected by PCMB.

3) Difference in Inducibility of Isozymes.

Various investigators have reported that renal glutaminase activity varies with the dietary protein level and also that administration of dilute hydrochloric acid or ammonium chloride to rats increases the renal glutaminase activity(Davies, R.M. and Yudkin, J., 1952; Rector, F.C., et al., 1955; Goldstein, L.

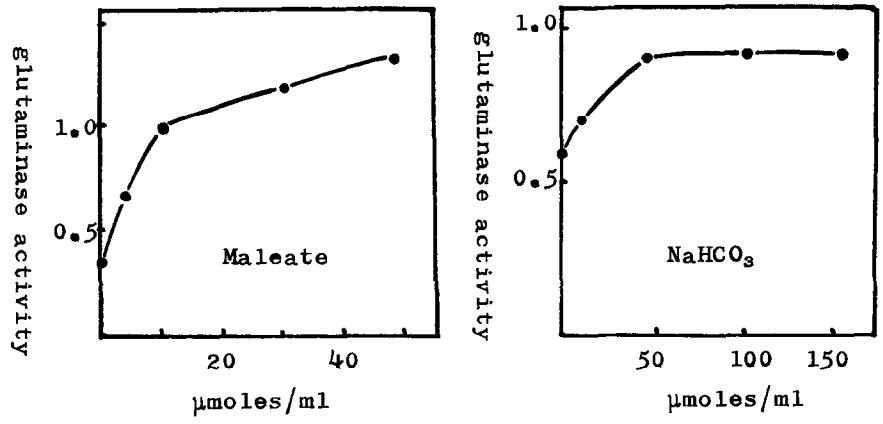

Fig. 1
Activation of Phosphate Independent Glutaminase by Maleate and Carbonate

---

et al., 1960). In the present experiments the relationship between the dietary protein level and the activity of the glutaminase isozymes was examined. As shown in Table III the phosphate dependent glutaminase was only induced when animals were fed on a high protein diet, but the carbonate activated glutaminase was almost the same in animals fed on a low and on a high protein diet. From the above observation, these glutaminases

Table III
Difference in Inducibility of Isozymes.

% Casein in diet	Specific activity		Total activity	
	Independent	Dependent	Independent	Dependent
5	0.8 ±0.1	2.5 ±0.3	80 ±10	300 ±20
50	0.7 ±0.2	9.5 ±0.2	90 ±10	2200 ±100

Male Donryu rats, weighing about 100 grams, were used. Rats were separated to two groups which were fed on 50 % and 5 % casein, respectively, for a week. The kidneys were homogenized in 0.02 M Tris-HCl buffer, pH 8.6, and sonicated at 10 KC for 5 minutes. After centrifugation the supernatant was used as the enzyme preparation.

seem to be regulated by different control systems. Since an increase in renal glutaminase is associated with an increased excretion of ammonia, the difference in the inducibility of these isozymes and the activation of the phosphate independent enzyme by maleate and carbonate ions may be significant in ammonium excretion under normal physiological conditions.

4) Physiological Roles of the Phosphate Independent Enzyme in Ammonium Excretion and its Diuretic Action.

Since an increase in renal glutaminase activity is associated with an increased excretion of ammonia, the effect of the administration of maleate, which is an activator of the phosphate independent glutaminase, on ammonium excretion was tested. Maleate, fumarate, citrate and diamox were intraperitoneally injected to rats, and the urine during 24 hours after the injection was collected and assayed for ammonia and urea. As shown in Table IV, it is clear that maleate shows a strong diuretic action and a remarkable increase in ammonium excretion. On the other hand, diamox which is an inhibitor of carbonic anhydrase, showed a strong diuretic action but its effect on ammonium excretion is not comparable to that of maleate. Fumarate and citrate had no effect on both diuretic action and ammonium excretion.

Table IV

Increase of Ammonium Excretion in Urine and Urine Volume by Injection of Maleate

Injected	Ammonia mmoles/day	Urine volume ml/day	Urea mmoles/day
Saline 1 ml.	0.4 ± 0.1	5 ± 1	4.0 ± 1.0
Maleate 50 mg/rat	1.4 ± 0.2	28 ± 4	5.9 ± 0.7
Diamox 50mg/rat	0.7 ± 0.1	21 ± 4	5.5 ± 0.9

Male Donryu rats, weighing about 100 grams, were used. Rats were fed on usual laboratory chow. After centrifugation the supernatant of the urine was used as the assay material of ammonia and urea. Urea was determined by diacetyl-monoxime method.

REFERENCES

Sayer, F.W. and Roberts, E., J. Biol. Chem., 233, 1128(1958)
Kvamme, E., Treit, B. and Svenneby, G., Biochem. Biophys. Res. Commun., 20, 566(1965)
Davies, R.M. and Yudkin, J., Biochem., J., 52, 407(1952)
Rector, F.C., Swldin, D.W., and Copenhaver, J. H., J. Clin. Invest., 34, 20(1955)
Goldstein, L. and Kensler, C.J., J. Biol. Chem., 235, 1086(1960)