ACTIVATION OF γ-GLUTAMYL TRANSPEPTIDASE BY MONOVALENT CATIONS

Marian ORLOWSKI, Paul Obiekwe OKONKWO and Jack Peter GREEN
Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. 10029, USA

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1. Introduction

γ-Glutamyl transpeptidase [1] catalyzes an amino acid dependent breakdown of glutathione as follows: Glutathione + amino acid ⇄ γ-glutamyl amino acid + + cysteinyl-glycine.

The enzyme is widely distributed in animal tissues and highly purified preparations have been obtained from beef and hog kidney [2, 3]. Histochemical studies have shown that high activity is associated with brush borders of the proximal convoluted tubules of the kidney, the choroid plexus and with the apical portion of the intestinal epithelium [4–6].

It was postulated that the interaction of membrane bound γ-glutamyl transpeptidase with amino acid and carrier (the γ-glutamyl residue of glutathione or other γ-glutamyl peptides) is one of the main steps in amino acid transport mediated by the γ-glutamyl cycle [7]. This interaction results in the formation of a γ-glutamyl amino acid, and is followed by its translocation into the cell. Inside the cell, the amino acid is released by the action of γ-glutamyl cyclotransferase with the concomitant formation of 5-oxoproline (pyrrolidone carboxylic acid).

Supporting evidence for the functioning of the γ-glutamyl cycle in amino acid transport was recently obtained in studies of a patient with a metabolic block who excretes large amounts of 5-oxoproline in urine [8].

The known sensitivity of some amino acid transport systems toward sodium ions [9] prompted the present study on the influence of monovalent cations on γ-glutamyl transpeptidase activity. The findings indicate that the enzyme is strongly activated by both sodium and potassium and that it is also sensitive to other monovalent cations.

2. Methods

L-γ-Glutamyl-*p*-nitroanilide was synthesized as previously described [3].

Tissue extracts were prepared by homogenizing at 0° freshly obtained bovine choroid plexus and bovine and guinea pig kidneys with nine volumes of Tris-HCl buffer (0.01 M; pH 9.0) using a Potter–Elvehjem glass homogenizer equipped with motor-driven teflon pestle. Similar extracts were prepared from freshly frozen hog and sheep kidney and from rabbit choroid plexus (obtained from Pel Freeze, Rogers, Arkansas). For determination of activity all extracts were diluted with nine volumes of buffer. Purified hog kidney γ-glutamyl transpeptidase was obtained as described previously [3]; the preparation was carried through step 4 of the isolation procedure. Enzyme activity was determined by adding 0.05 ml of a solution containing the enzyme to a reaction mixture (final volume 1.0 ml) containing L-γ-glutamyl-*p*-nitroanilide (0.005 M) and Tris-HCl buffer (0.08 M; pH 9.0) at 37°. The release of *p*-nitroaniline was determined as described previously [3]. Enzyme activity is expressed in units, one unit being the amount of enzyme that catalyzes the formation of 1 μmole of *p*-nitroaniline per min. Specific activity is in units per mg of protein, as determined by the method of Lowry et al. [10].

3. Results

Crude preparations of γ-glutamyl transpeptidase from kidneys and choroid plexuses are strongly activated by monovalent cations (table 1). Relatively high

Table 1
Activation by cations of γ-glutamyl transpeptidase from several animal tissues.

Tissue	Specific activity**	Percent increase in enzyme activity* Na^+	K^+	Cs^+	Li^+	Tetraethyl ammonium$^+$
Rabbit choroid plexus	0.22	40	40	38	27	22
Bovine choroid plexus	0.07	100	80	99	66	42
Sheep kidney	0.09	110	123	101	76	46
Bovine kidney	0.09	108	124	99	65	48
Guinea pig	0.10	100	105	96	62	47
Hog kidney	0.05	71	70	68	55	41
Hog kidney (purified)	18.0	67	67	63	48	12

* Cations (.150 M) were used as chloride salts.
** Activity was determined and defined as described under Methods.

concentrations are needed for maximal activation (fig. 1). Virtually the same stimulation of activity was obtained with equimolar concentrations of Na^+, K^+ and Cs^+, while Li^+ was significantly less effective. In experiments with guinea pig kidney homogenates Rb^+ activated to the same extent as Na^+ and K^+. At concentrations that caused maximal activation with Na^+, addition of K^+ did not cause a further increase of enzyme activity. However at suboptimal concentrations the effects of Na^+ and K^+ were additive.

A purified preparation of γ-glutamyl transpeptidase from hog kidney showed virtually the same reactivity towards the metal ions as a crude kidney homogenate. This observation indicates that the sensitivity to metal ions is a property intimately associated with the enzyme.

An examination of the activation by sodium showed that the effect is independent of substrate concentration. The same percent increase of enzyme activity was obtained at different substrate

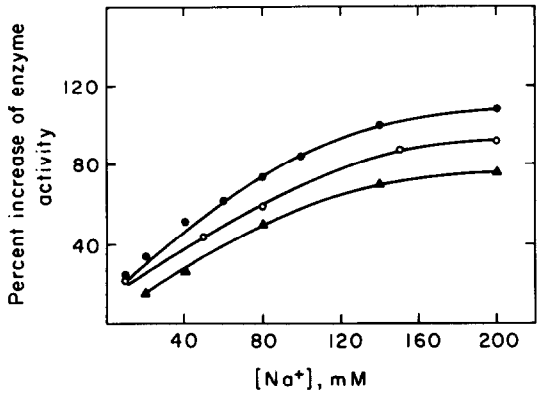

Fig. 1. Activation of γ-glutamyl transpeptidase by Na^+. Extracts from bovine kidney (•—•—•), bovine choroid plexus (○—○—○) and hog kidney (△—△—△) were assayed as described under Methods.

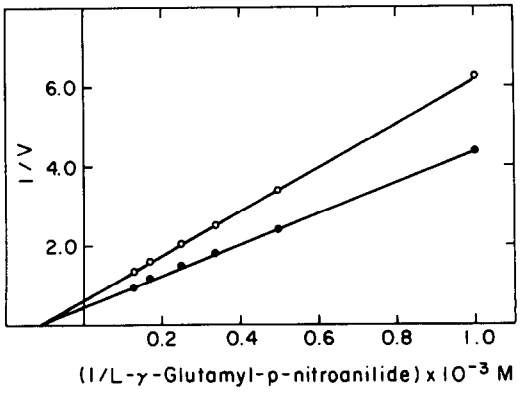

Fig. 2. Effect of Na^+ (0.15 M) on the activity of γ-glutamyl transpeptidase from rabbit choroid plexus at various substrate concentrations. Activity was determined as described under Methods. Double reciprocal plots of the data [13] obtained in the presence (•—•—•) and absence (○—○—○) of Na^+ are given.

concentrations and double reciprocal plots show that Na^+ does not affect the apparent K_m for the substrate but increases the V_{max} of the reaction (fig. 2). Similar results were obtained with K^+ as the activator. The apparent lack of specificity of the enzyme toward a particular inorganic cation prompted us to test the influence of quaternary ammonium salts on enzyme activity. Choline, tetramethylammonium and tetraethylammonium were tested as the chloride salts and all were found to activate the enzyme. The activation by tetraethylammonium chloride amounted to about 50% of the activation obtained by equimolar concentrations of Na^+ (table 1); somewhat higher activation was obtained with choline and tetramethylammonium chloride. Evidence was also obtained that the tris-hydroxy-methyl-aminomethane cation was activatory. Thus, enzyme activity increased when the concentration of Tris-HCl buffer was increased (both at pH 7.4 and pH 9.0). Furthermore the extent of activation at pH 7.4 by 0.15 M NaCl increased in a bovine kidney preparation from 40 to 250% when the concentration of Tris-HCl was decreased from 0.08 M to 0.005 M, indicating that both cations are activatory.

Qualitatively similar results were obtained with γ-glutamyl transpeptidase preparations that have been dialyzed either against sodium-EDTA (0.01 M; pH 7.2) or against deionized water, although lower concentrations of monovalent cations were required to achieve a given increase of activity compared with undialyzed preparations.

Activation of the enzyme was shown to be primarily dependent on the concentration of monovalent cations and not on the ionic strength or osmolarity of the solution. Thus 0.05 M Na_2SO_4 ($I = 0.15$) gave the same activation as a 0.1 M NaCl solution ($\gamma = 0.10$) and addition of sucrose (final conc. 0.3 M) did not affect enzyme activity.

4. Discussion

The present studies indicate that γ-glutamyl transpeptidase from different tissues is strongly activated by monovalent cations. Although the activation of the enzyme by Mg^{2+} has been previously observed, and indeed purified hog kidney γ-glutamyl transpeptidase has been reported to contain bound calcium and magnesium [3], the activating effect of monovalent cations remained unnoticed.

It is tempting to speculate that the activation of γ-glutamyl transpeptidase by monovalent cations is in some way related to the physiological function of the enzyme. The reaction catalyzed by the enzyme seems to be the only significant pathway by which glutathione is metabolized in the cell. Available evidence strongly supports the view that a major function of glutathione lies in its role as a donor of γ-glutamyl residues, which serve a carrier function in amino acid transport [7]. Several transport systems for amino acids have been reported to be coupled to sodium movement across cell membranes. Furthermore an inherited deficiency of glutathione in red blood cells of sheep has been found to be associated with low intracellular levels of Na^+ and K^+ and also with changes in intracellular concentration of amino acids [11].

At the present state of our knowledge, however, no conclusion can be made as to the significance of the interaction of γ-glutamyl transpeptidase with monovalent cations in the explanation of these observations. The enzyme is activated to the same extent by both Na^+ and K^+ and also by other monovalent cations, while Na^+ dependent amino acid transport systems were shown to be specific for this cation. The possibility however exists that the cation specificity of crude and purified preparations of the enzyme differs from that in intact cell membranes. More studies are clearly needed both on the cation requirements of various transport systems and on the relationship of the enzyme to these systems.

Examination of the literature indicates that several previous observations require re-evaluation in view of the present findings. Thus the reported activation of γ-glutamyl transpeptidase from kidney bean fruits by the sodium salts of citric acid, EDTA and several dicarboxylic acids [12] may have resulted at least in part from the presence of sodium ions rather than from the effect of organic acids. The observation that purified hog kidney γ-glutamyl transpeptidase is activated to the extent of 60% by EDTA (40 mM) [3], may now be explained by the use of the sodium salt of the chelating agent in these experiments, since it has now been demonstrated that at optimal Na^+ concentrations addition of EDTA did not further increase the activity of the enzyme. Further detailed studies

are needed on the mechanism of interaction of the enzyme with metal ions. In the present study no change in the apparent K_m of the enzyme for the substrate in the presence of Na^+ has been observed. In a study of the activating effect of sodium citrate on purified γ-glutamyl transpeptidase from kidney bean fruits, evidence has been presented that this effect is associated with a conformational change in the enzyme molecule [12]. It is likely that the present observations with several mammalian enzyme preparations are the result of a similar mechanism.

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