# EVIDENCE FOR TWO SPECIES OF MAMMALIAN PHOSPHATE-ACTIVATED GLUTAMINASE HAVING DIFFERENT REGULATORY PROPERTIES

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## 1. Introduction

Phosphate-activated glutaminase (PAG) (EC 3.5.1.2) has a metabolic key function in mammals as producer of ammonia and glutamate, the former being particularly important in kidney during acidosis, the latter having probable transmitter function in brain and is also the precursor of GABA. Phosphate is the main activator [1–3], but the enzyme is not phosphate-dependent since a great variety of anionic organic compounds may function as activators [2–5]. PAG has been reported to be localized to the inner part of the mitochondria in rat kidney [6–9]. Using *N*-ethyl maleimide (NEM) as a tool the present communication presents results of experiments suggesting that there exists two species of mitochondrial PAG, having distinct regulatory properties.

## 2. Methods

Mitochondria from pig kidney cortex were isolated by differential centrifugation, and rat brain synaptosomes were purified by the technique in [10]. The incubation was performed for 2 min at pH 7.4 and 25°C in 100 μl total vol. The incubation medium contained 2 mM [$^{14}$C]glutamine, 90 mM NaCl, Na-phosphate (usually 5 mM), 56 mM KCl, 100 mM D-mannitol, 30 mM sucrose, 4 mM Hepes and 10 mM $MgCl_2$. To prevent oxidation of glutamate 10 mg/l oligomycin and 0.6 mg/l antimycin were added. The incubation was terminated by adding 200 μl ice-cold ethanol. Following centrifugation (18 000 × *g*, 1 min), glutamine and glutamate were separated by paper chromatography in butanol/acetic acid/water, using the 'drip-and-dry' method [11]. The amino acids were colored with ninhydrin, the visible spots were cut out, decolorized with 2 drops of 5% $H_2O_2$ and counted by liquid scintillation counting. Phosphate-activated glutaminase from pig kidney was purified to apparent homogeneity as in [12].

## 3. Results and discussion

Pig kidney mitochondrial and rat brain synaptosomal glutaminase are inhibited ~40–60% by 0.5 mM NEM, and no further inhibition occurs on increasing the NEM concentration as shown in fig. 1. In contrast to NEM, 0.5 mM mersalyl inhibits glutaminase almost completely. Thus, we are either dealing with an enzyme which is partially inhibited by NEM or with two species of glutaminase, a NEM-sensitive and a NEM-insensitive one. However, NEM does not inhibit PAG purified to apparent homogeneity by methods developed in [11–13], as illustrated for kidney cortex PAG in fig. 1. This demonstrates that only the NEM-insensitive species of glutaminase survives the purification procedure, or that purified PAG is modified to prevent NEM binding to SH-groups essential for the activity. In this connection it should be mentioned that a small fraction of the total glutaminase present in the tissue is solubilized [11,12] and that the major fraction escapes purification. It is also possible that in intact structures NEM inhibits another glutaminase than PAG, such as microsomal maleate-activated glutaminase (MAG) [14,15] (which has been shown to be identical with γ-glutamyl transpeptidase (γ-GT) [16,17], and soluble ADP-sensitive glutaminase [18]. However, this possibility can be ruled out. Thus, total

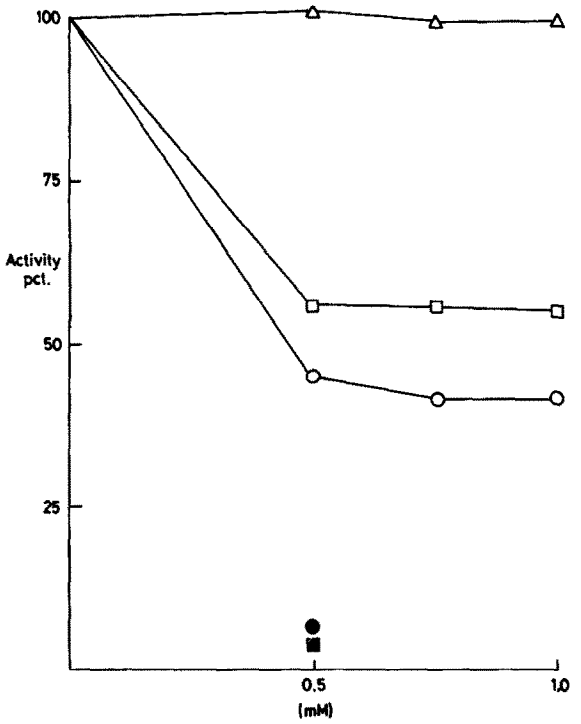


Fig. 1. The effect of *N*-ethyl maleimide and mersalyl on phosphate-activated glutaminase. Mitochondria from pig kidney cortex were isolated by differential centrifugation, and rat brain synaptosomes were purified by the technique in [10]. The mitochondria and synaptosomes were preincubated with the mercurial compound at 25°C for 5 min. Thereafter they were incubated at 25°C for 2 min with 2 mM [$^{14}$C]glutamine, and 5 mM phosphate buffer (pH 7.4) as in section 2. Rat brain synaptosomes (○,●), pig kidney cortex mitochondria (□,■) and purified enzyme from pig kidney cortex (△): open symbols, incubated with *N*-ethyl maleimide; filled symbols, incubated with mersalyl.

glutaminase activities both in brain microsomal preparations and in the high speed supernatant were found to be much too low to make any significant contribution to the synaptosomal glutaminase activity by contamination. Furthermore, by heat treatment of mitochondria and synaptosomes at 50°C for 10 min which inhibited PAG completely without affecting MAG and the ADP-sensitive glutaminase, these structures lost most of their glutaminase activity, as well as the property of being activated by phosphate and inhibited by NEM. We also found that both the NEM-sensitive and -insensitive glutaminases are activated by phosphate, the former enzyme being more sensitive to phosphate than the latter (shown for synaptosomal glutaminase in fig. 2). NEM-insensitive glutaminase is only activated by phosphate at >10 mM (not shown).

NEM-sensitive and -insensitive PAG appears to have distinct properties. Thus, total PAG is somewhat pH-sensitive, a phenomenon which has been largely overlooked, but fig. 2 demonstrates that only the NEM-sensitive PAG is affected by pH in the incubation medium. This suggests that at least the NEM-sensitive PAG is localized in a compartment to which $H^+$ have free access. $H^+$ (and NEM) are not freely permeable through the inner mitochondrial membrane and this structure, together with the matrix region, are reported to contain PAG [6–9]. If we assume that we are dealing with only one species of PAG which is partially inhibited by NEM, the enzyme is thus

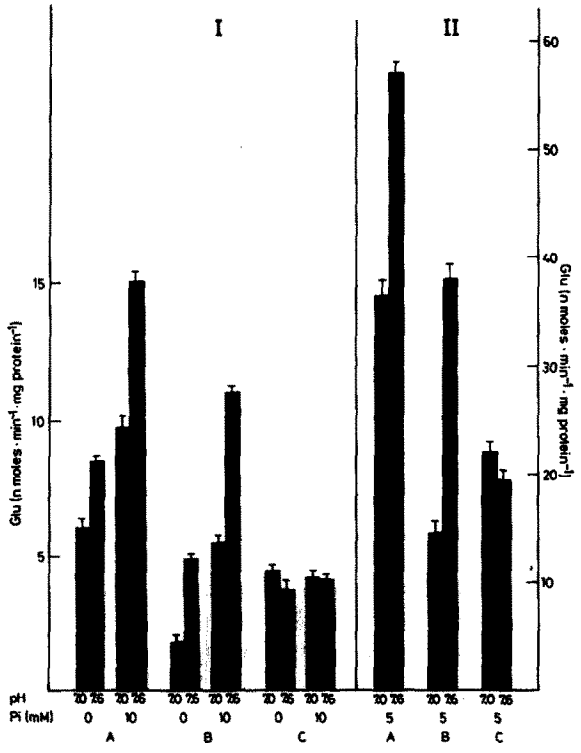


Fig. 2. The effect of pH and phosphate on phosphate-activated glutaminase. (I) Rat brain synaptosomes; (II) pig kidney cortex mitochondria. (A) Total PAG; (B) NEM-sensitive PAG; (C) NEM-insensitive PAG.

probably localized to the outer surface of the inner mitochondrial membrane where the pH-sensitivity is linked to NEM-sensitive SH-groups. However, this appears to be unlikely because the purified enzyme which is NEM-insensitive, is also strongly pH-dependent. Another possibility is that only the NEM-sensitive part of PAG is localized to the outer surface of the inner membrane. If this were the case, the NEM-sensitive and -insensitive PAG may either be localized to distinct compartments within the same mitochondrion or to different species of mitochondria. In support of the latter conclusion we found that non-synaptosomal mitochondria precipitating during the synaptosome purification on sucrose gradients, contain mostly NEM-insensitive PAG.

In further support of the two species theory we found that calcium has different effects on NEM-sensitive and -insensitive brain and kidney PAG (fig. 3). Thus, calcium (0.5–1.0 mM) activates ~2-fold NEM-insensitive PAG both from brain and kidney cortex and exerts a pronounced inhibition of NEM-sensitive PAG. Total kidney cortex PAG is ~40% activated by calcium, whereas total PAG from brain cortex is insignificantly affected. We have demonstrated (not shown) that calcium affects PAG by potentiating the activation by phosphate, but no effect of calcium has been found on purified enzyme preparations. The effect of calcium on synaptosomal PAG is also uninfluenced by high and low potassium concentrations in the external medium which is known to affect the state of polarization. It is thus of great interest that calcium has the ability to 'switch off and on' the NEM-sensitive and -insensitive PAG activity, respectively. Since the ionized portion of calcium increases during acidosis, this may be important to understand the regulation of kidney PAG in acidotic conditions. Moreover, calcium is essential for liberation of synaptosomal transmitter substances, and the arrival of the action potential at the nerve terminal leads to an influx of calcium and consequently to a transmitter release [19]. The effect of calcium on PAG may be secondary to the effect on transmitter release, triggering the filling up of depleted stores of transmitter glutamate and GABA. A prerequisite for the calcium regulation would probably be that one of the PAG species is constantly inhibited, or that the crucial synaptosomes and mitochondria involved contain only one of the PAG species. As

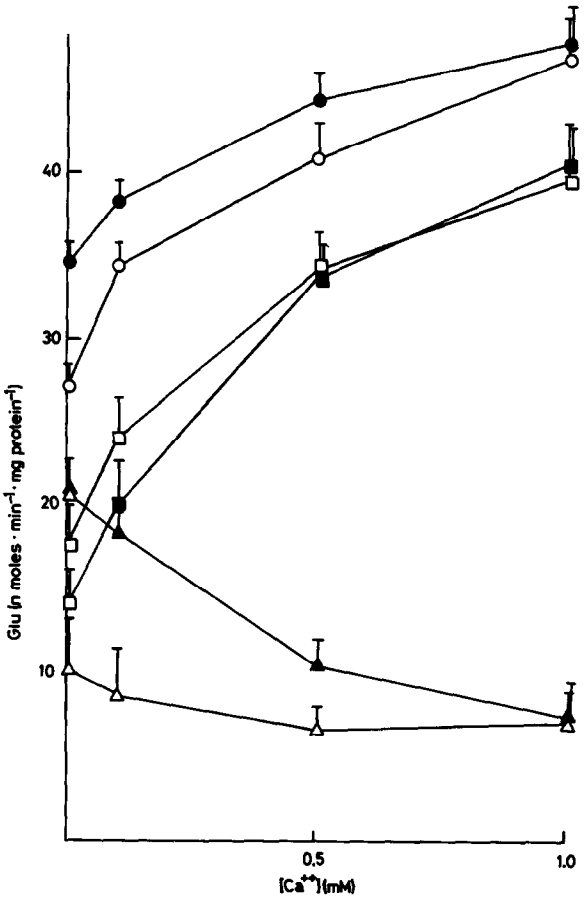


Fig. 3. The effect of calcium on phosphate-activated glutaminase. Total PAG (○,●), NEM-sensitive PAG (△,▲) and NEM-insensitive PAG (□,■): open symbols: pig kidney cortex mitochondria; filled symbols: rat brain synaptosomes.

described above, mitochondria containing mostly NEM-insensitive PAG have actually been isolated.

## Acknowledgements

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