ALLOSTERIC GLUTAMINASE IN RAT LIVER OF LIKELY REGULATORY SIGNIFICANCE

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1. Introduction

In the course of a survey of enzymes of amino acid catabolism in rat liver studied in near physiological conditions, we have found an allosteric property of the glutaminase (EC 3.5.1.2) Pi-independent isoenzyme (Katunuma et al. [1]), with characteristics of likely significance for metabolic regulation. This enzyme, that seems to be the major component of the total potential glutaminase activity of rat liver, presents a very marked sigmoidicity in its substrate concentration kinetics within the range of physiological values of glutamine in liver. The publication by Katsunuma et al. [2] of an observation qualitatively similar in a purified preparation, increases the interest of the prompt publication of our findings with the fresh enzyme *.

2. Materials and methods

Glutaminase activity was assayed in the particulate fraction of rat liver (or kidney) within one hour after killing the animal (the enzyme activity decreases markedly with ageing as will be described below).

Livers of white rats weighing 100–200 g were homogenized in 3 volumes of 0.3 M sucrose and centrifuged at 30000 × *g* for 20 min. The sediment was dispersed in water so as to give a final concentration of about 100 mg of protein per ml. The glutaminase assay mixture contained, as a rule, 50 μmoles of imidazole (HCl) buffer, pH 7.0, 5 to 30 μmoles of glutamine, and 0.2 ml of the enzyme preparation, in a final volume of 1 ml. The reaction was allowed to proceed at room temperature, usually for 15 min, and stopped by the addition of perchloric acid. After neutralization with KOH and centrifugation, the ammonium released was measured spectrophotometrically in aliquots of the supernatant with glutamate dehydrogenase (ammonium free glycerol solution from Boehringer) and excess of α-ketoglutarate. Glutamine was purchased from Sigma.

3. Results

Fig. 1 summarizes the main results obtained. The rat liver glutaminase shows markedly sigmoid kinetics, with respect to the substrate concentration with a half maximal activity, $S_{0.5}$ [4], of ca. 13 mM (12 to 15 mM values were obtained in different experiments). Similar results have been obtained at different pH values (in the range from 7.0 to 9.5), with different buffers (Tris-HCl and veronal), in the presence of 0.1 M KCl, 5 mM $MgCl_2$, or 0.5 mM $MnCl_2$, as well as in the standard assay conditions of Katunuma et al. [1].

The activity found at saturating concentrations of glutamine is 1.1 ± 0.4 μmoles/min/g (at ca. 22°). The activity disappears by mild heating (5 min at 40°) and markedly decreases by standing at 0° (75% loss in 8 to 10 hr).

In contrast with the liver enzyme, the rat kidney Pi-independent glutaminase, in the same conditions, shows a hyperbolic Michaelis-Menten type curve with a K_m of about 2.5 mM.

Fig. 1 also shows a Hill's plot [4] of the liver glu-

* The results reported here were presented at the 5th FEBS Meeting held in Prague in July 1968 [3].

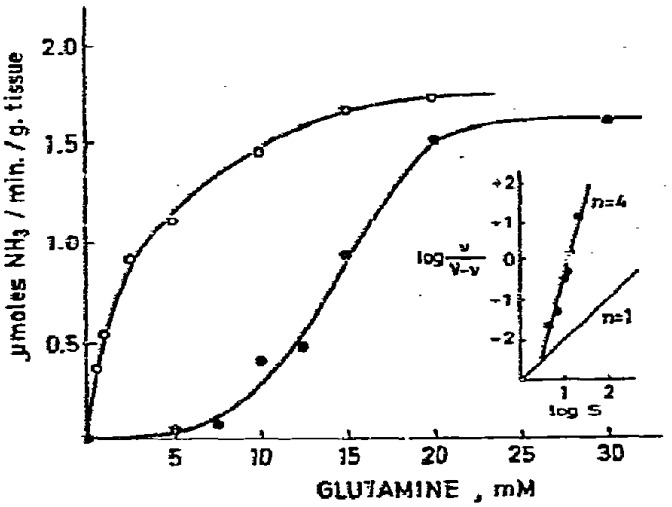

Fig. 1. Effect of the substrate concentration on the activities of the rat liver (●—●) and kidney (○—○) Pi-independent glutaminases. Activities were measured in fresh preparations as described in the text. A Hill's plot of the results with the liver enzyme is included as an insert.

taminase. From the experimental points a Hill coefficient n=4 is obtained, which suggests the existence of 4 or more interacting active sites.

Several attempts to confirm in our conditions the observations by Katunuma et al. [1] of activation of rat liver glutaminase by N-acetylglutamate and maleate, did not succeed both at high and at low substrate concentrations.

4. Discussion

The results presented above show a different kinetic behaviour of the Pi-independent glutaminases of rat liver and kidney. While the kidney isoenzyme presents typical Michaelis substrate kinetics, the liver enzyme exhibits marked sigmoidal kinetics. Qualitatively similar results have been independently observed by Katsunuma et al. [2] with a rat liver purified preparation. Nevertheless, our observations show some important features suggesting that this allosteric behaviour of the liver enzyme may well be of physiological significance in metabolic regulation. In contrast to the purified preparation with the rather high $S_{0.5}$ of about 50 mM [2], the liver glutaminase assayed in fresh preparations, shows an $S_{0.5}$ of about 13 mM, which corresponds to the range of physiological concentrations of glutamine in liver [5]. Moreover, the sigmoidicity of the enzyme kinetics is steeper in the fresh preparation: a Hill coefficient of 4 versus a value of 2.7 calculated from the figure in Katsunuma et al. [2]. This fact increases the potential regulatory significance of the allosteric character of the liver glutaminase. Another difference is that we have not observed any effect of maleate or N-acetylglutamate.

Total or partial desensitization of an allosteric protein is by no means a rare event in purification procedures [6]. This may well be the reason of the discrepancies between the results of Katsunuma et al. [2] and our own observations.

Kvamme et al. [7] had reported an allosteric effect of Pi and certain other ions on a purified pig kidney glutaminase, although the very high range of phosphate concentrations required tends to make uncertain any physiological significance of this allosteric property.

The activity of liver glutaminase at the lower range of physiological substrate concentrations could well keep the intrahepatic glutamine pool at steady concentrations sufficient for biosynthetic purposes [5]. The strong substrate activation of the enzyme might guarantee an effective disposal of any excess of glutamine that could flow in from other tissues in situations of enhanced amino acid catabolism [8]. The extent of the physiological significance in metabolic regulation of the allosteric behaviour of glutaminase here described, and its eventual interplay with the antagonistic glutamine synthetase [9, 10] deserve further studies.

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