REGULATION OF RAT LIVER GUANINE AMINO HYDROLASE BY GTP

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Guanine aminohydrolase (E.C. 3.5.4.3), an important enzyme in the catabolism of nucleic acids, is being shown for the first time to be regulated by GTP in rat liver. Guanine is not synthesized *in vivo* as such, but is formed from GMP which, in turn, is either formed from IMP or arises from nucleic acid break down. The enzymatic deamination of guanine could, therefore, be one of the means of controlling the concentration of guanine nucleotides *in vivo*. Such a control would be analogous to the ATP activation of AMP aminohydrolase of brain and other tissues of the rat (Setlow *et al.*, 1966), of calf brain (Setlow and Lowenstein, 1967) and the dCTP stimulation of dCMP aminohydrolase of donkey spleen (Scarano *et al.*, 1967).

MATERIALS AND METHODS

The assay system for enzyme activity determination, based on the method of Roush and Norris (1950), contained 50 μ moles Tris-HCl buffer, pH 8.0 and guanine, in a total volume of 1.0 ml.; the reaction was started by the addition of enzyme. GTP, when present, was added as the sodium salt. After incubating

for 10 minutes at 30°, the system was inactivated by the addition of 0.5 ml. 10 % perchloric acid and the volume diluted to 3.0 ml. with water. Following clarification, the extinction of the samples was read at 245 mμ in a Beckman spectrophotometer.

The 15,000 g supernatant of rat liver sucrose homogenate was fractionated with ammonium sulfate, as reported by Kumar et al. (1967) for the brain enzyme. The 40 to 70 % residue, containing the enzymic activity, was dialysed against water for 8 hours and the inert protein removed by centrifugation. The supernatant was used as such in the kinetic studies.

RESULTS AND DISCUSSION

With guanine concentration varied from 1.0 to 8.0×10^{-5} M and in the absence of effector, a plot of the reaction velocity against substrate concentration yielded a sigmoid curve and the double reciprocal plot a non-linear curve, the latter of which is shown in Figure 1. The extent of cooperativity as given by the slope of the Hill plot (log v/V_{max}-v versus log S) was 2.4 (Figure 2). The apparent Km value was 2.4×10^{-5} M.

When GTP was present in the assay system in 1.9×10^{-4} M concentration, the v and S plot and the double reciprocal plot gave classical Michaelis kinetics (Figure 1, where the latter plot is shown); the slope of the Hill plot was 1.1 (Figure 2) and Km 1.4×10^{-5} M. The V_{max} was increased 70 %.

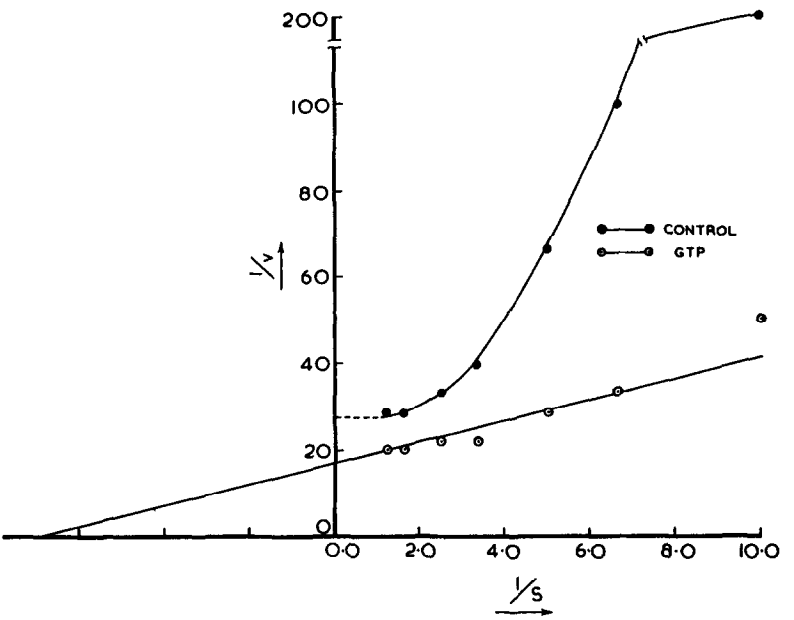

Figure 1: Effect of GTP on guanine aminohydrolase activity. The assay conditions are as described in text. The initial reaction velocity, v, given directly as Δ O.D. 245 mμ and substrate concentration, S, given in 10^{-4} M, are plotted as their reciprocals.

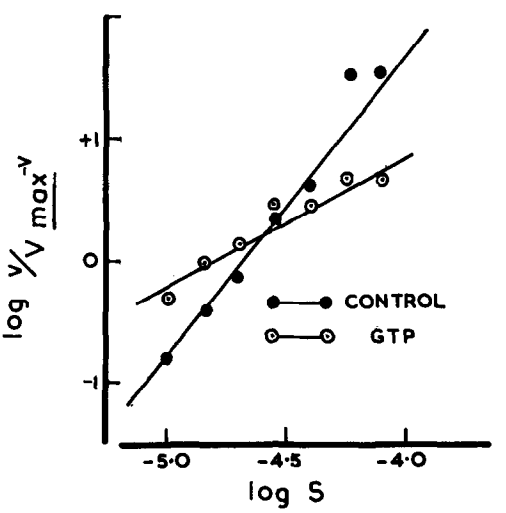

Figure 2: Data from Figure 1 plotted for Hill equation, that is, log v/V_{max}-v versus log S. V_{max} was calculated by extrapolating the plots given in Figure 1 to infinite substrate concentration.

The incubation of GTP alone with the enzyme preparation did not result in any altering of the extinction at 245 mμ. GTP activated guanine amino hydrolase by reducing the concentration of substrate needed for half saturation; it abolished practically completely the cooperativity in the binding of substrate in the absence of effector; it converted the apparent multimolecular reaction of guanine deamination into a practically unimolecular reaction.

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