Modulation of Guanine Deaminase

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1. Guanine deaminases purified from the 15000g supernatant fraction of iso-osmotic sucrose homogenates of rat and mouse liver and brain were tested for the influence of GTP and allantoin. 2. The suffixes A and B were assigned to the isoenzyme fractions eluted from DEAE-cellulose with the lower and the higher molarity of eluent respectively. Isoenzyme A from rat liver, the activity of which showed a sigmoid dependence on substrate saturation, was activated by GTP and inhibited by allantoin. Isoenzyme B, which had a hyperbolic substrate-saturation curve, was not influenced by GTP or allantoin. 3. Isoenzyme A from rat brain, the activity of which had a sigmoid dependence on substrate concentration, was stimulated by GTP. Isoenzyme B, which showed classical Michaelis–Menten kinetics, was inhibited by allantoin. 4. Mouse liver guanine deaminase was not influenced by either GTP or allantoin. 5. Isoenzyme A from mouse brain, which had a hyperbolic substrate-saturation curve, was not influenced by GTP or allantoin but isoenzyme B, with sigmoidal kinetics, was inhibited by allantoin. 6. Mg^{2+} activated, or inhibited or did not have an effect on guanine deaminase, depending on the source of the enzyme. 7. The bearing of the above findings on the possible regulation of guanine deaminase activity *in vivo* is discussed.

Earlier publications from this department dealt with guanine deaminase in brain and liver of rats and mice (Kumar *et al.*, 1967; Sree Kumar *et al.*, 1972). Except for mouse liver, the enzyme in the 15000g supernatant fraction was separable into two components, one of which was regulated in activity by substrate concentration. In rats and mice, guanine may be catabolized and excreted as allantoin, or it may be utilized in the elaboration of nucleotides by a 'salvage' pathway (Kornberg, 1957), either from guanine itself or from xanthine formed by guanine deaminase action. In the present experiments, we have tested the effect of GTP and allantoin on the activity of guanine deaminase from rat and mouse liver and brain. Since Mg^{2+} is one of the common non-metabolite effectors of allosteric enzymes, its influence on the enzyme was also tested. Some of the preliminary findings have been reported (Josan & Krishnan, 1968; Sree Kumar & Krishnan, 1970; Sitaramayya & Krishnan, 1970).

Materials and Methods

Enzymes

Isolation of enzymes. This was done as reported by Sree Kumar *et al.* (1972). The enzyme-activity peak appearing in the earlier eluate fractions from DEAE-cellulose columns was termed A and the one in the later fractions termed B.

Enzyme assay. This was as reported by Sree Kumar *et al.* (1972), a 2.0ml incubation mixture

consisting of 100 μ mol of Tris–HCl buffer, pH 8.0, water and enzyme preparation with 0.020–0.026 unit of guanine deaminase activity being used. The reaction was started with 0.06 μ mol of guanine. After incubation for 15 min at 30°C, the mixture was acidified with 1 ml of 10% (w/v) $HClO_4$ and E_{245} was measured. GTP, allantoin or Mg^{2+} was added to the assay system before the addition of substrate.

One unit of enzyme converts 1 μ mol of guanine to xanthine under the conditions of assay.

Enzyme protein. The protein concentration of the enzyme preparations employed in these experiments was determined spectrophotometrically (Kalckar, 1947).

Special reagents

The nucleotides used in the studies were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.

$S_{0.5}$ and K_m values

The K_m values for guanine were calculated from Lineweaver–Burk plots when the substrate saturation curve was hyperbolic. The K_m value of an isoenzyme showing a sigmoid substrate-saturation curve was calculated as $S_{0.5}$ from Hill plots (Atkinson, 1966); the same procedure was adopted even when the sigmoid substrate-saturation curve was transformed into a rectangular hyperbola in the presence of an effector. This plot also gave the Hill number, n .

The $V_{\max}$ values were always calculated from Lineweaver-Burk plots.

Results

Influence of effectors on guanine deaminase

The kinetic data reported are typical results, since the pattern of response was reproducible with different batches of the enzymes.

Guanosine triphosphate. (a) Rat liver. At a saturating concentration of guanine ($18 \mu\text{M}$), there was 40 and 60% activation of isoenzyme A by 10 and $20 \mu\text{M}$ -GTP respectively. The sigmoid shape of the substrate-saturation curve was abolished in the presence of GTP (Fig. 1a). The $S_{0.5}$ value for guanine was decreased from 5.3 to $3.9 \mu\text{M}$ and the n value from 2.3 to 1.3 in the presence of $20 \mu\text{M}$ -GTP (Fig. 1b). The $V_{\max}$ increased from 3.3 to $5.3 \mu\text{mol/min}$ per mg of protein (Fig. 1c).

The activation of isoenzyme A by GTP was independent of temperature over the range 10 – 70°C . Isoenzyme B of rat liver was unaffected by GTP.

(b) Rat brain. At a saturating concentration of substrate ($30 \mu\text{M}$), there was 20 and 40% activation of isoenzyme A by 45 and $60 \mu\text{M}$ -GTP respectively, and the sigmoid substrate-saturation curve became transformed into a rectangular hyperbola (Fig. 2a). In the presence of $60 \mu\text{M}$ -GTP the $S_{0.5}$ value was decreased from 6.6 to $6.3 \mu\text{M}$ and the n value decreased from 2.5 to 1.5 (Fig. 2b). The $V_{\max}$ was increased from 0.69 to $0.81 \mu\text{mol/min}$ per mg of protein (Fig. 2c). Isoenzyme B of rat brain was unaffected.

(c) Mouse liver and brain. The activities of mouse liver enzyme ($0.34 \mu\text{mol/min}$ per mg of protein) and mouse brain isoenzymes A and B (0.114 and $0.062 \mu\text{mol/min}$ per mg of protein respectively) were not affected by GTP (14 – $60 \mu\text{M}$).

Other nucleotides. ITP, ATP and UTP did not substitute for GTP in the activation of the enzyme of rat liver and brain.

Magnesium ions. (a) Rat liver. At a saturating concentration of guanine, 1 mM-MgCl_2 activated isoenzyme A by 45%, and the reaction rate–substrate concentration curve became less sigmoid (Fig. 1a). The $S_{0.5}$ value was decreased from 5.3 to $5.0 \mu\text{M}$ and the n value from 2.3 to 1.8 (Fig. 1b). The $V_{\max}$.

Fig. 1. Influence of $20 \mu\text{M}$ -GTP (●), 1 mM-Mg^{2+} (▲) and $200 \mu\text{M}$ -allantoin (□) on the substrate-saturation curve of rat liver supernatant guanine deaminase isoenzyme A

(a) Reaction rate–substrate concentration relationship; (b) Hill plot; (c) double-reciprocal plot. The incubation was at 30°C for 15 min, before acidification and measurement of E_{245} . ○, Control (substrate only added).

increased from 3.3 to 4.8 $\mu\text{mol}/\text{min}$ per mg of protein (Fig. 1c). Isoenzyme B was activated 22–33% by 1 mM-MgCl₂.

(b) Rat brain. Neither brain isoenzyme A nor isoenzyme B was affected by 1 mM-MgCl₂.

(c) Mouse liver. Mouse liver enzyme was unaffected by 1 mM-MgCl₂.

(d) Mouse brain. With a saturating concentration of guanine (18 μM), 100 μM -MgCl₂ inhibited isoenzyme B by 27%, and the sigmoid character of the substrate-saturation curve was abolished (Fig. 3a).

The $S_{0.5}$ value increased from 5.0 to 8.32 μM and the n value fell from 2.8 to 1.1 (Fig. 3b). The $V_{\max}$ remained unaltered at 0.062 $\mu\text{mol}/\text{min}$ per mg of protein (Fig. 3c).

Allantoin. (a) Rat liver. At a saturating concentration of guanine, 100 and 200 μM -allantoin inhibited isoenzyme A by 27 and 50% respectively. The sigmoid shape of the substrate-saturation curve was accentuated (Fig. 1a). The $S_{0.5}$ value was slightly increased from 5.3 to 5.6 μM but the n value remained unaltered at 2.3 (Fig. 1b). The $V_{\max}$ was lowered from 3.3 to 1.65 $\mu\text{mol}/\text{min}$ per mg of protein (Fig. 1c). Isoenzyme B of liver was unaffected by allantoin.

(b) Rat brain. Allantoin at 12 μM caused 40% inhibition of isoenzyme B of rat brain, at a saturating concentration of guanine. The presence of allantoin did not alter the hyperbolic nature of the substrate-saturation curve (Fig. 4a). The K_m value of the enzyme

Fig. 2. Influence of 60 μM -GTP on the substrate-saturation curve of rat brain supernatant guanine deaminase isoenzyme A

(a) Reaction rate-substrate concentration relationship; (b) Hill plot; (c) double-reciprocal plot. The incubation was as given in Fig. 1. $\circ$, Control (substrate only added); $\bullet$, 60 μM -GTP present.

remained unaltered at $25\mu\text{M}$ (Fig. 4b), suggesting that the inhibition was of non-competitive type (Fig. 4b). Isoenzyme A of rat brain supernatant was unaffected by allantoin.

(c) Mouse liver. Mouse liver enzyme was unaffected by allantoin ($30\mu\text{M}$ – 1.2mM).

(d) Mouse brain. At a saturating concentration of guanine, allantoin at $150\mu\text{M}$ inhibited isoenzyme B by 25–30%, and the substrate-saturation curve became less sigmoid (Fig. 3a). The $S_{0.5}$ value was increased from 5.0 to $7.9\mu\text{M}$ and the n value increased from 2.8 to 3.9 (Fig. 3b). The V_{max} remained unaltered at $0.062\mu\text{mol/min}$ per mg of protein (Fig. 3c).

Discussion

The possible modulation of guanine deaminase activity *in vivo* will depend on the concentration of the effector substances at the site of enzyme activity. The content of GTP has been reported to be $1.5\mu\text{mol/mg}$ of protein in rat liver (Mandel, 1964), that of allantoin in rat blood as $130\mu\text{M}$ (Byers *et al.*, 1947) and Mg^{2+} as 15.6mM in rat brain (McIlwain & Rodnight, 1962) and as $190\mu\text{g/g}$ fresh wt. of rat liver (Elson, 1961). Such reports do not permit the evaluation of the effective concentrations in the cytoplasm of whole brain or liver; however, the concentrations of GTP, allantoin and Mg^{2+} that affected the enzyme *in vitro* may well be met *in vivo*.

Fig. 3. Influence of $100\mu\text{M-Mg}^{2+}$ (Δ) and $150\mu\text{M}$ -allantoin ($\square$) on the substrate-saturation curve of mouse brain supernatant guanine deaminase isoenzyme B

(a) Reaction rate–substrate concentration relationship; (b) Hill plot; (c) double-reciprocal plot. The incubation was as given in Fig. 1. $\circ$, Control (substrate only added).

Fig. 4. Influence of 12 μM -allantoin on the substrate-saturation curve of rat brain supernatant guanine deaminase isoenzyme B

(a) Reaction rate-substrate concentration relationship; (b) double-reciprocal plot. The incubation was as given in Fig. 1. $\square$, Control (substrate only added); $\blacksquare$, 12 μM -allantoin present.

Guanine deaminase isoenzyme B of rat liver, isoenzyme A of mouse brain and the enzyme from mouse liver may perhaps have a catabolic role, as their activities are not under regulation by GTP, allantoin or Mg^{2+} (or guanine itself; Sree Kumar *et al.*, 1972). In contrast, isoenzyme A of rat liver and isoenzyme B of rat and of mouse brain may be biosynthetic, making xanthine in a regulated manner. The inhibition of isoenzyme A of rat liver and of isoenzyme B of mouse and rat brain by allantoin constitutes feedback inhibition (cf. Stadtman, 1966), perhaps especially significantly so in the first two cases where the enzyme itself is an allosteric protein regulated by guanine.

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