ALLOSTERISM IN RAT BRAIN SUPERNATANT GUANINE DEAMINASE

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Abstract — As in liver, one of the soluble guanine deaminase isozymes of rat brain is an allosteric protein, activated by GTP. Allantoin is an inhibitor, but only the non-allosteric component of the brain enzyme is inhibited, unlike in liver.

Introduction

Kumar, Josan, Sanger, Tewari and Krishnan (1967) reported separation of rat brain supernatant guanine deaminase into two chromatographically distinct components, which differed in their rate of utilization of 8-azaguanine, degree of inhibition by flouride and in the K_m value. Later work from this laboratory (Sree Kumar and Krishnan, 1970 a, b) revealed that guanine deaminase in 15,000 x g supernatant of rat liver also existed in isozymic forms. The most significant difference between the two components was the allosteric nature of one, exhibiting sigmoidal response to guanine concentration and activated by GTP and Mg^{2+} and inhibited by allantoin.

A reexamination of brain supernatant guanine deaminase has now revealed allosterism also in the enzyme from this tissue and activation by GTP. Allantoin, the end product, inhibited, but differently from the liver enzyme.

Materials and Methods

Purification

Purification of guanine deaminase from 15,000 x g supernatant of whole brain was according to Kumar, Josan, Sanger, Tewari and Krishnan (1967), with the modification that alumina gel treatment was left out and that 0.1 M phosphate buffer of pH 6.5 was not employed in eluting the loaded DEAE-cellulose column prior to eluting enzyme A with 0.1 M phosphate buffer, pH 7.5 and enzyme B with 0.2 M phosphate buffer, pH 7.5. Enzyme A was purified 50-fold and enzyme B 170-fold.

Assay

Enzyme assay was spectrophotometrically as reported by Kumar, Josan, Sanger, Tewari and Krishnan (1967), with modifications. The incubation was in 2.0 ml. volume; at the end of 15 minutes the mixture was acidified with 1.0 ml. 10 % perchloric acid and the absorption read at 245 mμ.

Results

Reaction rate-substrate concentration relationship

Guanine was used in the range of 1.5×10^{-6} to 3×10^{-5} M. Whereas enzyme B responded in a typical hyperbolic manner, enzyme A showed sigmoidal response to increasing substrate concentration (Fig. I). The apparent K_m value calculated from the reciprocal plot was in the range of 1.5 to 2.0×10^{-5} M for enzyme A; the K_m was in the range of 1.7 to 3.3×10^{-5} M for enzyme B.

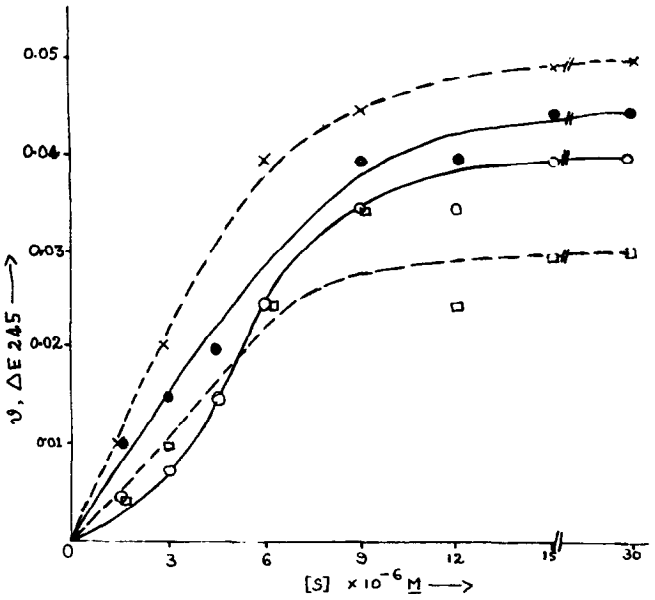

Fig. I. Substrate-concentration, reaction-rate relationship of guanine deaminase isozymes of rat brain supernatant. Guanine concentration was varied from 1.5 to 30 x 10^{-6} M. GTP was supplemented in 6 x 10^{-5} M and allantoin in 1.2 x 10^{-5} M concentration.

- Enzyme A without any additives
- Enzyme B without any additives
- Enzyme A with GTP
- Enzyme B with allantoin

Effect of GTP

GTP activated enzyme A in systems with saturating concentration of guanine. In the presence of 4 x 10^{-5} or 6 x 10^{-5} M GTP, the response of the enzyme to increasing substrate concentration shifted from sigmoidal to hyperbolic (Fig. I). The V_{max} was not altered markedly (-increased from 1.6 to 1.7 x 10^{-3} μ moles/minute of guanine-), but the K_m was decreased to a half or less (range 6.3 to 7.5 x 10^{-6} M). Enzyme B was unaffected at either concentration of GTP, nor was the kinetic response to increasing substrate

concentration influenced by the presence of GTP.

Effect of Mg^{2+}

Unlike in the case of the rat liver enzyme (Sree Kumar and Krishnan, 1970 b), either component of the brain enzyme was unaffected by Mg^{2+}.

Effect of allantoin

At a concentration of 1.2×10^{-5} M, allantoin inhibited enzyme B. The hyperbolic response of the enzyme to increasing substrate concentration was unaffected in the presence or allantoin (Fig. I). The V_{max} calculated from the double reciprocal plot was found to be almost halved (-1.9 instead of 3.6×10^{-3} µ moles/minute of guanine-); K_m was unaltered. Enzyme A was unaffected by allantoin under similar conditions.

Discussion

The present investigations have revealed similarities and differences between the supernatant guanine deaminases of rat brain and liver. In both tissues, one of the enzyme components was allosteric and activated by GTP. Whereas in rat liver the allosteric enzyme was inhibited by allantoin, in brain the non-allosteric component was inhibited. The activity of the two components of brain cytoplasmic guanine deaminase may be separately regulated *in vivo* by GTP and by allantoin.

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