INHIBITION OF BACTERIAL GLUTAMATE DECARBOXYLASE BY TRICARBOXYLIC ACID CYCLE INTERMEDIATES

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1. Introduction

Glutamate decarboxylase catalyzes the formation of 4-aminobutyric acid from L-glutamate and this enzyme plays a central role in maintaining the levels of 4-aminobutyric acid in the brains of animals [1–3]. Degradation of glutamate in microorganisms can take place by a pathway involving reversible deamination, catalyzed by glutamate dehydrogenase or by decarboxylation, catalyzed by glutamate decarboxylase. The dehydrogenase has been shown [4] to proceed only in the direction of glutamate synthesis when various metabolites including citrate, isocitrate, fumarate, fructose-1,6-diphosphate and ATP are present. Thus, the decarboxylase may be important in maintaining the concentration of glutamate in the organism.

Glutamate is synthesized via the tricarboxylic acid cycle and this raises the possibility that intermediates in the cycle modulate the activity of glutamate decarboxylase [5]. We report here that bacterial glutamate decarboxylase is competitively inhibited by intermediates in the Krebs cycle with citrate, α-keto-glutarate and *cis*-aconitate being especially effective. The results obtained with the last material stand in contrast to those given in [6]; these authors concluded that the beef brain enzyme is inhibited by *cis*-aconitate while the bacterial enzyme is not.

2. Materials and methods

All tricarboxylic acid intermediates were purchased from Sigma Chemical Co. except citric acid which was purchased from Allied Chemical Co. These compounds were of the best grade available and were used without purification. L-(+)-glutamic acid was obtained from Matheson, Coleman and Bell. Materials for the preparation of buffers were obtained from Mallinkrodt.

L-Glutamate decarboxylase (*Escherichia coli*) was obtained as the acetone powder from Sigma. The enzyme was purified from the powder by a procedure based on that in [7], modified as follows. After the protamine sulfate step, the enzyme was precipitated with $(NH_4)_2SO_4$ in the fraction at 50–70% of saturation. The protein was then subjected to a heat treatment (45°C for 1 h). Finally, chromatography on a DEAE–Sephadex A-50-120 column was carried out, elution being with a linear gradient (0.02–0.3 M) of phosphate buffer (pH 5.7). These procedures typically enhanced the specific decarboxylase activity 17-fold.

The kinetics of decarboxylation were determined using Warburg manometers. All assays were carried out at 37°C in 0.1 M pyridine–pyridine hydrochloride buffers. Usually the main chamber of the Warburg flask contained 2.8 ml buffer and 1 ml of a solution of L-glutamate in buffer. The side chamber contained 0.2 ml enzyme solution (1 mg protein/ml). After thermal equilibration the contents of the flask were mixed and the volume of gas evolved recorded at 30 s intervals for 5 min. The slope of a plot of CO_2 evolved against time was linear and is proportional to the initial rate of the reaction. For inhibition studies, the inhibitor was included in the main chamber of the manometer.

3. Results

A survey of the ability of tricarboxylic acid cycle intermediates to inhibit glutamate decarboxylase was carried out using fixed concentrations of substrate, inhibitor and enzyme (table 1). This revealed that citrate, *cis*-aconitate and α-ketoglutarate – those intermediates which occur at the beginning of the tricarboxylic acid cycle prior to the shunt leading to glutamate and 4-aminobutyrate [8] – are the most potent inhibitors of glutamate decarboxylase. When consideration is made of the fact that the isocitrate used in the assays was a racemic mixture, this compound would be considered strongly inhibitory as well.

The inhibition of glutamate decarboxylase by citrate, *cis*-aconitate and α-ketoglutarate is apparently competitive with substrate binding (fig.1). Treatment of initial reaction velocity data obtained as a function of substrate concentration in terms of Michaelis-Menton kinetics [9] gave dissociation constants for the binding of citrate, *cis*-aconitate and α-ketoglutarate of 1.3 mM, 0.52 mM and 0.43 mM, respectively, with the reaction conditions as described in table 1. The inhibition constant of citrate has been determined [10] to be 1.3 mM for the decarboxylase from *E. coli* while α-ketoglutarate has been demonstrated [11] to be a competitive inhibitor of the bacterial enzyme with a binding constant of 0.63 mM. Our results with these acids are, thus, in good agreement with these determinations.

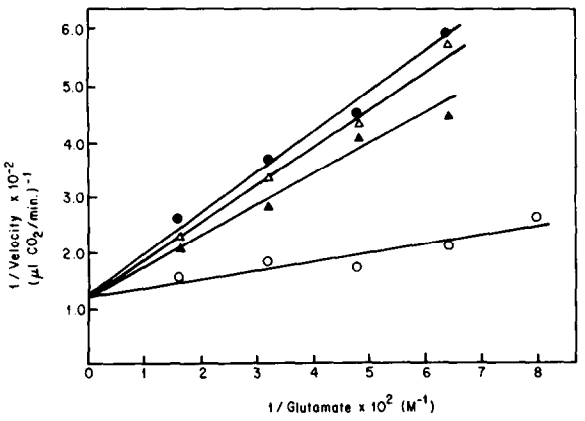

Fig.1. The effect of 3.12 mM citrate (solid triangles), 0.78 mM *cis*-aconitate (open triangles) and 1.56 mM α-ketoglutarate (solid circles) on the decarboxylation of L-glutamate by glutamate decarboxylase (*E. coli*). The open circles represent data obtained in the absence of inhibitors.

Table 1
Inhibition of L-glutamate decarboxylase by tricarboxylic acid intermediates

Acid	% Inhibition
Citric	34
cis-Aconitic	45
DL-isocitric	15
α-Ketoglutaric	30
Succinic	12
Fumaric	12
L-Malic	12
Oxalacetic	15

Assay carried out in 0.1 M pyridine–pyridine hydrochloride buffer (pH 5.0) at 37°C; 1 mg protein/ml; 6.25 mM glutamic acid; 3.12 mM inhibitor. Percent inhibition determined with respect to initial velocity in the absence of any inhibitor

4. Discussion

It has been shown that several controls operate to regulate the metabolic levels of glutamate in *E. coli* including:

(i) Inhibition of citrate synthetase by α-ketoglutarate [12];
(ii) Repression of *cis*-aconitase by glutamate [13];
(iii) Repression of α-ketoglutarate dehydrogenase by glutamate [13].

If the pool of glutamate becomes too large the controls cited regulate mainly the amount of intermediates which go into the synthesis of L-glutamate and not its removal. Our results suggest that *cis*-aconitate, citrate, α-ketoglutarate and probably isocitrate are effective inhibitors of glutamate decarboxylase; lowering the concentrations of these metabolites could activate decarboxylation by the enzyme thereby removing glutamate by means of its conversion to 4-aminobutyrate.

The most prominent feature of our observations is the significant effect of *cis*-aconitate on the activity of glutamate decarboxylase. When *E. coli* is grown in a medium containing 0.3 mM glutamate it was noted [13] that the synthesis of *cis*-aconitase is repressed.

Possibly some form of coordinate control which depends on the level of glutamate is operating to regulate both the activity of glutamate decarboxylase and *cis*-aconitase.

Reasons for the discrepancy between our results and those in [6] regarding the ability of *cis*-aconitic acid to inhibit glutamate decarboxylase from *E. coli* are not clear at present. These authors used acetate buffer and steps taken to purify the commercial acetone powder, if any, are not clear from [6]. A comparison of their results with those reported herein may thus not be appropriate.

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