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Short Communication

THE INTERACTIONS OF 2-AMINO-4-ARSONOBUTYRATE WITH GLUTAMATE DEHYDROGENASE

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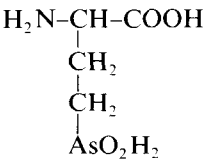
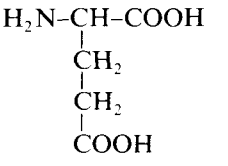
INTRODUCTION

The activity of glumate dehydrogenase [L-glutamate:NAD(P) oxidoreductase (deaminating), EC 1.4.1.3] (GDH) has been shown to be affected by a large number of different allosteric effectors (see reference[1]). These include the amino acid leucine which is a substrate for the enzyme as well as acting as an allosteric activator under some conditions.[2] Furthermore the interactions of the substrate glutamate with the enzyme show complex kinetic behaviour[3] which has been interpreted in terms of a mixture of positive and negative cooperativity that is further distorted by complexities in the kinetic mechanism of oxidative deamination.[4] Negative cooperativity was also observed for the inhibition of the enzyme by 2-oxoglutarate.[5] In order to provide a possible tool for the investigation of the interactions of the enzyme with substrate, we have studied the behaviour of D,L-2-amino-4-arsonobutyrate. The structure of this compound is compared to that of glutamate in Scheme 1. This compound is an arsono-analogue of glutamate and its interactions with ox brain glutamate dehydrogenase as a possible substrate, activator or inhibitor were investigated.



2-Amino-4-arsonobutyric acid



Glutamic acid

SCHEME 1

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MATERIALS AND METHODS

Glutamate dehydrogenase was purified from ox brain according to the method previously reported,[6] which avoids the limited proteolysis that has been shown to affect commercially-available preparations of the mammalian enzyme. D,L-2-amino-4-arsonobutyric acid was synthesized as described by Adams *et al.*[7]

Activities were determined spectrophotometrically at 30°C, by monitoring the change in absorbance at 340 nm resulting from the oxidation of NADH or the reduction of $NAD.^+$ Assays in the direction of oxidative deamination were performed in a mixture containing, in a total volume of 2.5 ml, 50 m*M* phosphate buffer, pH 7.9, 1 m*M* NAD^+, the enzyme (within the concentration range 0.2–0.8 $\mu g\,ml^{-1}$) and glutamate and/or 2-amino-4-arsonobutyrate. The reaction was started by the addition of the substrate, which did not modify the pH of the reaction medium. Assays in the direction of reductive amination were in a mixture containing, in a total volume of 2.5 ml, 50 m*M* phosphate buffer, pH 7.4, 5 m*M* 2-oxoglutarate, 100 m*M* NH_4Cl and 160 μM NADH.

The kinetics of inhibition were studied in the direction of glutamate oxidative deamination. Each set of initial rate data was analysed by non-linear hyperbolic regression to obtain slope, intercept and apparent K_m values, and also their standard errors. The secondary plot of K_m values *versus* the concentration of D,L-2-amino-4-arsonobutyrate which gave them was analysed by weighted linear regression to obtain its slope and intercept. The inhibitor constant K_i was equal to the ratio intercept/slope of this secondary plot and Fieller's theorem was used to estimate its standard error. The results are shown as a double-reciprocal plot for illustrative purposes.

RESULTS AND DISCUSSION

The addition of 2-amino-4-arsonobutyrate to the reaction medium in the concentration range 2–10 m*M* did not give rise to any significant time-dependent change of the absorbance at 340 nm in the assay for oxidative deamination, thus indicating that this compound was not a substrate for GDH. However, as shown in Figure 1, it was found to inhibit GDH activity in a strictly competitive manner relatively to L-glutamate, with an inhibitor constant, K_i of 7.3($\pm$0.5, s.e.)m*M*. The lack of any significant effects of the inhibitor on the apparent maximum velocity of the reaction is also consistent with the inhibition being reversible. Rates of reaction in the present or absence of 2-amino-4-arsonobutyrate were found to be linear over the same time periods, indicating that there were no pronounced time-dependent hysteretic or inhibitory effects.

The competitive inhibition by 2-amino-4-arsonobutyrate suggests that it can bind to the catalytic glutamate site on the enzyme. Its failure to act as a substrate is not likely to be the result of a difference of overall charge, since the pK values of the arsono group are about 4 and 9, but it might result from the size or shape of the tetrahedral $-AsO_3H^-$ group, as opposed to the planar $-COO^-$ group of glutamate. The latter group is rotationally symmetrical whereas the arsonate monoanion is not. This difference will result in different dipole orientations on interaction with a positively charged group on the enzyme. In glutamate the dipole would be along the bond between the $-CH_2$ and the $-COO^-$ groups, whereas in the $-CH_2-AsO_3H^-$ analogue the dipole would be at an angle to the corresponding bond. This difference might

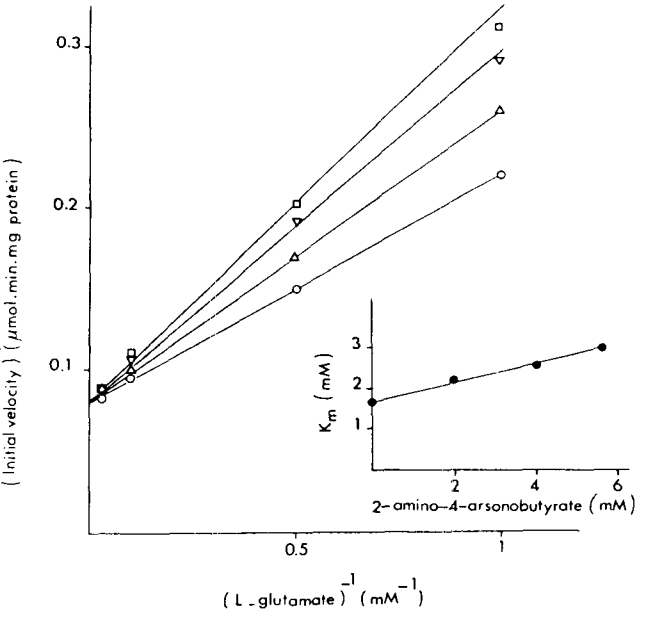

FIGURE 1 Inhibition of ox brain glutamate dehydrogenase by (D,L)2-amino-4-arsonobutyrate. The L-glutamate concentrations were varied in the range 1-40 m*M* while the concentration of D,L-2-amino-4-arsonobutyrate was held constant at the following values: O (O); 2 (Δ); 4 (∇); and 5.6 (□) m*M*. Each point represents the mean of two determinations. The secondary plot of K_m values *versus* the concentration of D, L-2-amino-4-arsonobutyrate which gave them is shown in the inset.

distort the interaction between the analogue and the enzyme and thus prevent catalysis. Such a model has been proposed to account for the observation that phosphonate analogues of acetoacetate were competitive inhibitors but not substrates for β-hydroxybutyrate dehydrogenase, whereas the corresponding sulphonate analogues were substrates.[8] The arsonate group would resemble the phosphonate in geometry but sulphonate would resemble the carboxylate.

The 2-amino-4-arsonobutyrate preparation used in this study was a racemic mixture of the D- and L -enantiomers, thus the observed effect could be ascribed either to both enantiomers or to only one of them. Inhibition of GDH by D-glutamate has been shown to be mixed with respect to L-glutamate.[5,9] In the case of 2-amino-4-arsonobutyrate, any non-competitive effect of the D-enantiomer would be very weak since there was no significant intercept variation in the data in Figure 1. This might suggest that the observed inhibitory effect could be ascribed to the L-enantiomer. If such was the case, the value of the inhibition constant for L-2-amino-4-arsonobutyrate would be half the experimental value obtained for the racemic mixture. This value may be compared to the K_m value for L-glutamate of 1.7 m*M*, that was determined under these conditions.

The potential activatory effects of 2-amino-4-arsonobutyrate were investigated by measuring the rates of reaction in the direction of reductive amination under assay conditions that were found to result in high activation by L-leucine.[2] Since any

activatory effects might be observed more easily at concentrations where inhibition is a minor effect, the concentration of 2-amino-4-arsonobutyrate was varied in the range 0.2–1 m*M*. However, at these concentrations, it was found to have no effect on activity, whereas L-leucine at the same concentrations activated the enzyme significantly.[2]

2-Amino-4-arsonobutyrate might interact with the enzyme as a dicarboxylic or as a monocarboxylic amino acid analogue. The lack of any activatory effects, such as those observed with L-leucine[2] and a number of other monocarboxylic amino acids, such as 2-aminovalerate,[10] might suggest the compound to be acting as a dicarboxylic amino acid analogue, although there was no indication of the negatively cooperative interactions that are observed with glutamate as substrate. It appears that mono- and dicarboxylic amino acids interact at the same catalytic site on glutamate dehydrogenase (see reference 1) but there is a separate binding site that may be responsible for the activatory effects of the monocarboxylic compounds.[11] Thus the lack of any activatory effect might be owing to failure to bind at this second site.

The fact that the arsono-analogue seemed to bind well to the enzyme is encouraging for further development of such compounds as inhibitors, or as substrates. The 2-amino-4-arsonobutyrate might be of interest for the study of glutamate receptors in the central nervous system, since 2-amino-4-phosphonobutyrate is known to be a potent antagonist of L-glutamate binding in the central nervous system (see reference 12).

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