GLYCINE AND SERINE INHIBITION OF D-GLYCERATE DEHYDROGENASE AND 3-PHOSPHOGLYCERATE DEHYDROGENASE OF RAT BRAIN

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1. Introduction

Glycine is probably a major inhibitory transmitter in the mammalian central nervous system [1, 2]. Hence the metabolism and metabolic control of glycine could be important for the efficient functioning of nervous tissue, particularly of the spinal cord. As glycine may be formed from serine by serine hydroxymethylase (EC 2.1.2.1) [3], enzymes necessary for the formation of serine from carbohydrate sources could be important for the production and regulation of glycine. Two major pathways of serine formation have been described in mammalian systems, the "phosphorylated" pathway from 3-phosphoglycerate [4], and the "non-phosphorylated" pathway from D-glycerate [5, 6]. We decided therefore to determine if glycine has any effect on either D(−)3-phosphoglycerate:NAD oxidoreductase (3-phosphoglycerate dehydrogenase) or D-glycerate: NAD (P) oxidoreductase (EC 1.1.1.29) (D-glycerate dehydrogenase).

The results recorded here show that glycine appears to inhibit D-glycerate dehydrogenase of rat cortex in a manner non-competitive with respect to D-glycerate, and to inhibit 3-phosphoglycerate dehydrogenase to a much smaller degree. Limited data for serine inhibition indicate that serine probably inhibits 3-phosphoglycerate dehydrogenase in a non-linear fashion, and much more strongly than glycine, and is only a poor inhibitor of D-glycerate dehydrogenase.

2. Materials and methods

Barium phosphoglycerate and calcium DL-glycerate purchased from Sigma Chemical Corporation, were converted to sodium salts by passage through a column of Dowex-50(H^+) and neutralization of the emerging acids. Phosphoglycerate concentration was estimated by the method of Czok and Eckert [7]. Glycerate was estimated by the method of Bartlett [8] with the concentration of chromotrophic acid raised to 0.025% as recommended by Dawkins and Dickens [9]. NAD was purchased from P-L Biochemicals, and NADP from Sigma.

Rat cortical tissue was homogenized in 0.32 M sucrose containing 0.5 mM dithiothreitol (DTT) and centrifuged at 105,000 *g* for 100 min. The 20–80% ammonium sulphate fraction of the supernatant was dissolved in 10 mM potassium phosphate buffer pH 6.8 with 0.5 mM DTT and EDTA, and dialysed against the same buffer until free from ammonium sulphate. The precipitate which formed during dialysis was removed and the supernatant used for enzyme analysis. With this preparation, no breakdown of phosphoglycerate or glycerate could be detected in the absence of nucleotides under the conditions of enzyme measurement.

The enzyme activities were measured by the method of Sallach [10], except that tris buffer was replaced by tris(hydroxymethyl)methylaminopropane sulphonic acid (TAPS) buffer, glutathione by DTT, and D-glycerate dehydrogenase was measured using NADP with DL-glycerate. Details are given in the legends to the figures. Reaction mixtures were incubated at 30° in a Shimadzu spectrophotometer and the absorbance at 3400 Å was read at 1 min intervals. Since there was

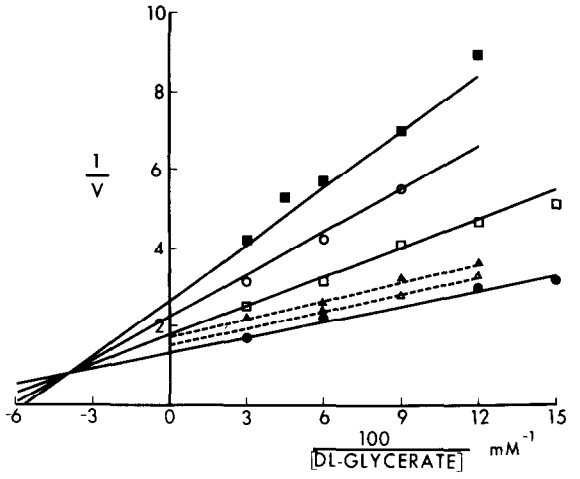

Fig. 1. Double reciprocal plot of the initial velocity of the reaction catalysed by rat brain D-glycerate dehydrogenase with DL-glycerate as variable substrate in the presence and absence of glycine or serine. Reaction mixtures contained 100 mM TAPS buffer pH 9.0, 167 mM hydrazine sulphate pH 9.0, 0.4 mM DTT and EDTA, 42 mM NaCl, 0.83 mM NADP, varying concentrations of DL-glycerate as shown, glycine or serine as indicated, and rat brain extract in a total volume of 1.2 ml. Concentrations of glycine were: •, 0 mM; □, 10 mM; ○, 20 mM; ■, 30 mM. Concentrations of serine were: △, 10 mM; ▲, 20 mM. Velocities are expressed as nmoles of NADPH formed per min per mg protein. The points are experimental; the lines represent the fit to the equation $v = VA/(K(1 + 1/K_{is}) + A(1 + 1/K_{ii})]$ for linear non-competitive inhibition using the computer program of Cleland [12].

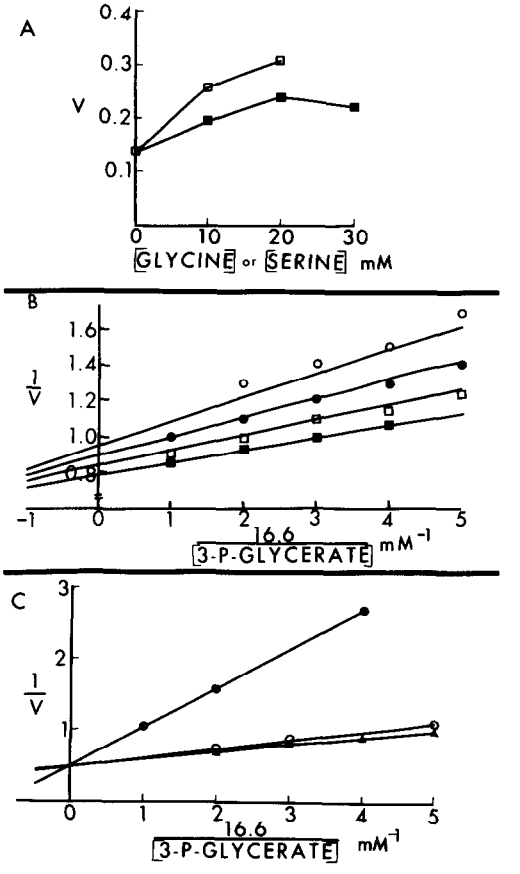

Fig. 2. Analysis of data for the measurement of 3-phosphoglycerate dehydrogenase. Velocities are expressed as nmoles NADH formed per min per mg protein. (A) Velocity of NADH formation in the absence of 3-phosphoglycerate plotted as a function of the concentration of glycine (■) or serine (□). Reaction mixtures contained 100 mM TAPS buffer pH 9.0, 167 mM hydrazine sulphate pH 9.0, 0.4 mM EDTA and DTT, 0.83 mM NAD, rat cortex extract, and glycine and serine at concentrations as indicated, in a final volume of 1.2 ml. (B) Double reciprocal plot of initial velocity with 3-phosphoglycerate as variable substrate in the presence or absence of glycine. Reaction mixtures were as in fig. 2A except that 3-phosphoglycerate was present at varying concentrations as shown. Concentrations of glycine were: ■, 0 mM; □, 10 mM; •, 20 mM; ○, 30 mM. The points are experimental. The lines represent the fit to linear non-competitive inhibition. (C) Double reciprocal plot of initial velocity with 3-phosphoglycerate as variable substrate in the presence and absence of serine. The reaction mixtures are as described in fig. 2B except that glycine is replaced by serine at concentrations of: ▲, 0 mM; ○, 10 mM; •, 20 mM. The points are experimental. The lines represent the fit of the data for each concentration of serine to equation $v = VA/(K + A)$, using the computer program of Cleland [12].

Table 1

Kinetic constants for the inhibition of D-glycerate dehydrogenase by glycine and serine. The values for K_{is} and K_{ii} for glycine inhibition are weighted means of two experiments. The values for weighted mean and standard error were calculated using the formula given in Morrison and James [13].

Variable substrate	Inhibitor	K_{is} mM	K_{ii} mM
D-Glycerate	Glycine	13.73 ± 1.64	33.84 ± 4.61
	Serine	120.7 ± 91.3	76.4 ± 22.2
3-Phospho-glycerate	Glycine	35.2 ± 9.9	154.9 ± 47.0

some reduction of NAD and NADP by rat brain extracts in the absence of either 3-phosphoglycerate or DL-glycerate, it was necessary to read these "background" velocities over the same time period as the velocities with both substrates to estimate the initial velocity of the reactions catalysed by the dehydrogenases.

Initial velocities have been analysed by the methods of Cleland [11, 12], and his notation has been used for the appropriate equations and the kinetic constants.

3. Results

The initial velocity of the reaction between D-glycerate and NADP, as catalysed by the rat cortex extracts, appears to be inhibited by glycine. Fig. 1 shows a double reciprocal plot with DL-glycerate as variable substrate at a fixed concentration of NADP and at increasing concentrations of glycine. The data are fitted to the equation which describes linear non-competitive inhibition (see legend to fig. 1). Values for the slope inhibition constant (K_{is}), and for the intercept inhibition constant (K_{ii}) derived from this and similar experiments are given in table 1. The dotted lines in fig. 1 indicate the inhibition by serine. Inhibition constants derived from this very limited data are shown in table 1 for comparison with those found for glycine inhibition.

Measurement of the initial velocity of the reaction of 3-phosphoglycerate and NAD as catalysed by rat cortex extracts was more difficult, both because there is a reasonable rate of NAD reduction in the absence of added 3-phosphoglycerate, and because this "background" rate of NAD reduction was considerably increased by the addition of glycine or serine (fig. 2A). When these increased "background" rates are taken into account, the initial velocity of the reaction catalysed by 3-phosphoglycerate dehydrogenase appears to be inhibited slightly by glycine (fig. 2B), and perhaps more significantly by serine (fig. 2C).

The inhibition by glycine with respect to 3-phosphoglycerate has been fitted to the equation for linear non-competitive inhibition to yield the constants listed in table 1. The inhibition by serine, as recorded in fig. 2C, is obviously non-linear, although insufficient data are present in this preliminary experiment to define the nature of the inhibition. It appears to be competitive.

4. Discussion

Because our aim was only to investigate possible end-product inhibitions of enzymes which may be involved in glycine synthesis, we have worked with a preparation of rat cortex which is, at best, only 10–15-fold purified over the homogenate. The physical and chemical properties of these brain enzymes have not yet been described. Therefore, without extensive enzyme purification and a study of reaction mechanisms, it is not possible to interpret mechanistically the inhibitions recorded here. Nevertheless it seems clear that, under the conditions of assay, both D-glycerate dehydrogenase and 3-phosphoglycerate dehydrogenase are inhibited by glycine and serine.

The inhibitions of 3-phosphoglycerate dehydrogenase are of interest because the initial velocities of animal enzymes so far examined, unlike those from *E. coli* [14] and plants [15, 16], have been found to be insensitive to glycine or serine [17, 18]. In addition, when serine inhibition has been examined by measuring the incorporation *in vitro* of ^{14}C-3-phosphoglycerate into phosphoserine and serine in the presence and absence of serine, concentrations of serine up to 10 mM did not inhibit the incorporation catalysed by either mouse brain [19] or KB cell [20] extracts. Therefore serine did not appear to inhibit any enzymes on the pathway to phosphoserine under these conditions. However, in a more recent paper by Davis and Fallon [21] it was found that 16.6 mM serine did reduce incorporation. These results are compatible with the initial velocity results of fig. 2C where serine inhibition is only readily apparent at concentrations over 10 mM. Significant inhibition by glycine (fig. 2B and table 1) requires high glycine concentrations and so may not be metabolically important.

NAD but not NADP reduction was stimulated by the addition of glycine or serine in the absence of glycerate or 3-phosphoglycerate (fig. 2A), indicating an NAD-dependent oxidation of either the amino acids or substances formed from them by the rat cortex extracts. Reactions which may be occurring are at present being investigated.

Although the conditions used to measure the enzyme activities are not physiological, the relative sensitivity of D-glycerate dehydrogenase to glycine indicates that this inhibition may be of metabolic importance. Although the inhibition constants of table 1 are higher than normal glycine concentrations, it must be borne in mind that they are only apparent constants, that they are almost certainly not simple dissociation constants, and that glycine concentrations in the central nervous system can be as high as 7 μmoles/g in the ventral grey part of the cat spinal cord [22]. Moreover, the non-competitive nature of the inhibition suggests that glycine is not acting simply as a substrate analogue but rather as an allosteric end-product inhibitor. The high concentrations of serine required to inhibit the enzyme suggest that serine inhibition may not be of physiological significance. This raises the question as to whether glycine formed from D-glycerate is made via serine, or whether it could be made via the decarboxylation of hydroxypyruvate as suggested by Sallach [23]. This possibility is under in vestigation.

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