Glutamate Decarboxylase. Substrate Specificity and Inhibition by Carboxylic Acids†

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ABSTRACT: A number of amino acids were investigated as substrates and substrate-competitive inhibitors of bacterial glutamate decarboxylase. Appreciable activity was obtained with only L-glutamic acid and α-methyl-DL-glutamic acid. L-Isoglutamine inhibits the enzyme, but shows very little activity with the enzyme. D-Glutamic, L- and D-aspartic, DL-α-aminoadipic, and DL-α-aminopimelic acids do not inhibit the enzyme and do not function as substrates. A variety of carboxylic acids were demonstrated to be substrate-competitive inhibitors. Aliphatic dicarboxylic acids are the most effective inhibitors. A chain-length effect is observed with the binding of the dicarboxylic acids with glutaric, pimelic, and adipic acids functioning as the most effective inhibitors. The inhibitor dissociation constants for the enzyme complex with glutarate or α-ketoglutarate are sensitive to pH. This pH dependence implicates the monoanion of the dicarboxylic acid as the ionic form which interacts most effectively with the enzyme.

Bacterial L-glutamate decarboxylase (EC 4.1.1.15) has been purified extensively and shown to contain pyridoxal phosphate as a prosthetic group (Shukuya and Schwert, 1960a; Anderson and Chang, 1965). At low pH the pyridoxal phosphate is bound as a Schiff base to a lysine residue at the active site (Strausbauch and Fischer, 1970). The enzyme has been reported to have a pH optimum between pH 4 and 4.5 (Shukuya and Schwert, 1960a,b; O'Leary *et al.*, 1970). Bacterial glutamate decarboxylase is most active toward L-glutamic acid, but has been reported to also decarboxylate γ-methyleneglutamic and *threo*-β-hydroxyglutamic acids (Homola and Dekker, 1967), glutamate γ-methyl ester (Lupo and Halpern, 1970), and α-methylglutamic acid (Sukhareva and Torchinsky, 1966; Huntley and Metzler, 1968).

Since there are so few substrates of glutamate decarboxylase, it is difficult to determine the types of interaction between the enzyme and glutamate from a study of substrate specificity. However, an investigation of the inhibition of the enzyme by dicarboxylic acids should be useful in understanding the basis for substrate specificity. Very little information has been obtained using substrate-competitive inhibitors of the bacterial glutamate decarboxylase. The enzyme from *Escherichia coli* sources has been demonstrated to be inhibited by α-ketoglutarate, glutarate, and α-methylglutarate (Lupo and Halpern, 1970). Glutamate decarboxylase from the squash *Curcurbita moschata* is inhibited competitively by monocarboxylic and dicarboxylic acids, the most effective inhibitor being pimelate (Ohno and Okunuki, 1962). Mammalian brain glutamate decarboxylase has been demonstrated to be inhibited by phenyl acids (Hanson, 1958), but not by aliphatic monocarboxylic or dicarboxylic acids (Roberts and Simonsen, 1963).

In the present study, the binding of a variety of carboxylic acids to bacterial glutamate decarboxylase was investigated in an attempt to evaluate the structural requirements for interaction of inhibitors and substrate with the enzyme. These data are compared to those obtained for the interaction of dicarboxylic acids with aspartate aminotransferase, another pyridoxal phosphate dependent enzyme utilizing glutamate as substrate. Such a comparison could provide information on the mechanism of the two reactions and on substrate specificity.

Materials and Methods

Materials. Pyridoxal phosphate, glutathione, L-glutamine, L-alanine, glycine, and α-methyl-DL-glutamic, L-aspartic, D-aspartic, D-glutamic, DL-α-aminoadipic, DL-α-aminopimelic, glutaric, adipic, pimelic, suberic, azeleic, maleic, fumaric, acetylenedicarboxylic, itaconic, L-malic, D-malic, L-α-hydroxyglutaric, D-α-hydroxyglutaric, oxaloacetic, and γ-aminobutyric acids were purchased from Sigma Chemical Co. Dithiothreitol and α-ketoglutaric acid were obtained from Calbiochem. Succinic and malonic acids were from Mallinckrodt Chemical Works. 4-Acetylpyridine, monomethylglutarate, oxalic, citraconic, mesaconic, methylsuccinic, phenylsuccinic, 2,2-dimethylsuccinic, 3,3-tetramethyleneglutaric, *meso*-tartaric, phthalic, isophthalic, and terephthalic acids were purchased from Aldrich. L-Glutamic acid was obtained from Nutritional Biochemicals. DL-*O*-Phosphoserine was purchased from Mann Research Laboratories. L-Isoglutamine, methyl γ-L-glutamate, and glutaconic acid were from K & K.

Isolation of Enzyme. Glutamate decarboxylase was isolated from *Escherichia coli* (ATCC 11246) as previously described (Huntley and Metzler, 1967; Fonda, 1971). The enzyme was stored at 4° at a concentration of approximately 10 mg/ml in 0.1 M pyridine hydrochloride (pH 4.6), containing 1×10^{-4} M pyridoxal phosphate and 1×10^{-3} M dithiothreitol.

Enzyme Assay. A Gilson differential respirometer was used for all kinetic studies. Unless otherwise stated, assays were conducted at 25° in 3-ml reaction mixtures containing 0.1 M pyridine hydrochloride buffer (pH 4.6), 0.2 M chloride, and 6.67×10^{-5} M pyridoxal phosphate. The concentrations of L-glutamic acid and the inhibitors are included in the specific descriptions of the experiments. The assay mixture was placed in the main chamber of the Warburg flask, and the enzyme, usually 2 μl, was placed in the side arm. After equilibration

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at 25° for 5 min, the contents of the flasks were mixed and the volume of the gas evolved was recorded at 1-min intervals for 6 min.

Initial velocities could not be measured at low-substrate concentrations since more than 20% formation of CO_2 (5 μl/min) was required for accurate measurement on the respirometer. A modification of the Eadie equation was used to calculate K_m and K_i values using the average velocity, $\bar{v} = (S_0 - S_t)/t$, and the arithmetic mean substrate concentration, $\bar{S} = (S_0 + S_t)/2$, during the time interval, t, in place of the initial velocity and initial substrate concentration. Such calculations have been shown to be quite accurate if not more than 50% of the substrate is utilized, if there is no product inhibition, and if the reaction is not significantly reversible (Lee and Wilson, 1971). *E. coli* glutamate decarboxylase is not inhibited by its product, γ-aminobutyric acid, at the concentrations used in this study, and the reaction catalyzed by the enzyme is not reversible. The K_m and K_i values calculated in this manner for glutamate decarboxylase are good approximations, and the K_i values are comparable since all measurements were made at inhibitor concentrations roughly equal to the K_i values.

pH Measurements. pH measurements were made at 25° with a Corning Model 12 research pH meter and with a Radiometer PHM 4d pH meter. Standard buffer solutions from Mallinckrodt and Fisher were used for calibration. Stock solutions of substrates, inhibitors, and pyridoxal phosphate were all prepared in 0.1 M pyridine hydrochloride and adjusted to the appropriate pH for the reactions to be studied. In the determination of the pH profiles, the pH of each assay mixture was determined immediately after the reaction.

TABLE I: Effect of Amino Acids on Glutamate Decarboxylase Activity.

Amino Acid	Concn (mM)	Act.[a] (%)	Inhibn[b] (%)
L-Glutamic	20	100	
D-Glutamic	35	1	0
α-Methyl-DL-glutamic	48	5	55 (K_i = 15 mM)
Methyl γ-L-glutamate	8.3	1	0
L-Glutamine	97	80 (34)	
L-Isoglutamine	66	2 (2)	14 (K_i = 95 mM)
L-Aspartic	33	2 (<1)	0 (0)
D-Aspartic	36	2	0
DL-α-Aminoadipic	46	<1 (<1)	0 (0)
DL-α-Aminopimelic	46	<1 (<1)	3
DL-*O*-Phosphoserine	33	<1	0
L-Alanine	87	<1	0
Glycine	87	<1	0

[a] Activity of glutamate decarboxylase was determined with each amino acid at the concentration given in 0.1 M pyridine hydrochloride (pH 4.6) and 6.67×10^{-5} M pyridoxal phosphate. The values given in parentheses were obtained in 0.1 M 4-acetylpyridine hydrochloride (pH 3.6). [b] Inhibition of glutamate decarboxylase was determined using 1.33×10^{-3} M glutamate and 6.67×10^{-5} M pyridoxal phosphate in 0.1 M pyridine hydrochloride (pH 4.6). The K_i values were obtained by measuring velocities at varying concentrations of glutamic acid and plotting the data according to Eadie.

Results

The specificity of *E. coli* glutamate decarboxylase was investigated by studying a number of amino acids as potential substrates or inhibitors of the enzyme. The results obtained in 0.1 M pyridine hydrochloride (pH 4.6) are presented in Table I. The enzyme shows much greater activity with L-glutamic acid than with any other amino acid. Glutamate decarboxylase does not decarboxylate and is not inhibited by D-glutamic, L- or D-aspartic, DL-α-aminoadipic, or DL-α-aminopimelic acids. L-Isoglutamine is a poor inhibitor and gives low activity; however, the γ-methyl ester of glutamate does not inhibit the enzyme. Carbon dioxide is released when L-glutamine is used as substrate; however, this activity may be due to the presence of trace amounts of L-glutamic acid. The rates obtained with L-glutamine fell off extremely rapidly with time suggesting rapid substrate depletion. Phosphoserine, L-alanine, and glycine do not bind to glutamate decarboxylase. α-Methyl-DL-glutamic acid does inhibit and is slowly decarboxylated by the enzyme. In addition, the α-methylglutamate inactivates the enzyme irreversibly.

Activation and inhibition of glutamate decarboxylase by these amino acids were also studied in 0.1 M 4-acetylpyridine hydrochloride (pH 3.6). The results were essentially the same as those obtained at pH 4.6 and are presented in Table I in parentheses.

The inhibition of glutamate decarboxylase by a variety of carboxylic acids was investigated to determine the binding requirements of the enzyme for these compounds. Inhibition by each of the compounds used was evaluated in two separate experiments. The inhibitors were first studied as a function of varying inhibitor concentration with two concentrations of substrate. The data obtained in this manner were plotted according to Dixon (1953). In a second set of experiments the inhibitors were studied as a function of varying substrate concentration and three constant inhibitor concentrations. The data obtained were plotted as v *vs.* v/S. For example, in Figure 1 is an Eadie plot showing the competitive inhibition of the enzyme by glutaric acid. The inhibitor dissociation constants (K_i) calculated from the Dixon plots and the Eadie plots were in good agreement for all inhibitors studied. The K_i value for glutaric acid calculated from the Eadie plot is presented in Table II.

The inhibition of glutamate decarboxylase by glutaric acid and the kinetic parameters of the enzyme-catalyzed decarboxylation of glutamic acid are dependent on the chloride concentration in the assay mixture. In 0.1 M pyridine hydrochloride (pH 4.6), containing 0.2 M chloride, the K_m for glutamate is approximately 1.0 mM, the maximum velocity is 8.28 μmoles of CO_2/min, and the K_i for glutarate is 0.68 mM. In 0.1 M pyridine phosphate (pH 4.6) and containing no chloride, the K_m for glutamate is 0.32 mM, the maximum velocity is 1.92 μmoles of CO_2/min, and the K_i for glutarate is 0.20 mM.

In an attempt to evaluate the intercarboxylate distance required for optimum binding of dicarboxylic acids to glutamate decarboxylase, an homologous series of dicarboxylic acids was studied. These compounds were all shown to inhibit the enzyme competitively with respect to glutamate, and the inhibitor dissociation constants obtained for these compounds are listed in Table II (column 2). In going from oxalic acid to glutaric acid there is an increase in affinity with chain length. Glutaric, adipic, and pimelic acids have approximately the same affinity for the enzyme, while suberic and azeleic acids have decreased affinity. Apparently the carboxyl groups

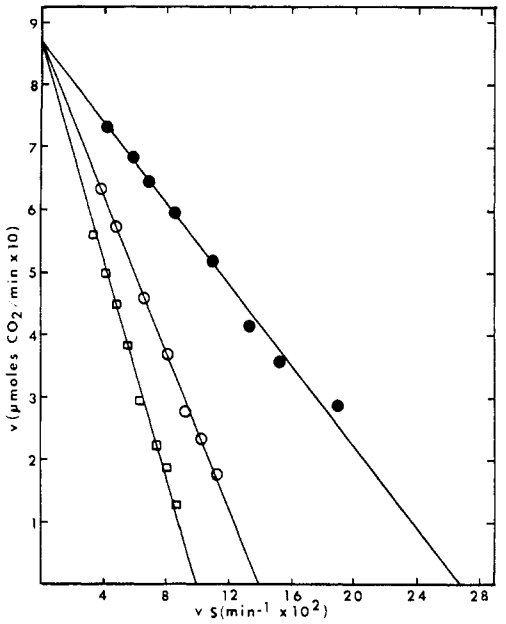

FIGURE 1: Eadie plot showing competitive inhibition of glutamate decarboxylase by glutaric acid. Glutamic acid concentration was varied from 6.67×10^{-4} to 6.67×10^{-3} M. Reaction mixtures contained 0.1 M pyridine hydrochloride (pH 4.6), 0.12 M sodium chloride, 6.67×10^{-5} M pyridoxal phosphate, approximately 10 μg of glutamate decarboxylase, glutamic acid, and glutaric acid as indicated, in a total volume of 3 ml. Line 1 (●), no inhibitor; line 2 (○), 5×10^{-4} M glutaric acid; line 3 (□), 1×10^{-3} M glutaric acid.

of the dicarboxylic acids should be approximately 6 Å apart for optimum interaction with the enzyme.

Maleic and fumaric acids were tested as inhibitors since the carboxyl groups of these compounds are rigidly held at a fixed distance from each other. Although the intercarboxylate distances of these compounds differ, their K_i values are very similar.

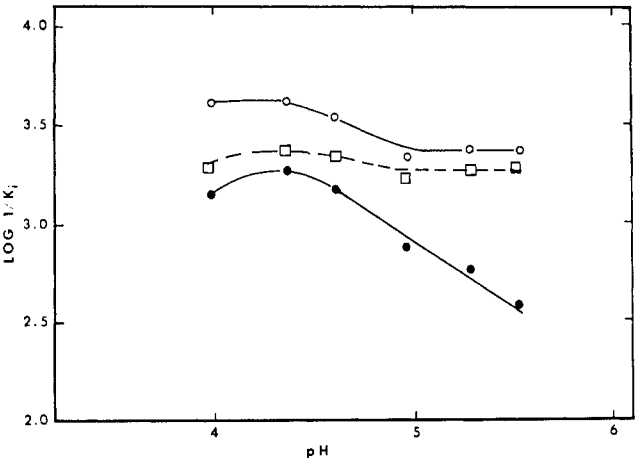

FIGURE 2: Effect of pH on log $(1/K_i)$ for glutaric acid. The reaction mixtures contained 0.1 M pyridine hydrochloride buffer adjusted to the required pH and to a chloride concentration of 0.2 M, 6.67×10^{-5} M pyridoxal phosphate, 10–20 μg of glutamate decarboxylase, glutamic acid, and inhibitor as indicated, in a total volume of 3 ml. At each pH, the rates were obtained as a function of varying substrate concentration and three constant inhibitor concentrations. The data were plotted as v *vs.* v/S, and the inhibitor dissociation constants were calculated from the Eadie plots. Bottom line (●), K_i values calculated on the basis of total concentration of glutaric acid. Line 2 (□), K_i values calculated on the assumption that only the monoanion and fully protonated forms of glutaric acid bind to the enzyme. Top line (○), K_i values calculated on the assumption that only the monoanion form of glutaric acid binds.

TABLE II: Inhibition of Glutamate Decarboxylase by Dicarboxylic Acids.

Compound	K_i (mM)	pK_a	K_i (Monoanion only)[e] (mM)	Max. Distance between (Carboxyls (Å)
Glutamic	0.88 (K_m)	2.19, 4.25[d]		6.0
Oxalic	480	1.09, 3.79[a]	65	2.42[a]
Malonic	36	2.58, 5.17[a]	29	3.28[a]
Succinic	5.9	3.95, 5.16[a]	3.5	4.75[a]
Glutaric	0.59	4.07, 4.93[a]	0.27	5.83[a]
Adipic	1.14	4.17, 4.95[a]	0.49	6.87[a]
Pimelic	0.81	4.23, 4.98[a]	0.33	7.88[e]
Suberic	4.1	4.27, 5.00[b]	1.6	9.07[e]
Azeleic	4.6	4.30, 5.02[b]	1.8	10.08[e]
Maleic	18	1.67, 5.75[a]	16.8	3.66[a]
Fumaric	18	2.85, 4.00[a]	3.32	4.87[a]
Glutaconic	1.9	3.33, 4.70[c]	0.94	5.64[e]
Acetylene-dicarboxylic	81	1.73, 4.40[d]	19	5.16[a]
Phthalic	32	2.98, 5.28[a]	26	3.55[a]
Isophthalic	8.0	3.46, 4.46[a]	2.8	5.84[a]
Terephthalic	19 (at pH 5.1)	3.51, 4.82[a]	6.2	7.33[a]

[a] Taken from Webb (1966). [b] Taken from Adell (1940). [c] Determined by titration in 0.2 M NaCl at 25°. [d] From Sober (1968). [e] The concentrations of the various ionic forms were calculated using the macroscopic pK values given. [f] The distances were calculated on the basis of bond lengths and angles given in Webb (1963). It was assumed that the center of negative charge lies midway between the oxygen atoms of the carboxyl groups.

Since the enzyme is assayed at pH 4.6 and these dicarboxylic acids all have pK values near pH 4.6, it was thought possible that one ionic form of the acid is preferentially inhibiting the enzyme and that the concentrations of the ionic forms should be considered in calculating the K_i values. Therefore, the K_i values for two dicarboxylic acids were determined as a function of pH. The effect of pH on log $(1/K_i)$ of glutaric acid is shown in Figure 2. The profile for the log $(1/K_i)$ of glutaric acid is very similar to the percentage monoanian present at each pH value. The open circles are the log $(1/K_i)$ calculated on the basis of the concentration of only the monoanion present at each pH. The squares are the log $(1/K_i)$ for glutaric acid calculated on the assumption that the monoanion and the fully protonated forms of glutaric acid bind equally well and that the dianion binds very poorly. The effect of pH on log $(1/K_i)$ of α-ketoglutarate is shown in Figure 3. Again the profile for the log $(1/K_i)$ is very similar to the percentage of α-ketoglutarate in the monoanion form. The K_i values were then calculated on the basis of the concentration of the monoanion present, and log $(1/K_i)$ was plotted against pH.

The K_i values for all the inhibitors studied were recalculated assuming that only the monoanion forms of each acid were binding and are presented in column 4 of Table II. Again

TABLE III: Inhibition of Glutamate Decarboxylase by Substituted Dicarboxylic Acids.

Compound	K_i (mM)	pK_a	K_i (Monoanion only) (mM)
Succinic	5.9	3.95, 5.16[a]	3.5
Methylsuccinic	10	3.93, 5.32[b]	6.3
Phenylsuccinic	55	3.43, 4.92[c]	34
2,2-Dimethylsuccinic	24	3.77, 5.82[a]	20
Itaconic (methylenesuccinic)	2.7	3.67, 5.19[a]	1.9
D-Malic	15	3.21, 4.62[a]	6.8
L-Malic	13	3.21, 4.62[a]	6.0
meso-Tartaric	16	3.07, 4.40[b]	5.7
Oxaloacetic	2.9	2.56, 4.37[b]	1.1
Maleic	18	1.67, 5.75[a]	16.8
Citraconic (methylmaleic)	27	2.23, 5.89[a]	26
Fumaric	18	2.85, 4.00[a]	3.32
Mesaconic (methylfumaric)	4.1	2.93, 4.82[a]	2.5
Glutaric	0.59	4.07, 4.93[a]	0.27
3,3-Tetramethyleneglutaric	3.0	3.30, 6.56[b]	1.8
L-α-Hydroxyglutaric	4.4	3.58, 4.89[c]	2.5
D-α-Hydroxyglutaric	3.4	3.58, 4.89[c]	2.0
α-Ketoglutaric	0.63	2.29, 4.68[c]	0.34
Methylglutarate	9.5		
γ-Aminobutyric	135		

[a] From Webb (1966). [b] From Sober (1968) and corrected for ionic strength and temperature. [c] Determined by titration in 0.2 M NaCl at 25°.

there is a chain-length effect on inhibition in going from oxalic acid to glutaric acid. The larger suberic and azeleic acids have less affinity for the enzyme than does glutaric acid; this is probably due to steric hindrance. When it is assumed that only the monoanion is binding, the enzyme has a greater affinity for fumaric acid than it has for maleic acid. These new K_i values for fumaric and maleic acids are consistent with the K_i values observed with the saturated dicarboxylic acids with similar intercarboxylate distances (succinic and malonic acids, respectively).

A number of substituted dicarboxylic acids were used to investigate the topography of the substrate binding site of glutamate decarboxylase. The uncorrected K_i, pK_a, and K_i values calculated on the basis of the concentration of only the monoanion form are presented in Table III. Generally substituents on succinic acid decrease its affinity for glutamate decarboxylase. However, itaconic and oxaloacetic acids are bound better than succinic acid.

Methylmaleic and methylfumaric acids have K_i values similar to those of maleic and fumaric acids, respectively. Methylglutarate ester is a very poor inhibitor of glutamate decarboxylase, and γ-aminobutyric acid, the product of the reaction, is an extremely ineffective inhibitor.

The effect of pH on the V_{max} and K_m of glutamate decarboxylase with L-glutamate is presented in Figure 4. There is a decrease in both V/K_m and V below pH 4 and a slight decrease in each above pH 4.6.

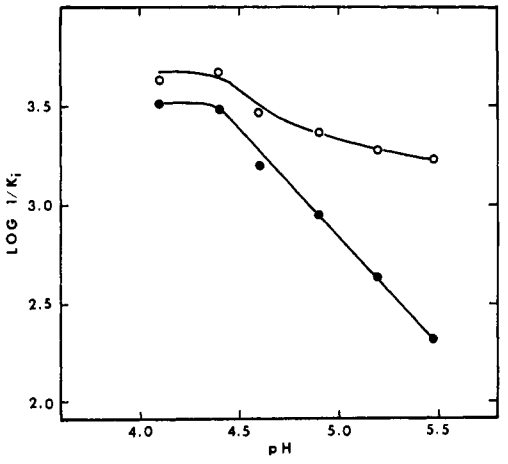

FIGURE 3: Effect of pH on log ($1/K_i$) for α-ketoglutaric acid. The conditions were the same as those described for Figure 3. Bottom line (●), K_i values calculated on the basis of the total concentration of α-ketoglutaric acid; Top line (○), calculation of the K_i values were made assuming that only the monoanion form of α-ketoglutaric acid binds.

Discussion

Bacterial glutamate decarboxylase has been demonstrated previously to be quite specific for L-glutamic acid. Besides L-glutamate only α-methyl-DL-glutamic acid gave appreciable activity in the present study. α-Methyl-DL-glutamic acid has been shown previously to be slowly decarboxylated by the enzyme, and it also inactivates the enzyme by transaminating the pyridoxal phosphate (Sukhareva and Torchinsky, 1966; Huntley and Metzler, 1968). It has been suggested that glutamate decarboxylase undergoes a conformational change upon interaction with glutamic acid (Sastchenko *et al.* 1971).

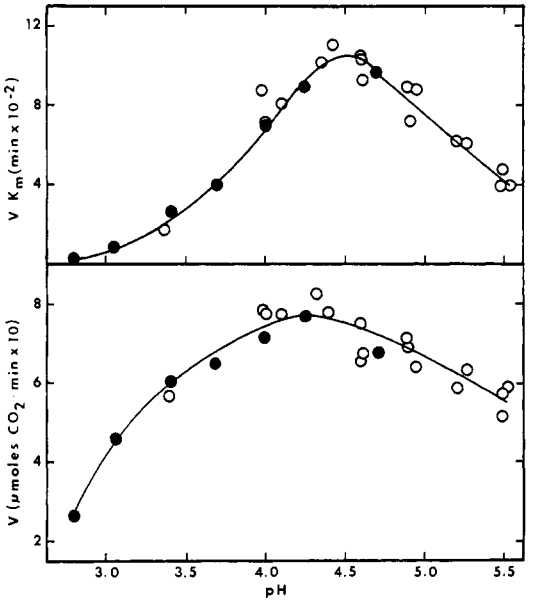

FIGURE 4: Effect of pH on V and V/K_m for the enzymatic decarboxylation of glutamic acid. The reaction mixtures contained 6.67×10^{-5} M pyridoxal phosphate, varying concentrations of glutamic acid, and either 0.1 M 4-acetylpyridine hydrochloride (●) or 0.1 M pyridine hydrochloride (○). The values for V were corrected for the presence of carbonate and bicarbonate in solution. Under the conditions used this correction is a function only of pH (Umbreit *et al.*, 1964).

The lack of activity observed with the aminodicarboxylic acids other than glutamate could be explained by their inability to induce this conformational change. However, it is quite surprising that L-aspartic and DL-α-aminoadipic acids do not inhibit glutamate decarboxylase, since succinic and adipic acids are quite effective inhibitors. Evidently the orientation of the amino group with respect to the carboxyl groups is critical, and improper orientation can lead to steric hindrance or electrostatic repulsion. Since methylsuccinic and D- and L-malic acids inhibit the enzyme, the lack of inhibition observed with L-aspartic acid is probably not due to steric hindrance alone, but may be attributed to the positively charged amino group.

A number of dicarboxylic acids inhibit glutamate decarboxylase competitively with respect to glutamic acid. Two free carboxyl groups are required for effective inhibition since methylglutarate and γ-aminobutyrate are quite poor inhibitors and γ-methylglutamate does not inhibit at all. Furthermore, an amino group does not appear to be involved in binding since γ-aminobutyrate is an extremely ineffective inhibitor. The inhibition of glutamate decarboxylase is dependent on chain length of the dicarboxylic acids. Evidently the carboxyl groups of these inhibitors are interacting with two groups at the active site of the enzyme that are approximately 6 Å apart.

Generally substituted dicarboxylic acids are less effective inhibitors than the unsubstituted acids. The introduction of a methyl group on the α-carbon atom of glutamic acid has an even greater effect on its binding than it has on the binding of succinic acid. α-Methyl-DL-glutamic acid has a K_i of 15 mM compared to a K_m of glutamic acid of 0.88 mM, whereas the K_i for methylsuccinic acid is only twice that of succinic acid. These data again point out the extreme specificity of the enzyme in binding only certain amino acids.

The dissociation constants obtained with glutaric and α-ketoglutaric acids vary significantly with pH. Analysis of these data based only on the pK values of the dicarboxylic acids is an oversimplification since the pK values of the groups of the enzyme that are involved in binding should affect this pH profile, and the pK's of the dicarboxylic acids could be significantly altered in the environment of the protein. However, it appears that the dianion of glutaric acid or α-ketoglutaric acid does not inhibit the enzyme. The inhibitor dissociation constants obtained with the dicarboxylic acids are consistent with this interpretation. Based on the K_i values determined from the total concentration of dicarboxylic acid, methylfumaric acid is a more effective inhibitor than fumaric acid. This is not consistent with the observations that methylsuccinic acid is a poorer inhibitor than succinic acid and that methylmaleic acid is a poorer inhibitor than maleic acid. The K_i values for fumaric and methylfumaric acids are essentially the same when calculated on the assumption that only the monanion form of each acid inhibits the enzyme.

Glutamic acid has pK_a values of approximately 2.19 and 4.25 (Sober, 1968). The profile obtained for V/K_m of glutamate plotted against pH is not consistent with only the zwitterion of glutamate binding to the enzyme. It appears that glutamate, unlike the dicarboxylic acid inhibitors, may also bind as a dianion (with a positively charged amino group). Possibly in the process of binding and/or in the formation of the Schiff base with the enzyme-bound pyridoxal phosphate the proton on the amino group of glutamate is transferred to the γ-carboxyl group of glutamate thus forming the monoanion. The decrease in V/K_m and in V below pH 4 would indicate the participation of an ionized group in binding and in catalysis. O'Leary *et al.* (1970) observed a twofold increase in V_{max} in going from pH 4 to 4.5 and attributed this to the influence of the γ-carboxyl group of glutamic acid.

It appears reasonable to assume that glutamate decarboxylase has the same specific binding sites for the carboxyl groups of L-glutamic acid as it has for the carboxyl groups of the competitive inhibitors. On the basis of the data obtained with the dicarboxylic acids, it may be postulated that the carbon chain of glutamic acid is oriented on the enzyme in the fully extended form to permit the maximum distance between the carboxyl groups. The binding of glutamic acid and its analogs to glutamine synthetase has been extensively studied, and apparently L-glutamic acid interacts with this enzyme with its carbon chain in the fully extended conformation (Meister, 1968; Gass and Meister, 1970).

Glutamate decarboxylases isolated from other sources also function specifically with L-glutamic acid. However, the specificity of inhibitor binding to bacterial glutamate decarboxylase differs from the binding of inhibitors to glutamate decarboxylase from other sources. Glutamate decarboxylase from the squash *Curcurbita moschata* is not inhibited by oxalic, malonic, succinic, or fumaric acids, but is inhibited by pimelic and maleic acids (Ohno and Okunuki, 1962). Glutamate decarboxylase from mouse brain is inhibited slightly by oxalic acid but not by 1 mM malonic, succinic, glutaric, adipic, or pimelic acids (Roberts and Simonsen, 1963). Unlike the bacterial glutamate decarboxylase, the mouse brain enzyme is weakly inhibited (12%) by 10 mM L-aspartic acid.

Most other amino acid decarboxylases that have been isolated show high substrate specificity. Among these are arginine decarboxylase (Blethen *et al.*, 1968) and histidine decarboxylase (Lawson and Quinn, 1967). Aspartate β-decarboxylase is highly specific in functioning as a decarboxylase; however, it also acts as a relatively nonspecific L-amino acid transaminase (Novogrodsky and Meister, 1964a) and catalyzes other β-elimination reactions (Tate *et al.*, 1969).

In determining the specificity of pyridoxal phosphate dependent enzymes, it may be of use to compare the binding requirements of those that function with the same amino acid substrate. Glutamate decarboxylase shows a much greater substrate specificity than does aspartate aminotransferase. Pig heart aspartate aminotransferase rapidly transaminates L-aspartic acid and L-glutamic acid, and very slowly transaminates a number of other L-amino acids (Jenkins, 1961; Novogrodsky and Meister, 1964b; Martinez-Carrion *et al.*, 1967). Dicarboxylic acids are competitive inhibitors of aspartate aminotransferase. The 4-carbon acids, such as maleate and succinate, are the most effective inhibitors of the mitochondrial transaminase (Michuda and Martinez-Carrion, 1970), whereas glutarate is a more effective inhibitor of the supernatant transaminase (Velick and Vavra, 1962; Jenkins, 1964; Haarhoff, 1969; Michuda and Martinez-Carrion, 1970). Apparently, at low pH values glutarate interacts through both its carboxylate groups with two positively charged groups on supernatant aspartate aminotransferase. However, at high pH values glutarate binds by only one carboxylate group (Jenkins and D'Ari, 1966). The two positively charged groups of aspartate aminotransferase that interact with glutarate must be closer together than the carboxyl binding sites on glutamate decarboxylase.

The inhibitory effect of chloride on the binding of glutaric acid to glutamate decarboxylase may be due to competition between the two compounds. The overall chloride effect on glutamate decarboxylation is not so simple as chloride alters both the glutamate K_m and V_{max} (Witte, 1971). Competition

between buffer anions and substrates or inhibitors has been observed previously with other enzymes. One of the positively charged sites on aspartate aminotransferase that interacts with the carboxylate group of glutarate is masked by the buffer anion which must be displaced by glutarate (Jenkins and D'Ari, 1966). Halide anions have been reported to inhibit mouse brain glutamate decarboxylase competitively with respect to the substrate (Susz *et al.*, 1966).

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