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Kinetic Studies of Glutamic Oxaloacetic Transaminase Isozymes*

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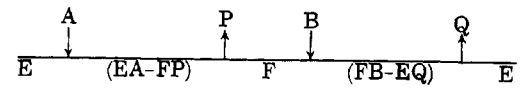
Initial velocity and product inhibition studies have been carried out on the anionic and cationic isozymes (separated by ion-exchange chromatography) of pig heart glutamic oxaloacetic transaminase at *p*H 7.4 and 37°, and Michaelis and inhibition constants for all reactants have been determined for both isozymes. The kinetic data are consistent only with a Ping Pong Bi Bi mechanism (an α-amino acid is bound to the enzyme and the corresponding α-keto acid is released, followed by binding of the second α-keto acid and subsequent release of the second α-amino acid). The measured kinetic constants for both isozymes are consistent with the predicted Haldane relationships for this mechanism. In addition, a dead-end α-ketoglutarate-enzyme complex is formed at high α-ketoglutarate concentrations. The dissociation constant for this dead-end complex is different for the two isozymes; the other kinetic constants are nearly the same. Substrates other than α-ketoglutarate do not show substrate inhibition.

The results of a number of workers (Schlenk and Fisher, 1947; O'Kane and Gunsalus, 1947; Nisonoff and Barnes, 1952; Meister *et al.*, 1954; Snell and Jenkins, 1959; Jenkins and Sizer, 1960; Turano *et al.*, 1960; Velick and Vavra, 1962) suggest that glutamic oxaloacetic transaminase has a Ping Pong Bi Bi mechanism (Mechanism 1):[1]



(1)

where E is the pyridoxal form of the enzyme, F is the pyridoxamine enzyme form, and A, B, P, and Q are L-aspartate, α-ketoglutarate, oxaloacetate, and L-glutamate, respectively. Recently, however, Evangelopoulos and Sizer (1963) proposed on the basis of spectral studies a sequential mechanism (both substrates must bind to the enzyme before release of either product).

Fleisher *et al.* (1960) observed in a crude pig heart preparation two electrophoretic fractions with trans-

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[1] Kinetic nomenclature used in this paper is that of Cleland (1963a,b).

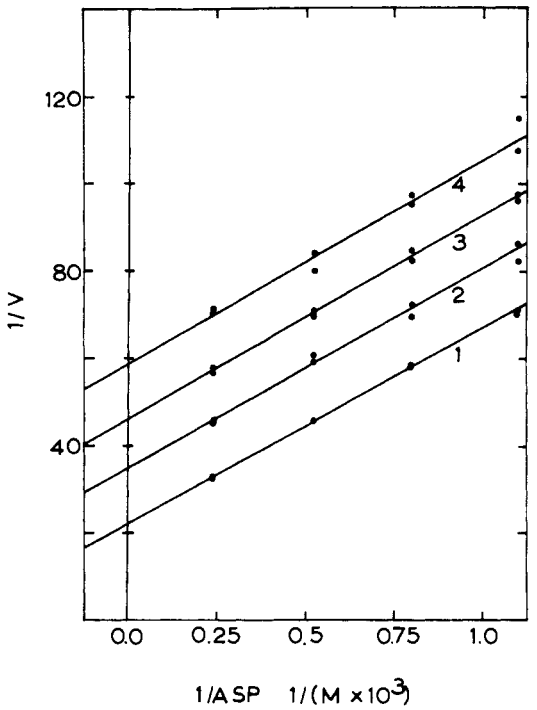

FIG. 1.—Initial velocity pattern for the anionic isozyme with L-aspartate as varied substrate. Velocity units are OD_{257}/minute. α-Ketoglutarate concentrations: (1) 0.333 mM, (2) 0.167 mM, (3) 0.111 mM, (4) 0.0833 mM. Enzyme concentration, 6.5 × 10^{-3} mg/ml.

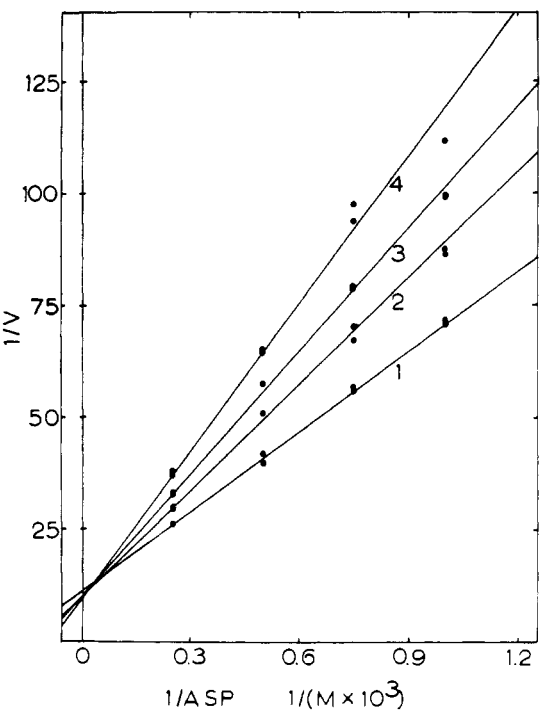

FIG. 3.—Reciprocal plots for the cationic isozyme with L-aspartate as varied substrate and high concentrations of α-ketoglutarate as substrate inhibitor. α-Ketoglutarate concentrations: (1) 6.0 mM, (2) 12.0 mM, (3) 18.0 mM, (4) 24.0 mM. Enzyme concentration, 7.6 × 10^{-4} mg/ml.

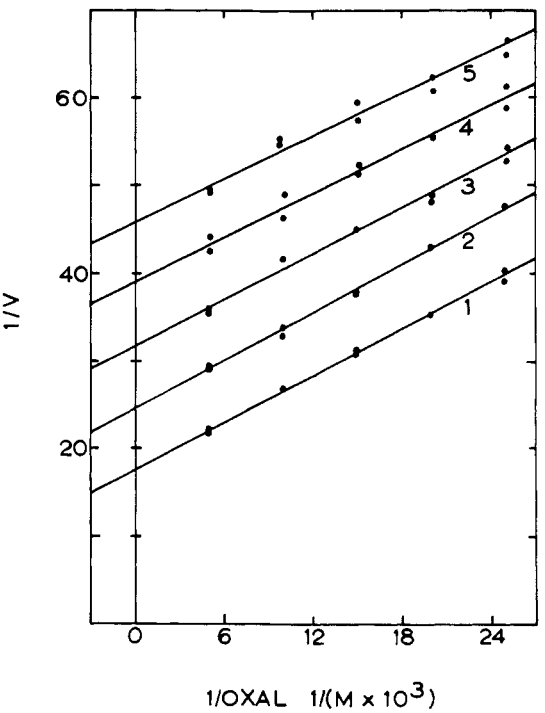

FIG. 2.—Initial velocity pattern for the cationic isozyme with oxaloacetate as varied substrate. L-Glutamate concentrations: (1) 11.31 mM, (2) 5.65 mM, (3) 3.77 mM, (4) 2.83 mM, (5) 2.26 mM. Enzyme concentration, 1.9 × 10^{-4} mg/ml.

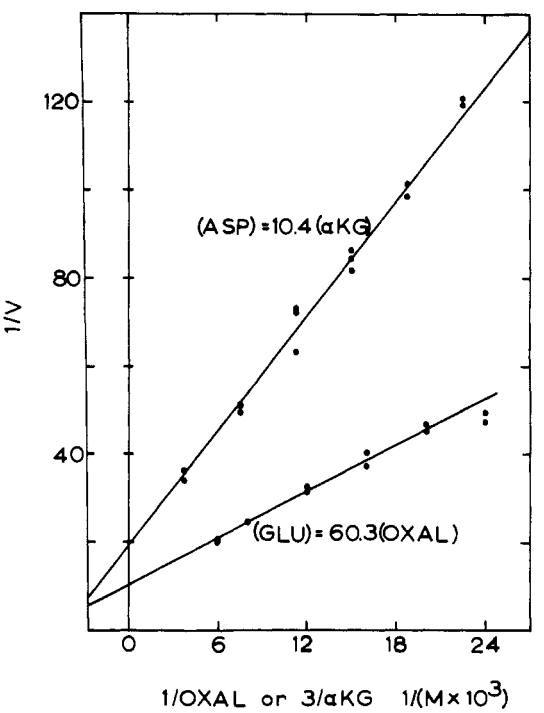

FIG. 4.—Reciprocal plots for the cationic isozyme with the concentrations of both substrates varied in a constant ratio. Enzyme concentration, 1.9 × 10^{-4} mg/ml.

aminase activity that have different apparent Michaelis constants for both α-ketoglutarate and L-aspartate. The work of Borst and Peeters (1961) showed that the cationic isozyme is present in the mitochondria and the anionic isozyme is in the cytoplasm. The present study was undertaken partly to investigate any differences in kinetic behavior between these two isozymes.

Various workers (Alberty, 1958; Fromm and Nelson, 1962; Cleland, 1963a,b) have described how the mechanisms of enzymic reactions can be distinguished by determination of the initial velocity and product inhibition patterns. The present study was designed to demonstrate the application of these methods to an enzyme for which the mechanism is presumably known, and to determine all the kinetic constants so that the complete rate equation could be written in terms of known constants.

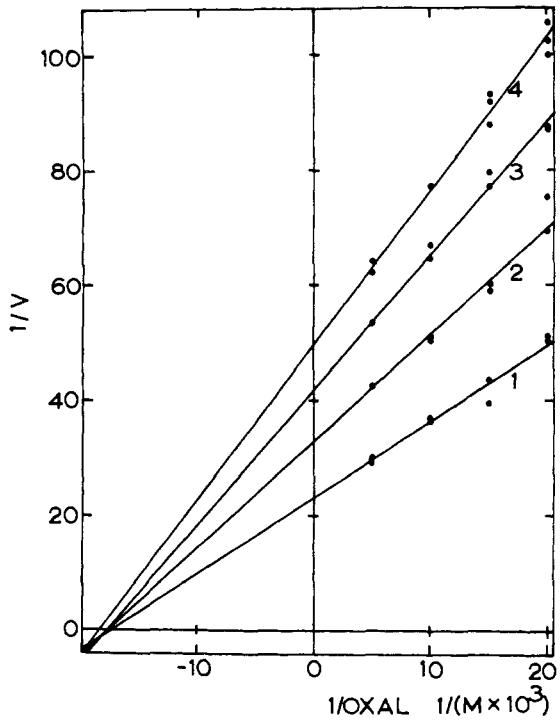

FIG. 5.—Reciprocal plots for the anionic isozyme with oxaloacetate as varied substrate and L-aspartate as inhibitor. L-Glutamate concentration, 12.5 mM. L-Aspartate concentrations: (1) 0, (2) 4.0 mM, (3) 8.0 mM, (4) 12.0 mM. Enzyme concentration, 2.43 × 10^{-3} mg/ml.

5.4, and centrifuged. The supernatant was placed on a column of CM-cellulose[2] (Bio-Rad Laboratories) which had previously been equilibrated with the same buffer. Additional 0.005 M sodium acetate, *p*H 5.4, was washed through the column until the optical density of the effluent at 280 mμ was equal to that of the buffer. About 60% of the enzymatic activity was not held on the column and was removed completely during the washing step (see below). Increasing the buffer concentration in an exponential gradient from 0.005 to 0.06 M eluted a protein peak between 0.035 and 0.045 M sodium acetate. This cationic isozyme was rechromatographed under the same conditions. Protein concentration in the effluent was estimated from the optical density at 280 mμ, assuming 1 mg/ml gives 1 OD_{280}/cm. Enzyme activity was determined by measuring oxaloacetate production at 280 mμ in a 3.0-ml reaction mixture containing 0.1 ml of the effluent, 0.2 mmole Tris, *p*H 8.3, 0.06 mmole L-aspartate, and 0.03 mmole α-ketoglutarate. Those fractions from the second CM-cellulose column which had a constant ratio of enzyme activity to OD_{280} were pooled and used for kinetic experiments.

The solution of the anionic isozyme, which was eluted from the first CM-cellulose column during washing with 0.005 M buffer, was dialyzed against several changes of 0.004 M potassium phosphate, *p*H 8.0, and adsorbed on a column of DEAE-cellulose (Schleicher and Schuell Co.) in the phosphate form. An increasing gradient from 0.004 to 0.1 M phosphate eluted the enzyme between 0.05 and 0.07 M phosphate. The fractions con-

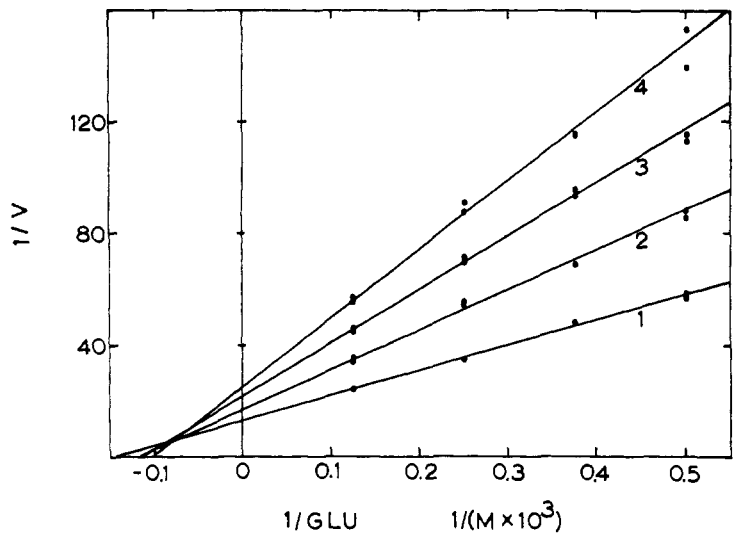

FIG. 6.—Reciprocal plots for the anionic isozyme with L-glutamate as varied substrate and α-ketoglutarate as inhibitor. Oxaloacetate concentration, 0.3 mM. α-Ketoglutarate concentrations: (1) 0, (2) 0.8 mM, (3) 1.6 mM, (4) 2.4 mM. Enzyme concentration, 3.65 × 10^{-3} mg/ml.

METHODS AND MATERIALS

Reagents.—L-Glutamic acid, L-aspartic acid, α-ketoglutaric acid, and *cis*-enol oxaloacetic acid (all of 99–100% purity) were obtained from Mann Research Laboratories. Except for oxaloacetic acid, neutral stock solutions of the potassium salts of the substrates were stored frozen. Oxaloacetic acid was dissolved and neutralized with potassium hydroxide immediately before each experiment, and the solution was kept at 0° during the experiment to minimize decarboxylation.

Enzyme.—Glutamic oxaloacetic transaminase was prepared from pig heart according to the method of Jenkins *et al.* (1959) through the ammonium sulfate fractionation. The redissolved enzyme was dialyzed against several changes of 0.005 M sodium acetate, *p*H taining most of the activity were combined and dialyzed against several changes of 0.01 M ammonium acetate, *p*H 7.0, and absorbed on another column of DEAE-cellulose in the acetate form. The enzyme was eluted near the upper limit of a gradient running from 0.04 to 0.1 M ammonium acetate, *p*H 7.0. The pooled fractions containing activity were concentrated by alternate pervaporation and dialysis against 0.02 M ammonium acetate, *p*H 7.0. Both isozymes were stable at 4° for at least a year.

Apparatus.—The reaction was followed by measuring the appearance or disappearance of oxaloacetate at

[2] Abbreviations used in this work: CM, carboxymethyl; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)-aminomethane.

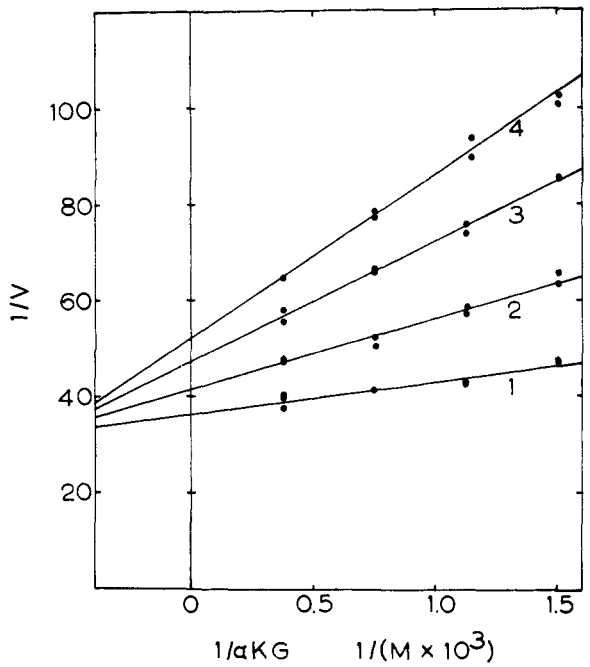

FIG. 7.—Reciprocal plots for the anionic isozyme with α-ketoglutarate as varied substrate and L-glutamate as inhibitor. L-Aspartate concentration, 10.0 mM. L-Glutamate concentrations: (1) 0, (2) 8.0 mM, (3) 16.0 mM, (4) 24.0 mM. Enzyme concentration, 6.5×10^{-3} mg/ml.

257 mμ using a Beckman DU monochromator with a deuterium lamp, a Gilford Model 200 optical density converter, and a 10-mv recorder equipped with an adjustable zero and a multispeed chart drive. Full scale sensitivity of 0.1–0.2 OD units, and chart speeds from 0.25 to 2.5 in./minute were used.

Most kinetic experiments were carried out using a 3.0-ml reaction mixture in 10 mm silica cuvets which were warmed to 37° in metal water-jacketed incubation boxes before each experiment. The prewarmed cuvets were filled with solutions which had been preincubated at 37°, and were then kept in the incubation boxes until used. The reaction cell compartment was also kept at 37° with thermospacers. All components of the reaction mixture except enzyme and oxaloacetate (when used) were placed in the prewarmed cuvets. Oxaloacetate solutions were kept at 0°, and 0.1 ml was added to cuvets just prior to the addition of enzyme. The reaction was started by addition of 0.1 ml of enzyme with an adder-mixer (Boyer and Segal, 1954). Initial velocities were determined by measuring the tangent to the recorded curve extrapolated to the time of addition of the enzyme.

Reaction Conditions.—The reaction was followed at 257 mμ, which is the maximum of the difference spectrum of oxaloacetate and α-ketoglutarate. However, the reaction rate showed an initial lag which made estimations of initial velocities difficult. This lag has been noted by other workers (Nisonoff and Barnes, 1952; Turano *et al.*, 1960; Velick and Vavra, 1962), when the reaction was followed at wavelengths greater than 250 mμ, where only the enol form of oxaloacetate absorbs appreciably (Kornberg *et al.*, 1948). Since the keto tautomer is probably the enzymatically active form, a lag would result if the enolization rate did not greatly exceed the reaction rate. This lag should be decreased either by raising the enzolization rate or by lowering the reaction rate. Both increasing the buffer concentration (enolizations are acid- and base-catalyzed) and decreasing the enzyme concentration greatly shortened the lag. Sodium arsenate, *p*H 7.4, lessened the lag to a greater extent than phosphate buffer of the same *p*H and ionic strength. All reaction mixtures for kinetic experiments were therefore made 0.1 M in arsenate, *p*H 7.4. For use, the enzyme was diluted with a solution containing 5 mg/ml Carbowax 1500 (a polyethylene glycol with a molecular weight of 500–600) and 0.025 M sodium arsenate, *p*H 7.4; these dilutions were stable for several hours at 0°. For the range of enzyme concentrations used in this work, a constant extent of reaction was observed when the product of enzyme concentration and time of incubation was held constant. Since the lowest reactant concentrations used always exceeded the enzyme concentration (based on a molecular weight of 110,000, Jenkins *et al.*, 1959) by a factor of at least 500, the existence of steady-state conditions was assumed.

Data Processing.—Reciprocal velocities were plotted graphically against the reciprocals of substrate concentrations and an occasional point which deviated greatly from a linear relationship was discarded. The remaining data were fitted to equation (2) using a least

$$v = \frac{VS}{K + S} \qquad (2)$$

squares method and assuming equal variance for the velocities (Wilkinson, 1961). All calculations were performed by a digital computer using a Fortran program which provides values of K, V, K/V, $1/V$, the standard errors of their estimates, and weighting factors (reciprocals of squares of standard errors) for further analysis.[3] Slopes (K/V) and intercepts ($1/V$) were then plotted graphically against inhibitor concentration for inhibition experiments and against reciprocal concentration of the second substrate for initial velocity experiments. All replots were linear. When the difference between slopes or intercepts was not great, t tests were made to determine the significance of the variation. On the basis of the replots and t tests, the proper pattern was chosen for each experiment and all data points used in the first analysis were fitted to equations relating the observed velocities to substrate and inhibitor concentration (Cleland, 1963c). Data for the Ping Pong initial velocity equation were fitted to equation (3), for linear noncompetitive inhibition to equation (4), and for linear competitive inhibition to equation (5).

$$v = \frac{V_1AB}{K_aB + K_bA + AB} \qquad (3)$$

$$v = \frac{VA}{K(1 + I/K_{is}) + A(1 + I/K_{ii})} \qquad (4)$$

$$v = \frac{VA}{K(1 + I/K_{is}) + A} \qquad (5)$$

RESULTS

Initial Velocity Studies.—At low substrate concentrations, plots of reciprocal velocities against the reciprocal concentration of one substrate at various fixed levels of the second substrate were parallel lines. Replots of intercepts versus reciprocal concentrations of the nonvaried substrate were always linear. Figure 1 depicts one such family of lines with L-aspartate as the variable substrate. The same pattern was obtained in four experiments with the cationic and in four experiments with the anionic isozyme. Figure 2 shows an initial velocity pattern obtained with oxaloacetate as the variable substrate. Three experiments with the cationic and two experiments with the anionic isozyme gave the same pattern.

[3] Copies of the Fortran programs used in this work may be obtained from the authors.

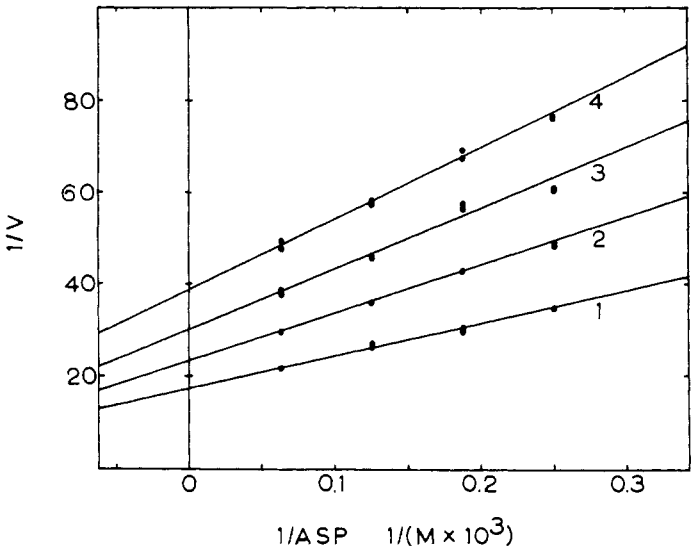

Fig. 8.—Reciprocal plots for the anionic isozyme with L-aspartate as varied substrate and oxaloacetate as inhibitor. α-Ketoglutarate concentration, 1.33 mM. Oxaloacetate concentrations: (1) 0, (2) 0.08 mM, (3) 0.16 mM, (4) 0.24 mM. Enzyme concentration, 1.27×10^{-2} mg/ml.

Several attempts were made to perform similar experiments with lower concentrations of α-ketoglutarate and L-aspartate than those used in Figure 1. When 100-mm cuvets were used at 25° with 0.04 M arsenate, *p*H 7.4, α-ketoglutarate from 0.04 to 0.3 mM, and L-aspartate from 0.2 to 1.7 mM (these conditions are essentially those of Figure 1 of Velick and Vavra, 1962), the initial lag was so great that accurate determination of initial velocities was impossible, even at low enzyme concentrations. When the buffer concentration was raised to 0.1 M the initial rates could be easily determined and the same pattern was obtained as that shown in Figure 1.

When L-asparate was the variable substrate high concentrations of α-ketoglutarate produced linear competitive inhibition (Fig. 3). Two experiments with the cationic and three with the anionic isozyme gave the same inhibition pattern. In experiments designed to detect substrate inhibition by L-asparate, L-glutamate, or oxaloacetate, concentrations of these substrates higher than those used in any of the other experiments reported in this paper were tried with low concentrations of α-ketoglutarate, oxaloacetate, or L-glutamate, respectively. At 0.2 mM L-glutamate, substrate inhibition by concentrations of oxaloacetate as high as 15 mM was not observed for either isozyme. One hundred twenty mM L-glutamate was not inhibitory with oxaloacetate concentrations of 0.05 mM for the cationic and 0.1 mM for the anionic isozyme. High concentrations of L-aspartate (144 mM for the cationic and 48 mM for the anionic isozyme) were not inhibitory with 0.0833 mM α-ketoglutarate.

The ratio of maximum velocities in forward and reverse directions was determined in a single experiment by varying substrate concentrations together in a constant ratio for both the forward and reverse reactions. If $B = xA$ is substituted into equation (3), for instance, the resulting equation has the form of equation (2), with $K = (K_a + K_b/x)$ and $V = V_1$. The straight lines resulting from plots of reciprocal velocity vs. the reciprocal of one substrate concentration are shown for such an experiment in Figure 4. Three experiments with the cationic and three with the anionic isozyme gave similar straight lines.

Product Inhibition Studies.—When oxaloacetate was the variable substrate, L-aspartate was a linear noncompetitive inhibitor (Fig. 5). Three experiments using the anionic and two using the cationic isozyme gave the same pattern. Figure 6 depicts linear noncompetitive inhibition by α-ketoglutarate with L-glutamate as the varied substrate. Three experiments with the cationic and four with the anionic isozyme gave the same pattern. With α-ketoglutarate as the variable substrate, L-glutamate was a linear noncompetitive inhibitor (Fig. 7). Four experiments using the cationic and three using the anionic isozyme gave the same pattern. Figure 8 shows linear noncompetitive inhibition by oxaloacetate when L-aspartate was the variable substrate. Two experiments using the cationic and two with the anionic isozyme gave the same pattern.

With L-glutamate as the variable substrate, L-aspartate gave linear competitive inhibition (Fig. 9). Three experiments with the cationic and two with the anionic isozyme gave the same pattern. Two experiments with the cationic and three with the anionic isozyme showed α-ketoglutarate to be a linear competitive inhibitor of oxaloacetate (Fig. 10). Figure 11 depicts linear competitive inhibition of L-aspartate by L-glutamate. Three experiments using the cationic and two using the anionic isozyme gave the same pattern. When α-ketoglutarate was the variable substrate, oxaloacetate was a linear competitive inhibitor, as shown in Figure 12. Four experiments using the cationic and three using the anionic isozyme gave the same pattern.

Discussion

The initial velocity patterns described in this paper and those obtained by Turano *et al.* (1960) and by Velick and Vavra (1962) show that, at low substrate concentrations, both isozymes of glutamic oxaloacetic transaminase obey the initial velocity equation:

$$\frac{1}{v} = \frac{1}{V_1}\left(\frac{K_a}{A} + \frac{K_b}{B} + 1\right) \qquad (6)$$

as shown by the parallel lines obtained when $1/v$ is plotted against $1/A$ or $1/B$ at various constant concentrations of B or A, respectively. If both substrates had to be bound to the enzyme before release of a product, the term $K_{ia}K_b/AB$ would be added to the terms in the parentheses of equation (6) and the initial velocity pattern would be a family of lines intersecting to the left of the vertical axis (Cleland, 1963b). Also, when the concentrations of both substrates are

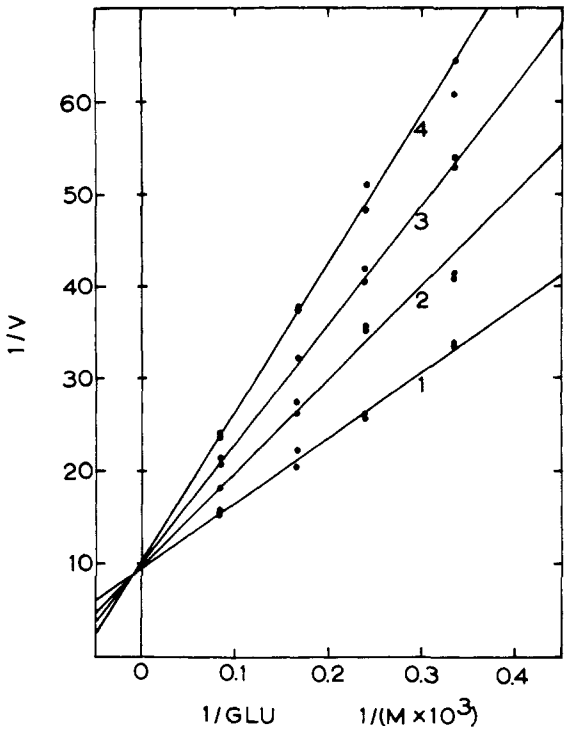

FIG. 9.—Reciprocal plots for the cationic isozyme with L-glutamate as varied substrate and L-aspartate as inhibitor. Oxaloacetate concentration, 0.5 mM. L-Aspartate concentrations: (1) 0, (2) 1.5 mM, (3) 3.0 mM, (4) 4.5 mM. Enzyme concentration, 2.55×10^{-4} mg/ml.

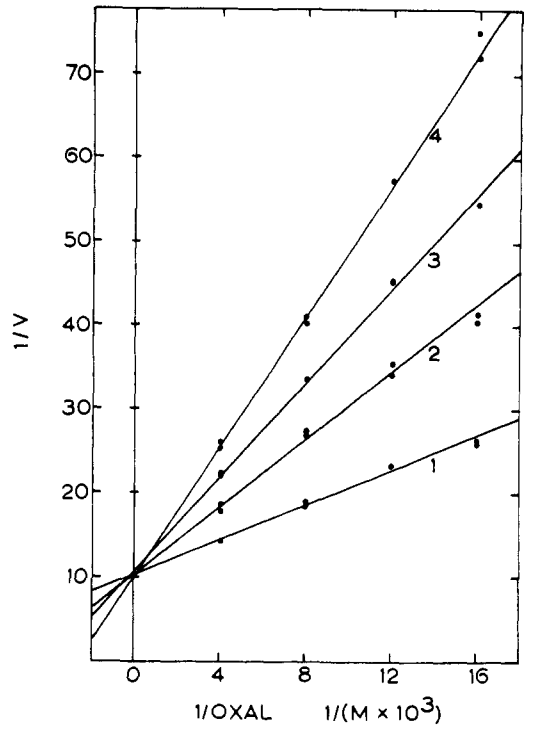

FIG. 10.—Reciprocal plots for the anionic isozyme with oxaloacetate as varied substrate and α-ketoglutarate as inhibitor. L-Glutamate concentration, 37.3 mM. α-Ketoglutarate concentrations: (1) 0, (2) 0.591 mM, (3) 1.182 mM, (4) 1.773 mM. Enzyme concentration, 4.86×10^{-3} mg/ml.

varied in a constant ratio, the presence of the $K_{ia}K_b/AB$ term would produce curved reciprocal plots rather than the straight lines obtained in Figure 4. These data are consistent only with a basic Ping Pong Bi Bi mechanism; that is, release of a product from the enzyme must occur between the times of addition of the two substrates to the enzyme (mechanism 1). The rate equation for this mechanism is (Cleland, 1963a):

$$v = \frac{V_1(AB - PQ/K_{eq})}{K_bA + K_aB + AB + \frac{K_{ia}K_bP}{K_{ip}} + \frac{K_aK_{ib}Q}{K_{iq}} + \frac{K_aK_{ib}PQ}{K_pK_{iq}} + \frac{K_bAP}{K_{ip}} + \frac{K_aBQ}{K_{iq}}} \quad (7)$$

At high α-ketoglutarate concentrations, Velick and Vavra (1962) demonstrated substrate inhibition which they attributed to formation of a dead-end α-ketoglutarate-pyridoxal enzyme complex. Figure 3 shows that the inhibition is linear competitive with L-aspartate. The dead-end complex is therefore with enzyme form E (pyridoxal form) of mechanism 1. The complete rate equation including this inhibition is the same as that given in equation (7), except that the B and P terms in the denominator are multiplied by $(1 + B/K_I)$, where K_I is the dissociation constant of the dead-end complex.

Equations for product inhibition by P or Q with A as the variable substrate are obtained from equation (7) by setting the concentration of Q or P, respectively, equal to zero:

$$\frac{1}{v} = \frac{K_a}{V_1}\left[1 + \frac{P}{\left(\frac{K_aK_{ip}}{K_bK_{ia}}\right)B}\right]\frac{1}{A} + \frac{1}{V_1}\left(1 + \frac{K_b}{B}\right) \times \left[1 + \frac{P}{K_{ip}\left(1 + \frac{B}{K_b}\right)}\right] \quad (8)$$

$$\frac{1}{v} = \frac{K_a}{V_1}\left[1 + \frac{Q}{\left(\frac{K_{iq}}{1 + K_{ib}/B}\right)}\right]\frac{1}{A} + \frac{1}{V_1}\left(1 + \frac{K_b}{B}\right) \quad (9)$$

Equation (8) describes linear noncompetitive inhibition, and equation (9) describes linear competitive inhibition. Equations analogous in form to (8) and (9) can be derived for the cases where B is the variable substrate and Q or P is the inhibitor, where P is the variable substrate and A or B is the inhibitor, and where Q is the variable substrate and B or A is the inhibitor. Mechanism 1 thus predicts linear noncompetitive inhibition when a keto acid is variable substrate and an amino acid is the product inhibitor, or vice versa; and linear competitive inhibition when one amino acid is variable substrate and the other is a product inhibitor, or when one keto acid is variable substrate and the other is the inhibitor. The patterns shown in Figures 5–12 are consistent with these predictions; thus all of the product inhibition patterns for both isozymes are those expected on the basis of mechanism 1.

The experiments reported in this paper allow calculations of all the kinetic constants for both isozymes. Michaelis constants were obtained directly from fits to equation (3), and maximum velocity ratios from fits to equation (2) of data obtained when the concentrations of both substrates were varied together in a constant ratio. The dissociation constant for the dead-end α-ketoglutarate-enzyme complex was obtained from fits to equation (5) of data from experiments like that shown in Figure 3 ($K_{is} = K_I$ in this case). Weighted averages of the values obtained in different experiments are shown in Table I.

Velick and Vavra (1962) reported Michaelis constants two to five times smaller than those reported in Table I, while Turano *et al.* (1960) reported Michaelis constants for L-aspartate and α-ketoglutarate which

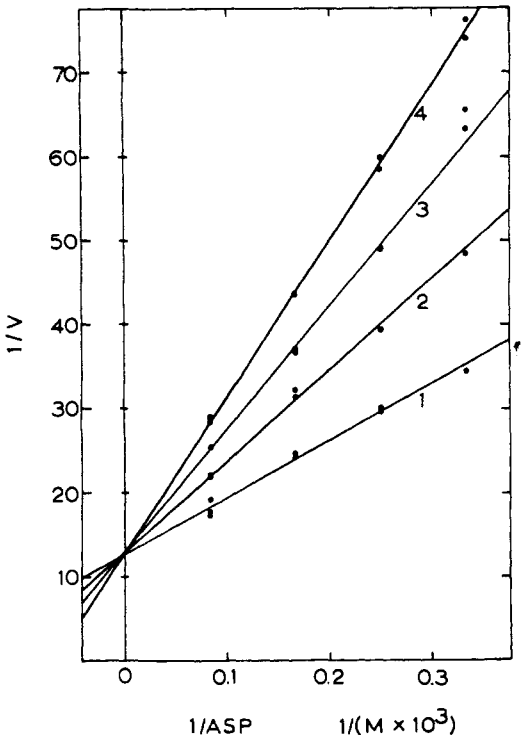

FIG. 11.—Reciprocal plots for the cationic isozyme with L-aspartate as varied substrate and L-glutamate as inhibitor. α-Ketoglutarate concentration, 12.0 mM. L-Glutamate concentrations: (1) 0, (2) 8.0 mM, (3) 16.0 mM, (4) 24.0 mM. Enzyme concentration, 3.8×10^{-4} mg/ml.

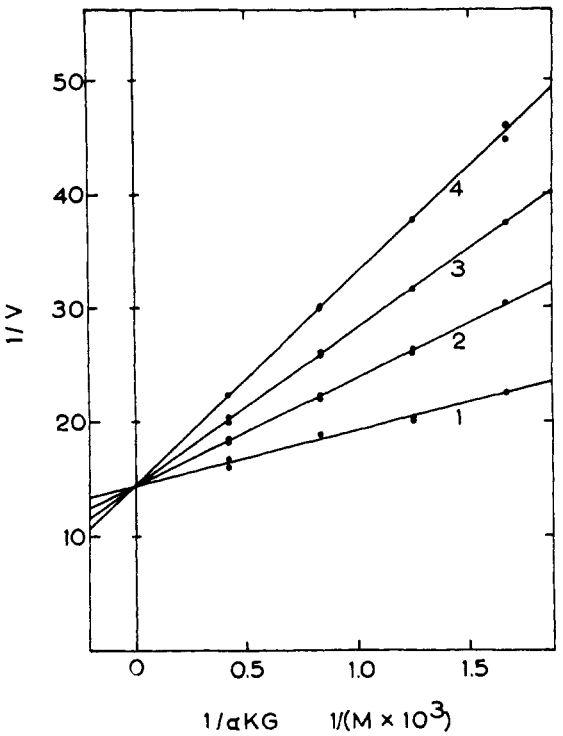

FIG. 12.—Reciprocal plots for the anionic isozyme with α-ketoglutarate as varied substrate and oxaloacetate as inhibitor. L-Aspartate concentration, 40.0 mM. Oxaloacetate concentrations: (1) 0, (2) 0.04 mM, (3) 0.08 mM, (4) 0.12 mM. Enzyme concentration, 8.3×10^{-3} mg/ml.

TABLE I
KINETIC CONSTANTS FOR GLUTAMIC OXALOACETIC TRANSAMINASE[a]

	Cationic Isozyme	Anionic Isozyme
K_a	3.9 ± 0.2[b]	4.4 ± 0.2
K_a[c]	3.6 ± 0.4	
K_b	0.43 ± 0.02	0.38 ± 0.01
K_b[c]	0.25 ± 0.03	
K_p	0.088 ± 0.003	0.095 ± 0.003
K_q	8.9 ± 0.3	9.6 ± 0.3
V_1/V_2[d]	0.51 ± 0.02	0.50 ± 0.02
K_{is}	3.48 ± 0.09	3.9 ± 0.1
K_{ib}	0.71 ± 0.02	0.73 ± 0.01
K_{ip}	0.050 ± 0.001	0.048 ± 0.001
K_{iq}	8.4 ± 0.2	10.7 ± 0.2
K_I[e]	16.6 ± 0.6	26.5 ± 0.7

[a] Michaelis and inhibition constants are mM. Identifying subscripts: a, L-aspartate; b, α-ketoglutarate; p, oxaloacetate; and q, L-glutamate. [b] These standard errors are a measure of the precision of the experiments rather than of the accuracy of the values. [c] Reaction conditions were those of Velick and Vavra (1962) with 0.1 M buffer. [d] V_1 is the maximum velocity for formation of oxaloacetate; V_2 is the maximum velocity in the reverse reaction. [e] Dissociation constant of dead-end α-ketoglutarate-enzyme complex.

were 1.2 and 9 times larger than those reported here. Although the low buffer concentration used by Velick and Vavra did not allow accurate rate determinations with our instrumentation, essentially the same Michaelis constants for α-ketoglutarate and L-aspartate were obtained when the conditions of Velick and Vavra (1962) were used except for a higher buffer concentration (Table I).

Comparison of equations (5) and (9) shows that $K_{is} = K_{iq}/(1 + K_{ib}/B)$. The four competitive inhibition patterns thus provide four equations with which to calculate the four inhibition constants K_{ia}, K_{ib}, K_{ip}, and K_{iq}. These equations can be solved simultaneously, or, since the concentrations of the nonvaried substrates were held high in these experiments, the values of K_{is} are very nearly equal to an inhibition constant in each case, so that a process o successive approximation could be used. Additional corrections were necessary when α-ketoglutarate was present at high concentrations as the nonvaried substrate (in other experiments the concentrations o α-ketoglutarate were low enough that substrate inhibition could be ignored). In this case, the value of K_{iq} obtained as outlined above was divided by $(1 + B/K_I)$ to give the correct value of K_{iq}, and this corrected value was used in further calculations. Weighted averages of K_{is} from different experiments were used for these calculations, and the resulting inhibition constants are shown in Table I.

Comparison of equations (4) and (8) shows that $K_{is} = K_a K_{ip} B/K_{ia} K_b$ and $K_{ii} = K_{ip}(1 + B/K_b)$. Calculation of the inhibition constants by equations of this type using data from the noncompetitive product inhibitions is not as precise as the methods outlined above; these equations were thus used only to check for consistency between observed K_{is} or K_{ii} values and the corresponding calculated values. In general the agreement was satisfactory.

When the rate equation for mechanism 1 is written in the form of equation (7), four additional equations relating the equilibrium constant and the various kinetic constants (Haldane relationships) must also hold (Cleland, 1963a). These relationships are not invalidated by dead-end combination of B with E. The values of the equilibrium constant calculated by these equations from the kinetic constants in Table I are shown in Table II for both isozymes. Agreement of the calculated values with the observed equilibrium constant is excellent; thus the steady-state kinetic behavior of both isozymes at *p*H 7.4 can be fully de-

TABLE II
VALUES OF THE EQUILIBRIUM CONSTANT CALCULATED FROM HALDANE RELATIONSHIPS[a]

$K_{eq} =$	$\left(\frac{V_1}{V_2}\right)^a \frac{K_p K_q}{K_a K_b}$	$= \frac{V_1 K_p K_{iq}}{V_2 K_a K_{ib}}$	$= \frac{V_1 K_{ip} K_q}{V_2 K_{ia} K_b}$	$= \frac{K_{ip} K_{iq}}{K_{ia} K_{ib}}$
Cationic isozyme	0.14 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
Anionic isozyme	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.18 ± 0.01

[a] The value of $K_{eq} = \frac{[\text{oxaloacetate}][\text{L-glutamate}]}{[\alpha\text{-ketoglutarate}][\text{L-aspartate}]}$ observed in this laboratory at *p*H 7.4, 37°, $\mu = 0.13$, was 0.16–0.17.

scribed by equation (7), with the kinetic constants replaced by their numerical values from Table I.

The kinetic data obtained in this study are completely consistent with a Ping Pong Bi Bi mechanism for both isozymes of glutamic oxaloacetic transaminase, and the recent suggestion of Evangelopoulos and Sizer (1963) that the enzyme forms a ternary complex with both amino acid and keto acid prior to exchange of amino and keto groups cannot be valid. Moreover, the kinetic constants for the two isozymes are nearly the same; the only significant difference is between the dissociation constants of the dead-end α-ketoglutarate-enzyme complexes. Thus the arrangement in the vicinity of the active site is probably the same for both isozymes, with the differences in charge resulting from altered amino acid composition in other parts of the molecule.

It is interesting to note that for both isozymes only α-ketoglutarate is capable of combining with both stable enzyme forms with resulting substrate inhibition. Velick and Vavra (1962) showed by spectral binding studies that complexes are formed with the pyridoxal enzyme by both α-ketoglutarate and oxaloacetate with dissociation constants of 3 and 6.7 mM, respectively. Comparison of these values with the kinetic data reported here shows that these complexes are not formed at the active site and do not influence the enzymatic reaction. Substrate inhibition by only α-ketoglutarate is not characteristic of all transaminases, however. Bulos and Handler (1963) have reported for glutamic alanine transaminase that substrate inhibition is given by both amino acids, but not by the keto acids.

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