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Glutamic-Oxaloacetic Transaminase of Cauliflower

2. KINETICS AND MECHANISM OF ACTION

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The kinetics of glutamic-oxaloacetic transaminase have been investigated with crude preparations from pig heart (Nisonoff & Barnes, 1952) and maize-root tips (Cook, 1957). In both cases, the kinetic data were explained in terms of a ternary complex formed between the enzyme and its two substrates. Nisonoff & Barnes derived a rate law by a modification of the treatment introduced by Van Slyke & Cullen (1914). Their assumption of irreversible combination of enzyme with substrate

could conceivably be valid for an irreversible reaction, but can hardly be justified for the readily reversible transamination. The general case of the mechanism proposed by Cook when treated by the steady-state assumption leads to a rate equation of quadratic form, but when treated by the equilibrium assumption leads to a rate equation which gives a linear Lineweaver-Burk plot (Haldane, 1930).

Both treatments are unsatisfactory in that they fail to recognize the participation of pyridoxal phosphate and pyridoxamine phosphate in transamination. On the basis of model experiments by Snell (1945), Schlenk & Fisher (1947) proposed a

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binary mechanism for transamination which may be represented as follows:

Sum:

where E_{PL} and E_{PM} represent the pyridoxal and pyridoxamine forms of the enzyme respectively. The rate law for this mechanism has been given by Alberty (1956). More recently, spectrophotometric evidence giving strong support to the binary mechanism has been obtained (Snell & Jenkins, 1959). In this paper an attempt is made to distinguish between the two mechanisms on the basis of enzyme kinetics, and some data on the cofactor requirements of a plant transaminase are reported.

METHODS AND MATERIALS

Materials. All the chemicals used except α -oxoglutarate were commercial preparations. The α -oxoglutarate was prepared by the method described in *Organic Syntheses* (26, 42, 1946), and had a melting point of 112° (uncorr.); the sample gave a negligible oxygen uptake when added to an active succinoxidase preparation from pigeon-breast muscle. Malic dehydrogenase was purified from pig heart by the method of Wolfe & Neilands (1956). Glutamic-oxaloacetic transaminase was prepared from cauliflower florets as described in the preceding paper (Ellis & Davies, 1961).

Measurement of enzyme activity. Initial velocities were determined by measuring the change in $E_{280\text{ m}\mu}$ due to the

production or disappearance of oxaloacetate. Measurements were made in a Unicam SP. 500 spectrophotometer fitted with a constant-temperature cell-holder, readings being taken at 5–10 sec. intervals for 2–3 min. after the addition of the last component to the assay system. On some occasions the production of oxaloacetate was determined by coupling with malic dehydrogenase and measuring the oxidation of reduced diphosphopyridine nucleotide at 340 m μ . The dehydrogenase preparation used contained a trace of transaminase, whose activity was allowed for by control assays. All assays were performed in potassium phosphate buffer (0.2M). A pH of 7.5 was used for the evaluation of kinetic constants, and a pH of 7.0 for other experiments, the pH-activity curve being almost flat between these values (Fig. 1). Kinetic constants were measured at 30° in cells of 1 cm. and 4 cm. light-paths; the effects of activators and inhibitors were studied at 20° or at 30°.

In the standard assay, L-aspartate (150 μ moles), potassium phosphate buffer (pH 7.0, 0.2M) and enzyme were incubated for 10 min. at 20° before the addition of α -oxoglutarate (75 μ moles) to start the reaction in a final volume of 3 ml. Under these conditions a unit of enzyme activity is defined as the amount of enzyme that will cause an increase in $E_{280\text{ m}\mu}$ of 0.01/min. Specific activity is defined as units/mg. of protein.

RESULTS AND DISCUSSION

Activation by pyridoxal 5-phosphate and pyridoxamine 5-phosphate

The activity in a crude extract of cauliflower was not appreciably increased by either of the cofactors, but a twofold to threefold activation was observed after ammonium sulphate fractionation. The time course of the activation was studied by incubating the partially resolved enzyme at 20° with an equilibrated mixture of aspartate (150 μ moles), pyridoxal phosphate (140 μ M) and phosphate buffer (pH 7.5, 0.2M) for measured time intervals before the addition of α -oxoglutarate (75 μ moles) to start the reaction. The plots of E against time were slightly convex to the time axis, presumably due to the continued combination of cofactor and apoenzyme during the period of assay. The initial velocity was taken to be the tangent to the curve. The results (Fig. 2) show that to obtain full activation an incubation period of approximately 10 min. at 20° is required. Similar experiments with pyridoxamine phosphate demonstrated that the rate of activation was much slower than that observed with an equal concentration of the pyridoxal form of the cofactor. Increasing the concentration of pyridoxamine phosphate increased the rate of activation (Fig. 2).

Fig. 1. pH-activity curve of cauliflower glutamic-oxaloacetic transaminase. Transaminase purified to step 2 (Ellis & Davies, 1961) was diluted with cold water until the concentration of phosphate buffer was 5 mM at pH 7.0. Neutralized L-aspartate (0.5 ml., 150 μ moles), 0.5 ml. of neutralized α -oxoglutarate (75 μ moles) and 1.5 ml. of potassium phosphate buffer (0.2M, at the required pH) were placed in a 1-cm. silica cell. Enzyme (0.5 ml.) was added to start the reaction, and the increase in $E_{280\text{ m}\mu}$ measured for 3 min. In this way, the effects of pH on the stability of the enzyme were minimized. The pH was checked after the assay had been completed. Initial velocity equals the increase in E /min.

Purified preparations of enzyme activated with either of the two forms of cofactor were dialysed against a running stream of phosphate buffer (0.01M, pH 7.0) at 3–4° at a rate of 1.2 l./hr. for periods up to 48 hr. No increase in activity was produced by incubating these dialysed solutions with either cofactor before assay. These results are similar to those which Meister, Sober & Peterson (1954) obtained with the transaminase from pig heart.

Fig. 2. Activation of transaminase by pyridoxal 5-phosphate and pyridoxamine 5-phosphate. Samples (0.1 ml.) of 67-fold purified transaminase were placed in a 1-cm. silica cell with 0.1 ml. of cofactor and 0.5 ml. of L-aspartate (150 μ moles) in phosphate buffer (0.2M, pH 7.5); after incubation at 20° for various intervals of time, α -oxo-glutarate (75 μ moles) was added to bring the volume to 3 ml. The rise in $E_{280\text{ m}\mu}$ was measured for 3 min. Initial velocity equals the rise in E /min. The concentration of pyridoxal 5-phosphate in the incubation mixture was 140 μ M (●); that of pyridoxamine 5-phosphate was 0.9 mM (○) and 140 μ M (▲). The square on the ordinate indicates the initial velocity in the absence of added cofactor.

Inhibition by isoniazid

The anti-tubercular drug isoniazid (isonicotinic acid hydrazide) inhibits a number of enzymes known to require pyridoxal phosphate for activity. Evidence has been presented which supports the view that inhibition of these enzymes is the result of interaction between the aldehyde group of pyridoxal phosphate and the hydrazide (Davison, 1956; Hicks & Cymerman-Craig, 1957).

A preparation of the enzyme, purified to step 2 (see preceding paper), was incubated with isoniazid (10 mM) for 30 min. at 37°, then cooled and diluted fourfold with phosphate buffer before the activity was measured under standard conditions. This activity was compared with that of controls in which isoniazid was omitted, and others in which the enzyme was incubated with pyridoxal phosphate before assay. A correction was made for the non-enzymic reaction between isoniazid and α -oxo-glutarate which leads to an increase in $E_{280\text{ m}\mu}$. The results (Table 1) show that isoniazid inhibits the transaminase, and that the inhibition is relieved by pyridoxal phosphate but not by pyridoxal. Inhibition was not observed when the incubation was carried out at 20°.

Relationship between initial velocity and enzyme concentration

Crude and purified preparations of the enzyme showed a linear relationship between low initial velocities and enzyme concentration, but the velocities at higher enzyme concentrations were less than those expected by extrapolation of the lower values. The non-linearity was observed when the transamination reaction was assayed in either direction, and the extent of linearity was greater in the purified than in crude preparations (Fig. 3). The enzyme was not inhibited by the addition of boiled enzyme, and prolonged dialysis did not relieve the non-linearity.

Table 1. *Inhibition by isoniazid*

Enzyme purified to step 2 (Ellis & Davies, 1961) was incubated with isoniazid (10 mM) for 30 min. at 37° in phosphate buffer (0.2M, pH 7.0); after being cooled in ice, and diluted fourfold with buffer, 0.5 ml. samples were assayed in 1-cm. silica cells by the standard method (see Methods and Materials section) with and without prior incubation with pyridoxal 5-phosphate (0.1 mM) or pyridoxal (0.1 mM) for 10 min. at 20°. The ratio α -oxoglutarate/isoniazid in the assay cell was 60. The non-enzymic reaction between isoniazid and α -oxoglutarate gave an apparent transaminase activity of 2.8 units; this figure must be subtracted from the figures given below for the isoniazid-treated enzyme to give the true number of transaminase units.

Treatment	Units/0.5 ml.		
	Without pyridoxal 5-phosphate	With pyridoxal 5-phosphate	With pyridoxal
3° for 30 min.	14.7	19.0	—
37° for 30 min.	13.5	18.8	—
37° for 30 min. + isoniazid (10 mM)	5.2	18.7	5.0

The reason for non-linearity, which is a common feature among transaminases, remains obscure. The most usual explanation for non-linearity is the presence of a dissociable inhibitor in the enzyme preparation. Banks, Oldham, Thain & Vernon (1959) consider this explanation unlikely for the pig-heart enzyme, because non-linearity is found at all levels of purification and the enzyme is not inhibited by the addition of a boiled extract of pig-heart muscle. However, if the inhibitor was a

protein, it would not appear in the boiled extracts or be removed on dialysis. It is possible that the enzyme shows self-inhibition; thus if the enzyme contains a γ -glutamyl end group, the end group of one molecule might react with the pyridoxal phosphate associated with the active site of another enzyme molecule.

Determination of kinetic constants

The purified transaminase was incubated with pyridoxal phosphate (0.5 mM) for 1 hr. at 20° to ensure complete reactivation; the preparation was then dialysed for 5 hr. at 3–4° against a running stream of phosphate buffer (0.05M, pH 7.5) to remove excess of cofactor. The enzyme concentration used in the kinetic experiments was kept low, so as to fall on the linear part of the curve relating velocity to enzyme concentration. The variation of initial velocity with substrate concentration was studied by keeping one substrate at a constant high concentration whilst varying the concentration of the other. Silica cells of light-path 4 cm. were used on some occasions to give increased sensitivity, and duplicate determinations were made at each concentration of substrate. Oxaloacetic acid was dissolved immediately before use, and calibration curves (Fig. 4) were used to correct for the amino acid-catalysed decarboxylation of oxaloacetate. Some of the results of these experiments are shown in Figs. 5–7.

The intercept on the $1/V$ axis is the reciprocal of the apparent maximum velocity, the true maximum velocity being attained when both substrates are at infinite concentration. In the plots shown in Figs. 5–7, the concentration of one substrate is extrapolated to infinity whilst the concentration of

Fig. 3. Variation of initial velocity with enzyme concentration. *A*. The glutamate-oxaloacetate reaction was assayed in a crude extract (specific activity 2.3) and a purified fraction (specific activity 568) prepared from it. The enzyme was incubated with L-glutamate (600 μ moles) and pyridoxal 5-phosphate (0.1 mM) for 10 min. at 20° in phosphate buffer (0.2M, pH 7.5); oxaloacetate (6 μ moles) was added to start the reaction in a final volume of 3 ml. at 20°. Initial velocity equals the decrease in $E_{280 \text{ m}\mu}$ /min. corrected for the non-enzymic reaction (see Fig. 4). *B*. The aspartate- α -oxoglutarate reaction was assayed in the same fractions as were used in *A*, L-aspartate (150 μ moles) replacing the glutamate and α -oxoglutarate (75 μ moles) replacing the oxaloacetate. Initial velocity equals the increase in $E_{280 \text{ m}\mu}$ /min. $\circ$, Purified enzyme; $\bullet$, crude extract. The values on the abscissae of graphs *A* and *B* refer to the crude extract; these figures must be divided by 100 to give the concentration of protein for the purified enzyme.

Fig. 4. Glutamate-catalysed decarboxylation of oxaloacetate. *A*. Varying amounts of L-glutamate were added to freshly dissolved oxaloacetate (6 μ moles) in a final volume of 3 ml. of phosphate buffer (0.2M, pH 7.5) in 1-cm. silica cells at 30°. The decrease in $E_{280 \text{ m}\mu}$ was measured for 3 min. *B*. Various amounts of freshly dissolved oxaloacetate were added to L-glutamate (450 μ moles) under the conditions described in *A*. V is the decrease in E /min. Similar calibration curves were made for cysteate and cysteine-sulphinate.

the second substrate is kept constant (B). If K_B represents the concentration of the second substrate which gives half-maximum velocity, then for both binary and ternary mechanisms, the ap-

parent maximum velocity ($V_{app.}$) is related to the true maximum velocity ($V_{max.}$) by the expression:

$$V_{max.} = V_{app.} \left(1 + \frac{K_B}{B} \right).$$

The evaluation of $V_{max.}$ thus requires the value K_B . The slopes of the double reciprocal plots shown in Figs. 5 and 6 give the ratio

$$\frac{\text{Concn. of substrate giving } 0.5 V_{app.}}{V_{app.}} = \frac{K_B (app.)}{V_{app.}}.$$

For the ternary mechanism proposed by Cook, $K_B (app.)$ equals K_B , but this is not so for the binary mechanism. Representing the transamination reaction as:

the rate law for the ternary mechanism is:

$$V = \frac{V_{max.}}{\left(\frac{1 + K_A}{A} \right) \left(\frac{1 + K_B}{B} \right)},$$

where V is the initial velocity and A and B are the concentrations of the two substrates. Let A be held constant, and let b be the concentration of the second substrate giving half the apparent maximum velocity. Then

$$V = \frac{V_{app.} \left(1 + \frac{K_A}{A} \right)}{\left(\frac{1 + K_A}{A} \right) \left(\frac{1 + K_B}{b} \right)},$$

from which $K_B = b$, and similarly $K_A = a$. The rate law for the binary mechanism is

$$V = \frac{V_{max.}}{1 + \frac{K_A}{A} + \frac{K_B}{B}}.$$

Fig. 5. Variation of initial velocity with substrate concentration. Reactivated transaminase (specific activity 148) was mixed with the substrates in a final volume of 3 ml. of phosphate buffer (0.2M, pH 7.5) in 1-cm. silica cells at 30°. The concentration of oxaloacetate when L-glutamate was varied was kept constant at 2 mM; that of L-glutamate when oxaloacetate was varied was 150 mM. V is the decrease in $E_{280} \text{ m}\mu/\text{min.}$ corrected for the non-enzymic reaction (see Fig 4).

Fig. 6. Variation of initial velocity with substrate concentration. Reactivated transaminase (specific activity 148) was mixed with the substrates, excess of malic dehydrogenase, and reduced diphosphopyridine nucleotide (0.3 mg.) in a final volume of 12 ml. of phosphate buffer (0.2M, pH 7.5) in 4-cm. silica cells at 30°. When L-aspartate was varied, the concentration of α -oxoglutarate was kept at 15 mM; when α -oxoglutarate was varied, the concentration of L-aspartate was kept at 20 mM. V is the decrease in $E_{340} \text{ m}\mu/\text{min.}$ corrected for a small blank reaction due to a trace of transaminase in the malic dehydrogenase.

Fig. 7. Variation of initial velocity with substrate concentration. Reactivated transaminase (specific activity 568) was mixed with the substrates in a final volume of 3 ml. of phosphate buffer (0.2M, pH 7.5) in 1-cm. silica cells at 30°. The concentration of oxaloacetate was kept constant at 2 mM, the compound being dissolved immediately before use. V is the decrease in $E_{280} \text{ m}\mu/\text{min.}$ corrected for the non-enzymic reaction.

For the same conditions as above, we have

$$V = \frac{V_{app.} \left(1 + \frac{K_A}{A}\right)}{1 + \frac{K_A}{A} + \frac{K_B}{b}},$$

therefore $K_B = b + b \frac{K_A}{A}.$

Similarly, $K_A = a + a \frac{K_B}{B}.$

Combining these two equations gives:

$$K_B = \frac{Bb(A+a)}{AB-ab} \quad \text{and} \quad K_A = \frac{Aa(B+b)}{AB-ab}.$$

It is thus possible to calculate the true Michaelis constants from the data provided in Figs. 5 and 6. The calculated values are shown in Table 2, which also includes a number of other determinations. The values of apparent maximum velocity were all obtained with the same enzyme preparation on the same day; the apparent Michaelis constants were obtained on different occasions. In those cases where the activity was measured by means of oxaloacetate reduction, excess of malic dehydrogenase was always present to ensure that the transamination was rate-determining.

Figs. 5 and 6 show that the enzyme activity is inhibited by high concentrations of the keto acids. It has been assumed that extrapolation of values obtained at low concentrations gives a valid

Table 2. *Kinetic constants of cauliflower glutamic-oxaloacetic transaminase*

A. The observed kinetic constants were obtained from Lineweaver-Burk double reciprocal plots. All assays were performed in potassium phosphate buffer (0.2M, pH 7.5) at 30°. The samples of enzyme were purified 67-fold (specific activity 148) and 224-fold (specific activity 568).

Substrate varied	Concn. of other substrate (mM)	Wavelength of assay method (mμ)	Light-path of assay cell (cm.)	Specific activity of enzyme	Apparent Michaelis constant (K_m) (mM)	Apparent maximum velocity ($V_{app.}$)
Glutamate	Oxaloacetate					
	2	280	1	148	37	0.110
	2	280	1	148	31	
	2	280	1	568	35	
	2	280	1	568	41	
Oxaloacetate	Glutamate					
	150	280	1, 4	148	0.11	0.097
	150	280	4	568	0.06	
Aspartate	α-Oxoglutarate					
	15	340	4	148	7.4	0.038
	15	280	4	568	7.1	
α-Oxoglutarate	Aspartate					
	20	340	4	148	0.50	0.037
	50	280	4	568	0.66	
Cysteate	Oxaloacetate					
	2	280	1	148	100	—
Cysteinesulphinate	Oxaloacetate					
	2	280	1	568	66	0.06
		280	1	568	58	

B. The observed kinetic constants were corrected by the equations for the binary and ternary complex mechanisms (see text). The values of $V_{app.}$ and $V_{max.}$ are given in terms of the change of $E_{280 \text{ mμ}}$ /min.

Substrate	Average apparent K_m (mM)	True K_m (corrected by binary equation) (mM)
Glutamate	36	38
Oxaloacetate	0.084	0.105
Aspartate	7.25	7.5
α-Oxoglutarate	0.66	0.76

Reaction	Average $V_{app.}$	$V_{max.}$ (corrected by binary equation)	$V_{max.}$ (corrected by ternary equation)
Glutamate-oxaloacetate	0.103	0.119	0.116
Aspartate-α-oxoglutarate	0.0375	0.042	0.041

estimate of the apparent maximum velocity and the apparent Michaelis constant. Another more difficult situation arises when the keto acid is maintained at a constant high concentration. Inhibition is greatest at low concentrations of the amino acid under these conditions, and this leads to an overestimate of the apparent maximum velocity and Michaelis constant. Theoretically, this effect can be minimized by using a lower constant concentration of the keto acid, but it then becomes technically difficult to measure true initial rates. In practice a compromise has to be made and the resultant uncertainty reduces the incisiveness of kinetic tests.

A possible explanation of the observed inhibition by keto acids may be made in terms of the binary mechanism. When the enzyme is in the pyridoxal form, a keto acid may nevertheless combine with the protein and thereby inhibit the formation of a Schiff base between the enzyme-bound pyridoxal phosphate and the amino acid substrate.

Kinetic tests to distinguish between binary and ternary complex mechanisms

Haldane relationships. Haldane (1930) first showed that there is a simple relationship between kinetic constants and the equilibrium constant. The Haldane relationships for the two mechanisms under discussion have been given by Alberty (1956). For the ternary complex mechanism

$$K_{\text{equil.}} = \frac{V_F}{V_R} \times \frac{K_C K_D}{K_A K_B}$$

and for the binary mechanism

$$K_{\text{equil.}} = \frac{(V_F)^2}{(V_R)^2} \times \frac{K_C K_D}{K_A K_B},$$

where V_F and V_R represent the maximum velocities of the forward and reverse reactions, and K_A , K_B etc., represent the Michaelis constants of the substrates. With the values given in Table 2, the ternary mechanism gives

$$K_{\text{equil.}} = \frac{0.116}{0.041} \times \frac{7.25 \times 0.66}{36.0 \times 0.084} = 4.5$$

and the binary mechanism gives

$$K_{\text{equil.}} = \frac{(0.119)^2}{(0.042)^2} \times \frac{7.5 \times 0.76}{38.0 \times 0.105} = 11.5.$$

The equilibrium constant determined by Krebs (1953) is 6.74, and the value determined by Banks *et al.* (1959) is 6.5. It follows that the kinetic data are not sufficiently accurate to discriminate between the two mechanisms.

In passing, mention may be made of the report by Grisolia & Burris (1954) that the aspartate- α -oxoglutarate transamination catalysed by purified pig-heart transaminase failed to reach comple-

tion when oxaloacetate was continuously decarboxylated by means of ethylenediamine-citrate buffer; pyruvate did not inhibit the transamination. Removal of oxaloacetate by malic dehydrogenase from an equilibrium mixture of substrates resulted in the continuous production of more oxaloacetate by the cauliflower enzyme, thus allaying any doubts about the effects of an 'immovable' equilibrium on the interpretation of the kinetic data.

Effect of varying both substrates simultaneously. When substrates A and B are varied simultaneously and A is always kept equal to B, the rate law for the binary mechanism may be written:

$$V = \frac{V_{\text{max.}}}{1 + \frac{K_A}{A} + \frac{K_B}{A}}.$$

Thus when $V_{\text{max.}}/V = 2.0$, then $A = K_A + K_B$. For the ternary mechanism,

$$V = \frac{V_{\text{max.}}}{1 + \frac{K_B}{A} + \frac{K_A}{A} + \frac{K_A K_B}{A^2}},$$

from which, when $V_{\text{max.}}/V = 2.0$,

$$A = \frac{1}{2}\{K_A + K_B + \sqrt{[(K_A)^2 + 6K_A K_B + (K_B)^2]}\}.$$

Substituting the values given in Table 2 leads to the following values of aspartate (or α -oxoglutarate) at which $V_{\text{max.}}/V$ equals 2.0: binary mechanism, 8.2 mM; ternary mechanism, 8.4 mM. These values are too close to provide a test between the two mechanisms.

Fig. 8. Variation of initial velocity when both substrates are varied simultaneously. A. Reactivated transaminase (specific activity 568) was mixed with equimolar solutions of L-aspartate and α -oxoglutarate in 12 ml. of phosphate buffer (0.2M, pH 7.5) in 4-cm. silica cells at 30°; V is the rise in $E_{280 \text{ m}\mu}/\text{min.}$ B. The assays were performed as in A, but excess of malic dehydrogenase and reduced diphosphopyridine nucleotide (1.0 mg.) were also added; V is the decrease in $E_{340 \text{ m}\mu}/\text{min.}$ corrected for a small blank reaction due to a trace of transaminase in the malic dehydrogenase. Curves 1 and 2 are the expected curves calculated on the basis of the ternary and binary complex mechanisms respectively (see text). The filled-in circles represent the points obtained by experiment.

However, the rate law for the binary mechanism gives a linear plot of $1/V$ against $1/A$ when A is kept equal to B :

$$\frac{1}{V} = \frac{1}{V_{\max.}} + \left(\frac{K_A + K_B}{V_{\max.}} \right) \times \frac{1}{A}.$$

The rate law for the ternary mechanism has a quadratic form, and hence the two mechanisms may be tested by comparing the experimental results with the two calculated curves. In Fig. 8A, some experimental points obtained by measuring the production of oxaloacetate at 280 $m\mu$ are plotted together with the calculated curves for the binary and ternary mechanisms. The greatest difference between the two curves is at low concentrations of substrate. Consequently the effect of varying the substrates together was studied by coupling the transamination to the reduction of oxaloacetate by malic dehydrogenase, since this method of assay is about 12 times as sensitive as the 280 $m\mu$ method. The results shown in Fig. 8B support the binary mechanism.

The binary mechanism for the action of the transaminase, which is supported by the available evidence, provides a role for the cofactors of the enzyme and also accounts for the phenomenon of the exchange of identical carbon chains. The observation that a number of amino acids will transaminate with either oxaloacetate or α -oxoglutarate (Ellis & Davies, 1961) is also in agreement with the binary mechanism, since this mechanism can be interpreted in terms of a single active site at which all the substrates combine to form a Schiff base with the cofactor. The ternary mechanism, on the other hand, requires separate sites for the amino and keto acid reactants, and it is difficult to account for the above observations on this basis; for example, it would have to be postulated that cysteic acid and cysteinesulphinic acid can react at both of the two sites, or that oxaloacetate and α -oxoglutarate can both react at one site. This is tantamount to supposing that the two sites are very similar, and it therefore seems simpler to suppose that they are identical, in accordance with the binary mechanism.

SUMMARY

1. Purified cauliflower transaminase is stimulated by a maximum of threefold when incubated with pyridoxal 5-phosphate or pyridoxamine 5-phosphate.

2. The enzyme is inhibited by isoniazid, and this inhibition is reversed by pyridoxal 5-phosphate.

3. Determinations of several kinetic constants are reported; these constants provide evidence which supports a mechanism of enzyme action involving a binary complex.

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