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STUDIES ON THE CATALYTIC AND REGULATORY PROPERTIES OF HOMOSERINE DEHYDROGENASE OF *ZEA MAYS* ROOTS

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SUMMARY

1. Homoserine dehydrogenase has been isolated and partially purified from the apical centimeter of *Zea mays* roots. It appears to be a single enzyme possessing dual coenzyme specificity. The apparent K_m for $NADP^+$ was significantly lower than that for NAD^+, although the v_{max} with NAD^+ was twice that observed with $NADP^+$. Two apparent K_m values for homoserine were calculated from double-reciprocal plots of initial velocity and homoserine concentration in the presence of excess $NADP^+$. Enzyme-catalyzed reduction of aspartic semialdehyde could be inhibited by either of the reduced coenzymes. When noninhibitory levels of coenzymes were employed, reaction rates were always greater with NADPH.

2. The enzyme was inhibited by the pathway product threonine, and by three other natural amino acids: serine, aspartic acid and cysteine. The $NADP^+$- and NAD^+-linked enzyme activities proved to be differentially sensitive to these amino acids. The results of kinetic and desensitization experiments suggest that the mechanism of action or the enzyme binding site of each inhibitory amino acid may be different.

3. Properties of the enzyme are discussed in relation to properties of microbial homoserine dehydrogenases and physiological conditions in developing roots.

INTRODUCTION

Several classes of regulatory mechanisms such as feedback inhibition and repression have been well characterized in microorganisms. In contrast, there have been comparatively few detailed investigations of the nature and extent of such mechanisms in higher plants. The present investigation is concerned with the reversible NAD(P)-linked reduction of aspartic semialdehyde to homoserine, catalyzed by homoserine dehydrogenase (L-homoserine:NAD(P) oxidoreductase, EC 1.1.1.3). The substrate aspartic semialdehyde is derived from aspartic acid and is a precursor of lysine; methionine, threonine, and isoleucine are ultimately derived from the product homoserine. Evidence for operation of this pathway of amino acid biosynthesis in multicellular plants was obtained by tracer studies[1,2] and by direct demonstration of some of the component enzymes[3,4]. SASAOKA[3] isolated homoserine dehydrogenase from pea seedlings but did not investigate possible regulatory characteristics of the enzyme.

The significant contribution of this enzyme to regulation of amino acid biosynthesis in microorganisms has, on the other hand, been extensively studied and recently reviewed[5-7].

In the present study homoserine dehydrogenase was isolated and partially purified from maize root tips and shown to share several characteristics with individual microbial homoserine dehydrogenases. Nevertheless, the plant enzyme differs from every microbial enzyme studied so far by virtue of its unique combination of regulatory and catalytic properties. A preliminary report of some of this work has appeared previously[8].

MATERIALS AND METHODS

Materials. Seeds of *Zea mays* var. earliking were purchased from Joseph Harris Seed Company, Rochester, N.Y. Homoserine, coenzymes, amino acids and keto acids were obtained from Calbiochem or Sigma. Aspartic semialdehyde was prepared according to the method of BLACK[9] and further purified by column chromatography with Dowex 50. All other chemicals used during this study were of reagent grade.

Preparation of the enzyme. Batches of about 9000 seeds were surface sterilized with a 5% solution of Clorox and germinated under axenic conditions at 28° in the dark. After three days, homoserine dehydrogenase was extracted from one centimeter root tips by homogenization in a Waring blender at 4° with 1.25 vol. of 0.2 M Tris–HCl buffer containing 1.4 mM 2-mercaptoethanol, 1.0 mM EDTA, 0.1 M KCl and 30% (v/v) glycerol, pH 8.5. All of the remaining procedures were carried out at 0° to 4°. After filtration of the extract through nylon mesh, cell debris were sedimented at 20 000 × *g* for 30 min. The protein fraction of the resultant supernatant solution precipitating between 40 and 55% saturation with $(NH_4)_2SO_4$ was collected by centrifugation. This fraction contained most of the enzyme and was dissolved in and dialyzed against 0.05 M potassium phosphate buffer containing 1.4 mM 2-mercaptoethanol, 1.0 mM EDTA, and 20% glycerol (pH 7.5). The enzyme was further purified by addition of 1.5 mg (dry wt.) calcium phosphate gel, prepared according to the method of TSUBOI AND HUDSON[10], per mg of protein. The enzyme was not adsorbed by the gel after 30 min and was recovered in the supernatant solution after centrifugation. This solution was diluted to 1 to 3 mg protein per ml with the phosphate buffer described above. Unless otherwise specified, all of the experiments were conducted with enzyme prepared in this manner. These procedures resulted in 20- to 30-fold purification of the enzyme with an overall recovery of about 60%.

Protein determinations. Protein was measured by the methods of KALCKAR[11] or LOWRY *et al.*[12].

Enzyme assays. Homoserine dehydrogenase activity was determined from the initial rates of coenzyme reduction or oxidation in the presence of homoserine or aspartic semialdehyde, respectively. Changes in absorbance at 340 mμ and 25° ± 1° were recorded at 15-second intervals after rapid addition of coenzyme or substrate to initiate the reaction utilizing an Hitachi Perkin–Elmer spectrophotometer equipped with a digital display attachment and a temperature-controlled cell compartment. Initial rates were linear for at least 3 min under most assay conditions and were extrapolated to express enzyme activity as $\Delta A_{340\ m\mu}$ per 10 min.

The reaction mixture employed to measure the enzyme activity in the reduction

of aspartic semialdehyde, hereafter referred to as the forward reaction, contained in μmoles: 200 potassium phosphate buffer, 0.7 2-mercaptoethanol, 0.5 EDTA (pH 6.7); reduced coenzyme and freshly neutralized aspartic semialdehyde (final concentrations of substrates and coenzymes are indicated in each table or figure), plus an appropriate amount of enzyme in a final vol. of 1.0 ml. Measurements of coenzyme oxidation were always corrected for small nonspecific changes in absorbance (0.01–0.05 per 10 min) by use of suitable control mixtures in which aspartic semialdehyde had been omitted. The reaction mixtures used to measure enzyme activity in the oxidation of homoserine designated the reverse reaction, contained in μmoles: 200 Tris–HCl, 150 KCl, 0.7 2-mercaptoethanol, 0.5 EDTA (pH 9.0); enzyme, oxidized coenzyme and homoserine in a final vol. of 1.0 ml. Corrections for nonspecific changes in absorbance in the absence of homoserine were only necessary when crude extracts were used as the source of the enzyme.

Inhibitors. The amino acids added to the reaction mixtures as potential inhibitors or activators were appropriately neutralized with HCl or KOH.

RESULTS

Specificity for substrates and coenzymes

The enzyme preparations did not exhibit aspartic semialdehyde dehydrogenase or aspartokinase activity. Several different assay conditions were used in tests for the presence of these enzymes including use of 0.8 mg of protein for assay of aspartic semialdehyde dehydrogenase and 4 mg of protein for assay of aspartokinase. No coenzyme reduction was detected upon addition of several hydroxy-amino acids to reaction mixtures in which homoserine had been omitted, nor did any of the compounds tested as potential enzyme modifiers stimulate either coenzyme oxidation or reduction in complete assay mixtures. Mixtures of D- and L-homoserine at unsaturating concentrations, supported reaction rates which were proportional to the concentration of the L-stereoisomer in the mixture. It is therefore concluded that the maize homoserine dehydrogenase exhibits a high degree of specificity for L-homoserine and that the partially purified preparations were substantially free of several contaminating enzymes which could influence the experimental results. Aspartic semialdehyde was prepared chemically from DL-allylglycine, but its concentration was determined enzymatically. The concentrations of aspartic semialdehyde expressed in the results are thus based on the assumption that the enzyme is specific for the L-stereoisomer.

The enzyme does not exhibit specificity for a single type of coenzyme, but can utilize either NADP or NAD coenzymes. Enzyme activity was proportional to protein concentration when measured in the reverse direction with either $NADP^+$ or NAD^+. The results of a typical experiment are shown in Fig. 1. Similar linear relationships were also obtained in assays of the forward reaction with either NADPH or NADH.

Kinetics of coenzymes and substrates

Apparent K_m values for the oxidized coenzymes were determined from the intercepts of double-reciprocal plots of initial velocity and coenzyme concentration in the presence of excess homoserine (Fig. 2A, B). The average values, calculated from five independent determinations, were 0.68 mM for NAD^+ and 0.024 mM for $NADP^+$. Although the efficiency of the enzyme in utilization of the different coenzymes in the

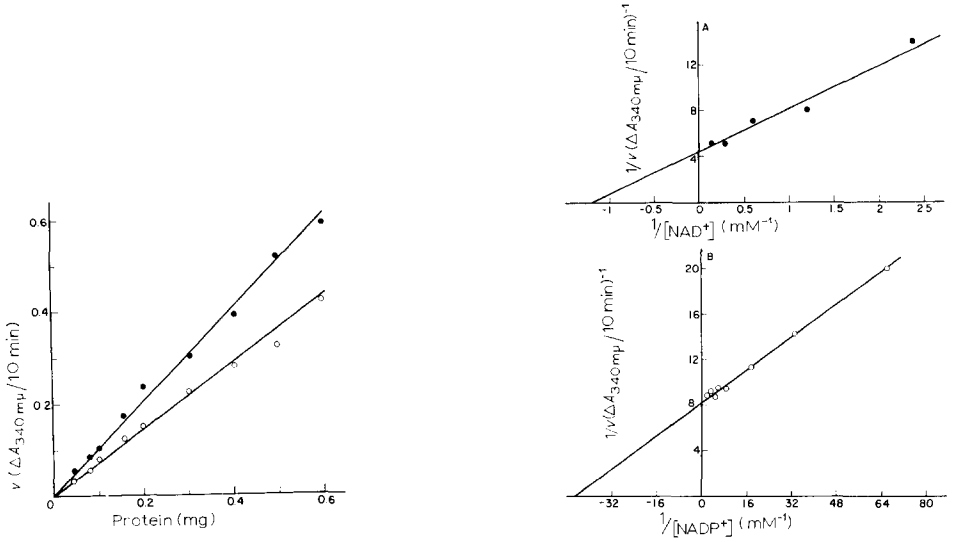

Fig. 1. The effect of protein concentration on the initial velocity of the enzyme-catalyzed oxidation of L-homoserine. Assay conditions were as described in MATERIALS AND METHODS with 20 mM homoserine and 6.7 mM NAD^+ (●), or 0.48 mM $NADP^+$ (○).

Fig. 2. Double-reciprocal plots of initial velocity and coenzyme concentration. Assay conditions were as described in MATERIALS AND METHODS with 20 mM homoserine. (A), NAD^+. (B), $NADP^+$.

reverse reaction appears significantly different as judged from the apparent K_m values, the v_{max} with $NADP^+$ is only one-half of the v_{max} with NAD^+.

The kinetics with respect to homoserine appear complex in the $NADP^+$-linked reaction. Two apparent K_m values can be calculated from double-reciprocal plots of initial velocity and homoserine concentration in the presence of excess $NADP^+$ (Figs. 3, 6B). These are designated K_m^L and K_m^H after DATTA AND GEST[13] who reported a similar phenomenon using highly purified preparations of *Rhodospirillum rubrum* homoserine dehydrogenase. The values for the homoserine K_m^L and K_m^H averaged 0.69 mM and 3.1 mM with the maize enzyme. Activation at high homoserine concentrations could indicate the presence of multiple binding sites for homoserine or reflect a change in protein conformation. When NAD^+ was utilized as the coenzyme, bimodal plots of reaction velocity and homoserine concentration were not observed. The single apparent K_m for homoserine in this reaction averaged 2.3 mM.

Kinetic analysis of the forward reaction is complicated by inhibition at concentrations of either coenzyme higher than 0.2 mM (Fig. 4). Nevertheless, it is apparent from these data that half-maximal velocities are obtained with similar concentrations of either coenzyme. The approximate K_m value for each coenzyme was also similar, but these values could not be regarded as accurate due to the nonlinear nature of double-reciprocal plots. This approximate equality is quite different from the results described above for the reverse reaction in which the K_m values for the oxidized coenzymes differ by more than an order of magnitude. NADPH is clearly the preferred coenzyme for the forward reaction since it supports reaction rates 5 to 7 times greater than those observed with NADH.

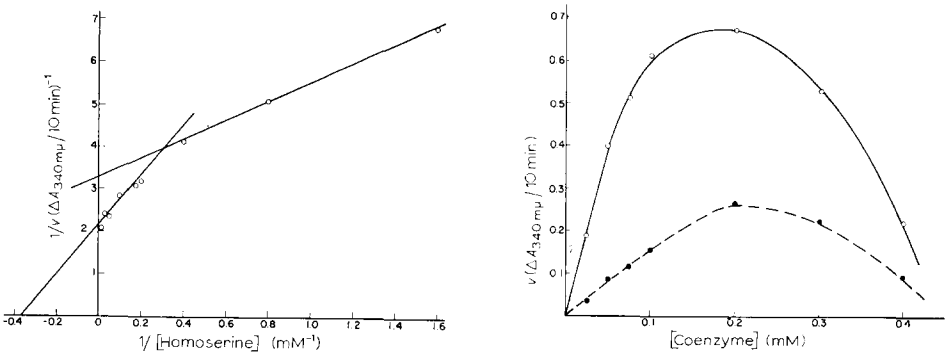

Fig. 3. Double-reciprocal plots of initial velocity and homoserine concentration. Assay conditions were as described in MATERIALS AND METHODS with 0.48 mM $NADP^+$.

Fig. 4. The effects of coenzyme concentration on the initial velocity of the enzyme-catalyzed reduction of aspartic semialdehyde. Assay conditions were as described in MATERIALS AND METHODS with 2.4 mM aspartic semialdehyde. (●), 0.1 ml enzyme with NADH. (○), 0.05 ml enzyme with NADPH.

Nonsaturating but noninhibitory coenzyme concentrations were employed to estimate the apparent K_m values for aspartic semialdehyde. Under these conditions, the enzyme still obeys normal Michaelis–Menten kinetics, and a single apparent K_m of approx. 0.25 mM for aspartic semialdehyde was calculated from double-reciprocal plots with either NADPH or NADH as the coenzyme.

Evidence for a single enzyme

The above data do not permit distinction between two homoserine dehydrogenases differing in their coenzyme specificity and a single enzyme with dual specificity. Precedents for either possibility are well established for other dehydrogenases[14,15]. The results of four different types of experiments are consistent with the concept that maize homoserine dehydrogenase is a single enzyme. First, the ratio of NAD/NADP-linked activity remained essentially constant during the partial purification of the enzyme. This has also been observed with individual preparations of enzyme that have been purified over 100-fold, and can be demonstrated in both the forward and reverse reactions. Second, a single homoserine dehydrogenase is suggested by similar rates of inactivation at 25°. Thus, the activities with NAD^+ and $NADP^+$ decreased at the same rate when the enzyme was incubated at 25° in 0.05 M phosphate buffer (pH 7.5), diluted 10–20-fold and assayed. After 4 h, a preparation retained 62% of its original activity with NAD^+ and 66% with $NADP^+$. If 5 mM threonine was included in the preincubation mixture, neither activity decreased within a period of several days. Third, when an excess of both NAD^+ and $NADP^+$ was added to reaction mixtures, the resulting enzyme activity was intermediate to the activities observed with NAD^+ or $NADP^+$ alone (Table I). Addition of optimal amounts of both reduced coenzymes resulted in significant inhibition of homoserine dehydrogenase activity, as would be predicted if both coenzymes were acting at the same catalytic site(s) on a single enzyme. Fourth, assays *in situ* of enzyme activity after disc gel electrophoresis at pH 9.3 revealed a single darkly staining band of homoserine dehydrogenase activity when either or both oxidized coenzymes were present in the incubation mixture with

TABLE I

EFFECTS OF DIFFERENT COENZYMES ON INITIAL VELOCITY

The assay conditions were as described in MATERIALS AND METHODS with 30 mM homoserine utilized for measurements of the reverse reaction velocity and 4.0 mM aspartic semialdehyde employed for the forward assays.

Coenzyme (mM)	*Reaction velocity ($\Delta A_{340\ m\mu}$ per 10 min)*
Reverse reaction	
$NADP^+$ (0.48)	0.110
NAD^+ (6.7)	0.205
$NADP^+$ (0.48) + NAD^+ (6.7)	0.150
Forward reaction	
NADPH (0.2)	1.170
NADPH (0.4)	0.370
NADH (0.2)	0.240
NADH (0.4)	0.095
NADPH (0.2) + NADH (0.2)	0.270

homoserine, phenazine methosulfate and nitroblue tetrazolium. In the absence of homoserine, no localized deposition of tetrazolium occurred.

Inhibitor studies

A large number of compounds were tested for their ability to influence the oxidation of homoserine with $NADP^+$ or NAD^+. The following compounds were tested at a concentration of 10.0 mM (unless a lower concentration is specified) and were neither stimulatory nor inhibitory: L-alanine, L-α-amino-N-butyric acid, γ-aminobutyric acid, α-amino-isobutyric acid, DL-β-amino-isobutyric acid, L-arginine, L-glucosaminic acid, L-glutamic acid (5.0 mM), L-glycine, DL-γ-hydroxybutyric acid, β-hydroxybutyric acid, L-isoleucine, L-leucine, L-lysine, L-methionine, S-methyl-L-cysteine, L-phenylalanine, O-phospho-L-serine (5.0 mM), O-phospho-L-threonine (2.0 mM), L-proline, L-tyrosine (1.0 mM), L-valine, D-aspartic acid, D-cysteine, D-histidine, D-serine, D-threonine. Five natural amino acids were, however, found to inhibit the enzyme during this series of experiments. These were L-cysteine, L-threonine, L-aspartic acid, L-serine, and L-histidine. Although histidine reproducibly inhibited $NADP^+$- or NAD^+-linked activity by 50 to 60%, the effects of this compound have not yet been studied extensively. The results presented in Table II demonstrate that the remaining four amino acids inhibit either the forward or reverse reaction.

Total inhibition or inactivation of the enzyme by 10 mM L-cysteine was confirmed repeatedly in measurements of the reverse reaction. It was also noted that lower concentrations of cysteine were, in general, more effective in inhibiting the reverse reaction than the forward reaction. Inhibition by cysteine does not reflect a general sulfhydryl effect, since neither D-cysteine nor 2-mercaptoethanol was inhibitory at 10 mM.

Threonine inhibition was neither alleviated nor enhanced by one or more of the other products of the pathway. Furthermore, the action of threonine appeared

to be independent of that of the pathway precursor aspartic acid; in that, addition of various concentrations of both threonine and aspartic acid to reaction mixtures resulted in greater inhibition than addition of either compound alone but less than would be predicted on the basis of completely additive effects. This finding tends to eliminate the possibility of isozymes with similar catalytic properties and different inhibitor specificity.

A given inhibitor might be expected to have equivalent effects on each coenzyme-dependent enzyme activity. However, the results presented in Fig. 5 demonstrate that the NAD^+-linked activity is significantly less sensitive to high concentrations of aspartic acid than the $NADP^+$-linked activity, whereas the opposite situation occurs with high concentrations of threonine. Similar quantitative differences between inhibition of NADPH- and NADH-linked activity by threonine or aspartic acid can also be observed in the data presented in Table II. Such differences in the extent of inhibition are clearly concentration dependent and may not be apparent in experiments which employ a fixed inhibitor concentration.

Kinetics with the inhibitors

Cysteine is a competitive inhibitor with respect to homoserine (Fig. 6A, B). This type of inhibition is observed with either $NADP^+$ or NAD^+ as the coenzyme, but the apparent inhibitor constants for cysteine differ significantly. The apparent K_i for cysteine, calculated from the slopes of the curves in Fig. 6A, B averaged 0.41 mM and 0.029 mM with NAD^+ and $NADP^+$, respectively. These results are applicable to the lower K_m for homoserine with $NADP^+$.

Threonine and aspartic acid also proved to be competitive inhibitors of the enzyme with respect to homoserine. Inhibitor constants are not presented for these amino acids due to the complex relationships between inhibitor concentration and

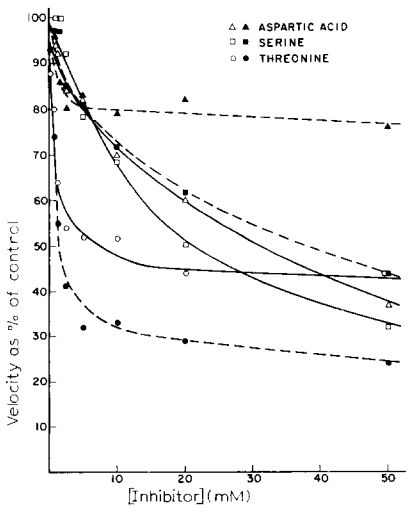

Fig. 5. The effects of increasing inhibitor concentrations on the initial velocity of the enzyme-catalyzed oxidation of homoserine. The solid figures and dashed lines refer to the NAD^+-linked activity with 6.7 mM NAD^+ and 10 mM homoserine. The open figures and solid lines refer to the $NADP^+$-linked activity with 0.48 mM $NADP^+$ and 2.0 mM homoserine.

degree of inhibition noted above. Additional kinetic experiments did, however, reveal differences between the type of inhibition caused by threonine and aspartic acid (Fig. 7A, B). Threonine results in an apparent mixed inhibition, whereas aspartic acid results in apparently uncompetitive or possibly non-competitive inhibition with respect to either coenzyme. At saturating levels of substrate, inhibition by aspartic

TABLE II

INHIBITION OF HOMOSERINE DEHYDROGENASE ACTIVITY BY SEVERAL AMINO ACIDS

Assay conditions were as described in MATERIALS AND METHODS utilizing 2.4 mM aspartic semialdehyde and 0.2 mM NADPH or NADH for measurements of the forward reaction. Homoserine was employed at about 3.5 times its K_m concentration or 2.0 mM with 0.48 mM $NADP^+$ and 10 mM with 6.7 mM NAD^+ for measurements of the reverse reaction except for the experiments with cysteine. Inhibition of the reverse reaction by cysteine was determined in independent experiments utilizing 20 mM homoserine and therefore, the actual changes in absorbancy are not specified. A bracketed value represents a % of the appropriate control velocity.

Additions	*Concentration (mM)*	*Reaction velocity ($\Delta A_{340\ m\mu}$ per 10 min)*			
		Forward reaction		*Reverse reaction*	
		NADPH	*NADH*	*$NADP^+$*	*NAD^+*
None	—	0.540 (100)	0.265 (100)	0.190 (100)	0.370 (100)
L-Cysteine	0.1	0.450 (83)	0.220 (83)	— (85)	— (91)
	1.0	0.260 (48)	0.145 (55)	— (22)	— (21)
	10.0			0	0
L-Threonine	0.1	0.260 (46)	0.034 (13)		
	1.0	0.180 (33)	0.020 (7)		
	10.0			0.091 (48)	0.130 (35)
L-Aspartic acid	0.1	0.460 (85)	0.255 (96)		
	1.0	0.260 (48)	0.195 (74)		
	10.0			0.138 (72)	0.330 (89)
L-Serine	0.1	0.510 (94)	0.240 (91)		
	1.0	0.470 (87)	0.220 (83)		
	10.0			0.140 (74)	0.260 (70)

acid is minimal, and therefore it is difficult to distinguish clearly between these two possibilities with the available data.

Enzyme desensitization

It has been possible to desensitize the maize enzyme by incubating it with high concentrations of phosphate buffer at 4°. Under the conditions described in Table III, the treated enzyme is significantly less sensitive to inhibition by threonine or serine but its ability to be inhibited by aspartic acid or cysteine is retained. The observed differences in sensitivity to various inhibitors were not altered within a five-day period, even though both the treated and untreated enzyme preparations progressively lost catalytic activity. Other experiments indicated that omission of glycerol, 2-mercaptoethanol or EDTA from the experimental buffer was not responsible for desensitization.

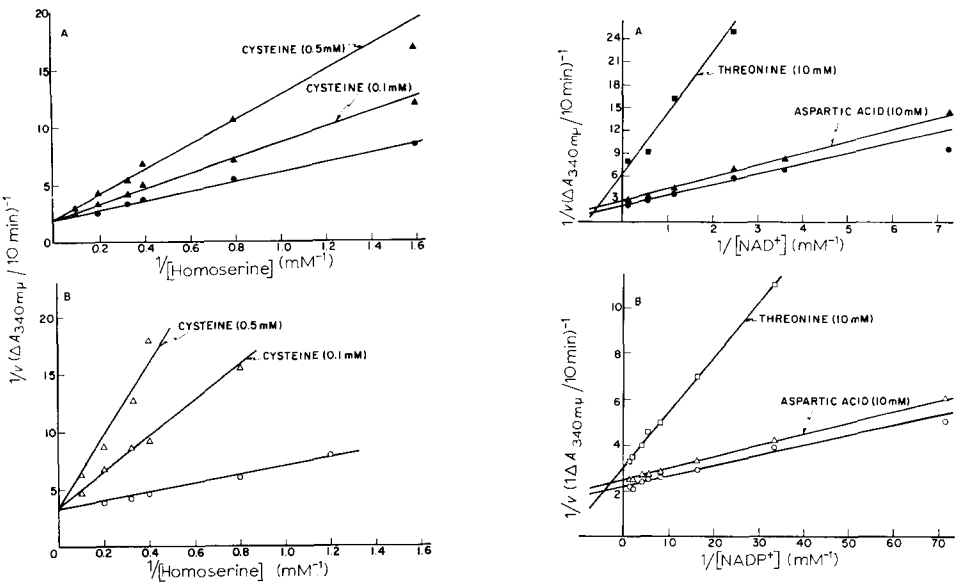

Fig. 6. Double-reciprocal plots of initial velocity and homoserine concentration in the presence or absence of L-cysteine. (A) Assays with 6.7 mM NAD^+. (B) Assays with 0.48 mM $NADP^+$. The remaining assay conditions were as described in MATERIALS AND METHODS.

Fig. 7. Influence of coenzyme concentration on inhibition of enzyme activity by threonine and aspartic acid. Double-reciprocal plots of initial velocity and coenzyme concentration. (A) Assays with 20 mM homoserine and NAD^+. (B) Assays with 20 mM homoserine and $NADP^+$. The remaining assay conditions were as described in MATERIALS AND METHODS.

TABLE III

ENZYME DESENSITIZATION

Aliquots of freshly prepared calcium phosphate-treated preparations of the enzyme were diluted 3-fold with either control buffer consisting of 0.05 M potassium phosphate, 1.0 mM EDTA, 1.4 mM 2-mercaptoethanol and 20% glycerol (v/v), pH 7.5, or an experimental buffer consisting of 0.5 M potassium phosphate, pH 7.5. These preparations were incubated at 4° for one day, diluted 20-fold (10-fold for NADH assays) and assayed with 2.4 mM aspartic semialdehyde and 0.2 mM NADPH or NADH. Phosphate buffer was employed for the assays as described in MATERIALS AND METHODS. A bracketed value represents a % of the appropriate uninhibited velocity.

Additions	*Concentration (mM)*	*Reaction velocity ($\Delta A_{340\,m\mu}$ per 10 min)*			
		Control		*Experimental*	
		NADPH	*NADH*	*NADPH*	*NADH*
None	—	0.400 (100)	0.165 (100)	0.330 (100)	0.196 (100)
Threonine	5.0	0.150 (37)	0.015 (9)	0.350 (106)	0.150 (77)
Cysteine	5.0	0.060 (15)	0.075 (45)	0.100 (30)	0.105 (54)
Serine	5.0	0.220 (55)	0.030 (18)	0.300 (91)	0.175 (89)
Aspartic acid	10.0	0.220 (55)	0.135 (82)	0.210 (64)	0.200 (102)

DISCUSSION

The foregoing results demonstrate that maize homoserine dehydrogenase is an example of a regulatory enzyme analogous to those widely described in microorganisms. The maize root enzyme is similar to microbial homoserine dehydrogenases in several respects. For example, all of the enzymes utilize NADP coenzymes and are inhibited by threonine[16–21]. The properties of *Escherichia coli* K12 homoserine dehydrogenase-I[22,23] appear to be most similar to those of the maize enzyme. Yet, the kinetics of threonine inhibition are quite different with the two enzymes. The maize and pea seedling[3] enzymes share the ability to utilize NAD coenzymes with only two of the seven different microbial enzymes so far described, and one of these is the enzyme from the eukaryiotic microorganism, yeast[24]. Methionine has no influence on the activity of the maize enzyme whereas it is a potent inhibitor of the yeast enzyme[17] and reverses threonine inhibition of the enzyme from *R. rubrum*[16]. Such differences in catalytic and regulatory properties may be of both physiological and evolutionary significance and should not be obscured by the general similarity between enzymes catalyzing identical reactions in different organisms.

Most compounds which are considered to be specific feedback inhibitors of particular enzymes are thought to act at specific regulatory sites rather than at the catalytic site[7]. This could also be applicable to the inhibitors of maize homoserine dehydrogenase. Nevertheless, the steric similarity between the effective inhibitors (excluding histidine) and the substrates of this enzyme does not *a priori* preclude direct involvement of the catalytic site. The observed competitive kinetics with respect to homoserine are clearly compatible with this possibility. However, the following observations support the contention that threonine, at least, acts at a specific regulatory site: the enzyme can be desensitized with respect to threonine without significant loss of catalytic activity. Inhibition is never complete, even when the ratio of threonine to homoserine is high. The observed competitive relationships could be explained if threonine induces conformational changes in the enzyme which in turn prevent formation of the enzyme substrate complex.

Serine may act at the same postulated regulatory site as threonine, as the enzyme simultaneously loses the capacity to be inhibited by serine or threonine. Serine may also act as a substrate analogue at the catalytic site since enzyme activity continues to diminish with increasing concentrations of serine; a result not obtained with threonine.

It is unlikely that aspartic acid or cysteine act at the threonine site, since they remain effective inhibitors under conditions in which the enzyme is no longer inhibited by threonine. Cysteine may act at a specific regulatory site on the enzyme, as suggested for several microbial homoserine dehydrogenases by DATTA[23]. Although the results obtained with the maize enzyme are not inconsistent with such an explanation, another mechanism appears to be equally plausible. Substrate-like mercaptans are known to form highly specific ternary complexes with certain dehydrogenases[25] and the oxidized coenzymes. If cysteine acts in an analogous manner with homoserine dehydrogenase, the relatively high sensitivity of the reverse reaction to inhibition by this amino acid might be expected, as would the competitive relationship between homoserine and cysteine. The physiological relationship between cysteine and homoserine is not totally clear in view of the recent evidence of multiple pathways of

methionine biosynthesis in higher plants[26]. Therefore the more general hypothesis involving a ternary complex is currently favored to explain the effects of cysteine on maize homoserine dehydrogenase activity.

Aspartic acid has been shown to inhibit one other homoserine dehydrogenase[22]. In this case the effects of aspartic acid are attributed to the existence of a multienzyme complex between aspartokinase and homoserine dehydrogenase. Since we have been unable to demonstrate aspartokinase activity either in crude extracts or purified preparations, and the results of the kinetic experiments do not eliminate direct involvement at the catalytic site, further experiments will be required before the mechanism of aspartic acid inhibition of the maize enzyme can be resolved.

The physiological significance of dual coenzyme specificity and differential sensitivity of the maize enzyme to various metabolites ultimately depends upon the concentration of the substrates, coenzymes and modifiers *in vivo*. Both aspartic acid[27] and homoserine[28] have been observed to occur in relatively high concentrations in some multicellular plants. Under such conditions oxidation of homoserine could serve as a source of NADH during growth, since aspartic acid would tend to inhibit $NADP^+$-linked activity with only a minimal effect on NAD^+-linked activity. Inhibition of the maize homoserine dehydrogenase by threonine also provides one explanation of the observation that a supply of exogenous threonine, prevented threonine biosynthesis in maize[27]. Thus regulation of amino acid biosynthesis by feedback mechanisms such as those reported here and those described by OAKS[29] appears to be operational in higher plants and particularly well adapted to the physiological needs of developing roots which are dependent upon the vascular system for a variable supply of metabolic precursors[30].

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