Phytochemistry, 1979, Vol 18, pp. 395–400. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0301-0395 $02 00/0

REGULATION OF HOMOSERINE DEHYDROGENASE IN DEVELOPING ORGANS OF SOYBEAN SEEDLINGS

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(*Revised received* 8 *August* 1978)

Key Word Index—*Glycine max*; Leguminosae; soybean; enzyme regulation; homoserine dehydrogenase; feedback inhibition; potassium stimulation; multiple enzyme forms; threonine; methionine.

Abstract—The regulation of homoserine dehydrogenase (HSD) activity (EC 1.1.1.3) by L-threonine, L-cysteine and K^+ was examined using extracts of organs of soybean seedlings harvested 3, 6, 11, and 19 days after germination. K^+ stimulated HSD activity from each source at least 2-fold. HSD activity was completely inhibited by 10 mM L-cysteine while 10 mM D-cysteine was not inhibitory. A progressive decrease in sensitivity of NAD-dependent HSD to inhibition by 10 mM L-threonine occurred in all organs except the leaf during the sampling period. This progressive decrease in sensitivity of the HSD to threonine inhibition was detected only when K^+ was present in the assay mixtures. Four major molecular forms, including one rapidly migrating form (form I) and three more slowly migrating forms (forms II, III, IV) of HSD, were identified in extracts of soybean organs by polyacrylamide electrophoresis. Chromatographic and electrophoretic data indicate that form I, which was not inhibited by threonine or stimulated by K^+, was of lower MW than forms II, III and IV which were of similar MW. These latter 3 forms were inhibited by threonine and stimulated by K^+. During soybean seedling development form II increased in amount and forms I and IV decreased in amount. This alteration in the amounts of the forms of HSD occurred during the same period as the decrease in the amount of threonine inhibition. Since K^+ stimulation of HSD decreased during soybean organ development and K^+ enhanced threonine inhibition, this might account for the observed decrease in threonine inhibition.

INTRODUCTION

Homoserine dehydrogenase (HSD) (EC 1.1.1.3) is the third enzyme in the pathway which synthesizes threonine, isoleucine and methionine from aspartate and is located at a branch point. It catalyses the reversible conversion of aspartic-β-semialdehyde to homoserine using NADH or NADPH as coenzyme and is feedback inhibited by the end product L-threonine in several higher plants [1–7].

In extracts of peas [3] and maize [5, 6] multiple molecular forms of HSD have been demonstrated. In maize a progressive decrease in the amount of inhibition of HSD by threonine was observed during seedling development. A change in the amount of the different molecular forms of HSD accompanied the observed decrease in threonine inhibition. However, the change in enzyme forms did not readily explain the change in regulation, since the insensitive form of maize HSD decreased instead of increased in amount in relation to the amounts of the two threonine-sensitive enzyme forms. No change in regulation of HSD from any other plant source during development has been reported.

In this study the distribution of HSD in soybean seedling organs, the sensitivity of the enzyme to threonine inhibition and K^+ stimulation and the molecular forms of HSD in extracts of roots, stems, cotyledons and leaves of soybean seedlings of different ages were examined.

RESULTS

Seedling analysis

The cotyledons of soybean seedlings harvested 3 days after germination contained more protein and were of greater fr. wt than stems, leaves or roots. By day-19 the leaves contained more protein and were of greater fr. wt than the other 3 organs. Similar trends were observed for HSD when measured on a unit per organ basis (Fig. 1a). The cotyledons of young seedlings contained the highest quantities of HSD, but by 19 days most of the activity was located in the leaves.

The level of HSD per g fr. wt (relative activity) in each organ remained *ca* constant (Fig. 1b) throughout seedling development except in leaves, in which the level decreased. The sp. act. of the enzyme (units of activity/mg protein) in each organ followed the same trends as organ relative activity (Fig. 1c). The sp. act. of the enzyme in the cotyledon was the lowest of the organs, since the cotyledons from young seedlings contained very large amounts of protein.

The effects of several compounds and their analogs upon HSD from 19-day-old soybean leaves were examined (Table 1). L-Threonine and L-cysteine inhibited the enzyme activity when assays were conducted in either the direction of homoserine or aspartic-β-semialdehyde formation. The inhibitory effect of threonine was enhanced by K^+, but K^+ had no effect on cysteine inhibition. Serine partially inhibited the enzyme activity. D,L-β-Hydroxynorvaline, a threonine analog, inhibited HSD substantially while the lysine analog, *S*(2-aminoethyl)-L-cysteine, and methionine analogs, D,L-ethionine and α-methyl-D,L-methionine, were ineffective.

L-Cysteine (10 mM) strongly inhibited HSD in all preparations obtained from each organ from seedlings harvested 3, 6, 11 and 19 days after germination. However, NAD-dependent HSD from organs of soybean

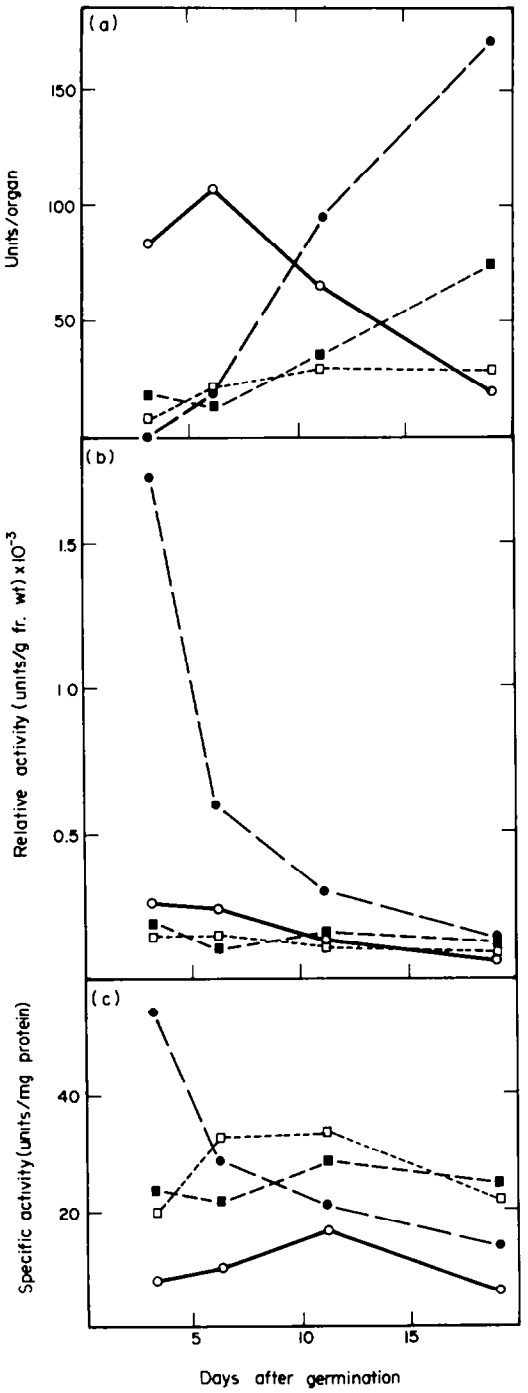

Fig. 1. Homoserine dehydrogenase activity (NAD-dependent) during soybean seedling development. Enzyme activity expressed as (a) units per organ, (b) relative activity and (c) specific activity. Cotyledon (○ ——), leaf (● – – –), stem (■ – – –) and root (□-----). The activity using NADP as coenzyme was about half that shown and followed similar trends. Seedling description: (seedling age; height); (3 days; 2.1 cm); (6 days; 5.2 cm); (11 days; 8.45 cm); (19 days; 13 cm). Cotyledons were attached to the seedling except for 19-day-old seedlings to which only 50% of the cotyledons were attached. The values reflect this loss. Averages of 3 experiments are given as described in Experimental.

Table 1. Effects of compounds on homoserine dehydrogenase activity from leaves of 19-day-old soybean seedlings*

Compound (10 mM)	% of original activity NAD	NADP
L-Threonine	46 (66)†	32 (85)†
L-Methionine	90	85
D,L-Homocysteine	88	82
D,L-Homocystine	111	100
L-Cystathionine	114	89
S-Adenosyl-L-methionine	93	91
L-Cysteine	0	0
D-Cysteine	87	89
L-Serine	41	65
L-Isoleucine	89	91
S(2-Aminoethyl)-L-cysteine	79	83
α-Methyl-D,L-methionine	79	100
D,L-Ethionine	108	110
D,L-β-Hydroxynorvaline	28	21

* Each assay averaged 1.2 mg of protein containing 16 units NAD^+-dependent homoserine dehydrogenase activity. Averages of at least 4 assays are given. All replicate assays were within 3% of the average given. KCl was added to the assay mixtures as described in Experimental.

† Number in parentheses was obtained when KCl was omitted from the assay mixture and compared with the control also lacking KCl.

seedlings grown for 3 days was more sensitive to threonine inhibition than was the activity from organs of 19-day-old soybean seedlings (Fig. 2). As the age of the soybean seedlings increased the NAD-dependent HSDs in extracts of stems, roots and cotyledons, became progressively less sensitive to inhibition by 10 mM threonine (Fig. 3a). The largest decrease in sensitivity occurred in stems, while little change occurred in leaves during development.

The above studies were carried out with 150 mM K^+ present in the assay mixture, because HSDs from several higher plants are known to be stimulated by K^+. Control enzyme assays in these studies contained low amounts

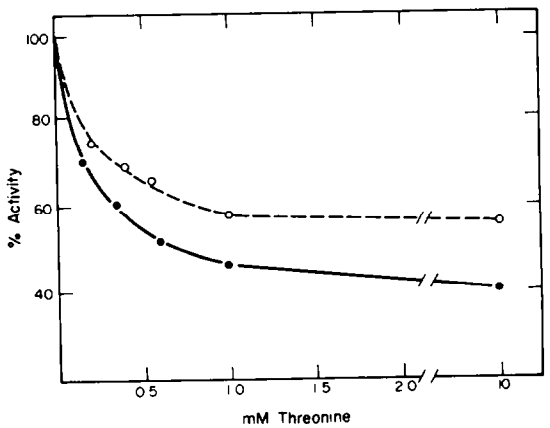

Fig. 2. Comparison of the effects of a range of threonine concentrations on NAD-dependent homoserine dehydrogenase activity from 3- (● ——) and 19- (○ — —) day-old cotyledons. Assays contained 150 mM KCl and 20 units of NAD-dependent homoserine dehydrogenase activity.

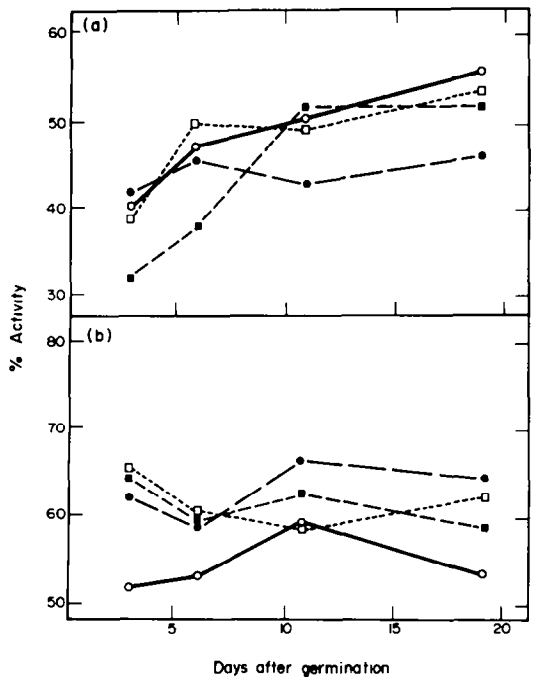

Fig. 3. Effect of 10 mM threonine on homoserine dehydrogenase activity (NAD) from organs of soybean seedlings harvested 3, 6, 11 and 19 days after germination. Assays were conducted in the presence (a) and absence (b) of 150 mM KCl using extracts of cotyledon (○ ——), leaf (● — —), stem (■ – – –), and root (□·····). Averages of at least 3 experiments are given. Standard deviation was ± 3% or less in all cases. Assays contained between 15 and 25 units of NAD-dependent homoserine dehydrogenase activity.

(5 μmol/assay) of K^+ from buffer A used for enzyme preparation. However, when preparations of HSD were dialysed for 18 hr against either buffer A or buffer A with 50 mM Tris substituted for KPi, the enzyme preparation lacking K^+ was stimulated only 5% more by 150 mM KCl (maximal stimulation). HSD was not as stable when stored in Tris containing buffer A so the remaining experiments were conducted using buffer A. Maximum stimulation of HSD from soybean seedlings was achieved using 150 mM K^+. Other monovalent cations, Na^+ and NH_4^+, did not stimulate HSD as greatly as K^+ (Table 2). NAD-dependent HSD was stimulated by monovalent cations more than NADP-dependent activity. The effects of threonine on NAD-dependent HSDs from each organ were then examined by assaying the enzyme without added K^+ (Fig. 3b). Although slight fluctuations in the sensitivity of the enzyme to 10 mM threonine did occur, no progressive decrease in the amount of threonine inhibition of HSD was noted without added K^+ (Figs. 3a and 3b).

Table 2. Effects of salts on homoserine dehydrogenase activity from leaves of 19-day-old soybean seedlings*

Salt (100 mM)	% of control activity NAD	NADP
KCl	480	260
NaCl	360	220
NH_4Cl	240	200

* Assays are as described in Table 1.

Table 3. Effect of K^+ on homoserine dehydrogenase activity derived from 3- and 19-day-old roots and stems

Organ	Coenzyme	3-day-old Specific activity* $-K^+$	K^+	19-day-old Specific activity $-K^+$	K^+
Stem	NAD	22.5	129	24.1	99.5
	NADP	16.6	52	16.3	36.2
Root	NAD	20.5	86	21.3	65.9
	NADP	12.3	24	12.2	25.9

* Specific activity = units/mg protein.

The effect of K^+ on NAD-dependent HSD from extracts of 3- and 19-day-old soybean stems and roots was compared (Table 3). Enzyme extracts of organs from the older seedlings were stimulated less by K^+. Thus a decrease in the amount of stimulation of HSD by K^+ also occurred during soybean development and may be related to the progressive decrease in the amount of inhibition of HSD by threonine.

Electrophoresis and gel filtration chromatography

Analysis of root, stem, cotyledon and leaf crude enzyme extracts on polyacrylamide electrophoretic gels using a range of acrylamide concentrations indicated that 3 and sometimes 4 major forms of HSD were present in extracts of the soybean organs (Fig. 4a). The 4 major forms were detected on gels containing extracts of 3-day-old soybean leaves, stem and cotyledons, as well as 19-day-old cotyledons. Only forms II, III and IV of

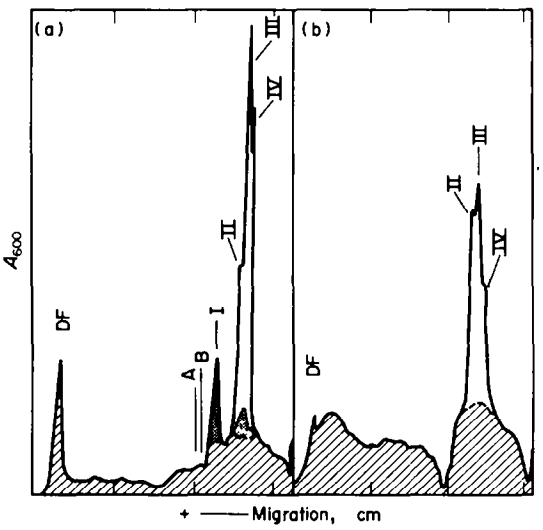

Fig. 4. Recorder trace of polyacrylamide gels stained for homoserine dehydrogenase activity (NAD) after electrophoresis of an enzyme extract from cotyledons of (a) 3-day-old and (b) 19-day-old soybean seedlings. Six units of homoserine dehydrogenase activity were applied to each gel. The positions of the 4 forms are noted from fastest to slowest migrating. The bromphenol blue dye front (DF) is indicated. Gels stained for NADP dependent enzyme activity yielded similar results. Shaded portion indicates gel incubated in assay mixture lacking homoserine. Dotted portion indicates gel incubated in the presence of 10 mM threonine and when K^+ was omitted from the gel incubation medium. All 4 forms were present when K^+ was present in the gel incubation medium.

the enzyme were detected on gels containing extracts of 3-day-old roots and 19-day-old leaves, stems, and roots. However, all extracts may contain amounts of form I which are too low to be readily detected on electrophoretic gels. Form I appears to stain less readily than forms II, III and IV on some gels (especially gels of low acrylamide concentrations) and at times can be detected only by prolonged incubation of the gel in the enzyme-specific staining mixture. This may be due to the low amount of form I enzyme activity in certain samples. Some crude enzyme extracts did not appear to have form I when analysed on gels after electrophoresis but did contain small amounts of form I when analysed on gels containing enzyme purified by gel filtration chromatography. Sometimes following prolonged staining of gels for HSD, 2 new, faint bands of enzyme activity (less than 1% of the total activity) were detected on the gels at the positions labeled A and B in Fig. 4a.

The rates of migration of enzyme forms I–IV were determined using gels of different acrylamide concentrations and were analysed according to the method of Hedrick and Smith [8]. By this method forms II, III and IV appear to be of similar MW, as indicated by parallel lines (Fig. 5), while the line representing the rates of migration of form I intersects these 3 parallel lines. Thus, form I differs from the other forms with respect to MW but possesses a similar charge-to-mass ratio as suggested by the intersection of the lines near the 2% acrylamide concentration [8].

Data obtained by chromatographing soybean organ extracts on gel filtration columns also indicate forms II, III and IV are of similar MW. Form I was separated from the other 3 forms on gel filtration columns as a lower MW component, and its identity confirmed by electrophoresis. When enzyme preparations of either 3-day-old or 19-day-old cotyledons were chromatographed on gel filtration columns containing Sephacryl S-200, similar elution patterns and recoveries (over 85%) were obtained. The enzyme activities were eluted in two distinct peaks. The early-eluting peak of HSD contained the higher MW forms II, III and IV. Fractions containing enzyme from the beginning, middle and end of the peak contained similar amounts of these forms as indicated by electrophoretic analysis so no apparent separation of these 3 forms was observed by gel filtration chromatography. Enzyme activities from the earlier-eluting peak (pool A) and later-eluting peak (pool B) were precipitated using $(NH_4)_2SO_4$, dialysed and analysed. After dialysis, pool A still contained 3 forms of HSD (forms II, III and IV), while pool B still contained only one form (form I) when analysed on polyacrylamide electrophoretic gels. No interconversion of these forms was observed during these procedures.

The properties of the forms separated by gel filtration chromatography were then further analysed. Enzyme activities from pool A (forms II, III and IV) of 3-day and 19-day-old cotyledons were more sensitive to threonine inhibition than enzyme activities of the B pools (form I), which were less than 5% inhibited by 10 mM threonine. The sensitivity of forms II, III, and IV to threonine inhibition was confirmed by adding 10 mM L-threonine to the incubation mixture used for enzyme-specific staining of electrophoretic gels. The addition of threonine to the mixture did not inhibit stain accumulation at the position where form I is located, but inhibited the staining of forms II, III and IV (Fig. 4a). Hence, form I is insensitive to threonine inhibition, while forms II, III and IV are more sensitive to threonine.

In a similar manner, differential effects of K^+ on the 4 forms were observed. HSD in pool A from either enzyme source was stimulated by K^+, whereas activity in pool B was not stimulated (Table 4). This was also demonstrable following gel electrophoresis where the presence of K^+ in the staining mixture gave the 4 forms while in the absence of K^+ only form I was clearly active.

Furthermore, the HSD in pool A (forms II, III and IV) of 3-day-old cotyledons was more greatly inhibited by threonine and stimulated by K^+ than was HSD of pool A of 19-day-old cotyledons (Table 4). This agrees with the observations that threonine inhibition (Fig. 3a) and

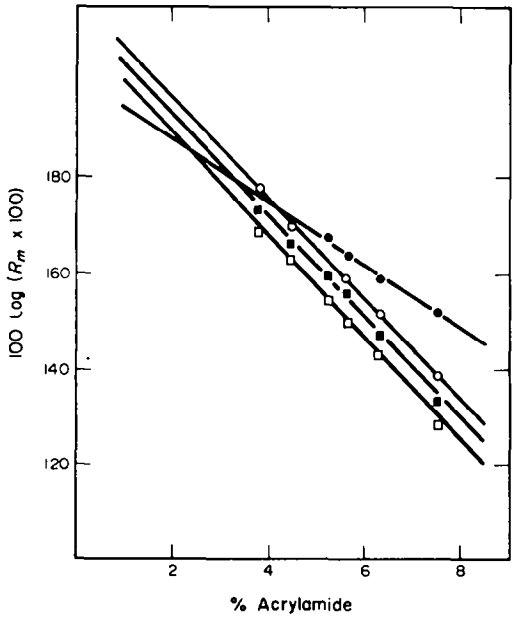

Fig. 5. Relative mobilities of the multiple forms of soybean homoserine dehydrogenase. Form I (●), II (○), III (■) and IV (□) were examined by electrophoresis of extracts from 3-day-old cotyledons on gels of 6 different acrylamide concentrations. The results were plotted according to the method of Hedrick and Smith [8].

Table 4. Characterization of enzyme recovered from Sephacryl S-200 chromatography*

	Enzyme source	
	3-day-old Cotyledon	19-day-old Cotyledon
% Activity in presence of 10 mM Threonine:		
Pool A	50	64
Pool B	95	97
K^+ added: % of −KCl activity:		
Pool A	1080	230
Pool B	86	100

* Chromatography as described in Experimental. Pool A contained the higher MW forms of homoserine dehydrogenase (forms II, III, IV), while Pool B contained the lower MW form (form I) in both cases. All data represent NAD-dependent activity.

K^+ stimulation (Table 3) of total HSD of unchromatographed extracts decrease during seedling development. The amount of stimulation of the major portion of HSD (forms II, III and IV) decreased almost 5-fold during the period examined. This occurred at the same time as the decrease in amount of threonine inhibition of forms II, III and IV.

The data indicate that a decrease in threonine inhibition and K^+ stimulation of HSD occurs during soybean seedling development. These decreases occur concomitantly with a shift in the amounts of forms II, III and IV. The relative amounts of forms I–IV from cotyledons of 3-day-old seedlings were estimated from the peak height of tracings of the electrophoresis gels. The development of color during the enzyme staining process was linear with time under defined conditions after an initial lag of *ca* 15 min. Color development was also proportional to the enzyme activity applied to the gel, when less than 23 units of NAD-dependent HSD were applied. Data obtained in this way using unchromatographed enzyme preparations of 3-day-old and 19-day-old cotyledons indicate a decrease in the relative amount of forms I and IV and an increase in the relative amount of form II during seedling growth (Table 5). Typical gel scans of electrophoretic gels containing extracts of 3-day and 19-day-old cotyledons are presented in Figs. 4a and 4b illustrating the increase in the relative amount of form II and decrease in the relative amount of forms I and IV during seedling growth. The individual regulatory properties of forms II, III and IV remain to be determined, but estimations from staining patterns on gels indicate that all 3 forms are stimulated by K^+ and inhibited by threonine.

DISCUSSION

A progressive decrease in the amount of inhibition of NAD-dependent HSD by threonine occurs in the roots, stem and cotyledons of soybean seedlings during development. This increase in inhibition of HSD activity by threonine was detected only when K^+ was present in the assay mixture. The amount of stimulation of HSD by K^+ also decreases during seedling development. Because K^+ enhances the inhibitory effect of threonine on HSD of soybean seedlings, a decrease in K^+ stimulation of HSD during soybean seedling development could account for the observed decrease in threonine inhibition. The amounts of stimulation by K^+ and inhibition by threonine of the activities of forms II, III and IV decrease during seedling development. With this decrease there is a shift in the relative amounts of forms II, III and IV. However, the individual regulatory properties of these forms are not yet known.

HSDs from maize [5, 6, 9, 10], pea [2], castor bean [2] and carrot [7] are also stimulated by K^+ and inhibited by threonine. HSD from maize, as in soybean, progressively decreases in its sensitivity to inhibition by threonine during seedling development [5]. In maize, HSDs from 3-day and 7-day-old shoots are inhibited by 10 mM threonine 82 and 35%, respectively. HSDs of 3-day and 19-day-old soybean cotyledons are inhibited 72 and 51% respectively by 10 mM threonine; thus a greater change occurs in the maize system. In both plants this alteration in regulation is accompanied by an alteration in the amounts of the molecular forms of the enzyme [5, 6, 9, 10]. However, the relationship between the alteration in the amounts of the different forms of maize HSD and the decrease in inhibition of HSD by threonine is not yet understood.

Electrophoretic analysis of HSD from extracts of 72-hr-old maize shoots indicated the presence of 3 enzyme forms [5, 6], while soybean HSD consists of at least 4 major forms. The fastest migrating form (form I) of maize HSD is similar to soybean HSD form I, since it is of lower MW than the other 2 forms of maize HSD (forms II and III) [10]. The higher two MW forms appear to be of similar MW, but differ in charge as is the case of forms II, III and IV of soybean HSD. Form I of maize seedlings could not be detected on polyacrylamide gels containing extracts of 168-hr-old maize shoots. However, a more slowly migrating diffuse band of activity was detected [6, 10]. No such diffuse band of activity was detected on polyacrylamide gels containing extracts from 11-day or 19-day-old soybean organs. The amount of the fastest migrating form (form I) decreases in older maize seedlings [6, 10] as it does in soybean seedlings. Both form I of maize and soybean seedlings are insensitive to threonine inhibition and are not stimulated by K^+. Throughout maize and soybean seedling development, total HSD becomes less sensitive to threonine inhibition.

Our data indicate that the progressive decrease in threonine inhibition of HSD and the change in the

Table 5. Comparison of the amounts of different homoserine dehydrogenase forms in extracts of 3- and 19-day-old soybean cotyledons

Measurement	Forms† I	II	III	IV
rm values:*				
3 day	0.339 ± 0.002	0.255 ± 0.004	0.232 ± 0.003	0.215 ± 0.004
19 day	N.D.‡	0.256 ± 0.005	0.236 ± 0.002	0.215 ± 0.005
% of Total activity§				
3 day	4 ± 2	20 ± 2	40 ± 1	36 ± 1
19 day	N.D.	34 ± 1	42 ± 2	24 ± 2

* Data are averages of at least 8 determinations. Standard deviation is presented as ± value.

† Forms A and B accounted for less than 1% of the total homoserine dehydrogenase activity and were not included.

‡ Not determined. Form I was present in 19-day-old cotyledon extracts (*ca* 2% of the total activity) as determined by gel filtration chromatography and by electrophoresis of the resultant pool B. However, staining of electrophoretic gels containing unchromatographed extracts of 19-day-old cotyledons did not reveal the presence of form I.

§ Average of 5 determinations.

amounts of the different enzyme forms are correlated with the decrease in K^+ stimulation of the higher MW forms (II, III and IV) of soybean seedlings. This interpretation may also apply to the maize system and is supported by the data of DiCamelli and Bryan [10] using maize HSD. Form I of maize HSD is insensitive to threonine inhibition and is not stimulated by K^+. Forms II and III, which are sensitive to threonine inhibition, are stimulated by K^+ 6.7-fold in extracts of 72-hr-old shoots and only 2-fold in extracts of 168-hr-old maize shoots. Maize HSD is more greatly inhibited by threonine in the presence of K^+ than when K^+ is absent. Thus, in maize, the decrease in the amount of inhibition of HSD by threonine occurs at the same time as, and perhaps may also be accounted for by, a decrease in K^+ stimulation of the threonine-sensitive forms of HSD.

Quantitation of the enzyme forms using enzyme-specific staining of electrophoretic gels has allowed us to observe a shift in the relative amounts of the forms of HSD during soybean seedling growth. Although the individual regulatory properties of forms II, III and IV remain to be elucidated, the decrease in the relative amount of form II and increase in form IV may be the basis for the decrease in threonine inhibition and K^+ stimulation of HSD.

Whether the change in threonine inhibition and K^+ stimulation is important biologically remains to be elucidated. It is possible that as soybean seedlings develop, less sensitive regulatory controls are needed. This might allow for an increase in the size of the cellular threonine pool as seedlings mature. Although different intracellular compartments may contain different forms of soybean HSD, this does not appear to be the case with the several forms of maize HSD, all of which have been detected in the chloroplast [9].

Soybean HSD activity is potently inhibited *in vitro* by L-cysteine. Cysteine also inhibits HSD from pea leaves [2], maize roots [1], barley seedlings [4] and carrot cell suspension cultures [7]. D-Cysteine and several other sulfur-containing compounds do not strongly affect HSD. L-Cysteine is a more effective inhibitor of carrot cell culture HSD in the direction of β-aspartate semialdehyde formation than in the direction of homoserine formation. Thus, regulation of HSD by cysteine may be important in the control of sulfur flow from cysteine to methionine by preventing the conversion of homoserine to aspartic-β-semialdehyde when cysteine levels are high. However, the *in vivo* effects of cysteine on HSD have not yet been assessed.

EXPERIMENTAL

Soybeans (*Glycine max* Merr. Var. Williams) grown in sand in the greenhouse were collected 3, 6, 11 and 19 days after planting. Shoot and root lengths of at least 20 seedlings were averaged. Organs of between 20 and 150 plants of each age were separated into roots, leaves, cotyledons and stems. The expts were conducted at least 3 times and the trends noted were always present, although up to 10% fluctuation in values was noted between expts.

Extraction of HSD. All extraction procedures were conducted at 0–5°. Weighed tissues were ground in 50 mM KPi buffer (pH 7.5) containing 1 mM EDTA, 10 mM 2-mercaptoethanol, 10 mM diethyldithiocarbamic acid and 20% glycerol using a mortar and pestle. The slurry was centrifuged (27000 *g*, 20 min) and the supernatant protein precipitating between 30 and 66% satn by $(NH_4)_2SO_4$ (pH 7.5) was collected by centrifugation, resolubilized and dialyzed for 18 hr against a 200-fold excess of buffer A [50 mM KPi buffer (pH 7.5) containing 1 mM EDTA, 1.4 mM 2-mercaptoethanol and 20% glycerol]. This prepn contained at least 95% of the HSD activity present in the crude unfractionated homogenate and was used for all protein determinations.

Enzyme assays and protein determination. HSD was measured in the direction of coenzyme reduction using NAD and NADP as described previously [7], using 15 mM homoserine and either 0.48 mM NADP or 15 mM NAD. KCl (150 mM) was included in the assay buffer except when stated otherwise. Compounds used for enzyme inhibition studies were neutralized with KOH. One unit of HSD activity is defined as the amount of enzyme necessary to produce a change in A_{340} of 0.001/min. HSD was measured in the direction of coenzyme oxidation as described previously [5], using 200 mM KPi buffer (pH 7.1), 0.7 mM 2-mercaptoethanol, 0.5 mM EDTA, 0.5 mM NADPH or 0.3 mM NADH, and 1.0 mM D,L aspartic-β-semialdehyde in a total vol. of 1 ml. Aspartic-β-semialdehyde was synthesized as described in ref. [11] and purified using a Dowex 50-X8 column. Protein determinations were by the biuret method after cold TCA precipitation [12].

Polyacrylamide gel electrophoresis. Prepns were analysed by discontinuous gel electrophoresis [13] with the enzyme activity being located on the gels as described in ref. [5]. Unless stated otherwise the assay mixture used to detect HSD on the gels contained 60 mM Tris HCl (pH 9), 0.15 mM EDTA, 0.21 mM 2-mercaptoethanol, 150 mM KCl, 24 mM homoserine, 0.266 mg/ml nitro blue tetrazolium, 0.025 mg/ml phenazine methosulfate and either 30 mM NAD or 0.96 mM NADP. After stain development, the gels were rinsed in H_2O and scanned at 600 nm using a gel scanning apparatus. In this way the intensity of the stain could be measured as peak ht on the recorder tracing. Peak hts were determined by comparing tracings of gels incubated in complete assay mixtures with those of control gels where homoserine was omitted.

Gel filtration chromatography. Enzyme prepns from soybean cotyledons were applied to a 1.5 × 50 cm gel filtration column (Sephacryl S-200) at 4° in buffer A. The enzyme was eluted at a flow rate of 4 ml/hr and 1 ml fractions were collected and assayed for enzyme activity. Aliquots of several fractions were analysed on polyacrylamide gels. Fractions were then combined as described in the text. The enzyme was precipitated using $(NH_4)_2SO_4$ at 66% satn, solubilized and dialysed against buffer A. The enzyme was purified at least 20-fold by these procedures.

Acknowledgements—This work was supported by the Illinois Soybean Program Operating Board, a National Science Foundation Grant PCM-76-11513, and the Illinois Agricultural Experiment Station and was conducted with the technical assistance of Shou Shye.

REFERENCES

1. Bryan, J. K. (1969) *Biochim. Biophys. Acta* **171**, 205.
2. DiMarco, G. and Grego, S. (1975) *Phytochemistry* **14**, 943.
3. Aarnes, H. and Rognes, S. E. (1974) *Phytochemistry* **13**, 2717.
4. Aarnes, H. (1977) *Plant Sci. Letters* **9**, 137.
5. Matthews, B. F., Gurman, A. W. and Bryan, J. K. (1975) *Plant Physiol.* **55**, 991.
6. Matthews, B. F. and Bryan, J. K. (1975) *Plant Physiol.* **56S**, 273.
7. Matthews, B. F. and Widholm, J. M. (1978) *Planta*, **141**, 315.
8. Hedrick, J. L. and Smith, A. J. (1968) *Arch. Biochem. Biophys.* **126**, 155.
9. Bryan, J. K., Lissik, E. A. and Matthews, B. F. (1977) *Plant Physiol.* **59**, 673.
10. DiCamelli, C. A. and Bryan, J. K. (1975) *Plant Physiol.* **55**, 999.
11. Black, S. and Wright, N. G. (1955) *J. Biol. Chem.* **212**, 39.
12. Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) *J. Biol. Chem.* **177**, 751.
13. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404.