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Immunological Characterization of *in Vitro* Forms of Homoserine Dehydrogenase from Carrot Suspension Cultures[1]

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ABSTRACT

Multiple forms of homoserine dehydrogenase (HSDH) from carrot (*Daucus carota* L.) have been identified. One form of HSDH (T-form) has a relative molecular weight of 240,000 and is strongly inhibited by threonine. Another form (K-form) has a relative molecular weight of 180,000 and is insensitive to inhibition by threonine. The interconversion of these two forms is dependent upon the presence or absence of threonine and potassium. Polyacrylamide electrophoretic gels stained for HSDH activity and protein, paralleled with Western blot analysis, verified the interconversion of the T- and K-forms in 5 millimolar threonine and 100 millimolar potassium, respectively. Carrot HSDH also aggregates to form higher molecular weight complexes of 240,000 up to 720,000 M_r. Polyclonal antibody from mouse was raised against the T-form (240,000 M_r) of carrot HSDH. Specificity of the mouse antisera to carrot HSDH was verified by immunoprecipitation and Western blot analysis. The T-form, K-form, and all of the higher molecular aggregates of carrot HSDH cross-reacted with the anti-HSDH antiserum. The antiserum also cross-reacted with soybean HSDH, but did not cross-react with either of the two HSDH forms found in *Escherichia coli*. A model for the *in vivo* regulation of threonine biosynthesis in the chloroplast is presented. The model is based on the interconversion of the HSDH forms by potassium and threonine.

HSDH[2] (EC 1.1.1.3) is a key branch-point enzyme in the biosynthetic pathway of the aspartate family of amino acids. In plants and bacteria the essential amino acids lysine, threonine, isoleucine and methionine are synthesized by this pathway (Fig. 1). HSDH catalyzes the reduction of aspartate semialdehyde to homoserine utilizing the cofactor NADPH or NADH. Most of our understanding of the structure and regulation of enzymes and genes involved in the biosynthesis of the aspartate family of amino acids is from research conducted in bacterial systems. In *Escherichia coli*, the gene encoding HSDH is fused with the gene encoding AK, yielding a fused protein product carrying both enzyme activities. There are two AK/HSDH isoenzymes in *E. coli*. Antibodies specific for each of the *E. coli* AK/HSDH proteins have been used to immunochemically compare and characterize the proteins (27, 28). Antibodies specific for each of the AK/HSDH forms cross-reacted with the denatured homologous and heterologous AK/HSDH proteins, but the antibodies did not cross-react with the heterologous native proteins (28). A more detailed immunological analysis has shown the distribution of various antigenic determinants between the two AK/HSDH polypeptides (27). Recently, both of the genes encoding these enzymes have been isolated and sequenced (14, 29). Amino acid homology between the HSDH region of the two *E. coli* proteins is 53%.

A plant HSDH gene has not yet been identified, and little is known about the regulation of HSDH gene expression in plants. However, the enzyme has been studied from several different plant species (1, 2, 4, 9, 19–22). HSDH has been localized in the chloroplast fractions of maize (4) and pea leaves (24, 25). To date, HSDH has been purified to homogeneity and well characterized from maize (18) and recently in our laboratory from carrot suspension cultures (23). In carrot, there are multiple *in vitro* forms of HSDH that form aggregates with relative molecular weights up to 720,000 (23). Two different mol wt forms have different regulatory properties yet appear to interconvert. The enzymatic activity of one form, the T-form (240,000 M_r), is sensitive to inhibition by threonine. The activity of the other form, the K-form (180,000 M_r), is not inhibited by threonine. In order to gain a better understanding of the relationships among the several *in vitro* forms of HSDH, we have prepared antiserum to the T-form of carrot HSDH. In this paper, we verify the interconversion of the T- and K-forms of HSDH. Additionally, we describe the relationships of the several different *in vitro* forms of carrot HSDH by immunological characterization using antiserum specific to carrot HSDH. Finally, a model is proposed for the *in vivo* regulation of threonine biosynthesis in the chloroplast.

MATERIALS AND METHODS

HSDH Isolation and Assay

HSDH was isolated from 5-d-old carrot (*Daucus carota* L.) suspension cultures as described previously by Matthews *et al.* (23). The high mol wt aggregated forms of HSDH were obtained by electroeluting the 240,000 M_r form of HSDH from a 9% polyacrylamide gel (23) in the absence of potassium. Interconversion of HSDH was accomplished by dialyz-

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[2] Abbreviations: HSDH, homoserine dehydrogenase; AK, aspartokinase; Ig, immunoglobulin G.

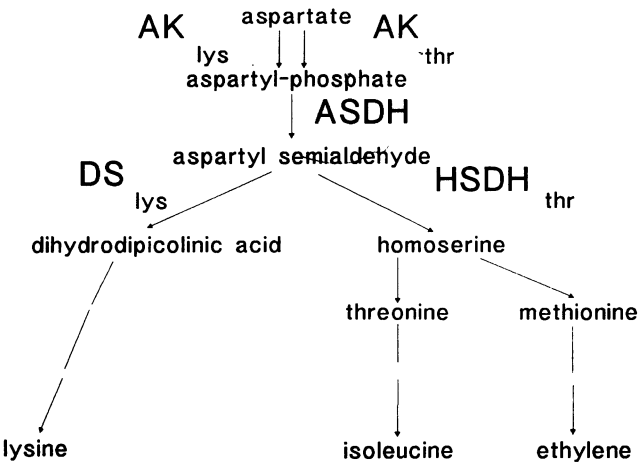

Figure 1. Pathway leading to the synthesis of the essential amino acids lysine, threonine, methionine, and isoleucine from aspartate. Aspartokinase (AK), aspartate semialdehyde dehydrogenase (ASDH), homoserine dehydrogenase (HSDH), and dihydropicolinic acid synthase (DS).

ing partially purified samples of HSDH (>500-fold) overnight (18 h) in TT buffer (25 mM Tris, 5 mM threonine [pH 7.6]) to product the T-form of HSDH or in TK buffer (25 mM Tris, 100 mM KCl [pH 7.6]) to produce the K-form. *E. coli* HSDH was isolated as described by Klungsoyr *et al.* (15), while HSDH from soybean was isolated as described by Matthews and Widholm (20). Proteins were separated by electrophoresis on 10 to 15% polyacrylamide gels using a pHast system (Pharmacia)[3] and visualized on the gels using silver according to the manufacturer's instructions. Proteins containing HSDH activity were visualized by incubating the gels at 37°C for 5 to 15 min in 50 mM Tris (pH 9.0), 150 mM KCl, 0.15 M NAD, 1 mM EDTA, 1.5 mM β-mercaptoethanol, 0.15 M homoserine, 0.15 M nitro blue tetrazolium, and 0.15 M phenazinemethosulfate. HSDH activity was determined using the reverse reaction, in which NAD is reduced and in the forward reaction in which NADH is oxidized (19).

Preparation of Polyclonal Antibody

A total of 40 μg of purified native carrot HSDH (240,000 M_r) was injected into the peritoneal cavity of a female BALB/c mouse. One hundred microliters (10 μg protein) of HSDH was mixed with Freund's complete adjuvant and injected into the peritoneal cavity every 10 d for 40 d. Ten days after the last injection NS-1 myeloma cells were injected into the peritoneal cavity. Five to 10 ml of ascites fluid were removed every 3 to 7 d starting 7 d after the injection of the myelomas. Cells and debris were removed from the ascites fluid by centrifugation (1000*g*) at 4°C for 5 min and lipids were removed by filtration through glass wool. Ascites fluid was diluted 1:1 with 1 × TBS (20 mM Tris, 150 mM NaCl [pH 7.5]) and stored at 4°C.

[3] Mention of trademark, proprietary product or vendor does not constitute a guarantee of warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of the products or vendors that may be suitable.

Western Blot Analysis

Proteins were separated by PAGE as described above and were transferred to nitrocellulose filters by electrotransfer in 25 mM Tris and 20 mM glycine (pH 8.3), at 25 to 35 V for 1 h at 4°C. HSDH activity on the nitrocellulose was visualized as described above for electrophoretic gels. Nonspecific protein binding sites were blocked by incubating the filters in 1 × TBS, 1.0% dry milk, and 0.5% BSA for 1 h at room temperature or overnight at 4°C. Nitrocellulose filters were incubated with primary antibody (anti-HSDH antiserum) in 1/10 × block solution for 1 h at room temperature or 16 h at 4°C. Filters were washed four times with 1 × TBS. Following the wash, filters were incubated with secondary antibody (goat anti-mouse) in 1/10 × block solution for 1 h at room temperature and washed 1 × TBS. The filters were incubated for 1 h at room temperature with tertiary antibody (alkaline phosphatase conjugated rabbit anti-goat) and 1/10 × block solution. Filters were washed in 1 × TBS. Bands were visualized by incubating the filters in 100 mM Tris (pH 9.5), 100 mM NaCl, and 5 mM $MgCl_2$, 0.035% nitroblue tetrazolium, and 0.0175% 5-bromo-4-chloro-3-indoyl phosphate for 10 min at room temperature. The phosphatase reaction was stopped by incubating the filters in 20 mM Tris (pH 7.5), and 1 mM EDTA for 5 to 10 min.

Immunoprecipitation

Mouse anti-HSDH IgG was purified from the ascites fluid (anti-HSDH antiserum) by ion exchange chromatography. Fifty mg of protein (from mouse ascites) was bound to a Protein-PAK DEAE 5PW column (Waters, 7.5 mm × 7.5 cm). Protein was eluted from the column with a 30 min linear gradient, buffer A (20 mM Tris [pH 8.5]) and buffer B (20 mM Tris [pH 7.0] and 0.3 M NaCl). Two ml fractions were collected and each fraction was tested for cross-reactivity with carrot HSDH on Western blots. Fractions were also analyzed on native SDS-PAGE gels to determine the IgG composition. Normal mouse IgG (Kirkgaard and Perry Laboratories) was used as a control.

One hundred μl of carrot HSDH (250 units) was incubated in half strength buffer with varying amounts of anti-HSDH IgG or normal mouse IgG for 16 to 24 h at 4°C. The concentration of the antibodies ranged from 2 to 20 μg of IgG per immunoprecipitation reaction. An equal volume of 1 × TBS buffer was incubated at each of the different IgG concentrations tested as a control. The samples were incubated in 100 μL of protein A agarose beads (300 μg/mL in 1 × TBS) at room temperature for 1 h. The HSDH/IgG/protein A complex was pelleted in a microfuge at 4°C for 10 min. The supernatant was analyzed for HSDH activity was previously described.

RESULTS

Mouse antiserum raised against native carrot HSDH (240,000 M_r) was able to immunoprecipitate carrot HSDH from solution, while normal mouse IgG did not (Fig. 2). Approximately 3 μg of anti-HSDH IgG precipitated 50% of the HSDH activity, while 90% of the HSDH activity was precipitated with 8 μg of anti-HSDH IgG.

The specificity of anti-HSDH antiserum was determined by Western blot analysis of carrot HSDH at various stages of purification (Fig. 3). The antiserum reacted with HSDH protein from a crude extract, an extract of enzyme purified over 100-fold and an extract purified over 5000-fold (to homogeneity). The antisera did not cross-react with any other band of protein in either the crude or partially purified extract, indicating a high specificity for carrot HSDH.

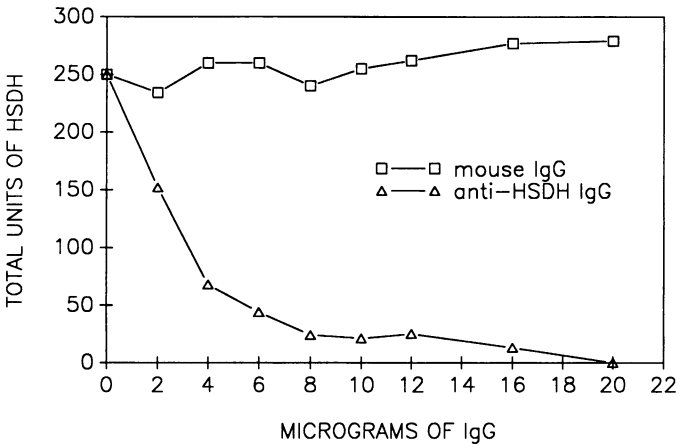

Figure 2. Immunoprecipitation of carrot HSDH with mouse antisera. Immunoprecipitation was performed with various concentrations (0 to 20 μg IgG) of mouse anti-HSDH IgG or normal mouse IgG as described in "Materials and Methods." The amount of carrot HSDH activity used to obtain each data point was kept constant (250 units) in all the experiments. The percentage of free enzyme was determined by normalizing the results from the anti-HSDH IgG samples to those of the normal mouse IgG control. The IgG concentration is expressed in total μg per reaction.

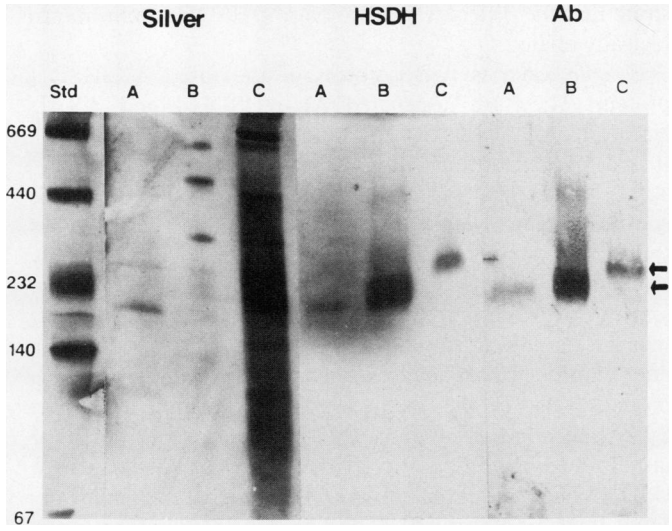

Figure 3. Analysis of mouse antisera specificity to carrot HSDH. HSDH samples of different degrees of purity, pure HSDH (>5000-fold) (A), HSDH extract purified >100-fold (B), and crude extract (C), were separated by PAGE. Samples were stained for protein with silver or for HSDH activity. An identical gel blotted for Western analysis (Ab). Each lane contains approximately 25 units of HSDH activity. Arrows indicate 180,000 and 240,000 M_r forms of HSDH. Fifty ng of each of the following proteins were added as mol wt (×1000) standards (Std); thyroglobulin (669), ferritin (440), catalase (232), lactate dehydrogenase (140), and BSA (67).

Further analysis of the specificity of the antiserum to HSDH showed that anti-HSDH antisera did cross-react with soybean HSDH but it did not cross-react with *E. coli* (Fig. 4). These results suggest that the two plant HSDH proteins are related. In contrast, there was no indication of cross-reactivity of the anti-HSDH antisera with extracts of *E. coli* containing HSDH activity, thus the HSDH forms in *E. coli* are not immunologically related to HSDH from carrot or soybean.

The antisera was tested for its ability to detect denatured carrot HSDH by reacting the antiserum with Western blots of SDS treated and boiled carrot HSDH. The antiserum was not able to detect denatured carrot HSDH (data not shown).

Highly purified (>2000-fold) HSDH can form aggregates ranging from 240,000 to 720,000 M_r as visualized on polyacrylamide electrophoretic gels (Fig. 5). These aggregated forms of HSDH were tested with HSDH antiserum to determine if all of the forms are immunologically related. PAGE gels were stained for HSDH activity. Identical gels were blotted to nitrocellulose and incubated in the HSDH antibody. Analysis of the western blots show that the antibody reacted with all of the polymerized HSDH forms present on a Western blot, indicating that they are all related.

Protein extracts containing HSDH activity were separately dialyzed overnight in TT buffer (5 mM threonine) or TK buffer (100 mM potassium). The enzyme forms were separated by PAGE. The gels were silver stained or stained for HSDH activity. Duplicate gels were transferred to nitrocellulose for Western blot analysis (Fig. 6). The T-form and K-form of carrot HSDH reacted equally strong with the antiserum. HSDH dialyzed in TT buffer only contained the T-form

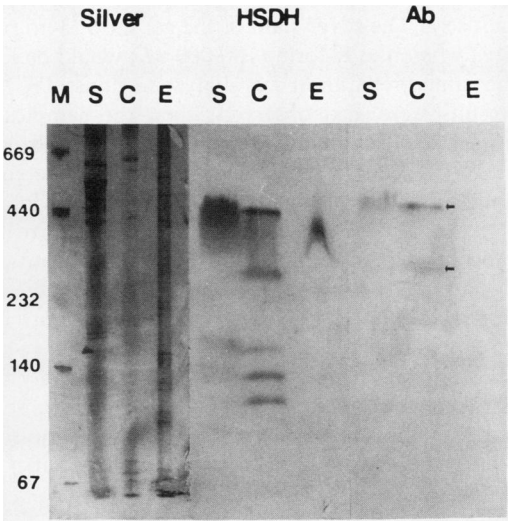

Figure 4. Analysis of mouse antisera specificity to plant HSDH. Crude extracts of HSDH were isolated from soybean (S), carrot (C), and *E. coli* (E) and separated on PAGE. Each lane contains approximately 25 units of HSDH activity. Samples were stained for protein with silver or for HSDH activity. Identical gels were blotted for western analysis (Ab). Molecular weights (×1000) are indicated. Molecular weight standards (Std) are as described in Figure 3.

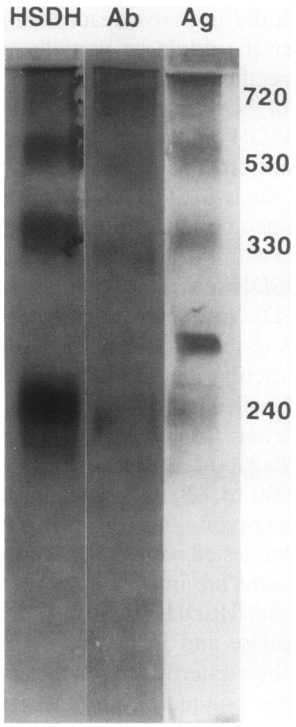

Figure 5. Polymerization of HSDH. HSDH was electroeluted from native polyacrylamide gels as described in "Materials and Methods." HSDH forms were separated by PAGE. Each lane contains approximately 75 units of HSDH activity. Samples were stained for protein with silver or for HSDH activity. Identical gels were blotted for Western analysis (Ab). Molecular weights are indicated (×1000).

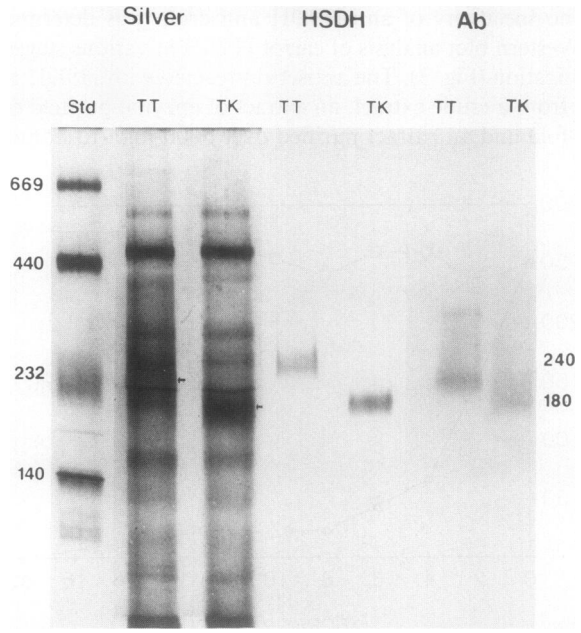

Figure 6. Interconversion of carrot HSDH forms. Carrot HSDH was dialyzed in TT-buffer (25 mM Tris, 5 mM threonine [pH 7.6]) or TK-buffer (25 mM Tris, 100 mM KCl [pH 7.6]). The two forms were separated by PAGE (3 μg protein/lane and 25 units of activity/lane). The samples were stained for protein by silver and for HSDH activity. Identical gels were blotted and used for Western blot analysis (Ab). Molecular weight standards (Std) are as described in Figure 3.

(240,000 M_r) of the enzyme as indicated by analysis of the enzymes and protein on gels stained for protein and HSDH activity and by western blot analysis using the anti-HSDH mouse antisera. The silver stain of the gel showed no protein in the area where the K-form (180,000 M_r) would be located when the same preparation was dialyzed against TK buffer. These results were also observed when the samples were stained for HSDH activity or when HSDH was localized with the antisera by Western blot analysis. Likewise, the gel lane containing the K-form of the enzyme showed no evidence of the T-form. This verifies the conversion of one form to the other and not merely an inhibition of one form under conditions where the other form is more active. Further experimentation verified the interconversion of the two forms. Enzyme samples were dialyzed in TT buffer and were shown by PAGE and Western blot analysis to contain only the T-form; these samples were then dialyzed in TK buffer and shown to contain only the K-form. Likewise, samples containing only the K-form after dialysis in TK buffer were converted to the T-form after dialysis in TT buffer.

DISCUSSION

Mouse polyclonal antiserum was prepared against the 240,000 M_r form of carrot HSDH. The specificity of the mouse antiserum toward carrot HSDH was demonstrated by immunoprecipitation and Western blot analysis. Western blot analysis also showed that the antiserum reacted with the various *in vitro* forms of HSDH. These data verify the specificity of the mouse antiserum to carrot HSDH and demonstrate that the different forms of carrot HSDH are immunologically related.

E. coli contains two isoforms of the fused AK/HSDH protein. They are designated AK-I/HSDH-I and AK-II/HSDH-II. AK-I/HSDH-I has a mol wt of 355,000 ± 27,000. It is composed of four subunits having mol wt of 86,000 ± 4000. AK-II/HSDH-II has a mol wt of 185,000, and it is composed of two identical subunits of 88,000 (6). Antibodies specific for each of the two *E. coli* AK/HSDH proteins only cross-react with the denatured heterologous *E. coli* protein and not with the native heterologous protein. This suggests that the serological similarities between the two proteins are due to amino acids sequences that are buried within the native proteins (28). In this study, we have prepared antiserum against the native 240,000 M_r form of carrot HSDH for immunological comparison of the *in vitro* forms of carrot HSDH. The antiserum cross-reacted with all enzymatically active forms of carrot HSDH and with the high mol wt forms of soybean HSDH. These data suggest that plant HSDHs are structurally similar and that the two *E. coli* forms are not closely related to the two plant HSDH forms. Unlike the antisera raised against the two *E. coli* HSDH forms, the mouse antiserum to carrot HSDH did not cross-react with the denatured carrot or soybean HSDH. Future immunological analysis of the relationship between carrot and soybean HSDH may reveal the regions of similarity between the two plant HSDH forms.

Carrot HSDH is composed of identical 85,000 M_r subunits, the T-form appears to be a homotrimer and the K-form appears to be a homodimer (23). The antiserum was tested for its ability to detect the 85,000 M_r subunits. SDS-treated and boiled HSDH was separated by SDS-PAGE and used for Western blot analysis. The antiserum was not able to detect denatured carrot HSDH. Since the antisera was raised against native HSDH, it must only react with sequences that are present on the native or folded protein.

In plants there are several molecular weight forms of HSDH. They are separated into classes of forms, designated I through IV, according to their related molecular weights on native PAGE. HSDH form I (HSDH I) has a mol wt of 70,000 to 80,000. HSDH II has a mol wt range of 150,000 to 190,000. HSDH III has a mol wt range of 300,000 to 400,000, and HSDH IV has mol wt greater than 400,000 (for review, see ref. 5). Often, some of the mol wt forms are not present in the plant, *i.e.* carrot does not contain an enzymatically active HSDH I (our unpublished observations). Usually all forms of HSDH, except form I, are sensitive to threonine. Generally threonine concentrations of 5 mM or higher totally inactivate the enzyme (5). Threonine concentrations as low as 0.2 mM have inhibited approximately 70% of the HSDH activity in carrot (19).

In our previous study (23) and here, we have shown that highly purified samples of carrot HSDH can aggregate into different forms of M_r from 240,000 to 720,000. Comparison of gels stained for protein, HSDH activity stain, and Western blots show that all of the enzyme forms react with the antiserum; therefore, it appears that there are no inactive intermediate forms of HSDH. The HSDH aggregates which form *in vitro* appear to be multiples of about 180,000. However, a 180,000 M_r form has not been identified among the aggregates. A 180,000 M_r form has been identified only after dialysis of the enzyme in a potassium containing buffer. These data suggest that potassium may aid in the formation or maintenance of a stable 180,000 M_r form *in vitro*. Aggregation of HSDH into various forms has been observed in *Rhodospirillum rubrum* in the presence of threonine (7), but this phenomenon may not occur *in vivo* in carrot. In carrot the 240,000 M_r form of HSDH is predominant.

The activation and interconversion of maize HSDH forms by changes in potassium, threonine, and glycerol concentrations has been proposed by Krishnaswamy and Bryan (16, 17), based on different initial rates of velocity of HSDH reactions. Previously, we have shown physical evidence for the interconversion of carrot HSDH by gel electrophoresis (23). However, these conversions were observed at potassium concentrations of 250 mM, which are approximately 2 to 2.5 times higher than the potassium concentrations that have been observed for isolated pea (3, 11) and spinach chloroplast (8, 26). Here we have shown that the interconversion of the T- and K-forms of HSDH occurs at physiologically significant concentrations, 5 mM threonine and 100 mM potassium, respectively. Additionally, the Western blot analysis shows that there is an interconversion and not an activation or inactivation of either of the two forms under different conditions.

Our data, combined with the data of others, support a model we have developed to describe the role of the different carrot HSDH forms in controlling threonine production *in vivo* (Fig. 7). We have considered several factors in the chloroplast that effect HSDH activity, *i.e.* potassium, threonine, and NADPH concentrations and pH changes occurring in the light and dark. Potassium is the major monovalent cation found in isolated chloroplasts (3, 26), ranging in concentration from 41 to 198 mM (3, 8, 10, 11, 26). Most, *i.e.* 90 to 93%, of the potassium in the isolated chloroplasts is in the stroma or is loosely associated with the thylakoid membranes (26). Diurnal changes in potassium concentrations occur in soybean leaves, with levels increasing during the day and decreasing during the night (13). Furthermore, when isolated chloroplasts are illuminated, potassium ions are transported across the chloroplast envelope (3, 8, 10) elevating potassium levels in the stroma. At the same time, light-stimulated chloroplastic protein synthesis would increase the demand for amino acids and decrease the concentrations of free amino acids, including threonine. Thus, high potassium and low threonine concentrations in the illuminated chloroplast would favor the conversion of existing HSDH into the K-form. The K-form is stimulated by potassium and is insensitive to threonine inhibition (23), thus allowing increased threonine production. Additionally, the pH in the stroma of the illuminated chloroplast would increase to approximately 8.0 (13), the pH optimum for carrot HSDH is *ca.* 8.0 (our unpublished observations). Also, NADPH, a cofactor for HSDH activity, is produced during photosynthesis. When the chloroplast is no longer illuminated, protein synthesis slows down, free threonine levels in the chloroplast increase, potassium levels decrease and the pH is lowered. Conditions in the chloroplast

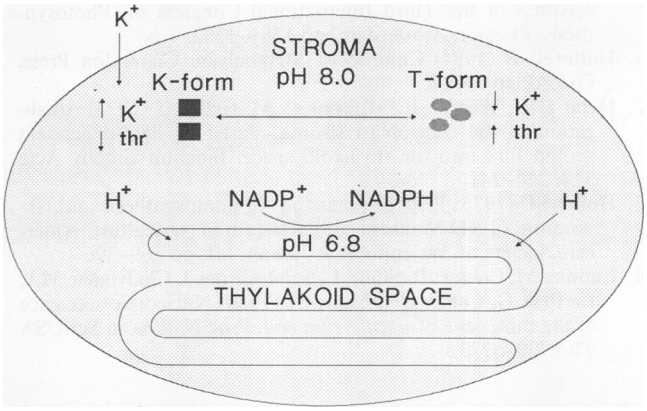

Figure 7. A model of the regulation between the K- and T-forms of HSDH in the chloroplast. In the light, protein synthesis utilizes amino acids, thus decreases the amount of threonine in the chloroplast. Illumination of chloroplast increases the K^+ concentration in the stroma via potassium pumps across the chloroplast envelope. The equilibrium of the HSDH forms is shifted towards the K-form, which is insensitive to feedback inhibition by threonine. Additionally, the pH in the stroma increases to 8.0 and NADPH (a cofactor for HSDH) is produced resulting in more rapid synthesis of threonine. In the dark, threonine levels increase as protein synthesis decreases and K^+ leaves the chloroplast. The equilibrium shifts back towards the T-form, which is sensitive to threonine inhibition, resulting in a decrease in threonine synthesis.

would then favor the formation of the T-form, which is threonine sensitive and is less responsive to stimulation by potassium ions than the K-form (23). Under these conditions, HSDH activity and threonine biosynthesis would be curtailed.

LITERATURE CITED

1. **Aarnes H, Rognes SE** (1974) Threonine-sensitive aspartate kinase and hormoserine dehydrogenase from *Pisum sativum*. Phytochemistry **13:** 2712–2724
2. **Aarnes H** (1977) A lysine-sensitive aspartate kinase and two molecular forms of homoserine dehydrogenase from barley seedlings. Plant Sci Lett **9:** 137–145
3. **Barber J** (1980) Ionic regulation in chloroplast and its control of photosynthesis. *In* RM Spanswick, WJ Lucas, J Dainty, eds, Plant Membrane Transport, Current Conceptual Issues. Elsevier, Amsterdam, pp 83–96
4. **Bryan JK, Lissik EA, Matthews BF** (1977) Changes in enzyme regulation during growth of maize. III. Intracellular localization of homoserine dehydrogenase in chloroplasts. Plant Physiol **59:** 673–679
5. **Bryan JK** (1980) Synthesis of the aspartate family and branched-chain amino acids. *In* BJ Miflin, ed, The Biochemistry of Plants: A Comprehensive Treatise, Vol 5. Academic Press, New York, pp 403–452
6. **Cohen GN** (1983) The common pathway to lysine, methionine, and threonine. *In* KM Herrmann, RL Somerville, eds, Amino Acids: Biosynthesis and Genetic Regulation. Addison-Wesley, Reading, MA, pp 147–171
7. **Datta P, Gest H, Segal HL** (1964) Effects of feedback modifiers on the state of aggregation of homoserine dehydrogenase of *Rhodospirillum rubrum*. Proc Natl Acad Sci USA **51:** 125–130
8. **Demming B, Gimmler H** (1983) Properties of the isolated intact chloroplast at cytoplasmic K^+ concentrations. Plant Physiol **73:** 169–174
9. **DiCamelli CA, Bryan JK** (1980) Comparison of sensitive and desensitized forms of maize homoserine dehydrogenase. Plant Physiol **65:** 176–183
10. **Gimmler H, Schafer G, Heber U** (1974) Low permeability of the chloroplast envelope towards cations. *In* M Avron, ed, Proceedings of the Third International Congress on Photosynthesis. Elsevier, Amsterdam, pp 1381–1392
11. **Halliwell B** (1984) Chloroplast Metabolism. Clarendon Press, Oxford, pp 31–62
12. **Heldt HW, Werdan K, Milovancev M, Geller G** (1973) Alkalization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. Biochim Biophy Acta **314:** 224–241
13. **Huber SC** (1985) Role of potassium in photosynthesis and respiration. *In* RD Munson, ed, Potassium in Agriculture. American Society of Agriculture, St Joseph, MI, pp 369–396
14. **Katinka M, Cossart P, Sibilli L, Saint-Girons I, Chalvignac MA, Le Bras G, Cohen GN, Yaniv M** (1980) Nucleotide sequence of the thrA gene of *Escherichia coli*. Proc Natl Acad Sci USA **77:** 5730–5733
15. **Klungsoyr L, Hagemen JH, Fall L, Atkinson DE** (1968) Interaction between energy charge and product feedback in the regulation of biosynthetic enzymes. Aspartokinase, phosphoribosyladenosine triphophate synthetase, and phosphoribosyl pyrophosphate synthetase. Biochemistry **7:** 4035–4040
16. **Krishnaswamy S, Bryan JK** (1983) Ligand-induced interconversions of maize homoserine dehydrogenase among different states. Arch Biochem Biophys **222:** 449–463
17. **Krishnaswamy S, Bryan JK** (1983) Characterization of ligand-induced states of maize homoserine dehydrogenase. Arch Biochem Biophys **227:** 210–224
18. **Krishnaswamy S, Bryan JK** (1986) Use of monoclonal antibodies for the purification and characterization of the threonine-sensitive isozyme of maize homoserine dehydrogenase. Arch Biochem Biophys **246:** 250–262
19. **Matthews BF, Widholm JM** (1978) Regulation of lysine and threonine synthesis in carrot cell suspension cultures and whole carrot roots. Planta **141:** 315–321
20. **Matthews BF, Widholm JM** (1979) Regulation of homoserine dehydrogenase in developing organs of soybean seedlings. Phytochemistry **18:** 395–400
21. **Matthews BF, Widholm JM** (1979) Enzyme expression in soybean cotyledon, callus, and cell suspension culture. Can J Bot **57:** 299–304
22. **Matthews BF, Widholm JM** (1979) Expression of aspartokinase, dihydrodipicolinic acid synthase and homoserine dehydrogenase during growth of carrot cell suspension cultures of lysine- and threonine-supplemented media. Z Naturforsch **34c:** 1177–1185
23. **Matthews BF, Farrar MJ, Gray AC** (1989) Purification and interconversion of homoserine dehydrogenase from *Daucus carota* cell suspension cultures. Plant Physiol **91:** 1569–1574
24. **Mills WR, Wilson KG** (1978) Amino acid biosynthesis in isolated pea chloroplasts: metabolism of labeled aspartate and sulfate. FEBS Lett **92:** 129–132
25. **Mills WR, Lea PJ, Miflin BJ** (1980) Photosynthetic formation of the aspartate family of amino acids in isolated chloroplasts. Plant Physiol **65:** 1166–1172
26. **Robinson SP, Dowton JS** (1984) Potassium, sodium and chloride content of isolated intact chloroplasts in relation to ionic compartmentation in leaves. Arch Biochem Biophys **228:** 197–206
27. **Sibilli L, Le Bras G, Fazel A, Bertrando O, Dautry-Varsat A, Cohen GN, Zakin MM** (1982) Distribution of the common antigenic determinants in two isofunctional proteins from *Escherichia coli* K 12. Biochem Int **4:** 331–336
28. **Zakin MM, Garel JR, Dautry-Varsat A, Cohen GN, Boulot G** (1978) Detection of the homology among proteins by immunochemical cross-reactivity between denatured antigens. Application to the threonine and methionine regulated aspartokinase homoserine dehydrogenase from *Escherichia coli* K 12. Biochemistry **17:** 4318–4323
29. **Zakin MM, Duchange N, Ferrara P, Cohen GN** (1983) Nucleotide sequence of the metL gene of *Escherichia coli*. J Biol Chem **258:** 3028–3031