Expression of Aspartokinase, Dihydrodipicolinic Acid Synthase and Homoserine Dehydrogenase During Growth of Carrot Cell Suspension Cultures on Lysine- and Threonine-Supplemented Media

Benjamin F. Matthews and Jack M. Widholm

Department of Agronomy, University of Illinois, Urbana, Illinois 61801

Z. Naturforsch. **34 c,** 1177 – 1185 (1979); received June 18, 1979

Daucus carota, Suspension Cultures, Amino Acids, Enzyme Regulation

Reduction in the amounts of activity of the first enzyme, aspartokinase (EC 2.7.2.4) and two branch-point enzymes, dihydrodipicolinic acid synthase (EC 4.2.1.52) and homoserine dehydrogenase (EC 1.1.1.3), located in the pathway for the synthesis of aspartate-family amino acids, occurred when cell suspension cultures of *Daucus carota* L. var. Danvers were grown in media containing 2 mM threonine or 2 mM lysine, endproducts of the pathway. Activity of the lysine-sensitive form of aspartokinase was decreased when cells were grown in medium containing lysine and the activity of the threonine-sensitive form was decreased when cells were grown in medium containing threonine. Activity of the branch-point enzyme leading to threonine synthesis, homoserine dehydrogenase, was decreased up to 70% in specific activity (units/mg protein) and relative activity (units/g fresh weight) when cells were grown in media containing lysine or threonine. Threonine had no effect on the relative activity of dihydrodipicolinic acid synthase, but decreased its specific activity. Lysine decreased the relative activity of the synthase by up to 40%, but had little effect on its specific activity. The decreased activities of the enzymes were apparently not due to binding of the inhibitory amino acids to the enzymes since homogenization of cells in buffer with 2 mM lysine and threonine did not decrease the measurable enzyme activities. These and other results presented suggest that both forms of the aspartokinase activity and homoserine dehydrogenase activity can be altered by supplementing the growth medium with lysine or threonine.

Introduction

In higher plants, regulation of the concentrations of endproducts in amino acid biosynthetic pathways can occur by feedback inhibition [1, 2]. Thus, enzymes located at a branch in the pathway are commonly inhibited by the ultimate endproduct of that particular branch. Repression of the synthesis of enzymes in amino acid biosynthetic pathways is well documented in bacteria [3 – 5], but has not been commonly observed in higher plants. Our laboratory has been interested in regulation of amino acid biosynthesis in higher plants, especially control of the pathways involved in asparate-family [6 – 8] and aromatic amino acid formation [9 – 12]. Tobacco and carrot cell cultures have been selected which are resistant to several aromatic and aspartate-family amino acid analogs [9, 10]. To understand the mechanisms involved in resistance of selected cell lines to these analogs and to develop new screening methods for the selection of cell lines which overproduce amino acids, a thorough understanding of the basic regulatory mechanisms involved in amino acid metabolic control is important. In carrot we have demonstrated three key enzymes to be subject to feedback inhibition by endproducts in the pathway for the synthesis of lysine, threonine, isoleucine and methionine [6].

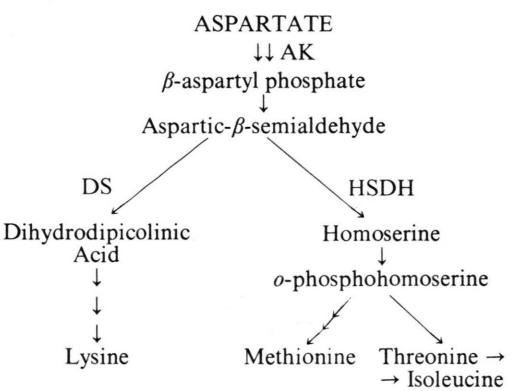

Diagram 1

Reprint requests to Dr. B. F. Matthews, Dept. of Agronomy, Univ. of Maryland, College Park, MD; USA 20742.

0341-0382/79/1200-1177 $ 01.00/0

The first enzyme in the pathway, aspartokinase (AK) (EC 2.7.2.4) is present in two forms, a lysine-sensitive and a threonine-sensitive form, as demonstrated in several laboratories [6, 13, 14]. The branch-point enzyme dihydrodipicolinic acid synthase (DS) (EC 4.2.1.52) is inhibited strongly by lysine [6]. The other branch-point enzyme, homoserine dehydrogenase (HSDH) (EC 1.1.1.3) is composed of several molecular forms in many higher plants [15 – 17]. In carrot only one form, which is strongly inhibited by threonine and stimulated by K^+, has been detected thus far [6].

Recently, Sakano [18] has suggested that repression and derepression of the lysine-sensitive form of aspartokinase occurs in carrot root discs. However, Bright *et al.* [19] suggest that there is no correlation between aspartokinase isoenzyme levels and amino acid levels in carrot cell suspension cultures. In this paper, we wish to describe the effects of supplementing the endproducts, lysine and threonine to cell culture media on aspartokinase and on two other key enzymes in this pathway, homoserine dehydrogenase and dihydrodipicolinic acid synthase.

Materials and Methods

Plant material

Garden carrot (*Daucus carota* L. cv. Danvers) root cells were cultured in 100 ml defined liquid medium using 0.5 g fresh weight of cells as inoculum [11]. This cell line has been described previously [6] and was initiated in 1967 from callus of carrot root tissue. Cells from two or more flasks were harvested at each time point examined during the growth cycle. All flasks were examined for microbial contamination by plating cells and medium on Difco nutrient agar. Whole carrots were grown in soil in the greenhouse, irrigated daily with water or Hoagland's nutrient solution on alternate days.

Enzyme extraction and assay

Unless otherwise indicated, chemicals were obtained from Sigma (St. Louis, MO, USA). Enzyme extraction and assay procedures have been described in full detail previously [6]. Briefly, vacuum filtered cells were ruptured in cold 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM ethylenediaminetetraacetic acid (EDTA) (Hach Chem., Ames, IA, USA), 10 mM 2-mercaptoethanol (2-ME), 10 mM diethyldithiocarbamic acid (DDCA) and 20% glycerol, using a French pressure cell. After clarification by centrifugation, the supernatant was brought to 66% saturation using $(NH_4)_2SO_4$. Precipitated protein was collected by centrifugation, resuspended and dialyzed for 18 h against the same buffer lacking DDCA.

Aspartokinase activity was assayed as described in Matthews and Widholm [6] using the hydroxamate assay procedure. Assay mixtures contained 50 mM L-aspartate, 40 mM ATP and 20 mM $MgSO_4$ and were incubated at 30 °C for 60 min. One unit of activity was equal to the amount of enzyme necessary to produce 1.0 µmol of β-aspartyl phosphate/h. Controls lacking aspartate were included for all assays. Homoserine dehydrogenase activity was measured as described in Matthews and Widholm [6] using 0.1 M KCl, 15 mM homoserine, and 15 mM NAD. One unit of enzyme activity is the amount of enzyme necessary to produce a change in A_{340} of 0.001/min. Dihydrodipicolinic acid synthase activity was measured using the O-aminobenzaldehyde assay method of Yugari and Gilvarg [20] as modified by Matthews and Widholm [6]. Assay mixtures contained 0.01 M Tris buffer (pH 8.2), 1.5 mM D,L-aspartic-β-semialdehyde, 37 mM pyruvate and 0.5 mg O-aminobenzaldehyde. One unit of enzyme activity is the amount which produces a change in A_{520} of 0.001/min at 37 °C. Reaction mixtures lacking pyruvate were used as controls.

Protein was determined by the biuret method [21] after precipitation with cold TCA.

Amino acid analysis

Two independent extractions and determinations for amino acid content were made for each point examined during the growth cycle. Amino acids were extracted using methanol-chloroform-water (12 : 5 : 3) according to the method of Bieleski and Turner [22]. The extracts were evaporated to dryness, resolubilized in 0.2 M lithium citrate (pH 2.2) and analyzed using a Beckman model 119 CL amino acid analyzer using physiological fluid methodology.

Polyacrylamide gel electrophoresis

Homoserine dehydrogenase activity was analyzed by discontinuous gel electrophoresis using 5% polyacrylamide gels. Enzyme activity was visualized as described previously [6] by incubating the gels in a

medium containing 60 mM Tris-HCl (pH 9.0) 0.15 mM EDTA, 0.21 mM 2-ME, 150 mM KCl, 24 mM homoserine, 0.266 mg/ml nitro-blue tetrazolium, 0.025 mg/ml phenazine methosulphate and 30 mM NAD. Identical gels serving as controls were incubated in medium containing no homoserine.

Results

The presence of either 2 mM lysine or 2 mM threonine in the culture medium inhibited the increase in cell fresh weight (Fig. 1 A). Final fresh weight accumulation was decreased about 25% by lysine and 50% by threonine. Cells grown in the absence of lysine and threonine contained more protein during the middle portion of the growth cycle (Fig. 1 B) than

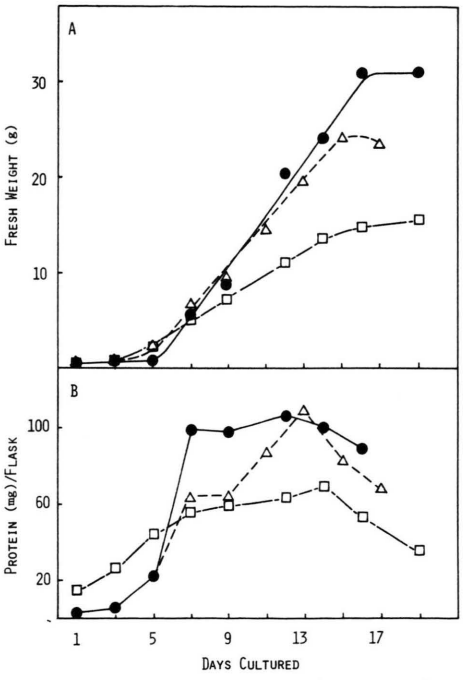

Fig. 1. Growth of carrot cells in suspension culture. A. Fresh weight accumulation of cells grown normally (●) or in the presence of 2 mM lysine (△) or threonine (□). Averages of analysis of two or more flasks are represented by each point.

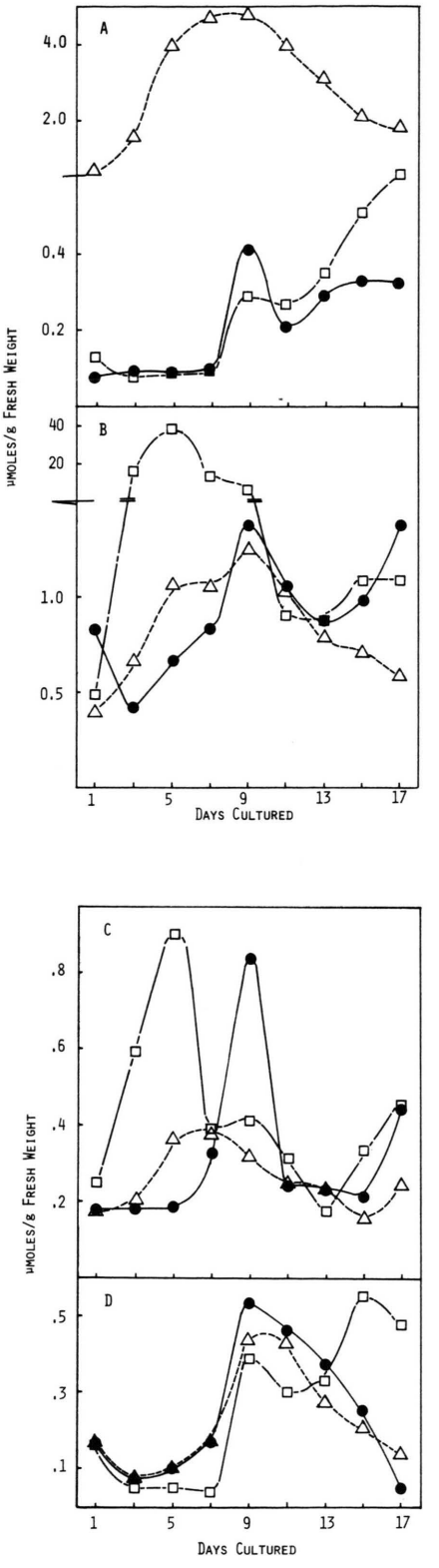

Fig. 2. Free amino acid pools present in carrot cells during suspension culture. Control (●), 2 mM lysine supplemented in medium (△); 2 mM threonine supplemented (□). A, Lysine; B, Threonine; C, Isoleucine; D, Methionine. Flasks were harvested independently in duplicate and analyzed. The average of the two analyses are presented. Maximum variation between duplicate samples was 30%. Variation between most duplicate samples was less than 15%.

did the treated cells. However, cells grown on lysine eventually did achieve a maximum amount of protein per flask similar to that of the control while cells grown on threonine contained approximately 30% less protein during the latter stages of the growth cycle.

The free lysine level within control cells and those grown in threonine-supplemented medium remained constant for the first 7 days of culture and then rapidly increased between day 7 and day 9 (Fig. 2 A). During the entire culture period, cells grown on 2 mM lysine contained between 5 and 48-fold more lysine than controls. Cells grown in the presence of 2 mM threonine contained increased levels of threonine between days 3 and 9 (Fig. 2 B). Growth of cells on lysine did not increase threonine levels, and growth of cells on threonine did not increase lysine levels. At the very beginning and at 11 days of culture and thereafter the threonine values were not elevated within threonine-supplemented cells. Elevation of threonine levels may have caused isoleucine levels to rise prematurely at days 5 through 7, but isoleucine levels in controls rose to a comparable level two days later (Fig. 2 C). The supplementation of either threonine or lysine to the medium yielded no strong effect on cellular methionine levels (Fig. 2 D).

These free amino acid levels can be compared to those found in 3-month-old whole carrot roots where the following levels were determined in nmol/g fresh weight: lysine, 118; threonine, 124; isoleucine, 247; methionine, 8.5. Duplicate samples were analyzed and data varied as described in the legend for Fig. 2. Thus, the amounts of lysine, isoleucine and methionine are similar to those found in cell suspension culture. The threonine concentration in whole carrot roots is about 20% of that found in carrot cell suspension cultures.

After 3 days of culture, cells grown in medium containing lysine or threonine contained less aspartokinase activity than the control cells when comparisons were made on a unit per g fresh weight basis (relative activity) (Fig. 3 A). The specific activity (units/mg protein) of aspartokinase was less in cells grown in medium containing either lysine or threonine than in controls. For the first 10 days of culture on 2 mM lysine, cells contained up to 25% less aspartokinase activity than controls (Fig. 3 B). This decrease in activity coincided with a decrease in sensitivity of the enzyme to inhibition by lysine and an in-

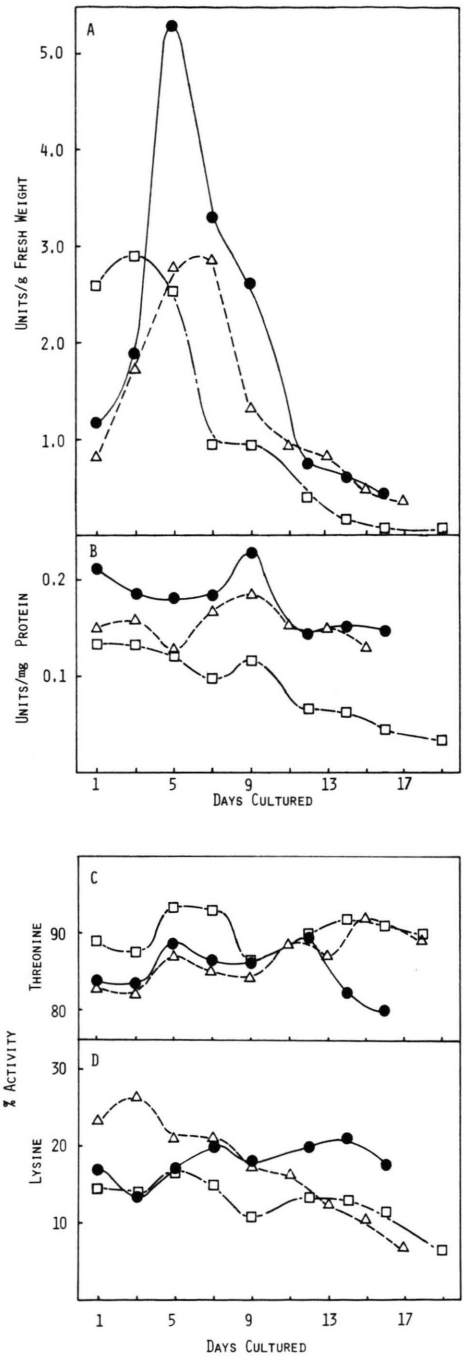

Fig. 3. Aspartokinase activity in carrot cells grown under different conditions. In all figures cells were grown as controls (●) or with 2 mM lysine (△) or threonine (□) supplemented in the medium. A, Units of enzyme per gram fresh weight (relative activity). B, Units per mg protein (specific activity). C, Percent activity remaining when assayed in the presence of 10 mM threonine. D, Percentage of the enzyme activity remaining when assayed in the presence of 10 mM lysine.

crease in its sensitivity to inhibition by threonine (Fig. 3 C). Thus, there is a decrease of approximately 12% in the amount of the lysine-sensitive form of aspartokinase in relation to total protein and to total aspartokinase activity (Table I). The amount of threonine-sensitive aspartokinase activity remained constant under these conditions.

When cells were grown in medium containing 2 mM threonine the lysine-sensitive aspartokinase activity was 72% of control values (Table I), while the specific activity of the threonine-sensitive form of aspartokinase was only 43% of the control value during the first 9 days of culture. This leads to a decrease of the total activity of the enzyme. During this period the threonine levels within cells were greatly elevated (Fig. 2 B). Thus, the threonine-sensitive form of aspartokinase can be decreased by supplementing the growth medium with threonine.

The relative activity of homoserine dehydrogenase from cells grown on threonine or lysine was usually less than half of the values from control cells (Fig. 4 A). The specific activity of homoserine dehydrogenase at its maximum in cells grown either on lysine or threonine was less than one-third that of the control cells (Fig. 4 B). During the initial portion of the

Table I. The amount of lysine-sensitive and threonine-sensitive forms of aspartokinase in carrot cell suspension cultures during the first 9 days of culture in the absence and presence of 2 mM lysine or 2 mM threonine *.

	Control	Lysine	Threonine
Units/mg protein	0.189	0.170	0.130
% activity with 10 mM			
Threonine	85	84	91
Lysine	18	22	15
Units/mg protein			
Lysine sensitive	0.154	0.133	0.111
Threonine sensitive	0.028	0.027	0.012

* Data are the average from cells cultured 1 – 9 days in each case, as described in Fig. 3.

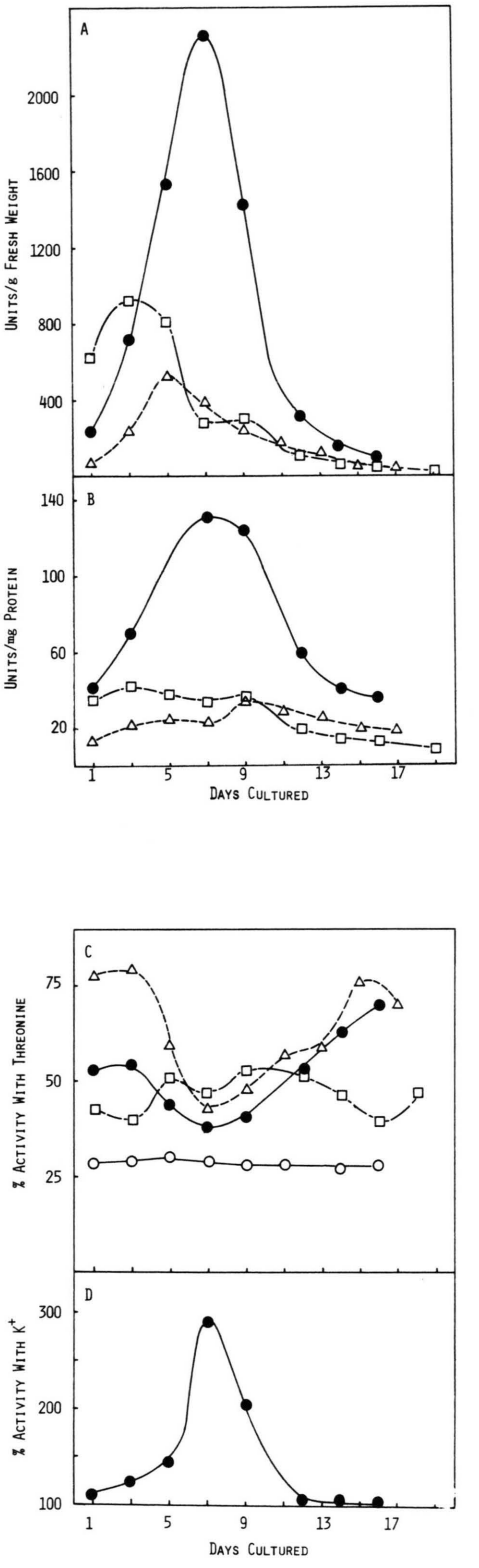

Fig. 4. Homoserine dehydrogenase activity in carrot cells grown in suspension culture under different conditions. Control (●), 2 mM lysine (△), 2 mM threonine (□) supplemented in the medium. A, Units of enzyme per g fresh weight (relative activity). B, Units per mg protein (specific activity). C, Percent enzyme activity in the presence of 10 mM threonine and 100 mM K^+ or without K^+ (○) added to the assay mixture. D, Percent enzyme activity in the presence of 100 mM K^+. Each point represents the analysis of two or more flasks.

culture period, cells grown on lysine contained less homoserine dehydrogenase activity than cells grown on threonine. Amino acid analysis revealed that threonine within the cells was not accumulating and did not account for low enzyme activity when cells were grown in 2 mM lysine (Fig. 4 B). In fact, isoleucine levels within the cells were lower than in the controls (Fig. 2 C). This suggests that less threonine is present in the pathway when cells are grown on lysine.

The sensitivity of homoserine dehydrogenase activity to regulation by feedback inhibition was dependent upon conditions of cell growth and period of the cell cycle when analyzed (Fig. 4 C). The alteration of homoserine dehydrogenase sensitivity to inhibition in control cells during growth was detected only when KCl was included in the assay medium. When K^+ was deleted, no alterations were noted. The amount of stimulation of homoserine dehydrogenase activity by K^+ also varies throughout the culture period (Fig. 4 C). Thus, the alteration in regulation by threonine in the presence of K^+ appears to be coincidentally with the amount of stimulation effected by K^+. This is consistent with observations using soybean [7] and corn [23], which indicate a coincidental high sensitivity of homoserine dehydrogenase activity to inhibition by threonine when the enzyme is most stimulated by K^+. A low sensitivity of homoserine dehydrogenase activity to inhibition by threonine occurs when there is little stimulation of the enzyme activity by K^+.

Cells grown on threonine contained homoserine dehydrogenase activity which was between 40 and 55% inhibited by threonine during the growth cycle. However, cells grown in the presence of lysine or threonine contained much less homoserine dehydrogenase activity than was present in control cells. This wide fluctuation of sensitivity of homoserine dehydrogenase to feedback inhibition by threonine may suggest the existence of multiple molecular forms of the enzyme or a very plastic enzyme which can assume more than one regulatory conformation. Polyacrylamide gel electrophoresis of some of these samples containing widely differently regulated homoserine dehydrogenase did not reveal any differences in electrophoretic mobility patterns and showed only one peak of activity. However, the presence of threonine in the upper buffer of the PAGE system decreased the electrophoretic mobility of homoserine dehydrogenase (Fig. 5).

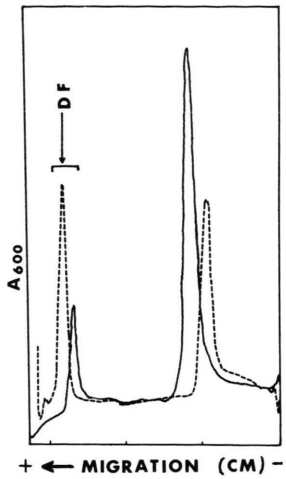

Fig. 5. Gel scan of 5% polyacrylamide gels containing carrot cell suspension culture extracts and stained for homoserine dehydrogenase activity (NAD) after electrophoreses. DF indicates the location of the bromphenol blue dye marker. No threonine present (————) and 2 mM threonine present (– – –) in enzyme extraction buffer and/or electrophoresis upper buffer.

Cells cultured in medium containing lysine possessed homoserine dehydrogenase activity which was quite insensitive to threonine inhibition at the beginning and end of the culture period (Fig. 4 C). Under all culture conditions examined, homoserine dehydrogenase activity was always 50 to 60% inhibited by 10 mM threonine during the middle portion of the culture period (days 7 to 9).

The relative activity of the other branch-point enzyme, dihydrodipicolinic acid synthase, is not greatly affected by the addition of threonine to the growth medium (Fig. 6 A) except the peaking of activity is earlier. When cells are grown on lysine, the enzyme activity peaks at a time similar to the control but the maximum relative enzyme activity is only 65% of that of the control. The specific activity of dihydrodipicolinic acid synthase is not affected much by the lysine supplement, however threonine decreases it by 50% (Fig. 6 B). Under all growth conditions dihydrodipicolinic acid synthase activity was inhibited over 90% by 1.0 mM lysine (Fig. 6 C). Thus, there is no suggestion of the existence of multiple forms of this enzyme. However, these data imply that threonine supplemented in the medium alters the amount of dihydrodipicolinic acid synthase present in the cell.

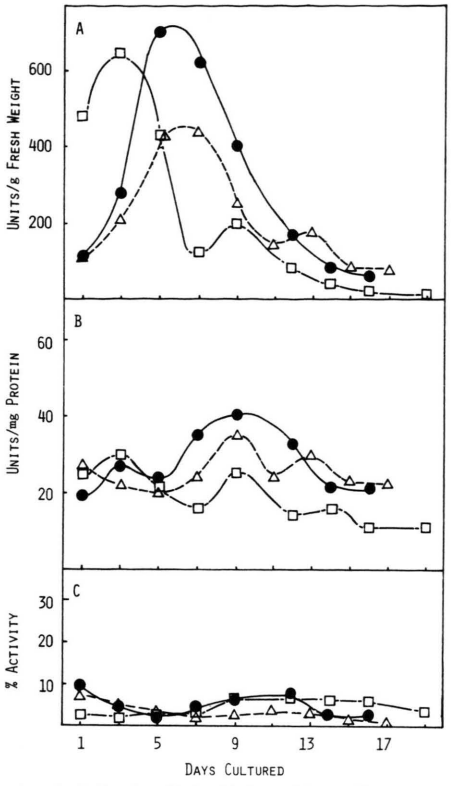

Fig. 6. Dihydrodipicolinic acid synthase activity in carrot cell suspension cultures grown under different conditions. Control (●), 2 mM lysine (△), 2 mM threonine (□) supplemented in the medium. A, Units of enzyme activity per g fresh weight (relative activity). B, Units of activity per mg protein (specific activity). C, Percent enzyme activity in the presence of 2.5 mM lysine. Each point represents averages of duplicate samples.

Table II. Effect of harvesting cells in the presence of 2 mM threonine and 2 mM lysine on enzyme activities *.

	Normal Buffer	Normal Buffer +Thr, +Lys	% of control
Aspartokinase			
total units	15.1	14.5	96
% activity (thr)	85	87	
% activity (lys)	18	18	
Homoserine dehydrogenase			
total units	2200	2600	124
% activity (thr)	62	60	
Dihydrodipicolinic acid synthase			
total units	1700	2100	128
% activity (lys)	2	1	

* Data are expressed as an average of triplicate experiments. Each enzyme preparation was assayed in duplicate. Variations in recoveries between experiments were less than 10%. Cells were harvested after 5 days. Endproduct concentrations for feedback inhibition analysis were 10 mM.

If the feedback inhibition of any of these three enzymes were irreversible, the activities would appear to be lacking when cells were grown on threonine or lysine. Thus, an apparent decrease in the amounts of these enzymes might be explained by inactivation of enzyme activity by irreversible binding of the endproduct to the enzyme. Therefore, cells grown in the absence of threonine and lysine supplements were harvested and ruptured in the presence of 2 mM lysine and 2 mM threonine in the buffer. The crude homogenate was allowed to incubate on ice for 30 min. Then the enzyme was treated the same as the control enzyme preparation harvested in the absence of amino acid additives (Table II). In the case of aspartokinase, the experimental and control contained virtually the same amount of enzyme activity and possessed the same regulatory characteristics. The sensitivity of homoserine dehydrogenase activity and dihydrodipicolinic acid synthase activity to inhibition by threonine and lysine, respectively, did not change either but the activity was increased slightly. Thus, no apparent irreversible binding of endproduct inhibitors appears to occur. This supports the conclusion that when carrot cells are grown in the presence of lysine or threonine, levels of key regulatory enzymes in the pathway are altered.

Discussion

Carrot cell suspension cultures grown in the presence of 2 mM lysine contain a low amount of the lysine-sensitive form of aspartokinase. Using carrot disks Sakano [18] observed that levels of this enzyme form appear to be inversely correlated with lysine levels within the cell and is regulated by repression and derepression. If so, then we suggest that the lysine-sensitive form of this enzyme is already substantially repressed in cell cultures under our growth conditions before lysine is supplemented since only small decreases in the amount of the lysine-sensitive form of aspartokinase can be observed when lysine is added to the culture medium. Using carrot cell suspension cultures Bright *et al.* [19] found no correlation between amino acid levels and aspartokinase

activity. However, minor fluctuations in amino acid content would not readily reveal changes in an already greatly repressed enzyme activity. Our data also suggest that the threonine-sensitive form of aspartokinase is slightly decreased when cells are grown in the presence of 2 mM threonine. If repression of aspartokinase activity occurs in carrot cell cultures then under our culture conditions aspartokinase must be substantially repressed and additions of lysine or threonine can repress the activity of the individual forms only a small amount more.

Sakano [18] was able to demonstrate that an increase in the activity of the lysine-sensitive form of aspartokinase occurred in carrot disks when low internal concentrations of lysine was supplemented in the culture medium. In culture systems using cell suspensions it may be possible to manipulate the medium to reduce the internal cell concentration of lysine thereby allowing repression and derepression to be investigated more easily.

The intracellular amounts of the different free amino acids examined under our cultures conditions are similar to those reported by Bright *et al.* [19] for carrot suspension cultures grown under somewhat different conditions. Our threonine determinations are slightly higher, however. Our analysis of free amino acids in whole carrot root tissue falls within the range of that reported by Sakano [18] with one exception. Threonine levels from our carrot root tissues were 5 to 20 fold lower than reported by Sakano. No reason for this has been determined. However, we obtained good resolution of threonine from serine, a common contaminant, using physiological fluid columns for amino acid analysis. It is interesting that, according to our data, threonine levels in carrot culture are at least 4 to 8 fold higher than in whole carrot root tissue. Thus, if repression of the threonine-sensitive form of aspartokinase does occur in carrot, then the threonine-sensitive form should be expressed more in whole carrot tissue and less in cell suspension culture. Our previous data [6] demonstrates this to be true. Aspartokinase activity from whole carrot roots contains 45% threonine-sensitive aspartokinase activity, while carrot cell suspension cultures contain only 15% threonine-sensitive aspartokinase activity.

This and other evidence presented suggest that the lysine-sensitive (as reported by Sakano [6]) and threonine-sensitive form of aspartokinase are influenced by the presence of lysine and threonine in the carrot culture medium.

There are no published reports indicating possible repression of homoserine dehydrogenase activity in higher plants. However, in several bacteria, such as *Escherichia coli*, homoserine dehydrogenase activity is repressed by threonine [3 – 5]. Carrot cell suspension cultures grown in the presence of threonine possess a reduced amount of homoserine dehydrogenase activity. This activity is not as strongly inhibited by threonine as is the enzyme activity derived from control cells or from cells grown in medium containing lysine. Cells grown in the lysine-containing medium possessed a low level of homoserine dehydrogenase activity which was quite insensitive to threonine inhibition as compared to control values. Although activity was too low in some enzyme preparations to be detected on electrophoretic gels, preparations which possessed enough activity contained only one form of homoserine dehydrogenase as demonstrated using enzyme specific stain. Homoserine dehydrogenase from other plant sources such as corn [15, 24] and soybean [7, 8] have more than one form of homoserine dehydrogenase which are detectable on polyacrylamide electrophoretic gels. The differences in the sensitivity of carrot homoserine dehydrogenase to inhibition by threonine when cells are grown under different conditions suggest either the existence of more than one form of the enzyme or a very "plastic" single form which can exist in more than one state of sensitivity to threonine inhibition.

Several plant species possess multiple forms of homoserine dehydrogenase [7, 15 – 17] and it would be interesting to determine if all of the forms are repressed by threonine. For example in corn a threonine-insensitive and several threonine-sensitive forms of homoserine dehydrogenase exist and are derived from at least two distinctly different genes [24]. It is not known if one of these gene products can be repressed by threonine, while another might be repressed by isoleucine or methionine.

Cells grown on lysine also contain much lower amounts of homoserine dehydrogenase activity than controls. This is not due to an increase in the internal concentration of threonine, because cells grown on lysine contain no more threonine than controls, nor do they contain higher concentrations of isoleucine or methionine. At this time it is unclear why lysine would decrease homoserine dehydrogenase activity in this pathway. This decrease could be due to an in-

crease in the nitrogen supply to the cell or it may be related to the observed inhibition of the reverse direction of homoserine dehydrogenase by cysteine [6]. Perhaps, homoserine is stored or transported as an intermediate of the pathway, rather than aspartate. Then homoserine might be used as a precursor for lysine as well as for threonine synthesis. In this case lysine repression might control backflow of stored or transported homoserine in cases of lysine abundance.

Lysine repression of homoserine dehydrogenase might also be an energy saving device for the plant cell. If sufficient lysine is present within the cell, then dihydrodipicolinic acid synthase would be feedback inhibited but not repressed since only slight if any decrease in the level of this enzyme was detected when carrot cells were grown in lysine-supplemented medium. Less homoserine dehydrogenase would be needed by the cell, since there would be less competition for the common substrate, aspartate semi-aldehyde. Therefore, this may explain why lysine may reduce the amount of homoserine dehydrogenase activity. A similar proposition could be envisioned for the observed partial decrease in dihydrodipicolinic acid synthase activity by threonine. With sufficient threonine in the cell, homoserine dehydrogenase activity would be feedback inhibited and repressed. Competition for aspartate semialdehyde would be decreased, thus reducing the required amount of dihydrodipicolinic acid synthase needed to maintain normal lysine levels. This type of "cross" repression may save cellular energy. Other experiments are needed to determine the mechanism involved in both of these cases.

When carrot cells are grown in the presence of lysine or threonine, the internal concentrations of the other pathway endproducts are not greatly altered during the first 11 days of culture. In wheat, Bright *et al.* [25] have demonstrated that although 1 mM lysine inhibited [^{14}C]acetate incorporation into lysine it had little affect on the other endproducts. Similarly, 1 mM threonine inhibited [^{14}C]acetate incorporation into threonine while having little effect on methionine synthesis. Their data and ours indicate that large increases in the concentration of one endproduct in this pathway does not strongly influence the amount of synthesis of other pathway endproducts. However, when cells were grown in the presence of lysine, although threonine levels were not greatly affected, isoleucine levels were only about 50% of control values. This may reflect a decrease in the availability of threonine in the precursor pool for isoleucine synthesis.

Acknowledgements

This work was supported by the Illinois Soybean Operating Board, a National Science Foundation Grant PCM-78-11105 and the Illinois Agricultural Experiment Station. We wish to thank Shou Shye for technical assistance and Ming Tung for operating the amino acid analyzer.

[1] B. J. Miflin and P. J. Lea, Ann. Rev. Plant Physiol. **28,** 299 (1977).
[2] J. K. Bryan, Amino Acid Biosynthesis and its Regulation. Plant Biochemistry (Bonner and Varner, eds.), Academic Press **1976,** p. 525.
[3] G. N. Cohen and J.-C. Patte, Cold Spring Harbor Symp. Quant. Biol. **28,** 513 (1963).
[4] M. Freundlich, Biochem. Biophys. Res. Commun. **10,** 277 (1963).
[5] E. R. Stadtman, G. N. Cohen, G. LeBras, and H. de Robichon-Szulmajster, J. Biol. Chem. **236,** 2033 (1961).
[6] B. F. Matthews and J. M. Widholm, Planta **141,** 315 (1978).
[7] B. F. Matthews and J. M. Widholm, Phytochem. **18,** 395 (1979).
[8] B. F. Matthews and J. M. Widholm, Can. J. Bot. **57,** 299 (1979).
[9] J. M. Widholm, Can. J. Bot. **54,** 1523 (1976).
[10] J. M. Widholm, Biochim. Biophys. Acta **261,** 44 (1972).
[11] J. M. Widholm, Biochim. Biophys. Acta **261,** 52 (1972).
[12] J. M. Widholm, Physiol. Plant. **25,** 75 (1971).
[13] K. Sakano and A. Komamine, Plant Physiol. **61,** 115 (1978).
[14] H. M. Davies and B. J. Miflin, Plant Sci. Lett. **9,** 323 (1977).
[15] B. F. Matthews, A. W. Gurman, and J. K. Bryan, Plant Physiol. **55,** 991 (1975).
[16] H. Aarnes and S. E. Rognes, Phytochemistry **13,** 2717 (1974).
[17] H. Aarnes, Plant Sci. Lett. **9,** 137 (1977).
[18] K. Sakano, Plant Physiol. **63,** 583 (1979).
[19] S. W. J. Bright, M. M. Leggatt, and B. J. Miflin, Plant Physiol. **63,** 586 (1979).
[20] Y. Yugari and C. Gilvarg, J. Biol. Chem. **240,** 4710 (1965).
[21] A. G. Gornall, C. J. Bardawill, and M. M. David, J. Biol. Chem. **177,** 751 (1949).
[22] R. L. Bieleski and N. A. Turner, Anal. Biochem. **17,** 278 (1966).
[23] C. A. DiCamelli and J. K. Bryan, Plant Physiol. **55,** 999 (1975).
[24] T. J. Walter, J. A. Connelly, B. G. Gegenbach, and F. Wold, J. Biol. Chem. **254,** 1349 (1979).
[25] S. W. J. Bright, P. R. Shewry, and B. J. Miflin, Planta **139,** 119 (1978).