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Purification and Properties of 5-Enolpyruvylshikimate-3-Phosphate Synthase from Dark-Grown Seedlings of *Sorghum bicolor*

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ABSTRACT

5-Enolpyruvylshikimate-3-phosphate (EPSP) synthase (3-phospho-shikimate 1-carboxyvinyltransferase; EC 2.5.1.19) was purified 1300-fold from etiolated shoots of *Sorghum bicolor* (L.) Moench. Native polyacrylamide gel electrophoresis revealed three barely separated protein bands staining positive for EPSP synthase activity. The native molecular weight was determined to be 51,000. Enzyme activity was found to be sensitive to metal ions and salts. Apparent K_m values of 7 and 8 micromolar were determined for the substrates shikimate-3-phosphate and phosphoenolpyruvate (PEP), respectively. The herbicide glyphosate was found to inhibit the enzyme competitively with respect to PEP (K_i = 0.16 micromolar). Characterization studies support the conclusion of a high degree of similarity between EPSP synthase from *S. bicolor*, a monocot, and the enzyme from dicots. A similarity to bacterial EPSP synthase is also discussed. Three EPSP synthase isozymes (I, II, III) were elucidated in crude homogenates of *S. bicolor* shoots by high performance liquid chromatography. The major isozymes, II and III, were separated and partially characterized. No significant differences in pH activity profiles and glyphosate sensitivity were found. This report of isozymes of EPSP synthase from *S. bicolor* is consistent with other reports for shikimate pathway enzymes, including EPSP synthase.

The enzyme EPSP[2] synthase (3-phosphoshikimate-1-carboxyvinyl transferase, EC 2.5.1.19) catalyzes the sixth step in the shikimate pathway leading to the biosynthesis of the aromatic amino acids L-phenylalanine, L-tyrosine, and L-tryptophan in bacteria, fungi, and plants (9). In higher plants, this pathway is common to the formation of a host of secondary metabolites including alkaloids, coumarins, flavonoids, lignin precursors, indole derivatives, and other phenolic compounds (7). Several studies suggest the intact shikimic acid pathway is localized primarily in the chloroplasts of green tissue; however, there is evidence that part of the pathway may exist in the cytosol as well (11). Distinct isozymes of several shikimic acid pathway enzymes have been separated and characterized (11).

EPSP synthase catalyzes the reversible transfer of the intact enolpyruvyl (carboxyvinyl) group from PEP to the 5-hydroxyl group of S3P. Interest in EPSP synthase has increased significantly since it was found to be the primary biochemical target of the broad-spectrum herbicide glyphosate (*N*-[phosphonomethyl]glycine) (25). This enzyme has been purified from several bacterial sources, fungi, plant cell cultures, and dicotyledonous plant seedlings (1). There are no reports of the purification and characterization of EPSP synthase from a monocotyledonous plant. Furthermore, it is not known to what degree natural diversity in plant EPSP synthase sensitivity to glyphosate inhibition can account for the spectrum of glyphosate tolerances displayed by field-grown plants. A review of the past literature on the kinetics of this enzyme (1) does not provide a consensus as to the degree of diversity of EPSP synthase from different plants.

In the present study, we report the first purification and characterization of EPSP synthase from a monocotyledonous plant source, *Sorghum bicolor*, and discuss its similarity to the enzyme from other sources. We also report the discovery of isozymes of *S. bicolor* EPSP synthase and describe some of their properties.

MATERIALS AND METHODS

Enzyme Purification. *Sorghum bicolor* (L.) Moench seeds were treated with a 1% (w/v) solution of NaOCl for 2 min, rinsed thoroughly with deionized water, sown in metal trays (7 × 12 × 23 cm) filled with vermiculite, and placed in the dark at 24°C for 6 d. Shoots were cut just above the vermiculite surface with a razor blade, weighed, and cooled to 4°C. All subsequent purification steps were carried out at 4°C unless specified otherwise. Etiolated shoots were homogenized (Polytron, Brinkman Instrument Co.) for 3 to 5 min in four volumes of buffer (20 mM Hepes/KOH, 10 mM glutathione, 0.1 mM EDTA, 1% [w/v] polyvinylpolypyrrolidone [pH 7.0]), filtered through a single layer of Miracloth and centrifuged 20 min at 20,000*g*.

S. bicolor EPSP synthase was purified by a sequential application of ammonium sulfate precipitation, Sephadex G-25, DEAE-cellulose, hydroxyapatite, phenylagarose, and Sephacryl S-200 chromatography as described by Steinrücken *et al.* (28). Fractions containing EPSP synthase activity from Sephacryl S-200 chromatography were pooled, concentrated by filtration (Amicon cell with PM10 membrane), and stored in 40% (v/v) glycerin at −20°C. Enzyme activity is stable at least 1 year under these conditions.

Isozyme Separation. Isozymes of *S. bicolor* EPSP synthase were monitored and purified by anion exchange HPLC using a Synchropak AX-300 column (SynChrom, Inc., Linden, Ind.) equilibrated with 20 mM Tris/HCl, 1 mM DTT, and 1% (v/v) glycerin (pH 7.5). Prior to injection, protein samples were adjusted to a lower ionic strength. This was achieved through either dilution with buffer or desalting over Sephadex G-25 (10), without using the described column wash with 0.1% BSA. Highly purified EPSP synthase was not desalted as it was found to strongly adsorb to Sephadex G-25. Proteins were eluted at a flow rate of 2 ml/min using a multistep, computer-controlled KCl gradient generated with two pumps (Waters Associates, Milford, Ma.).

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[2] Abbreviations: EPSP, 5-enolpyruvylshikimate-3-phosphate; S3P, shikimate-3-phosphate; PEP, phosphoenolpyruvate; PMSF, phenylmethylsulfonylfluoride.

Table I. *Purification of EPSP synthase from S. bicolor shoots*

Step	Total Activity	Protein	Specific Activity	Purification	Recovery	Phosphatase Specific Activity
	nkat	*mg*	*nkat/mg*		%	*nkat/mg*
Crude extract	1149	4220	0.27	1	100	1.4
40–65% $(NH_4)_2SO_4$	1259	1750	0.72	2.7	110	6.2
DEAE-Cellulose	908	324	2.80	10.4	79	7.2
Hydroxyapatite	402	51.7	7.77	29	35	—
Phenylagarose	366	4.01	89.6	332	32	0.54
Sephacryl S-200	241	0.68	352	1304	21	0.00

Protein was monitored at 215 and 280 nm. Fractions were collected and assayed for EPSP synthase activity.

Electrophoresis. SDS-PAGE was performed according to the procedure of Laemli (14) in 10% polyacrylamide gels at 20°C. Native gels were run under the same conditions in the absence of SDS. Gels were stained with Coomassie blue. EPSP synthase activity in native gels was detected with an activity stain according to the procedure of Nimmo and Nimmo (18) in the presence of 10 mM $CaNO_3$.

Enzyme Assays. EPSP synthase activity during the purification was determined by measuring enzymic organic phosphate release in the presence of 0.1 mM ammonium heptamolybdate and using 10 mM glyphosate for assay blanks (28). For kinetic studies, this procedure was modified slightly. The purified enzyme was diluted into buffer (20 mM Hepes/KOH, 2 mM glutathione, 5% [w/v] glycerin, and 0.1 mM S3P [pH 7.0]). Ten μl (28 ng protein) of temperature-equilibrated enzyme (5 min at 30°C) was added to a temperature-equilibrated (30°C) assay solution consisting of 50 mM Hepes/KOH (pH 7.0), 50 mM KCl, 5 mM glutathione, PEP, and S3P in a final volume of 0.2 ml. The reaction was initiated with the addition of enzyme, incubated 2 min at 30°C, and terminated by fivefold dilution with 1 N HCl. In control reactions, enzyme was added directly to the mixture of assay reagents and 1 N HCl.

Phosphatase activity was determined as described for EPSP synthase, except S3P and molybdate were omitted from the reaction mixture (28). Control reactions were stopped immediately following the addition of enzyme.

Protein concentration was determined by the method of Bradford (4) using bovine γ-globulin for a standard.

Chemicals. The barium salt of S3P was prepared as described by Knowles *et al.* (13). Inorganic phosphate was removed by anion exchange chromatography (Bio-Rad AG-1) (12). The barium salt was converted to the potassium salt by the addition of cation exchange resin (Bio-Rad AG-W). S3P levels were monitored by HPLC and were quantified by inorganic phosphate determination after incubation with acid phosphatase.

Mol wt marker proteins for SDS-PAGE were obtained from Bio-Rad and from Boehringer-Mannheim (Indianapolis, IN) for gel filtration.

All other chemicals were obtained in reagent grade from Sigma or Fisher Scientific.

RESULTS

Purification of EPSP Synthase. A sequential application of ammonium sulfate precipitation, DEAE-cellulose, hydroxyapatite, phenylagarose, and Sephacryl S-200 chromatography resulted in a 1300-fold enrichment of *S. bicolor* EPSP synthase with a 21% recovery of initial activity (Table I). A total of 0.65 mg protein was recovered from 450 g of dark-grown sorghum shoots. No phosphatase activity was detected in the purified enzyme sample. The final specific activity of purified EPSP synthase was 352 nkat/mg (21.1 μmol/min/mg) at 30°C.

SDS-PAGE of the purified sample revealed two barely separated major bands and several minor bands upon staining with Coomassie blue (Fig. 1). Electrophoresis under nondenaturing conditions yielded a similar pattern upon staining (Fig. 2, left). Subsequent staining of the native gel for EPSP synthase activity revealed three bands, two major and one minor, that corresponded to the bands detected with Coomassie blue (Fig. 2, right). Thus, three proteins with EPSP synthase activity, distinguishable by electrophoresis, were co-purified from the *S. bicolor* shoot extract.

Properties of *S. bicolor* EPSP synthase. The mol wt of purified *S. bicolor* EPSP synthase was determined to be 57,000 by gel filtration (Sephacryl S-200). Mol wt of two of the EPSP synthase bands were determined to be 50,600 (major band) and 51,900 (minor band) by SDS-PAGE. A mol wt of the third EPSP syn-

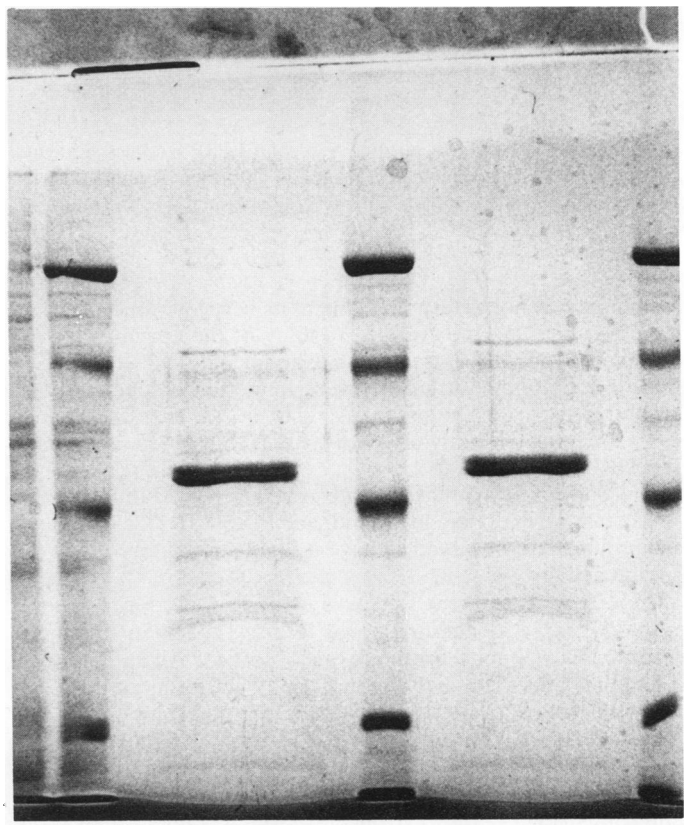

FIG. 1. SDS-PAGE of purified *S. bicolor* EPSP synthase. Lanes 2 and 4: Sorghum EPSP synthase (4.7 μg); lanes 1, 3, and 5: mol wt markers, from the bottom, of phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,500).

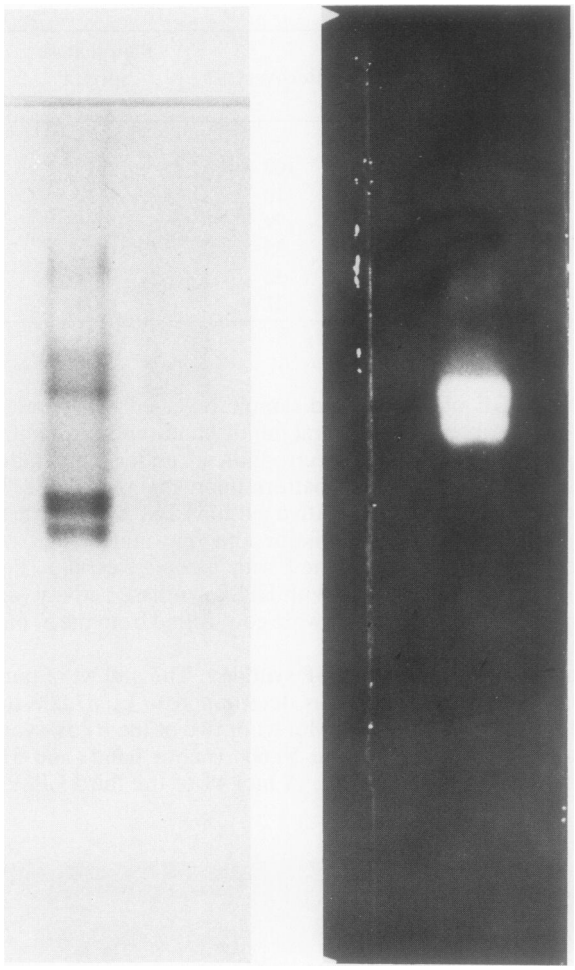

FIG. 2. Native PAGE of purified *S. bicolor* EPSP synthase. Enzyme (4.7 μg) run on gel and stained for protein with Coomassie blue G (left) or stained for EPSP synthase activity (right) using the procedure of Nimmo and Nimmo (18).

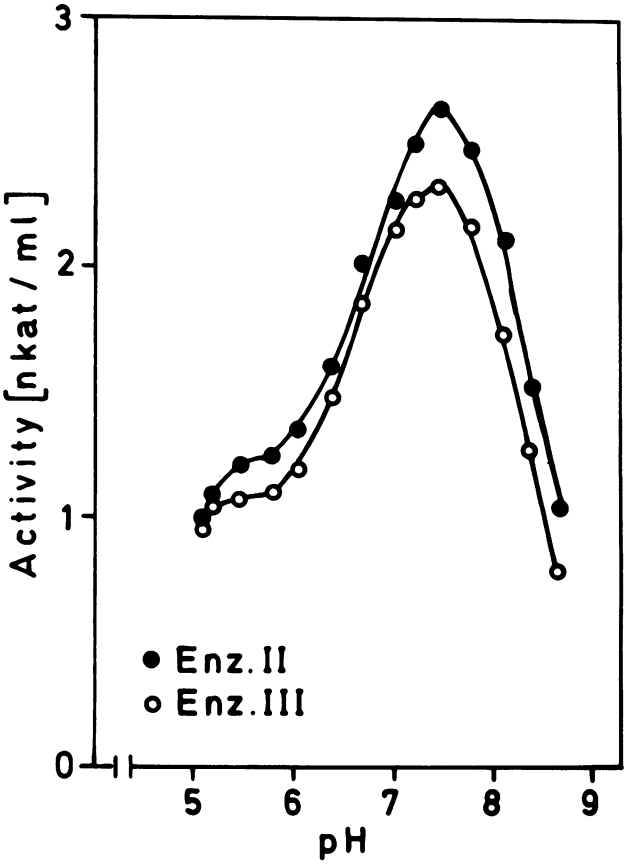

FIG. 3. Effect of pH on EPSP synthase activity of purified *S. bicolor* isozymes II and III.

thase band was not determined due to its very low concentration in the purified enzyme sample. A study of the enzyme activity dependence on pH suggests a maximum at pH 7.4 (Fig. 3). Enzyme activity measurements were found to be dependent on the buffer used for the assay. Activity in 100 mM Tris/maleic acid/KOH was 37% higher than activity measured in 50 mM Hepes/KOH at pH 7.0.

S. bicolor EPSP synthase was observed to be sensitive to metals, particularly copper, lead, and zinc (Table II). Calcium and magnesium were observed to have a slight stimulatory effect on enzyme activity.

The effect of different salts was examined at concentrations up to 200 mM. A general response to enzyme activity enhancement by low salt concentrations was observed. Alkali chlorides had similar effects on activity, maximally activating at levels of about 100 mM (Fig. 4, left). The alkali earth chlorides examined, $CaCl_2$ and $MgCl_2$, showed a different response. Maximal activity (150% of control levels) was reached at salt concentrations of 20 mM followed by a distinct inhibition of activity at higher concentrations (Fig. 4, right). Concentration-dependent activation was also observed when the anion was varied, with activation decreasing in the order of $Cl^- > NO_3^- > F^- > SO_4^{2-}$ (Fig. 4, right).

Ammonium heptamolybdate was found to inhibit *S. bicolor* EPSP synthase activity with an I_{50} of 1.1 mM. No inhibition was observed at 0.1 mM, the concentration we routinely used to inhibit interfering phosphatase activity in the inorganic phosphate assay for EPSP synthase.

Table II. *Effect of Metal Ions on Purified S. bicolor EPSP Synthase Activity*

All salts were evaluated at 5 mM. A control treatment for each assay without enzyme was subtracted to correct for nonenzymatic phosphate release.

Salt	EPSP Synthase Activity (% of control)
$CuSO_4$	0
$Pb(Acetate)_2$	3
$ZnCl_2$	0
$FeSO_4$	58
$SnCl_2$	40
$FeCl_3$	32
$MnCl_2$	58
$CoCl_2$	94
$CaCl_2$	110
$MgCl_2$	121

Kinetics of the forward reaction of *S. bicolor* EPSP synthase were investigated. Apparent K_m values were determined from double-reciprocal plots of enzyme velocity dependence on substrate concentration to be 7 and 8 μM for S3P and PEP, respectively. Substrate concentrations were varied from 5 to 50 μM at a fixed concentration (1 mM) of the other substrate. Varying both substrates yielded a double-reciprocal plot of lines con-

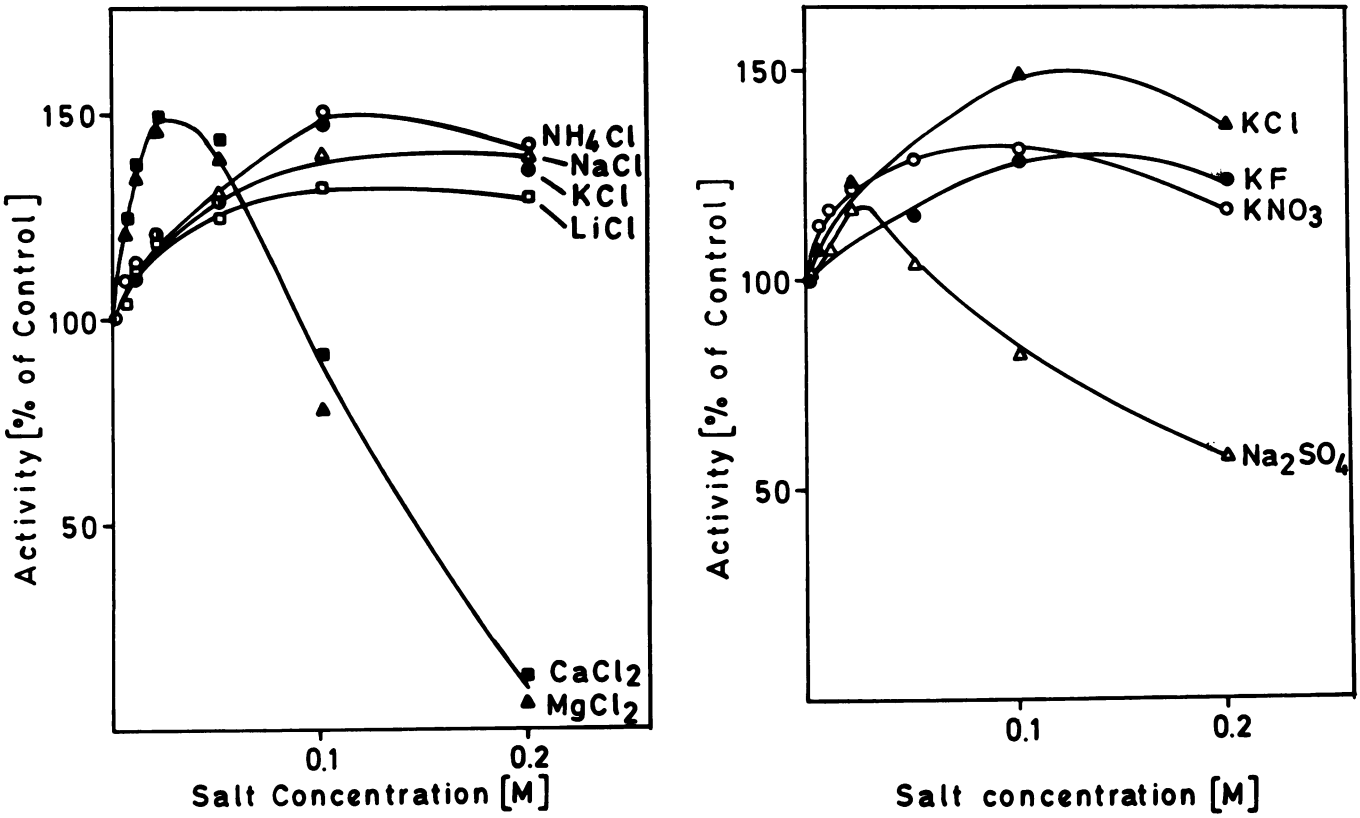

FIG. 4. Effect of salts on *S. bicolor* EPSP synthase activity. Purified enzyme (6.2 μg) was incubated 4 min at 30°C with 1 mM S3P and PEP in varying concentrations of the indicated salts. The effect of cations (left) and anions (right) on enzyme activity is shown.

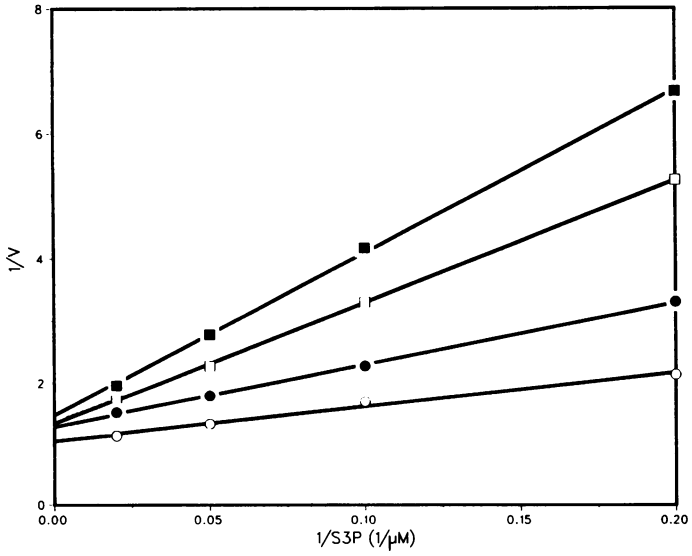

FIG. 5. Double reciprocal plot of initial enzyme velocity dependence on S3P concentration at several fixed concentrations of PEP. PEP was fixed at 5μM (○), 10 μM (●), 20 μM, (□), and 50 μM (■).

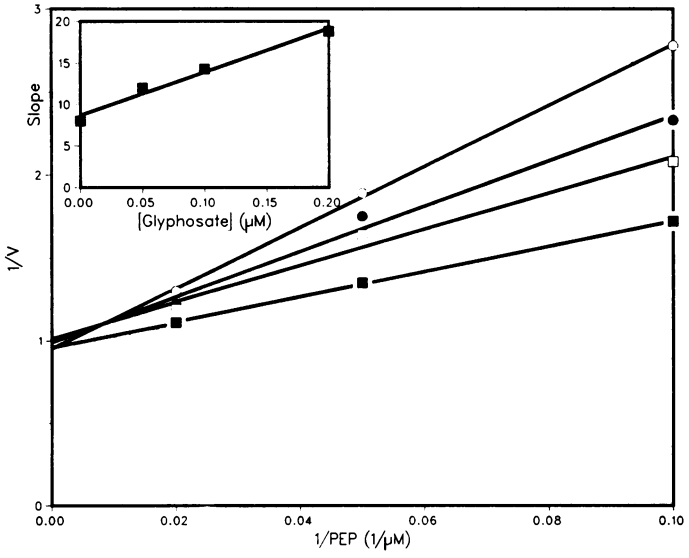

FIG. 6. Double reciprocal plot of initial enzyme velocity dependence on PEP concentration at several fixed concentrations of glyphosate. S3P was fixed at 1 mM. Glyphosate was fixed at 0.00 μM (■), 0.05 μM (□), 0.10 μM (●), and 0.20 μM (○). Inset: Secondary replot of slope *versus* glyphosate.

verging toward the *y*-axis (Fig. 5) suggesting a sequential mechanism for this enzyme (6). Glyphosate inhibition of EPSP synthase activity with respect to PEP gave a pattern suggesting competitive inhibition (Fig. 6). A K_i value of 0.16 μM was determined by a secondary replot of these data (Fig. 6, inset). The results from experiments examining glyphosate inhibition with respect to S3P were consistent with uncompetitive inhibition (data not shown).

Isozymes of *S. bicolor* EPSP Synthase. Electrophoresis of purified *S. bicolor* EPSP synthase suggested that three separate proteins with enzyme activity were present. Only a single peak corresponding to EPSP synthase was observed throughout the

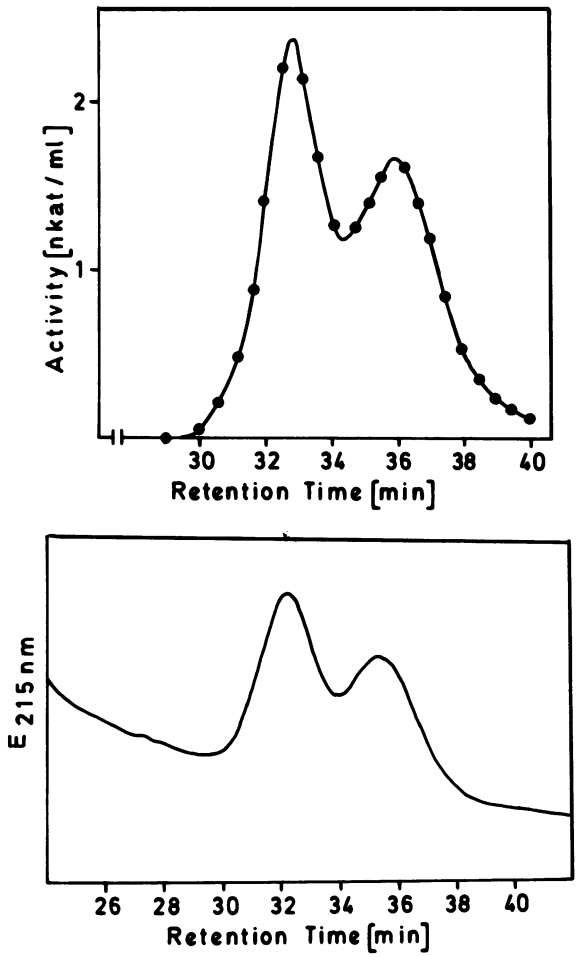

FIG. 7. Anion exchange HPLC of purified *S. bicolor* EPSP synthase. Enzyme (56 μg) was loaded on an HPLC column and eluted with a three-step KCl gradient in buffer: step 1 = 0 to 0.2 M KCl from 0 to 5 min; step 2 = 0.2 to 0.3 M KCl from 5 to 41 min; and step 3 = 0.3 to 1.0 M KCl from 41 to 45 min. Fractions were collected and assayed for EPSP synthase activity (top) and the elution was monitored continuously for protein (bottom).

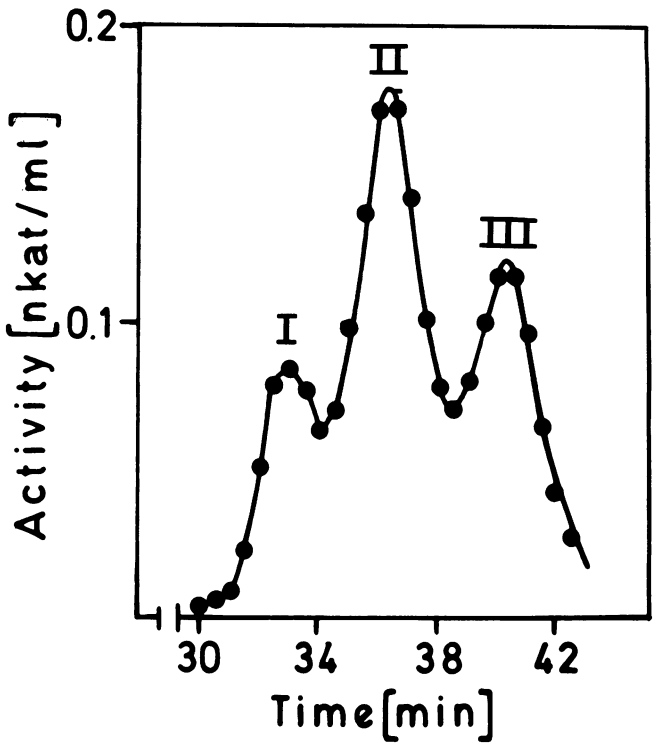

FIG. 8. Anion exchange HPLC of *S. bicolor* isozymes from partially purified enzyme extract. EPSP synthase was extracted from etiolated sorghum shoots by homogenization and ammonium sulfate precipitation. Extract was desalted, filtered, and eluted from the HPLC column using the conditions described for Figure 6.

purification procedure; however, the use of a high resolution anion exchange HPLC procedure could resolve two enzyme activity peaks that corresponded to protein peaks in the purified sample (Fig. 7). When partially purified extracts of dark-grown *S. bicolor* shoots were analyzed, three distinct peaks of EPSP synthase were detected (Fig. 8). These were designated isozymes I, II, and III for their order of elution from the anion exchange column. No significant change in the observed ratio of these isozymes was observed when a partially purified enzyme extract was incubated 2 h at 4°C or when PMSF was added to the enzyme extraction buffer. The purified enzyme preparation only clearly showed isozymes II and III, identified as such by their relative elution order and relative peak height after resolution by HPLC. Isozyme I was observed in this sample occasionally at extremely low levels as a slight shoulder on the front side of isozyme II. Small amounts of II and III were separated using the anion exchange HPLC procedure. No attempt was made to isolate isozyme I. The activity *versus* pH curves were nearly identical (Fig. 3) and could not be distinguished from that of the mixture. Both isozymes were completely inhibited by 10 mM glyphosate, and preliminary kinetic studies suggested no significant differences between them (data not shown).

DISCUSSION

The purification procedure described for EPSP synthase from *S. bicolor* is similar to those described for *Pisum sativum* (16) and *Petunia hybrida* (28). A powerful cellulose-phosphate chromatography step was used in the purification from *P. sativum* (16). It was not used in our preparation because of the presence of phosphatase activity after every step except the last. The use of cellulose-phosphate was successfully utilized in the *P. hybrida* EPSP synthase purification by including sodium tungstate, a phosphatase inhibitor, in the chromatography buffer (28). Specific elution of the *P. hybrida* EPSP synthase was achieved by elution with S3P and glyphosate.

The native mol wt (51,000) and subunit mol wt (57,000) determined for *S. bicolor* enzyme are generally similar to those reported from other sources (1). A native mol wt of 55,000 and a subunit mol wt of 49,000 are reported for both *Esherichia coli* (15) and *P. hybrida* (28) EPSP synthase. These results are consistent with *S. bicolor* EPSP synthase existing as a monomeric protein.

S. bicolor EPSP synthase yields a typical bell-shaped activity *versus* pH curve with a pH maximum at 7.4 (Fig. 3). A similar result was reported for the enzyme from *Nicotiana silvestris* (22). Two pH maxima (5.4 and 6.8) were reported for *Klebsiella pneumoniae* EPSP synthase (26); however, the 5.4 maximum was only present when very high (5 mM) substrate concentrations were used.

The sensitivity of *S. bicolor* EPSP synthase activity to different salts and their concentration is generally similar to that reported for the enzyme from *K. pneumoniae* (26). We are not aware of similar studies from another plant enzyme. In the case of *K.*

Table III. *A Comparison of Kinetic Parameters Determined for EPSP Synthase Purified From S. bicolor and E. coli*

Enzyme kinetics determined as described in "Materials and Methods." Purified *E. coli* EPSP synthase was generously supplied by Dr. R. Douglas Sammons.

Enzyme Source	K_m (S3P)	K_m (PEP)	K_i (Glyphosate)
		μM	
S. bicolor	7	8	0.16
E. coli	15	11	0.10

pneumoniae, the inhibition of activity by high salt concentration at low substrate concentrations (0.2 mM) was speculated to be due to competition of anions for the active site of the enzyme. At a procedural level, the sensitivity of enzyme velocity to salt concentration suggests that nonlinear kinetic plots could be obtained because of varying substrate concentration (26). In our investigation of the kinetics of *S. bicolor* EPSP synthase, 50 mM KCl was added to the assay buffer to minimize this effect. Potassium chloride at 50 mM was also included for the assay of EPSP synthase from *P. sativum* (16).

The sensitivity of *S. bicolor* EPSP synthase to heavy metals (Table II) and the activity-stabilizing effects of thiol reagents and EDTA (data not shown) are consistent with there being at least one sulfhydryl group on the enzyme required for activity. A sulfhydryl group at or near the active site of *K. pneumoniae* and *E. coli* has been proposed based on inactivation studies (2, 20, 26).

Molybdate inhibits acid phosphatases competitively with regard to their substrates (24). It is, then, not surprising to observe inhibition of *S. bicolor* EPSP synthase by molybdate as it uses phosphorylated substrates also. In our assay procedure, heptamolybdate at 0.1 mM is a selective inhibitor of phosphatase activity with no effect on EPSP synthase activity.

The kinetics of EPSP synthase have been reported for the enzyme from diverse sources (1). A direct comparison of kinetic parameters reported with those we obtained for *S. bicolor* is not possible due to the wide variety of assay conditions and substrate levels used. Reported K_i (glyphosate) values with respect to PEP vary from 0.08 to about 40 μM (1). In those studies where similar conditions were used, a comparison can be made. In kinetic studies of enzyme from *P. sativum* (16) and *P. hybrida* (28), apparent K_m values for S3P and PEP—7.7 and 5.2 μM from *P. sativum* and 14 and 8 μM from *P. hybrida*—were similar to those we determined for *S. bicolor*. The K_i values for glyphosate inhibition with respect to PEP from these sources—0.08 μM from *P. sativum* and 0.17 μM from *P. hybrida*—are also similar to the value of 0.16 μM determined from *S. bicolor*. This result, along with similarities in pH optima and mol wt, supports a high degree of similarity between EPSP synthases purified from different plant sources. Furthermore, we have examined the kinetic parameters for the forward reaction of EPSP synthase from a bacterial source, *E. coli*, under nearly identical conditions used for sorghum studies. The K_m (S3P), K_m (PEP), and K_i (glyphosate) values determined are very similar to those from *S. bicolor*. (Table III). This result supports a strong similarity between bacterial and plant EPSP synthases. Mol wt determination, pH, salt concentration, and heavy metal effects we observe for *S. bicolor* are generally consistent with similar studies on EPSP synthase from the bacteria *K. pneumoniae* (26).

Our report of a sequential mechanism for *S. bicolor* EPSP synthase, competitive inhibition by glyphosate with respect to PEP, and uncompetitive inhibition with respect to S3P is consistent with the proposal that the enzyme proceeds by an ordered sequential mechanism whereby S3P must bind before PEP and that glyphosate inhibits by binding in the PEP site to the enzyme:S3P binary complex to form a ternary dead-end complex (3).

We report the finding of three isozymes of EPSP synthase from dark-grown shoots of *S. bicolor*, of which two are present in our purified enzyme sample, one major isozyme (II) and one minor (III) isozyme with a slightly higher mol wt. It is unlikely that the minor isozyme (III) is an artifact from enzyme manipulation during purification, because (a) isozymes could be distinguished in extracts prior to purification (Fig. 8); (b) incubation of *S. bicolor* homogenate did not change the relative ratio of isozymes; (c) the homogenization of sorghum shoots in the presence of PMSF, a serine protease inhibitor, did not significantly alter the HPLC profile of the isozymes; and (d) of the two isozymes found in the purified preparation, the minor form had a higher mol wt than the major form, a result inconsistent with the minor peak being formed by protease activity during enzyme extraction.

A high degree of similarity between the isozymes II and III is supported by the following observations: (a) They co-purify through five different purification steps; (b) their pH profiles are virtually identical (Fig. 3); (c) both are completely inhibited by 10 mM glyphosate; and (d) preliminary kinetic studies did not suggest any significant differences. The latter observation is reinforced by the acquisition of linear double-reciprocal plots when the kinetics of the isozyme mixture were studied. Two peaks of EPSP synthase activity were reported recently for the enzyme from *P. sativum* that were detected by HPLC (17). A second peak of EPSP synthase activity was also reported from *N. silvestris* (21). A single enzyme form was described in the preparation of EPSP synthase from *P. hybrida* (27). No characterization studies were reported for the isozymes of EPSP synthase from *P. sativum* (17). The two forms in *N. sylvestris* were reported to be similarly inhibited by glyphosate (22). The minor isozyme from *P. sativum* was suggested to be cytosolic because of its absence in chloroplast preparations (17).

Isozymes have been reported for some other aromatic amino acid biosynthesis pathway enzymes, *e.g.* DAHP synthase, dehydroquinate dehydratase, shikimate oxidoreductase, and chorismate mutase (11). These isozymes have been reported to be differentially localized, allowing the proposal that at least part of the shikimic acid pathway consists of two separate pathways, one localized in the plastids and another in the cytosol (11). If so, this would be the third metabolic pathway in plants reported to be differentially compartmentalized with isozyme pairs, the others being glycolysis and the oxidative pentose phosphate pathway (8). We did not investigate the localization of the isozymes of *S. bicolor* EPSP synthase; however, a major plastidic and minor cytosolic isozyme were reported for EPSP synthase from *P. sativum* (17).

It has recently been reported (5) that EPSP synthase in *P. hybrida* is synthesized from a nuclear gene as a 55 kD precursor protein, transported into the chloroplast via a 72 amino acid transit peptide, and processed there into its mature form. This precursor protein is reported to be catalytically active and to bind glyphosate equally to that of the mature form. Thus, EPSP synthase in plants can exist in a higher mol wt cytosolic form and lower mol wt plastidic form. One could, then, speculate that the minor, higher mol wt isozyme of *S. bicolor* is the precursor enzyme, although this seems unlikely. The mol wt difference between the two sorghum isozymes is not sufficient to account for 77 amino acids. Co-purification of the precursor and mature protein through all steps used is inconsistent with the reported large charge difference from the highly basic transit peptide (5). Finally, the observation of a significant amount of pre-EPSP synthase seems unlikely because of its reported rapid translocation and processing (5).

Inherent differences in EPSP synthase sensitivity to glyphosate between plants is one possible explanation for the differential

tolerance to glyphosate *in vivo* (1). Glyphosate tolerance achieved in bacterial and plant cell cultures has been attributed to either an increased expression of EPSP synthase or an altered EPSP synthase which binds glyphosate less effectively (1). Naturally occurring differences in EPSP synthase sensitivity to glyphosate inhibition have been reported for bacteria (23). A limited survey of several plant EPSP synthase activities indicated no substantial differences in glyphosate sensitivity (21); however, in an extension of that study, enzyme from monocot sources was generally found to be more sensitive to glyphosate inhibition than that from dicot sources (J Ream, unpublished data). In the field, monocot perennials are, in general, more sensitive to glyphosate effects than dicot perennials (19). The present study of EPSP synthase from the monocot *S. bicolor* addresses whether there is a fundamental difference between EPSP synthases from monocots and dicots. The high degree of similarity between K_i values from *S. bicolor* and those reported from the dicots *P. hybrida* and *P. sativum* suggests this is not the case. Other factors (uptake, metabolism, enzyme levels, etc.) are more likely candidates to account for differential glyphosate tolerance between monocots and dicots in the field.

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