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The Metabolism of Quinate in Pea Roots[1]

Purification and Partial Characterization of a Quinate Hydrolyase

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A quinate (QA) hydrolyase was isolated from pea (*Pisum sativum* L.) roots. The enzyme converts QA into shikimate by elimination of water. The enzymatic reaction is independent of cofactors and divalent cations. The QA hydrolyase was purified about 1,600-fold to apparent electrophoretic homogeneity in three steps, including bovine serum albumin-affinity chromatography. The enzyme forms oligomers and/or complexes with bovine serum albumin and ovalbumin. The monomer molecular weight of the enzyme is about 15,000. The hydrolyase shows regular Michaelis-Menten kinetics with a K_m of 2.0 mM for QA. Compartmentation studies reveal that the QA hydrolyase is localized in plastids. The QA hydrolyase may function in channeling imported QA into the shikimate pathway to support aromatic amino acid biosynthesis in plastids.

QA and SK are compounds that often occur in relatively high concentrations in green and nongreen tissues of herbaceous (Yoshida et al., 1975) and woody angiosperms (Boudet, 1973). For example, in the spring, high amounts of QA are formed in developing coniferous needles (2–10% of dry weight) in the course of photosynthetic CO_2 fixation (Dittrich and Kandler, 1971). This QA pool is metabolized during the lignification process in the summer. QA is also a precursor for chlorogenic acids and is converted into other secondary products like protocatechuic acid (Haslam, 1974). Although QA is formed by a QA:NAD(P)$^+$ oxidoreductase from DHQ, an intermediate in the SK pathway (Bentley, 1990), its involvement in AAA biosynthesis is not clear. ^{14}C-labeled QA is incorporated into free and protein-bound AAA in different herbaceous species and members of the Rosaceae (Weinstein et al., 1959, 1961) and in mung bean (Minamikawa et al., 1969). However, the actual enzymatic steps for channeling QA into the SK pathway are not known.

In pea (*Pisum sativum* L.) epicotyls, an irreversible conversion of QA into SK has been found (Tazaki et al., 1974), whereas the reaction appears to be reversible in mung bean (Minamikawa et al., 1969; Minamikawa and Yoshida, 1972; Minimakawa, 1976). Three different reactions for the conversion of QA into SK pathway intermediates have been reported (Fig. 1). The first reaction is the above-mentioned QA:NAD(P)$^+$ oxidoreductase, catalyzing the conversion of QA to DHQ using NAD$^+$ in mung bean (Gamborg, 1966; Minamikawa, 1977; Kang and Scheibe, 1993) or NADP$^+$ in pine (Ossipov and Shein, 1990). The second reaction is catalyzed by a QA:NAD$^+$ oxidoreductase/DHQase II complex in corn (Graziana et al., 1980) converting QA via DHQ to DHS. Also in corn, indications have been obtained for a third enzyme, the QA hydrolyase, converting QA directly to SK (Graziana and Boudet, 1983). Although QA may be formed outside the chloroplast, investigations on intact leucoplasts from pea roots have provided evidence that QA is able to pass the plastidic envelope to serve as an intermediate for the biosynthesis of AAA in plastids (Leuschner and Schultz, 1991b).

In this paper we report the purification, partial characterization, and subcellular compartmentation of a QA hydrolyase of pea roots. We also discuss QA as a precursor for AAA biosynthesis in plastids.

MATERIALS AND METHODS

Biochemicals and Chemicals

[U-^{14}C]Quinic acid and [U-^{14}C]shikimic acid were isolated as described (Leuschner and Schultz, 1991b). Dowex 1 × 8 (200–400 mesh) Cl^--form was purchased from Serva (Heidelberg, Germany). DHQ and DHS were synthesized by the method of Grewe and Haendler (1966) and Grewe and Jeschke (1956), respectively. All other chemicals were of analytical grade and obtained commercially.

Plant Material and Leucoplast Isolation

Peas (*Pisum sativum* L. cv Kleine Rheinländerin) were grown in a growth chamber on vermiculite for 8 to 10 d. Washed roots were cut into 1- to 2-mm pieces and ground in a mortar in buffer A (300 mM sorbitol, 50 mM Tricine-

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Abbreviations: AAA, aromatic amino acids; DHQ, 3-dehydroquinate; DHQase, 3-dehydroquinate dehydratase, 3-dehydroquinase; DHS, 3-dehydroshikimate; kat, katal (mol s^{-1}); QA, quinate; QORase, quinate oxidoreductase, quinate dehydrogenase; SK, shikimate; SORase, shikimate oxidoreductase, shikimate dehydrogenase.

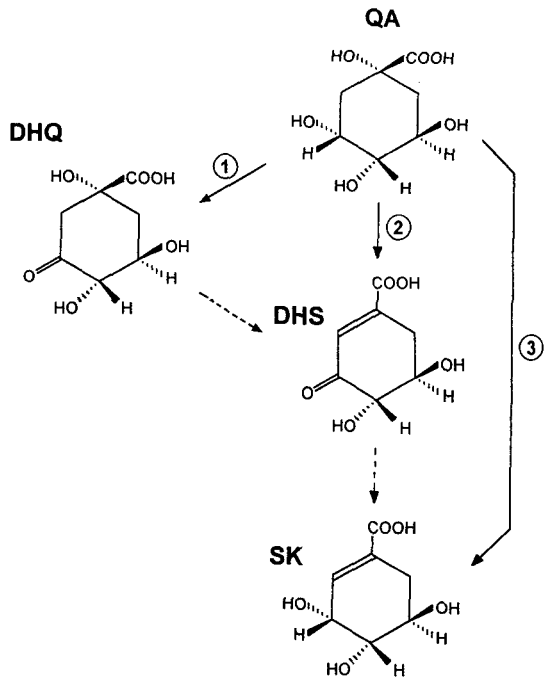

Figure 1. The reactions for the conversion of QA into SK pathway intermediates found in some plants. 1, Conversion of QA to DHQ by the QA:NAD(P)$^+$ oxidoreductase using either NAD$^+$ or NADP$^+$ as coenzyme, depending on the species. 2, Conversion of QA via DHQ to DHS by the QA:NAD$^+$ oxidoreductase/DHQase II complex. 3, Conversion of QA directly to SK by the QA hydrolyase, an enzyme of the EC 4.1.2.– class. The broken lines indicate the reactions from DHQ via DHS to SK of the common SK pathway generally distributed among plants and microorganisms.

KOH, pH 7.9, 1 mM EDTA, 1 mM $MgCl_2$, and 0.1% BSA). The brei was filtered through a nylon gauze (30 μm; Züricher Beuteltuchfabrik, Rüschlikon, Switzerland) to remove cell debris. The filtrate was then centrifuged at 15,000*g* for 10 min. Intact leucoplasts were prepared from the homogenate via a three-step Percoll gradient as described (Leuschner and Schultz, 1991b). Stroma was obtained from intact leucoplasts resuspended in buffer B (50 mM Tricine-KOH, pH 8.0, 1 mM EDTA, 1 mM $MgCl_2$, and 0.1% BSA). For control experiments, samples were denatured by heating at 100°C for 10 min. Intactness and contamination of leucoplasts by other organelles were measured as described (Leuschner and Schultz, 1991b).

Analysis of Cyclohexylcarbonic Acids

Intact plastids, stroma, or homogenate, equivalent to 0.1 to 0.6 nkat SORase mL^{-1}, were incubated for 15 min at 23°C in a final volume of 200 to 500 μL in buffer A for plastids or buffer B for stroma. [U-^{14}C]QA (specific activity 1.9–2.6 MBq mmol^{-1}) was added and the reactions were terminated with 0.5 mL of chloroform:methanol (1:1, v/v). The cyclohexylcarbonic acids derived from [U-^{14}C]QA were extracted successively with 3 mL of 0.1 M HCl and 3 mL of H_2O-KOH, pH 8.0. The aqueous phases were combined, adjusted to pH 8.0, and loaded onto a Dowex 1 × 8 column (acetate form, 3 mL). After washing with 18 mL of H_2O-KOH, pH 8.0, the cyclohexylcarbonic acids were eluted with 3 mL of 2.5 M acetic acid followed by 3 mL of 4 M acetic acid. The eluate was evaporated to dryness at a temperature of 25 to 30°C. The residue was dissolved in 500 μL of methanol:H_2O (1:1, v/v). Twenty microliters of a solution of 10 mg mL^{-1} each of QA, SK, DHS, and DHQ were added as carriers. The cyclohexylcarbonic acids were separated by TLC on cellulose MN 300 (Machery and Nagel, Düren, Germany) and identified by color reactions and photometry (Leuschner and Schultz, 1991a). The distribution of radioactively labeled cyclohexylcarbonic acids was analyzed by scanning the thin-layer plate (Berthold 2760, Wildbad, Germany).

Measurements of Enzyme Activities

For a rapid photometric measurement of QA hydrolyase activity from desalted fractions obtained from ammonium sulfate precipitation, gel filtration, ion-exchange, or affinity chromatography, incubation mixtures containing 250 to 400 μL of the enzyme, 100 μL of 50 mM QA (pH 8.0), 500 μL of buffer B, and distilled water in a total volume of 1 mL were employed. The mixtures were incubated for 15 min at room temperature and the absorption was measured at 250 nm against a reference containing buffer only. The extinction coefficient for SK in buffer B (pH 8.0) is 0.485 L mmol^{-1} cm^{-1}. Each measurement was calibrated by adding a known amount of SK.

SK:NADP$^+$ oxidoreductase (EC 1.1.1.25) and DHQase (EC 4.2.1.10) activities were measured according to Fiedler and Schultz (1985).

QA:NADP$^+$(NAD$^+$) oxidoreductase (EC 1.1.1.24) activity was measured photometrically essentially as described by Ossipov and Shein (1990). Each incubation mixture consisted of 100 mM Tris-HCl, pH 8.6, 200 μL desalted enzyme, 0.5 mM NAD$^+$ (NADP$^+$), and 5 mM QA in a final volume of 1 mL.

Purification of QA Hydrolyase

All steps were carried out at 4°C.

Precipitation by Ammonium Sulfate

The protein from either homogenate or leucoplast stroma of pea roots was precipitated by saturated ammonium sulfate, pH 8.0. The salt concentration was raised in increments of 10%. At each step, the protein precipitated within 30 min was collected by centrifugation at 15,000*g* for 10 min. The QA hydrolyase from the 60 to 70% ammonium sulfate precipitate was resuspended in buffer B and subjected to ion-exchange or affinity chromatography.

Ion-Exchange Chromatography

Desalted fractions of the 60 to 70% ammonium sulfate precipitate were loaded onto a Q-Sepharose Fast Flow (Pharmacia) column (1 × 16 cm) equilibrated with buffer C (50 mM Tris-HCl, pH 8.0, 1 mM EDTA). The flow rate was 0.3 mL min^{-1}. After washing with 100 mL of buffer C, the

QA hydrolyase was eluted with a linear 0 to 0.6 M KCl gradient in buffer C. Fractions of 3 mL were collected.

Affinity Chromatography

Affigel 10 (Bio-Rad) was activated with BSA and packed in a column (1 × 6 cm). The gel was equilibrated with buffer D (buffer B minus BSA) at a flow rate of 0.4 mL min^{-1}. Desalted fractions of the 60 to 70% ammonium sulfate precipitation resuspended in buffer C were loaded onto the affinity column. After washing with 50 mL of buffer D the QA hydrolyase was eluted with 10 mL of 50 mM KCl followed by 10 mL of 100 mM KCl, both equilibrated with buffer D. Fractions of 2.5 mL were collected. Fractions were desalted by filtration through Sephadex G 25–80 equilibrated with buffer D.

Molecular Mass Estimations by Gel Filtration

Desalted fractions after ammonium sulfate precipitation (60–70% saturation) were loaded onto a Sephacryl S 200 HR (Pharmacia) column (2.5 × 30 cm, flow rate 0.5 mL min^{-1}) equilibrated with buffer E (50 mM Tricine-KOH, pH 8.0, 1 mM EDTA). Fractions of 4 mL were collected. Bovine liver catalase (210 kD), BSA (67 kD), chicken ovalbumin (43 kD), and horse heart Cyt *c* (12.5 kD) were used for standardization. Protein concentrations were determined according to the method of Bradford (1976) using BSA as standard.

SDS-PAGE

Pooled fractions obtained after ammonium sulfate precipitation, ion-exchange, or affinity chromatography were desalted and concentrated to yield a minimum protein concentration of 0.25 mg mL^{-1}. Protein extracts were concentrated on 10-kD Macrosep units (Filtron, Karlstein, Germany) or 10-kD Centricon units (Amicon, Witten, Germany). SDS-PAGE was performed according to Laemmli (1970) with 3% acrylamide for the stacking gel and 12% for the resolving gel. Proteins on gels were visualized by silver staining (Schmidt et al., 1990).

RESULTS

QA Is Directly Converted to SK

To investigate by which enzymatic reaction QA is channeled into the SK pathway (i.e. QORase, QORase/DHQase II, or QA hydrolyase), we analyzed the product obtained after incubation of desalted leucoplast stroma, which is free of cofactors, with [U-^{14}C]QA. Figure 2 shows the distribution of the radioactive products after separation by cellulose TLC using two different solvents. The only detectable labeled product is SK; neither DHQ nor DHS is seen even after 20 min of incubation time. We also studied the effect of cofactors on the QA conversion. Figure 3 shows that the QA-to-SK conversion is independent of cofactors such as NAD^+, $NADP^+$, or NADPH, added alone or in combination. Also, the QA hydrolyase appears not to require any divalent cation for enzymatic activity, since EDTA does not affect the QA-to-SK conversion. Since DHQase is obligatorily dependent on Mg^{2+} (Schmidt et al., 1991), our results appear to exclude the participation of any of the enzymes of the following reaction sequence:

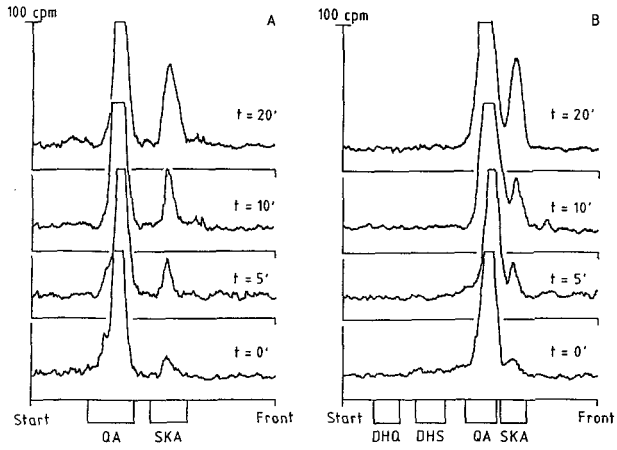

Figure 2. Radioscans of thin-layer chromatographic separations of the products of [U-^{14}C]QA (specific activity 2.3 MBq $mmol^{-1}$) incubations for 0, 5, 10, and 20 min with desalted leucoplast stroma from pea roots. Separation of the cyclohexylcarbonic acids was achieved on cellulose MN 300 in 2-butanol:acetic acid ethyl ester:glacial acetic acid:formic acid:H_2O (35:35:8:2:20, v/v/v/v/v) (A) and ethanol:isopentyl alcohol:1 M acetic acid (2:1:1, v/v/v) (B). The positions of reference standards are indicated on the abscissa.

$$\text{QA} \xrightleftharpoons[+\text{NAD}^+/\text{NADP}^+]{\text{QORase}} \text{DHQ} \xrightarrow[\text{Mg}^{2+}]{\text{DHQase}} \text{DHS} \xrightleftharpoons[+\text{NADPH}]{\text{SORase}} \text{SK}$$

The results of Figures 2 and 3 provide good evidence for a divalent cation-independent QA hydrolyase in pea root leucoplasts that catalyzes the direct conversion of QA into SK. Caution was taken during the determination of the product, since this reaction also occurs nonenzymatically at higher temperatures (>50°C).

Stability of the QA Hydrolyase

Stroma or root homogenates stored at −20°C lose up to 80% of their enzyme activity within 48 h. Neither glycerol (3%, w/v), protease inhibitors like PMSF (0.1–1 mM), benzamidine (0.5–1 mM), nor thiol protectives like DTE or mercaptoethanol (10 mM each) stabilized the QA hydrolyase activity. In contrast, the activity is stabilized when stored at 4°C in the presence of 0.1 to 0.2% (w/v) BSA. The protective effect of BSA is also observed during enzyme assays. Omitting BSA causes a 50% loss of the enzyme activity at once (Fig. 3), but this inactivation is reversible within 12 h.

The Molecular Mass of the QA Hydrolyase

The molecular mass was determined by gel filtration on Sephacryl S 200 HR. Figure 4 shows typical elution profiles for a fraction precipitated at 60 to 70% ammonium sulfate saturation and stabilized with either BSA (A) or with ovalbumin (B). In the BSA-stabilized fraction, molecular species with apparent masses of 100 ± 3 kD and 29 kD

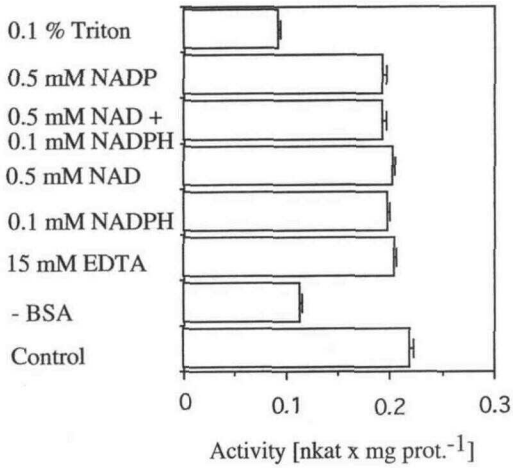

Figure 3. Effect of cofactors, EDTA, BSA, and Triton on the QA hydrolyase activity. Desalted stroma was incubated in buffer B with [U-^{14}C]QA (specific activity 2.6 MBq mmol^{-1}) and the indicated additions for 15 min. The control sample contained desalted stroma in buffer B (50 mM Tricine-KOH, pH 8.0, 1 mM EDTA, 1 mM $MgCl_2$, and 0.1% BSA). Enzyme assays were done in triplicate.

were seen. In the ovalbumin-stabilized fraction, we found species with masses of 91 ± 1 kD, 39 ± 1 kD, and 15 ± 0.4 kD. Apparently, the QA hydrolyase is a monomer of 15 kD that is stabilized by BSA or ovalbumin through oligomerization and/or complex formation with these proteins. The 15-kD monomer size is in good agreement with the results obtained from denaturing SDS-PAGE (Fig. 5).

Purification of the QA Hydrolyase

In a typical purification, extracts from 200 g of pea roots were subjected to ammonium sulfate precipitation and BSA affinity gel chromatography (Table I). During the ammonium sulfate precipitation, the QA hydrolyase was completely separated from SORase. The fraction precipitated at 40 to 60% ammonium sulfate saturation contained the majority of the SORase activity, whereas the 60 to 70% fraction contained the QA hydrolyase. No DHQase or QORase activity could be found in the 60 to 70% fraction (Table II). Since BSA was present in the isolation buffer, protein concentrations of the homogenate or stroma fraction were calculated by using the specific activity of SORase from a BSA-free preparation as an internal standard. Q-Sepharose fast-flow chromatography led to a 300-fold purification. The recovery was at least 40%. Since the QA hydrolyase was stabilized by BSA and therefore seemed to have an affinity for BSA, further purification was performed by fractionation on a BSA-activated Affigel 10 column (Table I). The fraction obtained at 60 to 70% ammonium sulfate saturation was resuspended in a BSA-free buffer that resulted in an approximately 50% loss of enzyme activity (Fig. 3) and was applied to a BSA-activated gel. The QA hydrolyase bound tightly enough to the BSA-activated gel and was not eluted during the washing procedure. The enzyme was eluted from the gel with a salt gradient. The purification factor of 1600 and a recovery of more than 30% demonstrate that affinity chromatography is a very useful

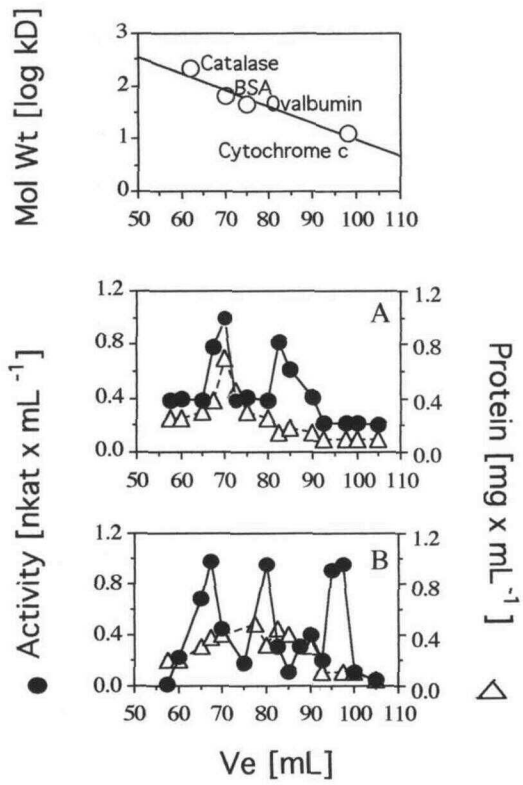

Figure 4. The molecular mass of the QA hydrolyase. For calibration of the Sephacryl S 200 HR column (30 × 2.6 cm, flow rate 0.5 mL min^{-1}), catalase (210 kD), BSA (67 kD), ovalbumin (43 kD), and Cyt *c* (12.5 kD) were used as standards at a concentration of 3 mg mL^{-1} each. The protein obtained after precipitation with 60 to 70% ammonium sulfate was resuspended in buffer E plus 0.1% (w/v) BSA (A) or 0.1% (w/v) ovalbumin (B). Molecular masses of active proteins in the BSA-stabilized fraction (A) were 100 ± 3 kD and 29 kD, and in the ovalbumin-stabilized fraction (B) were 91 ± 1 kD, 39 ± 1 kD, and 15 ± 0.4 kD.

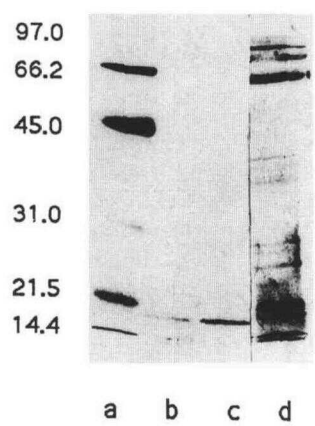

Figure 5. SDS-PAGE of QA hydrolyase purified from pea roots. Lane a, Molecular mass standards, in kD; lanes b and c, QA hydrolyase purified from pea roots by affinity chromatography, elution with 100 mM KCl (b) or 50 mM KCl (c); lane d, 60 to 70% ammonium sulfate fraction. Protein was detected by silver staining.

Table I. *Purification of QA hydrolyase from pea roots*

Purification Step	Total Activity	Total Protein	Specific Activity	Recovery	Purification Factor
	nkat	*mg*	*nkat mg^{-1}*	*%*	
Homogenate	6.15	248.4	0.025	100	1.0
60–70% $(NH_4)_2SO_4$ precipitate	2.55	23.5	0.109	41	4.5
BSA-Affigel: elution with					
50 mM KCl	1.22	0.03	40.7	20	1600
100 mM KCl	0.78	0.03	26.0	13	1040

purification step. On denaturing SDS-PAGE a single protein band corresponding to a mass of 15 kD could be detected by silver staining (Fig. 5).

Properties of the Pea Root QA Hydrolyase

Pea root QA hydrolyase has a pH optimum of 8, similar to those of other stromal enzymes. Kinetic studies of the purified enzyme show regular Michaelis-Menten kinetics for the QA hydrolyase (Fig. 6, A and B). The apparent K_m is 2.0 mM for QA with a maximal velocity of 0.072 nkat mg^{-1} related to the stromal fraction.

The QA hydrolyase is activated by SK as product (Fig. 6, C and D). Increasing SK concentrations up to 2.5 mM in the presence of 5 mM QA lead to a 3-fold stimulation of QA hydrolyase activity (Fig. 6, C and D). An apparent K_a value of 0.6 mM SK can be estimated. The activation is lost progressively at SK concentrations in excess of 2.5 mM (Fig. 6). DHQ and DHS have no effect on the activity of QA hydrolyase.

Compartmentation of SORase, QORase, DHQase, and QA Hydrolyase in Pea Roots

Table II shows the distribution of SORase, QORase (NAD^+), DHQase, and QA hydrolyase after ammonium sulfate precipitation obtained from pea root homogenate and stroma from pea root plastids. Leucoplasts isolated from pea roots had an intactness of 83% and were free from cytosolic contamination, but mitochondrial and peroxisomal contamination were about 2% each (Leuschner and Schultz, 1991b). Similar to spinach (Fiedler and Schultz, 1985) and other species (Schmidt et al., 1991), the SORase of pea roots is localized in the plastid stroma. No evidence for a cytosolic isoenzyme was obtained in our studies. The ratio of SORase to QA hydrolyase activity in pea remains constant at 2.3 ± 0.5:1 for homogenate and stroma preparations (Table II). This is strong evidence for a plastidic localization of QA hydrolyase.

Interestingly, a QORase activity is seen in the 40 to 50% fraction of the homogenate, but not in the stroma fraction. The occurrence of QORase activity only in the homogenate indicates its localization in the extraplastidic compartment. The QORase was tested only in the reverse direction. Its activity is low compared to that of the QA hydrolyase. However, in the forward reaction it may be adequate to account for the conversion of DHQ to QA.

Table II. *Distribution of enzyme activities in pea root homogenate and stroma obtained from pea root plastids*

SORase, DHQase, QQRase, and QA hydrolyase were assayed in various fractions obtained during ammonium-sulfate precipitation of homogenate and plastid stroma. n.d., Not detectable. Four independent experiments were performed.

$(NH_4)_2SO_4$ Saturation	Enzyme Activities			
	SORase	DHQase	QORase	QA hydrolyase
%		*nkat*		
Homogenate				
30–40	1.03	n.d.	0.08	n.d.
40–50	29.24	n.d.	2.11	n.d.
50–60	27.20	3.52	n.d.	n.d.
60–70	0.32	0.05	n.d.	24.70
Stroma				
30–40	0.03	n.d.	n.d.	n.d.
40–50	0.08	0.01	n.d.	n.d.
50–60	0.13	0.03	n.d.	n.d.
60–70	0.01	n.d.	n.d.	0.07

DISCUSSION

The aim of this paper was to search for an enzyme that effectively links QA metabolism with the plastidic SK path-

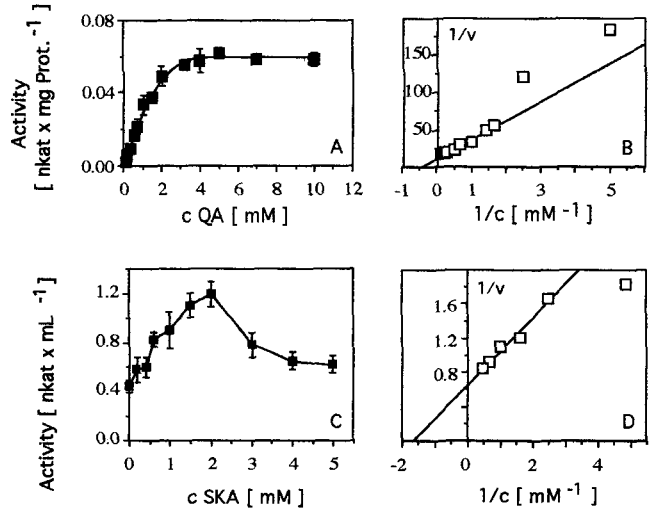

Figure 6. Kinetics of the QA hydrolyase. A, Michaelis-Menten plot; B, Lineweaver-Burk plot. Desalted stroma were incubated with [U-^{14}C]QA (specific activity 2.6 MBq mmol^{-1}) at the given concentrations for 15 min. Protein was determined indirectly via the specific activity of SORase. The experiment was repeated five times. C and D, Effect of SK on the QA hydrolyase activity. The QA hydrolyase activity is assayed with 5 mM QA and various SK concentrations between 0 and 5 mM (C). From the Lineweaver-Burk plot (D) for SK concentrations between 0 and 2.5 mM, a $K_{a(app)}$ of 0.6 mM is estimated. The experiment was repeated three times.

way. Such a link could be suggested from the studies of Weinstein et al. (1959, 1961). More direct experiments from our laboratory (Leuschner and Schultz, 1991b) showed that QA is taken up by intact leucoplasts from pea roots and that QA supplies carbon to the SK pathway for AAA biosynthesis to a similar extent as SK would itself. The present study shows the direct conversion of QA to SK by a plastidic QA hydrolyase that catalyzes the one-step reaction. Other QA-utilizing enzymes, such as QORase or the QORase/DHQase II complex detected in corn (Graziana et al., 1980), could be excluded for the conversion of QA to SK, since the QA hydrolyase reaction was independent of cofactors and divalent cations. Any participation of a SORase activity was excluded as well.

The QA hydrolyase has a distinct affinity for BSA as demonstrated by affinity chromatography. It is very unlikely that BSA functions as a protease inhibitor in its protection against inactivation of QA hydrolyase, since no protease inhibitor or thiol reagent could replace it. We could explain the BSA function, and a similar function of ovalbumin, through protein-protein interactions, as described for SK kinase from spinach leaves (Schmidt et al., 1990). This finding is consistent with results obtained from gel filtration. Protein species of different molecular mass dependent on the stabilizing protein were obtained. In a BSA-stabilized fraction, species of 100 and 29 kD were detected, whereas in ovalbumin-stabilized fractions, 91-, 39-, and 15-kD species were seen. These findings suggest oligomeric forms of the enzyme and/or complexes with the stabilizing agents. The molecular mass of the monomer is in good agreement with the result obtained by SDS-PAGE.

The QA hydrolyase shows a pH optimum of 8 that is typical for stroma enzymes. The maximal velocity of 260 nmol SK mg^{-1} protein h^{-1} in the stroma fraction demonstrates that the capacity of the QA hydrolyase is sufficient to serve all AAA synthetic needs in intact leucoplasts. The V_{max} exceeds the biosynthetic rate by 1 to 2 orders of magnitude, which suggests that even at low substrate concentrations the QA hydrolyase could satisfy the needs for AAA synthesis as calculated in vitro.

The QA hydrolyase is activated by SK as product, with an apparent K_a of about 0.6 mM. Above 2.5 mM SK, this cyclohexylcarbonic acid inhibits the enzyme. In contrast, DHQ and DHS are neither activating nor inhibitory. These effects have been observed in vitro; their in vivo relevance remains to be seen. If surplus SK is not metabolized in the SK pathway, it might be exported from the plastid compartment to the vacuole (Holländer-Czytko and Amrhein, 1983). This hypothesis is supported by the observation that QA supplied to pea epicotyls causes an accumulation of SK (Tazaki et al., 1974). Such a conversion to SK could be due to an irreversible reaction catalyzed by the QA hydrolyase in pea roots.

We assume that the QA hydrolyase plays a role in the biosynthesis of AAA in pea roots. The ratio of SORase/QA hydrolyase activity in plastid stroma is 2.3:1. In contrast, in corn this ratio is 82:1 (Graziana and Boudet, 1983). Additionally, a NAD^+-dependent QORase has been detected in the cytosol of pea roots. The enzyme may function in the biosynthesis of QA from DHQ that was exported from the plastids. This hypothesis is supported by the fact that QA is synthesized from erythrose-4-phosphate in bean (Tazaki, 1979).

QA is transferred into the leucoplast via a step mediated by a transporter (Leuschner and Schultz, 1991b) that may be similar to the Glc transporter (Schäfer et al., 1977), because transport protein for quinate from *Neurospora crassa* (Giles et al., 1991) has been found to be similar to the human Glc transporter from liver (Marger and Saier, 1993).

In conclusion, our studies suggest that QA plays a role not only as a precursor for chlorogenic acids, but also for the biosynthesis of AAA in nongreen tissues in providing building blocks for protein, for lignin, and for related compounds. Very recent studies on the organ-specific expression of genes encoding SK pathway isoenzymes in green and nongreen tissues (Eberhard et al., 1993; Görlach et al., 1993a, 1993b) suggest an organ-specific regulation of AAA synthesis. Of interest is the elevated transcription of genes of SK pathway enzymes in nongreen parts (Görlach et al., 1994). Such elevated transcription could mean an intensive synthesis in these parts and an export of surplus pre-aromatic and aromatic compounds. QA could be one of the pre-aromatic compounds, since it is extremely water soluble and it appears to be transportable in the phloem sap stream (Kluge, 1964; Ziegler, 1975). It would be intriguing to establish QA as a transport metabolite suitable to shuttle carbon for the biosynthesis of AAA and aromatic secondary metabolites.

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NOTE ADDED IN PROOF

Just recently, another QA:$NAD(P)^+$-oxidoreductase was isolated and characterized from *Larix sibirica* [Ossipov V, Chernov A, Zrazherskaga G, Shein I (1995) Quinate:$NAD(P)^+$-oxidoreductase from *Larix sibirica*: purification, characterization and function. Trees (in press)]

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