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Degradation of 2-Carboxyarabinitol 1-Phosphate by a Specific Chloroplast Phosphatase[1]

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ABSTRACT

The catalytic degradation of 2-carboxyarabinitol 1-phosphate (CA 1-P), a naturally occurring inhibitor of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), was investigated by chromatographic and spectroscopic analyses of the reaction products. Carboxy-labeled [^{14}C]CA 1-P was incubated with a partially purified tobacco (*Nicotiana rustica*) chloroplast protein that has been shown previously to catalyze metabolism of CA 1-P to a form incapable of inhibiting Rubisco (ME Salvucci, GP Holbrook, JC Anderson, and G Bowes [1988] FEBS Lett 231: 197-201). In the presence and absence of NADPH, ion-exchange chromatography showed a progressive conversion of [2′-^{14}C]CA 1-P to a labeled compound which coeluted with authentic carboxyarabinitol. Parallel assays with unlabeled CA 1-P showed a concomitant decrease in the ability of reaction samples to inhibit Rubisco activity. In separate experiments, a 1:1 stoichiometry was found between the release of inorganic phosphate from [2′-^{14}C]CA 1-P and accumulation of the ^{14}C-labeled product. Liberation of inorganic phosphate was not observed when the tobacco enzyme was incubated with ribulose-1,5-bisphosphate, fructose-1,6-bisphosphate, glucose-1-phosphate, glucose-6-phosphate, or 6-phosphogluconate. Proton nuclear magnetic resonance spectroscopy of the labeled CA 1-P reaction product established its identity as carboxyarabinitol. We therefore propose that light-stimulated degradation of CA 1-P is catalyzed *in vivo* by a specific phosphatase, 2-carboxyarabinitol 1-phosphatase. Carboxyarabinitol 1-phosphatase activity was detected in the absence of NADPH, but increased threefold when 2 millimolar NADPH was present. Thus, while not required for the reaction, NADPH may play an important role in the regulation of CA 1-P degradation.

Carboxyarabinitol 1-phosphate (CA 1-P[2]) accumulates in the chloroplasts of numerous plant species when plants are placed in darkness or at low irradiance (2, 5–7, 14, 16–18). Because of structural similarities to the carboxylation reaction intermediate, 2-carboxy-3-ketoarabinitol-1,5-bisphosphate, CA 1-P binds tightly to the active site of Rubisco (K_D = 32 nM) (2). CA 1-P accumulation is readily detected in leaf extracts by measuring the inhibition of Mg^{2+}/CO_2-activated Rubisco activity, since the decrease in maximal catalytic rate is a function of the proportion of active sites occupied by the inhibitor (14, 15). Among species in which CA 1-P accumulates, maximal synthesis of the inhibitor in the dark can cause a decrease in carboxylation activity ranging from 25 to 95% (7, 14–16, 18). When leaves are subject to high irradiance, CA 1-P is metabolized, causing inhibition of Rubisco activity to be fully reversed within 10 min (5, 11, 14).

The metabolic reactions that synthesize CA 1-P in the chloroplast of some plant species have not been elucidated. However, we have shown that a soluble protein fraction from tobacco chloroplasts catalyzes conversion of CA 1-P to a form incapable of inhibiting Rubisco activity (13). In these experiments, metabolism of CA 1-P by the stromal protein exhibited an apparent requirement for NADPH. Additionally, free CA 1-P was degraded more rapidly than CA 1-P bound to Rubisco (13). Partial purification of the protein by ion-exchange FPLC showed this catalytic activity to be associated with a minor constituent of the total stromal preparation, which required DTT to preserve activity (13). Purification of the enzyme that metabolizes CA 1-P is reported in a companion study (12).

In the present study, we report the results of chromatographic and spectroscopic analyses of the reaction products from CA 1-P metabolism. Here we show that a specific chloroplast phosphatase catalyzes the conversion of CA 1-P to carboxyarabinitol and Pi and that NADPH acts as a positive effector of the reaction.

MATERIALS AND METHODS

Synthesis of [2′-^{14}C]CA 1-P

[2′-^{14}C]Carboxyarabinitol-1,5-bisphosphate was synthesized from ribulose-1,5-bisphosphate and K^{14}CN according to the method of Pierce *et al.* (10). Unlabeled KCN was used in the synthesis of carboxyarabinitol-1,5-bisphosphate. The cyanohydrin reaction was performed in the presence of 1 M LiCl, a condition which causes preferential formation of the carboxyarabinitol epimer (S Gutteridge, J Pierce, personal communication). [2′-^{14}C]CA 1-P was synthesized from [2′-^{14}C]carboxyarabinitol-1,5-bisphosphate by limited treatment with alkaline phosphatase (2, 5). Reaction products were treated on a 2 mL column of Dowex 50[3] (H^+) and separated

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[2] Abbreviations: CA 1-P, 2-carboxyarabinitol 1-phosphate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; FPLC, fast protein liquid chromatography.

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by chromatography on Dowex 1-X8 (HCOOH). Following elution with 0.5 N HCOOH, [2′-^{14}C]CA 1-P was eluted with 10 N HCOOH. Chromatography on Sephadex G-10 was used to remove HCOOH from this material. [2′-^{14}C]CA 1-P from the Sephadex G-10 column was concentrated *in vacuo*, and the pH of the resuspended solution was adjusted to 7.8. [2′-^{14}C]CA 1-P was purified further by chromatography on a 1 × 9 cm Polyanion S-17 (Pharmacia) column. The column was equilibrated with 10 mM NH_4HCO_3, pH 7.2, and eluted with a linear gradient from 0.075 to 0.25 M NH_4HCO_3. Fractions containing [2′-^{14}C]CA 1-P were pooled, concentrated *in vacuo*, and the concentration of CA 1-P determined by measuring esterified phosphate (8).

Purification of the CA 1-P Metabolizing Enzyme

The protein which catalyzes CA 1-P degradation was partially purified from intact tobacco (*Nicotiana rustica*) chloroplasts as described previously (13). The enzyme was purified further by hydrophobic interaction chromatography (12). For the phosphohydrolytic experiments, the enzyme was isolated from tobacco leaves and purified through the phenyl-Superose and dye-ligand chromatographic steps (12).

Direct Assay of CA 1-P Degradation

Unless specified otherwise, enzymic degradation of CA 1-P was assayed at 25°C in 25 μL reactions containing 20 mM Tris-HCl (pH 8), 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 4 mM DTT, 2 mM NADPH, 19.2 μM [2′-^{14}C]CA 1-P (22.4 Ci/mol), and enzyme. Assays were initiated by addition of either the enzyme or [2′-^{14}C]CA 1-P and were terminated after 10 min by the addition of 75 μL of 0.5 N HCOOH. The entire acidified reaction mixtures were applied to Dowex 1-X8 (HCOOH) columns (0.56 mL bed volume) contained in Pasteur pipettes and were eluted with 3 mL 0.5 N HCOOH. Unreacted [2′-^{14}C]CA 1-P was retained on the columns, whereas the ^{14}C-labeled reaction product was eluted (see below). The eluant, which was collected in a 7 mL scintillation vial, was taken to dryness *in vacuo* and ^{14}C dpm were determined by scintillation spectroscopy.

Indirect Assay of CA 1-P Degradation

A separate quantitative measure of CA 1-P levels was obtained by measuring inhibition of the activity of purified tobacco Rubisco (13). Degradation of CA 1-P was determined from the decrease in the extent of inhibition (13).

Separation of CA 1-P and Reaction Products

Ion-exchange chromatography on a 7 × 0.36 cm Polyanion S-17 column was used to separate CA 1-P from the compound(s) formed during its catalytic breakdown. Assays were conducted as described above under direct assay for CA 1-P degradation. Reactions were terminated and deproteinized by the addition of methanol to 80% (v/v) and the supernatant taken to dryness *in vacuo*. The dried material was resuspended in 0.2 mL of 10 mM NH_4HCO_3, pH 7.3. This buffer was also used to equilibrate the column. Labeled compounds in the assays were separated using a progressive series of linear gradients of differing steepness from 0 to 0.075 M, 0.075 to 0.225 M, and 0.225 to 0.5 M NH_4HCO_3, pH 7.3, generated by twin FPLC pumps.

Assay of Phosphohydrolytic Activity

Phosphohydrolytic activity of the CA 1-P metabolizing enzyme was measured by determining substrate-dependent release of Pi. [2′-^{14}C]CA 1-P at 250 μM was incubated for 0 to 1 h in a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 4 mM DTT, 2 mM NADPH and CA 1-P metabolizing enzyme purified by dye-ligand chromatography from tobacco leaves (12). One-half of the reaction mixture was analyzed for Pi (3), and the remainder was chromatographed on Dowex 1-X8 as described above.

^{1}H NMR Spectroscopy

[2′-^{14}C]CA 1-P was incubated with the CA 1-P metabolizing enzyme purified from intact chloroplasts. After 10 h at 22°C, the reaction mixture was treated on a 2 mL Dowex 50 (H^+) column, and the labeled reaction product was separated from [2′-^{14}C]CA 1-P by chromatography on Dowex 1-X8 (HCOOH). The reaction product was purified further by chromatography on a 0.5 × 5 cm FPLC Mono Q column as described previously (9). Labeled material from this column was taken to dryness *in vacuo* and resuspended in D_2O. The pD of the solution (-log [D^+]) was adjusted to 8.0 with NaOD.

The 400 MHz NMR spectrum was recorded at ambient temperature in 17,000 transients pulsing with a flip angle of 35° and a cycle time of 4.2 s using 48,000 double precision data points over a 6,000 Hz spectral window. The spectrum was Fourier-transformed using an exponential function with a time constant of 1.0 Hz and zero filling to 64,000 data points. The spectrum was referenced to sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4.

RESULTS AND DISCUSSION

Chromatographic Separation of CA 1-P from the Reaction Products

In a previous study (13), determinations of CA 1-P degradation relied upon measurements of Rubisco inhibition as an indirect method for determining CA 1-P levels. To facilitate procedures for the purification and characterization of the chloroplast enzyme that catalyzes CA 1-P degradation (12), as well as for the definitive analysis of the reaction product(s), it was necessary to elucidate the chromatographic behavior of the CA 1-P degradative product(s). These experiments required the synthesis of [2′-^{14}C]CA 1-P and the development of a suitable chromatographic procedure for separating monophosphates. Because of problems with lactonization of the carboxy sugar-phosphates at low pH, the published procedures for separating phosphorylated compounds by HPLC (4) did not give satisfactory results. Therefore, separation was conducted at pH 7.3 using a medium pressure ion-exchange resin, Polyanion S-17 (HCO_3^-).

Unlabeled CA 1-P, detected by inhibition of Rubisco activ-

ity (Fig. 1A), eluted in the same position on the chromatogram as authentic [2′-^{14}C]CA 1-P (Fig. 1B). Treatment of [2′-^{14}C]CA 1-P with alkaline phosphatase caused a change in the elution pattern that was consistent with a decreased charge from the loss of the C-1 phosphate (Fig. 1B). Thus, chromatography of CA 1-P and carboxyarabinitol (Fig. 1, A and B) and authentic ^{14}C-labeled glucose-1-P and glucose (Fig. 1B) established the efficacy of the Polyanion S-17 ion-exchange column for separating sugars, sugar-monophosphates and carboxy-sugar-phosphates.

Degradation of CA 1-P to a Nonphosphorylated Reaction Product

The initial observation that CA 1-P degradation was detectable only in the presence of NADPH (13) led to the proposal that potential reaction products may include hamamelose 1-phosphate or hamamelose. To evaluate this proposal, the chromatographic behavior of the [2′-^{14}C]CA 1-P degradation product was examined (Fig. 2). Data presented in Figure 2, A, B and C, show the chromatographic separation of [2′-^{14}C]CA 1-P and its degradation product in samples taken at selected time points during a 60-min incubation with partially purified CA 1-P metabolizing enzyme and 1 mM NADPH. Chromatography on the Polyanion column showed a time-dependent diminution of the [2′-^{14}C]CA 1-P peak occurring concurrently with the increased appearance of a ^{14}C-labeled peak early in the chromatogram. The changes in the elution profile of [2′-^{14}C]CA 1-P were similar to those presented in Figure 1 following treatment with alkaline phosphatase. The accumulation of label in a peak early in the chromatogram indicated that metabolism of CA 1-P did not involve decarboxylation of the carbonyl group, but may have involved the hydrolysis of the C-1 phosphate.

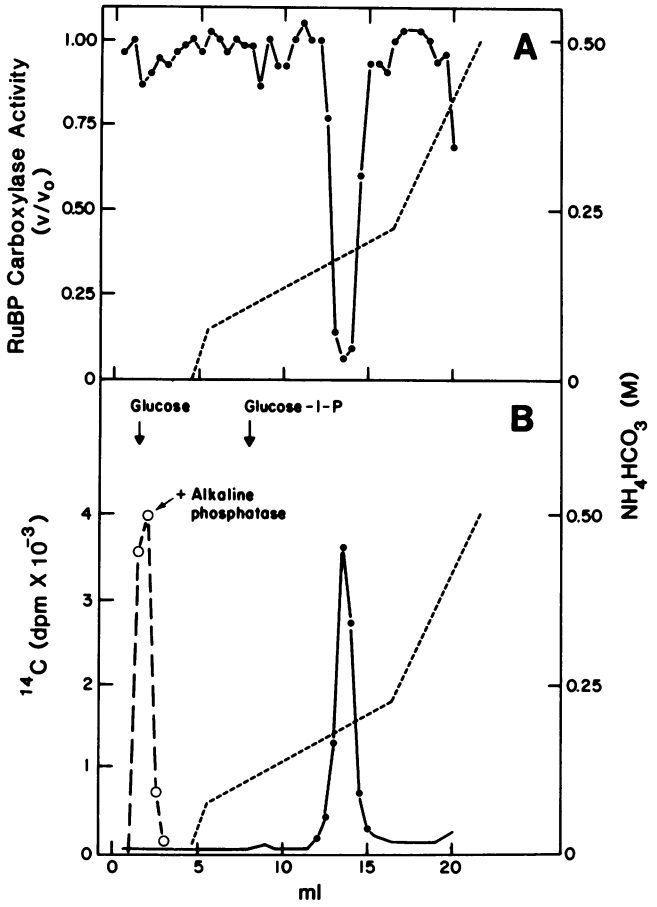

Figure 1. Elution profile of CA 1-P on a Polyanion S-17 ion-exchange column. A, Unlabeled CA 1-P was chromatographed on the column and the fractions assayed for inhibition of Rubisco activity (13). Activity is expressed as v/v_o, the ratio of the rate divided by the control rate without the column fraction. B, Separation of [2′-^{14}C]CA 1-P before (●) and after (○) treatment with alkaline phosphatase. For reference, the elution positions of [^{14}C]-labeled glucose and glucose-1-phosphate standards are indicated by the arrows. The dashed line indicates the NH_4HCO_3 gradient.

The chromatographic behavior of the [2′-^{14}C]CA 1-P reaction product on the Polyanion column led to the development of an ion-exchange-based separation system using small columns of Dowex 1-X8 (HCOOH) for the routine assay of CA 1-P degradation. Application of standards to these columns confirmed that charge-neutral sugars such as glucose eluted with water, whereas CA 1-P required elution with >6 N HCOOH. The reaction product formed during incubation of [2′-^{14}C]CA 1-P with the CA 1-P metabolizing enzyme proved to be weakly charged, requiring 0.2 N HCOOH for elution. For assays of CA 1-P degradation, the labeled product produced in a fixed-time assay was separated from unreacted [2′-^{14}C]CA 1-P by chromatography on Dowex 1-X8 and elution with 0.5 N HCOOH.

Effect of NADPH on CA 1-P Degradation

Catalytic degradation of CA 1-P in the presence (Fig. 2, B and C) and absence (Fig. 2, D and E) of NADPH generated products with identical elution profiles. In addition, there was a concomitant decrease in the capacity of the reaction mixture to inhibit the activity of purified Rubisco (Fig. 3). CA 1-P levels present at zero time were sufficient to inhibit 75% of the Rubisco carboxylation activity ($v/v_o = 0.25$). The extent of inhibition was reduced to 20%($v/v_o = 0.8$) after 60 min in the presence of NADPH (Fig. 3) when 61% of the CA 1-P had been degraded. When NADPH was omitted from the reactions, only 43% of the CA 1-P was converted in 60 min and the extent of Rubisco inhibition was reduced to 45% ($v/v_o = 0.55$). Similarly, up to a threefold stimulation of the CA 1-P degradation rate by NADPH was measured using the 10 min fixed time assay (Fig. 4). The concentration dependence of NADPH in the reaction showed that 2 to 5 mM was optimal (Fig. 4). $NADP^+$ at 0.1 and 0.5 mM had no effect on the rate of the reaction when added either alone or in the presence of varying concentrations of NADPH (data not presented). Thus, while the CA 1-P degradative reaction did not require NADPH, it was markedly stimulated in the presence of the reduced pyridine nucleotide.

Phosphohydrolytic Activity during CA 1-P Degradation

Seemann *et al.* (14) speculated that the phosphorylated inhibitor of Rubisco may be degraded *in vivo* by a light-activated chloroplast phosphatase. Ion-exchange chromatog-

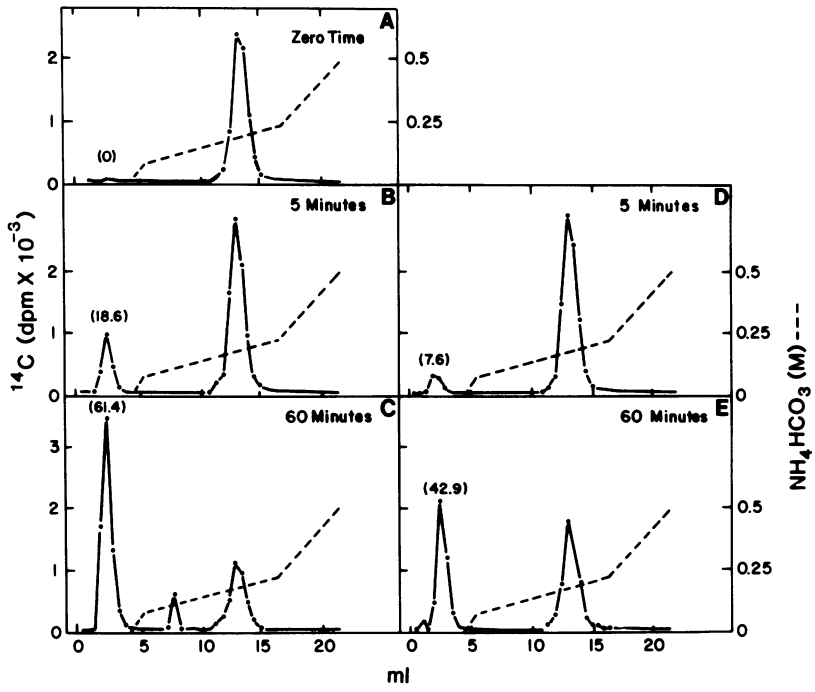

Figure 2. Chromatographic separation of [2′-^{14}C]CA 1-P from its degradation product. Samples were taken for analysis by ion-exchange chromatography after incubation for 0, 5, and 60 min with partially purified CA 1-P metabolizing enzyme in the presence (A, B, and C) or absence (D and E) of 1 mM NADPH. The numbers in parentheses indicate the percentage of ^{14}C label in the peak corresponding to the nonphosphorylated reaction product.

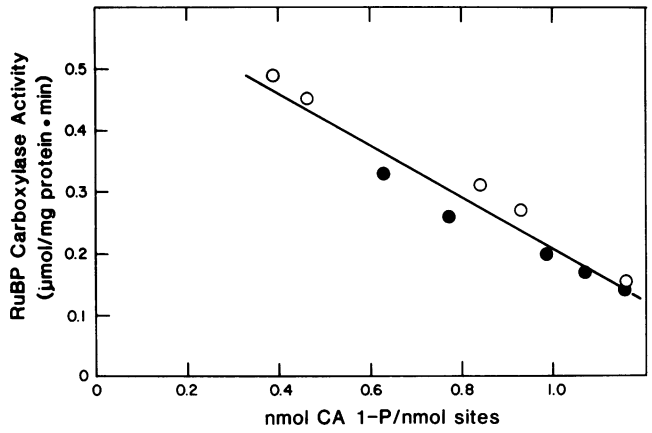

Figure 3. Correlation of [2′-^{14}C]CA 1-P degradation with a decrease in the capacity to inhibit Rubisco activity. The results are from a time-course experiment in which parallel assays were conducted with [^{14}C]- or unlabeled CA 1-P in the presence (○) or absence (●) of 1 mM NADPH. The concentration of [2′-^{14}C]CA 1-P remaining after incubation for various lengths of time with partially purified CA 1-P metabolizing enzyme was determined by ion-exchange chromatography as in Figure 2. Rubisco inhibition was measured as described previously (13) in parallel assays containing the identical concentration of unlabeled CA 1-P. The data are normalized to the active-site concentration of Rubisco.

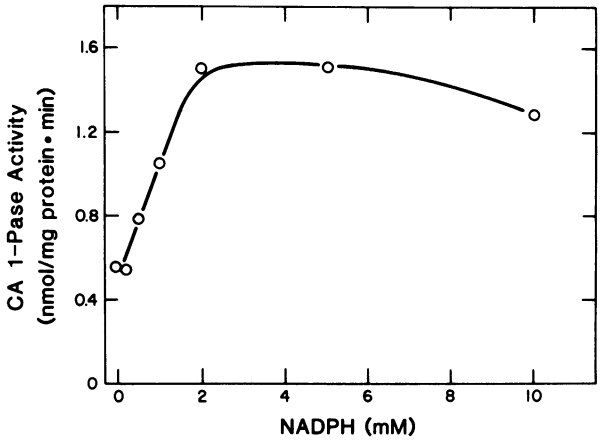

Figure 4. Effect of NADPH concentration on the rate of [2′-^{14}C]CA 1-P degradation by the CA 1-P metabolizing enzyme. 2-Carboxyarabinitol 1-phosphatase (CA 1-Pase) activity refers to the rate of formation of the dephosphorylated [2′-^{14}C]CA 1-P reaction product.

raphy of [2′-^{14}C]CA 1-P degradative products on the Polyanion and Dowex columns provided experimental evidence that the degradative reaction did indeed involve hydrolysis of the C-1 phosphate (Figs. 2 and 3). More direct evidence for a phosphohydrolytic reaction was obtained by conducting parallel measurements of Pi release and labeled product formation. The data presented in Table I show an approximate 1:1 stochiometric relationship between the amounts of Pi released

Table I. *Comparison of Pi Release and ^{14}C-Product Formation during Degradation of [2′-^{14}C]CA 1-P by the CA 1-P Metabolizing Enzyme from Tobacco*

Experiments were conducted for varying lengths of time with 250 μM [2′-^{14}C]CA 1-P in the presence of 2 mM NADPH.

Experiment No.	Pi Released	^{14}C-Product Formed
	nmol	
1	0	0
2	0.9	0.5
3	1.8	2.1
4	2.3	2.9
5	3.1	3.8

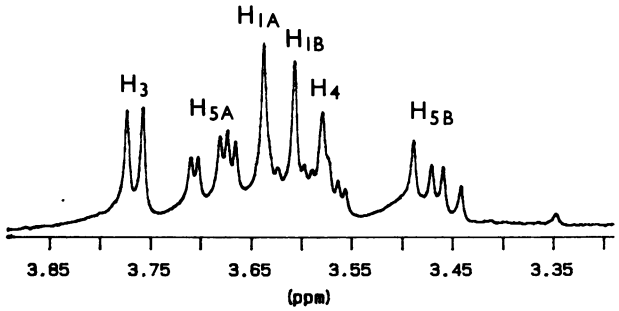

Figure 5. Proton NMR spectrum of the CA 1-P degradation product at 400 MHz. Peak assignments for the chemical shifts follow the nomenclature of Gutteridge *et al.* (5).

and ^{14}C product formed during enzymatic degradation of [2'-^{14}C]CA 1-P. No release of Pi occurred when the CA 1-P metabolizing enzyme from tobacco was incubated with either ribulose bisphosphate, fructose bisphosphate, glucose-1-phosphate, glucose-6-phosphate or 6-phosphogluconate, all at 0.5 mM. Thus, the phosphohydrolase activity of the enzyme was specific for CA 1-P.

Proton NMR Spectroscopy of the CA 1-P Degradative Product

Gutteridge *et al.* (5) have shown that carboxy pentitol epimers and related compounds can be distinguished on the basis of their proton NMR spectrum. Therefore, this technique was used for the definitive identification of the dephosphorylated product of the CA 1-P degradative reaction (Fig. 5). The relative positions of the chemical shifts in the spectrum of the reaction product were identical to those reported by Gutteridge *et al.* for authentic carboxyarabinitol (5). Assignments for the individual hydrogen atoms were as follows: H_3, 3.77; H_{5A}, 3.69; H_{1B}, 3.64; H_{1b}, 3.61; H_4, 3.57; H_{5B}, 3.47. Thus, in addition to Pi, 2-carboxyarabinitol also was identified as one of the products formed during catalytic degradation of CA 1-P.

CONCLUSIONS

Several lines of evidence presented in this study indicate that catalytic degradation of CA 1-P by the tobacco enzyme involves hydrolysis of the C-1 phosphate group. First, chromatographic analyses of the reaction product identified a labeled compound with an elution profile identical to that of authentic carboxyarabinitol. Since only the carbonyl carbon of CA 1-P was labeled, the formation of a labeled product with this elution behavior indicated that the carboxy carbon was retained in the product while the phosphate was lost. Second, measurements of inorganic phosphate proved that stoichiometric release of inorganic phosphate accompanied the formation of the labeled product. Third, spectroscopic analysis of the labeled reaction product by proton NMR definitively identified the product as carboxyarabinitol. Thus, tobacco chloroplasts contain a specific phosphatase which catalyzes the degradation of CA 1-P to carboxyarabinitol and Pi. This enzyme, carboxyarabinitol 1-phosphatase (2-carboxy-D-arabinitol1-phosphatephosphohydrolase), appears to be the major regulator of CA 1-P levels *in vivo*.

Our initial measurements of CA 1-P degradation used an indirect assay to determine CA 1-P levels (13). The data from these measurements indicated that NADPH was required for CA 1-P metabolism. A requirement for NADPH suggested that CA 1-P metabolism involved reductive conversion to hamamelose 1-phosphate (phosphohydroxymethyl D-ribose) or hamamelose. The feasibility of these reactions was supported by previous reports of light dependent formation of hamamelose and hamamelose-2,5-bisphosphate in the chloroplasts (1). In the present study, analysis of the reaction products by ion-exchange chromatography and proton NMR clearly showed that carboxyarabinitol and Pi were the products of CA 1-P degradation. Thus, the existence of a metabolic relationship between CA 1-P and hamamelose has yet to be determined. Furthermore, the use of an improved assay for the direct determination of CA 1-P made it possible to show that NADPH was not a required component in the CA 1-P degradative reaction. Instead, NADPH was found to be an activator of carboxyarabinitol 1-phosphatase, one which along with other stromal metabolites (12) may play a role in the light/dark regulation of CA 1-P levels.

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LITERATURE CITED

1. **Beck E, Stransky H, Furbringer M** (1971) Synthesis of hamamelose diphosphate by isolated spinach chloroplasts. FEBS Lett **13:** 229–234
2. **Berry JA, Lorimer GH, Pierce J, Seemann JR, Meek J, Freas S** (1987) Isolation, identification, and synthesis of 2-carboxyarabinitol 1-phosphate, a diurnal regulator of ribulose-bisphosphate carboxylase activity. Proc Natl Acad Sci USA **84:** 734–738
3. **Chifflet S, Torriglia A, Chiesa R, Tolosa S** (1988) A method for the determination of inorganic phosphate in the presence of labile organic phosphate and high concentrations of protein. Application to lens ATPases. Anal Biochem **168:** 1–4
4. **Giersch C** (1979) Quantitative high-performance liquid chromatographic analysis of ^{14}C-labelled photosynthetic intermediates in isolated intact chloroplasts. J Chromatogr **172:** 153–161
5. **Gutteridge S, Parry MAJ, Burton S, Keys AJ, Mudd A, Feeney J, Servaites JC, Pierce J** (1986) A nocturnal inhibitor of carboxylation in leaves. Nature **324:** 274–276
6. **Holbrook GP, Campbell WJ, Bowes G** (1987) Effect of light intensity during soybean growth on the activation state of RuBP carboxylase from leaves in the light and dark. *In* J Biggins, ed, Progress in Photosynthesis Research, Vol 3. Martinus Nijhoff, New York, pp 399–402
7. **Kobza J, Seemann JR** (1988) Mechanisms for the light regulation of ribulose-1,5-bisphosphate carboxylase activity and photosynthesis in intact leaves. Proc Natl Acad Sci USA **85:** 3815–3819
8. **Penney CL** (1976) A simple micro-assay for inorganic phosphate. Anal Biochem **75:** 201–210
9. **Pierce J, Andrews TJ, Lorimer GH** (1986) Reaction intermediate partitioning by ribulose-bisphosphate carboxylases with differing substrate specificities. J Biol Chem **261:** 10248–10256

10. **Pierce J, Tolbert NE, Barker R** (1980) Interaction of ribulosebisphosphate carboxylase/oxygenase with transition state analogues. Biochemistry **19:** 934–942
11. **Salvucci ME, Anderson JC** (1987) Factors affecting the activation state and the level of total activity of ribulose bisphosphate carboxylase in tobacco protoplasts. Plant Physiol **85:** 66–71
12. **Salvucci ME, Holbrook GP** (1989) Purification and properties of 2-carboxy-D-arabinitol 1-phosphatase. Plant Physiol **90:** 679–685
13. **Salvucci ME, Holbrook GP, Anderson JC, Bowes G** (1988) NADPH-dependent metabolism of the ribulose bisphosphate carboxylase/oxygenase inhibitor 2-carboxyarabinitol 1-phosphate by a chloroplast protein. FEBS Lett **231:** 197–201
14. **Seemann JR, Berry JA, Freas SM, Krump MA** (1985) Regulation of ribulose bisphosphate carboxylase activity in vivo by a light-modulated inhibitor of catalysis. Proc Natl Acad Sci USA **82:** 8024–8028
15. **Servaites JC** (1985) Binding of a phosphorylated inhibitor to ribulose bisphosphate carboxylase/oxygenase during the night. Plant Physiol **78:** 839–843
16. **Servaites JC, Parry MAJ, Gutteridge S, Keys AJ** (1986) Species variation in the predawn inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiol **82:** 1161–1163
17. **Vu CV, Allen LH, Bowes G** (1983) Effect of light and elevated atmospheric CO_2 on the ribulose bisphosphate carboxylase activity and ribulose bisphosphate level in soybean leaves. Plant Physiol **73:** 729–734
18. **Vu CV, Allen LH, Bowes G** (1984) Dark/light modulation of ribulose bisphosphate carboxylase activity in plants from different photosynthetic categories. Plant Physiol **76:** 843–845