2-Carboxyarabinitol 1-phosphate (CA1P) formation through a phosphate exchange reaction catalysed by the CA1P phosphatase from French bean (*Phaseolus vulgaris* L.)

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Transfer of the phosphate group of 2-carboxy-D-arabinitol 1-phosphate (CA1P) to ^{14}C -labelled 2-carboxy-D-arabinitol (CA) was catalysed by extracts from leaves of *Phaseolus vulgaris*. This phosphotransferase activity co-purified with CA1P phosphatase, described previously. This activity was increased, up to 16-fold, by addition of bicarbonate ions at pH 9–10, suggesting rate

enhancement by enzyme carbamylation. A V_{max} of $1.5 \mu\text{mol/min}$ per mg of protein and a K_m (for CA) of 1.8 mM were estimated for the exchange reaction, with the purified phosphatase. 2-Carboxy-D-arabinitol 1,5-bisphosphate and 2-carboxy-D-ribitol 1,5-bisphosphate, but not D-ribulose 1,5-bisphosphate, could replace CA1P as phosphate donor to $[^{14}\text{C}]\text{CA}$.

INTRODUCTION

2-Carboxy-D-arabinitol 1-phosphate (CA1P) is a naturally occurring, tightly binding inhibitor of D-ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) [1,2]. The total Rubisco activity [3,4] and the amount of CA1P in leaf extracts of many plant species [5] show a diurnal variation. Typically, the amount of CA1P at night is higher than during the day [5] whereas the night-time activity of Rubisco is lower than during the day [3,4]. It has recently been confirmed that CA1P is bound to Rubisco *in vivo* at night [6]. In species such as French bean (*Phaseolus vulgaris*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*) and soya bean (*Glycine max*) the amounts of CA1P bound to Rubisco at night are sufficient to reduce the total Rubisco activity in leaf extracts by over 50% [1,4,5,7]. Furthermore, the progressive increase in CO_2 assimilation in an ensuing period of daylight is correlated with the progressive breakdown of CA1P [8]. CA1P, tightly bound to Rubisco, is released in the light by means of Rubisco activase [9]. Unbound CA1P is then dephosphorylated by a specific CA1P phosphatase (CA1Pase) [10,11], yielding 2-carboxy-D-arabinitol (CA) and inorganic phosphate.

The decrease in total and chloroplastic CA in darkened leaves of *P. vulgaris* [12] is consistent with the metabolism of CA in the chloroplast in the dark. Leaves supplied with ^{14}C -labelled 2-carboxy-D-arabinitol (CA) produce $[^{14}\text{C}]\text{CA1P}$ (and no other ^{14}C -labelled product) in an ensuing period of darkness ([13] and J. Andralojc, unpublished work). It therefore seems likely that CA1P is derived from CA by a single-step interconversion catalysed by a kinase. Carbon recently assimilated by photosynthesis was incorporated into CA1P, especially when the period of assimilation was at low photosynthetic photon flux density [14]. However, it is not known whether CA is an intermediate in the synthesis of CA1P *de novo*.

During attempts to detect a kinase responsible for phosphorylating CA in the dark, a reaction catalysed by the CA1Pase was identified, by which the phosphate group of unlabelled CA1P was transferred to $[^{14}\text{C}]\text{CA}$. The properties and implications of this activity are presented here.

EXPERIMENTAL

Plant material

Phaseolus vulgaris L. (cv. Tendergreen) was grown as described previously [14], and leaves were harvested between 14 and 21 days after sowing. Where appropriate, plants were kept in a darkened cabinet for 4 h before use to provide 'dark-adapted leaves'.

CA1Pase purification

The method of Kingston-Smith et al. [11] was modified to minimize anomalous losses of CA1Pase activity. Initial extraction and purification (up to and including affinity chromatography with the Dyematrix Green A column) were as described previously [11], except that 1 kg batches of leaf material were used and buffer A was changed to 50 mM Tris/HCl, pH 7.5, 1 mM EDTA and 2 mM CaCl_2 . Active fractions from the Dyematrix column were precipitated with 80% -satd. ammonium sulphate, then redissolved in buffer A with the addition of PMSF (1 mM), *p*-aminobenzamidine (2.5 mM) and 2-mercaptoethanol (50 mM) and clarified by centrifugation. This material was then applied to a Toyopearl HW-55F size-exclusion column (1.6 cm $\times$ 60 cm) operated at 1 ml/min, using buffer A plus 7.5% (v/v) ethanediol and 2 mM dithiothreitol (DTT). Active fractions were frozen in liquid nitrogen until further processing. Four such preparations were combined and subjected to isoelectric focusing with a 110 ml capacity column (LKB) and a sucrose gradient containing ampholytes (Sigma) for the pH range 4.0–6.5. The separation was conducted at 4 °C for 18 h at constant power (15 W, 1600 V maximum). Fractions containing the CA1Pase activity (pI approx. 5.2) were pooled, the pH was adjusted to 7.5 with NaOH, and the solution was then diluted with an equal volume of buffer A. After clarification by centrifugation the solution was loaded on to a Resource Q anion-exchange column (Pharmacia). The column was operated at 0 °C with a flow rate of 1 ml/min. A gradient of 0–0.3 M KCl in buffer A [plus 7.5% (v/v) ethanediol and 2 mM DTT] was developed over 150 min. Fractions with phosphatase activity contained pure CA1Pase,

Abbreviations used: BTP, Bis-Tris Propane; CA, 2-carboxy-D-arabinitol; CA1P, 2-carboxy-D-arabinitol 1-phosphate; CA1Pase, CA1P phosphatase; CABP, 2-carboxy-D-arabinitol 1,5-bisphosphate; CRBP, 2-carboxy-D-ribitol 1,5-bisphosphate; DTT, dithiothreitol; Rubisco, D-ribulose 1,5-bisphosphate carboxylase/oxygenase; E, free enzyme; E.P, enzyme-bound phosphate; E-64, L-*trans*-epoxysuccinyl-leucylamido(4-guanidino)butane.

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which was either used immediately or frozen in liquid nitrogen. Typically, 110 μg of pure protein was obtained from 4 kg fresh weight of leaves.

Partly purified CA1Pase

This refers to material obtained after chromatography with Dymatex Green A (above), followed by dialysis against buffer A. Unlike that obtained after further purification, the CA1Pase at this stage retained its full activity for at least 10 h at 0 °C, and was unaffected either by repeated freezing or by desalting. Just before use, a 200 μl aliquot (150–200 μg of protein) was desalted by centrifugation through a polystyrene column (0.6 cm $\times$ 6 cm; Pierce & Warriner) containing Sephadex G-25 (medium), equilibrated in 20 mM Tris/HCl, pH 8.0, 1 mM benzamidine, 1 mM DTT and 1 mM EDTA. Protease inhibitors, pepstatin A, chymostatin and *L-trans*-epoxysuccinyl-leucylamido(4-guanidino)butane (E-64; Sigma) were added immediately to the desalted protein (to final concentrations of 24, 28 and 50 μM respectively).

Crude extract

Dark-adapted leaf material (1–2 g) from *P. vulgaris* was ground in ice-cold buffer (2.5 ml) containing 0.1 M Bis-Tris Propane (BTP)/HCl, pH 6.5, 5 mM MgCl_2 , 1 mM EDTA, 25 mM KCl, 5 mM P_i , 1 mM DTT, 1 mM benzamidine, 1 mM PMSF, 1 μM pepstatin A, 50 μM methylviologen and 0.1 g/ml insoluble polyvinylpyrrolidone. Insoluble material was sedimented (14000 g, 5 min, 4 °C) after which the protein in 1 ml was precipitated by adding solid ammonium sulphate to 80% (w/v) saturation. After 15 min at 0 °C the protein was sedimented (14000 g, 5 min, 4 °C), redissolved in 300 μl of 20 mM BTP/HCl, pH 8.0, 1 mM EDTA, 1 mM benzamidine, 1 mM PMSF, 0.1 μM pepstatin A and 50 μM methylviologen at 0 °C, and a 200 μl aliquot was desalted (as above) with a column equilibrated in the same buffer. This sample was assayed immediately.

Phosphate exchange assays

The assay medium contained 26 μM [^{14}C]CA (2.072 MBq/ μmol), 1.3 mM CA1P, 50–150 mM buffer and either 4 μg (crude extract), 450 ng (partly purified) or 7 ng (pure CA1Pase) of protein per μl , in a total volume of 10–50 μl . Assays were conducted at room temperature. Assay duration was varied according to pH, being sufficient for hydrolysis of 15–30% of the available CA1P. Aliquots containing approx. 800 Bq were quenched by addition to 200 μl of 50 mM NaOH at 0 °C, and analysed by HPLC within 3 h. The extent of exchange was deduced from the relative area of the CA and CA1P peaks. Samples for TLC were loaded directly on to the TLC plates, dried by hot air and resolved immediately.

CA1Pase assays

Aliquots from the exchange reaction were either quenched by addition to 3.5% (v/v) trifluoroacetic acid at 0 °C, and the P_i determined [15], or subjected to HPLC, which allowed the simultaneous determination of exchange and phosphatase activities.

HPLC

Quenched samples were loaded onto a CarboPac PA1 column (4 mm $\times$ 250 mm; Dionex U.K.) attached to a Bio-LC chromatography system (Dionex U.K.). The column was pre-equilibrated in 0.1 M NaOH/0.1 M sodium acetate. A linear 0.1–0.6 M sodium acetate gradient, developed over 15 min, was used to

elute CA and CA1P. Column effluent was monitored with a radioactivity detector (Berthold) either alone or in conjunction with a pulsed amperometric detector (Dionex U.K.), using a 50:50 effluent stream splitter (Berthold).

Other materials

D-Ribulose 1,5-bisphosphate, CA, CA1P, 2-carboxy-D-arabinitol 1,5-bisphosphate (CABP) and 2-carboxy-D-ribitol 1,5-bisphosphate (CRBP) were synthesized as described previously [11]. K^{14}CN (Amersham) was used in the synthesis of the ^{14}C -labelled compounds. All other chemicals were of analytical grade and used without further purification.

Other methods

Protein concentrations were determined by the dye binding method of Bradford [16], with BSA as standard. TLC was performed on 20 cm $\times$ 20 cm $\times$ 250 μm cellulose plates (Whatman) with the solvent n-propanol/ammonia/water (6:3:1, by vol.). Resolved ^{14}C -labelled compounds were detected by autoradiography. Cellulose in the vicinity of each radiolabelled spot was scraped off the TLC plate and the associated radioactivity quantified by liquid-scintillation counting.

RESULTS AND DISCUSSION

During attempts to detect a kinase capable of catalysing the phosphorylation of CA (forming CA1P), desalted crude extracts from the leaves of *P. vulgaris* were incubated with ^{14}C -labelled CA, in the presence of a variety of potential phosphate donors. These included nucleoside triphosphates, ADP, PEP, glucose 1-phosphate, 6-phosphogluconate, PP_i and Calvin cycle intermediates, at concentrations of 2–5 mM. In addition, NADPH, DTT and GSH, or NADP^+ and GSSG, were added, to create a reducing or oxidizing environment. Incubation times were varied between 10 and 300 min at 25 °C, and the pH between 6 and 9. Extracts from both light- and dark-adapted leaves were tested. Reaction products were analysed by TLC. None of these trials, even with protease inhibitors (EDTA, pepstatin A, chymostatin, E-64, PMSF or benzamidine) or phosphatase inhibitors (NaF , P_i) resulted in the detectable formation of [^{14}C]CA1P (results not shown).

Failure to detect phosphorylation of CA could have been due to the presence of phosphatase activity in the leaf extracts, destroying any CA1P produced. Further attempts to preserve such newly synthesized CA1P involved the addition of activated Rubisco (to sequester CA1P [14]) or the inclusion of non-radiolabelled CA1P (more than could be degraded by the phosphatase activity present). Although the addition of Rubisco had no effect, incubations in the presence of [^{14}C]CA1P caused considerable production of [^{14}C]CA1P (up to 7.4% of the radiolabel), particularly at high pH (Figure 1). However, the appearance of radiolabelled CA1P depended solely on the presence of [^{14}C]CA1P as phosphate donor. We concluded that a CA–CA1P phosphate exchange was taking place. This exchange was concentration- and time-dependent, and was abolished by boiling the extract before incubation (Figure 1), suggesting an enzyme-catalysed reaction.

Further studies of the exchange reaction involved anion-exchange HPLC with a radioisotope detector. In all the experiments described below, ^{14}C was only detected in peaks with the same retention time as CA and CA1P.

By analogy with glucose 6-phosphatase, which promotes the exchange of phosphate between glucose 6-phosphate and glucose [17], it seemed possible that the CA–CA1P exchange was due to

Figure 1 CA–CA1P phosphate exchange activity in a crude extract of *P. vulgaris* shown by TLC

The exchange reaction was monitored by the phosphorylation of [^{14}C]CA by non-radiolabelled CA1P. Unless otherwise indicated, assays were conducted at pH 10.0 for 90 min. Buffer concentration was 50 mM throughout, using Mes/NaOH, pH 6.0; BTP/HCl, pH 7.0–9.0, or $\text{NaHCO}_3/\text{NaOH}$, pH 10.0. Boiled extract was incubated at 100 °C for 5 min, then cooled before assay. The proportion of total ^{14}C (50 000 d.p.m.) incorporated into CA1P was: pH 7.0, 1.1%; pH 10.0, 7.6%; 0 μl , 2.0%; 2 μl , 5.1%; 4 μl , 6.8%; 6 μl , 7.4%; 5 min, 2.9%; 25 min, 4.4%; 50 min, 6.0%; 130 min, 7.4%; and boiled, 1.8%.

Figure 2 pH dependence of the phosphatase and exchange activities of CA1Pase

Partly purified (●, ○) or pure CA1Pase (△) were assayed for phosphate exchange (○, △) or phosphatase activity (●), as described in the Experimental section, at the indicated pH values. Buffer concentration was 65 mM throughout, using: sodium acetate/acetic acid, pH 5; Bis/Tris/HCl, pH 6; BTP/HCl, pH 7 and 8; and $\text{NaHCO}_3/\text{NaOH}$, pH 9 and above. Catalytic activities are expressed relative to the highest value in each set, which were 380 (●); 2.1 (○); and 2.2 (△) pmol/min.

the incomplete turnover of a CA1P-dependent phosphatase. This was tested using a partly purified preparation of the CA1Pase (as detailed in the Experimental section), for which results very similar to those in Figure 1 were obtained (results not shown). The pH dependence of both the phosphatase (Figure 2, ●) and

Figure 3 A combination of alkaline conditions and the presence of NaHCO_3 promote the CA–CA1P phosphate exchange reaction

The exchange capacity of partly purified CA1Pase was determined. BTP/HCl (100 mM) was present throughout, with (▲) or without (△) 50 mM NaHCO_3 . The exchange reaction was stopped when 20–30% of the CA1P had been hydrolysed.

Table 1 Effect of sodium salts on the extent of CA–CA1P exchange at pH 9.5

Assays were conducted in 0.1 M BTP/HCl, pH 9.5, with partly purified CA1Pase. After 50 min an aliquot was withdrawn for measurement of P_i , the remainder being used to determine the distribution of ^{14}C after product separation by HPLC as described. Amounts of [^{14}C]CA1P are expressed as a percentage of the total label detected. Numbers in parentheses show the extent of CA1P hydrolysis, expressed as a percentage of the total CA1P.

Concentration (mM) ...	0	10	25	50	100
Chloride	2.3 (19)	—	—	4.8 (21)	5.1 (22)
Bicarbonate	2.5 (21)	11.8 (20)	25.8 (19)	36.2 (20)	40.5 (25)
Acetate	—	3.5 (21)	—	5.4 (23)	7.0 (28)
Formate	—	—	—	5.8 (24)	6.8 (28)
Sulphate	—	3.7 (24)	—	5.4 (33)	—

exchange activities (Figure 2, ○) associated with this preparation were then determined simultaneously. The optima are separated by at least 2 pH units (Figure 2): pH 7 for the phosphatase, as reported for the purified CA1Pase [13], and pH 9–10 for the exchange reaction. A preparation of CA1Pase purified to homogeneity also possessed an exchange activity (Figure 2, △) with a pH dependence similar to that of the partly purified preparation. Thus the exchange activity was a property of the CA1Pase itself. The high pH optimum for the exchange was not merely a reflection of the conversion of CA (or CA1P) from a lactone into the open-chain form (the true substrate) at elevated pH, because these samples were neutralized (promoting delactonization) beforehand. HPLC analysis showed that all the CA1P, and at least 85% of the CA, was in the open-chain form at pH 7.0.

Elevated pH alone did not increase the exchange capacity of the CA1Pase. As shown in Figure 3, there was a requirement for added bicarbonate as well. Thus the stimulation of exchange activity at alkaline pH seen in Figures 1 and 2 was due to both the pH and the use of a bicarbonate buffer. Stimulation of exchange activity by sodium chloride, acetate, formate and sulphate, by up to 3-fold, was also observed (Table 1). However, over the range of concentrations tested, the stimulation by bicarbonate was 3–7-fold greater than by any other anion (Table

Figure 4 Simultaneous measurement of the phosphatase and exchange activities of CA1Pase

The reaction mixture contained [^{14}C]CA, unlabelled CA1P and partly purified CA1Pase (see the Experimental section) and 65 mM $\text{NaHCO}_3/\text{NaOH}$, pH 10.4. At the indicated times an aliquot was analysed by HPLC, to determine both the total CA1P remaining (●) and the proportion of [^{14}C]CA that had been converted into CA1P (○).

1). Thus, although sodium salts stimulated the CA–CA1P exchange, the much greater effect of sodium bicarbonate could be due to a distinct mechanism. The dual requirement of high pH and bicarbonate is reminiscent of the activation of Rubisco by carbamylation of the ϵ -amino group of an essential lysine residue [18]. By analogy, carbamylation of CA1Pase might explain the effect of bicarbonate on phosphate exchange activity. A $c_{1/2}$ [the concentration (of bicarbonate) which is required to achieve one-half the maximum effect (stimulation of catalysis by bicarbonate)] for bicarbonate of approximately 34 mM can be deduced from Table 1.

Although the maximal rate of exchange occurred at pH 9–10 (Figure 2), the maximal extent of exchange was observed above pH 10. Indeed, at pH 10.4, phosphorylation of up to 49 % of the radiolabelled CA was observed (Figure 4). This is possibly due to a smaller relative contribution of the phosphatase activity at higher pH. The time dependence of these competing processes is illustrated in Figure 4, where the phosphatase (●) and exchange (○) activities were measured in parallel. A linear rate of exchange was observed at first, but this gradually diminished after longer intervals. The fall in the rate of exchange was presumably due to (1) dilution of the [^{14}C]CA with unlabelled CA, produced by the hydrolysis of unlabelled CA1P, and (2) hydrolysis of newly formed [^{14}C]CA1P.

To characterize the exchange capacity of the pure CA1Pase further, a fixed amount of [^{14}C]CA was diluted with increasing quantities of unlabelled CA, and the effect on the rate of exchange was determined, at pH 9.2 (Figure 5). Total CA1P hydrolysis during this experiment was less than 20 %. The relationship can be described by the Michaelis–Menten equation, with a V_{max} of 1.5 $\mu\text{mol}/\text{min}$ per mg and a K_m for CA of 1.8 mM (Figure 5). A similar K_m (1.7 ± 0.3 mM) was determined by using the partly purified CA1Pase preparation (results not shown). The V_{max} for the phosphatase activity at its pH optimum (6.8 $\mu\text{mol}/\text{min}$ per mg [11]) exceeds that of the exchange activity approx. 4-fold. In Figure 2 (see legend) the maximal phosphatase activity exceeded that of the exchange 200-fold. This is largely because the CA1P concentration exceeded the K_m for the phosphatase activity 3-fold, whereas the CA concentration was one-fiftieth of the K_m for the exchange activity. [The low CA concentration – but high specific radioactivity – was considered

Figure 5 Dependence of the exchange activity on the concentration of CA

Reaction mixtures (volume 16 μl) contained 26 μM [^{14}C]CA (2.072 MBq/ μmol), 1.3 mM CA1P, 150 mM $\text{NaHCO}_3/\text{NaOH}$, pH 9.2, 0.008 $\mu\text{g}/\mu\text{l}$ pure CA1Pase and unlabelled CA, to the concentration indicated. After 25 min at 25 $^\circ\text{C}$, 10 μl aliquots were quenched by addition to 200 μl of ice-cold 50 mM NaOH, and analysed by HPLC. The estimates of K_m (1.8 mM) and V_{max} (1.5 $\mu\text{mol}/\text{min}$ per mg) and the corresponding curves were obtained by nonlinear regression analysis (with Enzfitter, Elsevier Biosoft).

Table 2 Ability of other substrates of CA1Pase to substitute for CA1P in the exchange reaction

The partly purified CA1Pase was used with a phosphate donor concentration of 1.3 mM in the presence of 65 mM $\text{NaHCO}_3/\text{NaOH}$, pH 9.2. The extent of phosphorylation of [^{14}C]CA after the indicated times was determined by HPLC. Abbreviation used: RuBP, D-ribulose 1,5-bisphosphate.

Assay duration (min)	^{14}C]CA phosphorylated (%)			
	CA1P	CABP	CRBP	RuBP
30	27	13	6	0
60	38	19	5	0

advantageous in the initial search for (trace) levels of kinase activity.]

Just as the CA1Pase was shown to hydrolyse 1,5-bisphosphates of CA and 2-carboxy-D-ribitol [11], so did these bisphosphates act as phosphate donors to [^{14}C]CA in the exchange reaction (Table 2). CA1P seemed to be the best phosphate donor, followed by CABP and then CRBP. D-Ribulose 1,5-bisphosphate, however, did not support any [^{14}C]CA phosphorylation (Table 2). Our preparation of CABP was completely free of either CA1P or CRBP, and our CRBP was free of CA1P and contained less than 1 % CABP. It is therefore unlikely that the phosphate exchange observed with either CABP or CRBP is due to sample contamination. The lower levels of exchange observed with CABP and CRBP could be because they are more rapidly dephosphorylated, by the CA1Pase, than CA1P itself [11].

A model for the phosphatase and exchange activities consistent with our observations is:

where E is free CA1Pase, E·CA1P is enzyme-bound CA1P, E·CA·P are enzyme-bound products of CA1P hydrolysis, and E·P is enzyme-bound phosphate. We propose that at elevated pH

the rate of phosphate release is decreased, allowing sufficient time for an enzyme-bound phosphate intermediate (E.P) to react with [^{14}C]CA from the surrounding medium. The formation of [^{14}C]CA1P is conceived as a reversal of the hydrolytic step, followed by product (CA1P) release.

As suggested above, bicarbonate might be involved in enzyme carbamylation, causing a higher exchange activity. (Table 1 shows that the corresponding effect of bicarbonate on phosphatase activity is relatively small.) Alternatively, bicarbonate, and possibly other anions, might stabilize an E.P complex. If bicarbonate (and/or carbonate) exerted its effect by competing with the carboxylate moiety of CA1P (and/or CA) for binding at the catalytic site (promoting CA1P/CA release), then acetate or formate would be expected to have a similar effect. Evidently this is not the case (Table 1).

Moore and Seemann [13] have shown that [^{14}C]CA introduced through the petiole is converted into [^{14}C]CA1P in an ensuing period of darkness. This interconversion could, in principle, be mediated by the exchange reaction described here. The V_{max} of 1.5 $\mu\text{mol}/\text{min}$ per mg suggests that the rate is adequate. However, it is unlikely that a sufficiently high pH or bicarbonate concentration would exist *in vivo* for the maximum rate to be achieved. Moreover, this exchange phenomenon cannot account for the net synthesis of CA1P. These reasons argue against the physiological relevance of this activity. In future searches for a CA-kinase activity, care should be taken to avoid (or control for) conditions that might permit the CA1P-CA exchange reaction.

Differences in the intracellular distribution of CA from light- and dark-adapted leaves are consistent with the movement of CA between chloroplast and cytosol after the onset or cessation of daylight [12]. If the passage of CA into the chloroplast and its phosphorylation to CA1P occur simultaneously, as in the bacterial glucose phosphotransferase system for sugar uptake [19], then several components (some of which may be membrane-associated) might be involved in the phosphorylation of CA. Therefore the notion of a simple 'kinase' may be misleading.

Because CABP and CRBP also acted as phosphate donors (Table 2) it is possible that 2-carboxy-D-arabinitol 5-phosphate and 2-carboxy-D-ribitol 5-phosphate will act as phosphate acceptors in the exchange reaction. If branched-chain sugars,

such as hamamelose and hamamelose phosphates, which may be intermediates in CA1P metabolism [14,20,21], are also able to donate/accept phosphate groups under the mediation of the CA1Pase, this property could be of use in the synthesis of putative intermediates of CA1P metabolism *in vitro*. Whether such a phosphate transfer is involved in CA1P synthesis *in vivo* remains to be seen.

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