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Research review

Avoiding and allowing apatite precipitation in oxygenic photolithotrophs

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Summary

The essential elements Ca and P, taken up and used metabolically as Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ respectively, could precipitate as one or more of the insoluble forms calcium phosphate (mainly apatite) if the free ion concentrations and pH are high enough. In the cytosol, chloroplast stroma, and mitochondrial matrix, the very low free Ca^{2+} concentration avoids calcium phosphate precipitation, apart from occasionally in the mitochondrial matrix. The low free Ca^{2+} concentration in these compartments is commonly thought of in terms of the role of Ca^{2+} in signalling. However, it also helps avoids calcium phosphate precipitation, and this could be its earliest function in evolution. In vacuoles, cell walls, and xylem conduits, there can be relatively high concentrations of Ca^{2+} and inorganic orthophosphate, but pH and/or other ligands for Ca^{2+}, suggests that calcium phosphate precipitates are rare. However, apatite is precipitated under metabolic control in shoot trichomes, and by evaporative water loss in hydathodes, in some terrestrial flowering plants. In aquatic macrophytes that deposit $CaCO_3$ on their cell walls or in their environment as a result of pH increase or removal of inhibitors of nucleation or crystal growth, phosphate is sometimes incorporated in the $CaCO_3$. Calcium phosphate precipitation also occurs in some stromatolites.

Introduction

Phosphorus (P) and calcium (Ca) are essential macronutrients for all photosynthetic organisms (Williams & Fraústo da Silva, 1996). P occurs in the environment almost entirely as oxidised $P^{(+5)}$ state, that is $H_2PO_4^-/HPO_4^{2-}$ depending on pH, and this is also the case inside cells except for phosphonolipids (P^{+3} state) in cyanobacteria such as some *Trichodesmium* strains (Dyhrman *et al.*, 2009). Calcium occurs in the environment as free and ligated Ca^{2+}. In photosynthetic organisms, the free Ca^{2+} and inorganic orthophosphate (as $H_2PO_4^-/HPO_4^{2-}$) concentrations in N and P compartments are as defined by Mitchell (1966; republished in a more accessible form as Mitchell, 2011; see also Nicholls & Ferguson, 2013). N compartments are electrically negative, have pH 7–8, and have a large diversity of proteins; P compartments are electrically positive, have pH generally < 7, and a low protein diversity. N compartments include the cytosol, chloroplast stroma, and mitochondrial matrix, and P compartments include intracellular lysosomes/vacuoles/acidicalcisomes, golgi lumen, thylakoid lumen, and apoplasmic compartments such as shoot cell walls and the lumen of mature xylem elements compartments (Mitchell, 2011; Nicholls & Ferguson, 2013; Pivato & Ballotari, 2021). The intracellular compartments are bounded by membranes of low ion permeability, as is also is the plasma membrane (Mitchell, 2011; Nicholls & Ferguson, 2013).

This paper considers the concentrations of free Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ in N and P compartments and their metabolic implications, and particularly the general avoidance of super-saturation with respect to a calcium phosphate solid phase, and the occurrence of such supersaturation. This solid phase is typically hydroxylapatite ($Ca_5(PO_4)_3OH$) or a related calcium phosphate (Case *et al.*, 2007; Hayes *et al.*, 2019; Nikolenko *et al.*, 2020) hereinafter apatite, in N and P compartments (Chow, 2001; Espie & Dorozhkin, 2002; Case *et al.*, 2007; Pan & Darvell, 2009; Zhu *et al.*, 2009; Hayes *et al.*, 2019; Jasielec *et al.*, 2020; Nikolenko *et al.*, 2020). The paper then considers the cases in which photosynthetic organisms have apatite precipitation in P and N compartments, and the biological and environmental implications of this precipitation. Finally, the implications of free Ca^{2+} and

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$H_2PO_4^-/HPO_4^{2-}$ concentrations in the environment for the origin and early evolution of life are considered.

Interpreting the measured free Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ concentrations in relation to the likeliehood of precipitation of solid phases such as amorphous calcium phosphate (ACP) and crystalline apatite (hydroxylapatite, fluoroapatite) requires knowldge of the solubilty product of the relevant solid phase. The problems with determining these solubility products are well known and only partially resolved (Chow, 2001; Espie & Dorozhkin, 2002; Pan & Darvell, 2009; Zhu *et al.*, 2009; Recillas *et al.*, 2012; Jasielec *et al.*, 2020; Nikolenko *et al.*, 2020). Accordingly, while published calculations of calcium phosphate solubility in compartments of photosynthetic organisms are cited, no attempt is made to make more calculations of calcium phosphate solubility.

Concentration of free Ca^{2+} in N compartments

The free Ca^{2+} concentrations in the N compartments in photosynthetic organisms are 100 μmol m^{-3} in the cytosol (Logan & Knight, 2003; Wagner *et al.*, 2016; Costa *et al.*, 2018; Pivato & Ballotari, 2021), 200 μmol m^{-3} in the mitochondrial matrix (Logan & Knight, 2003; Logan, 2006; Wagner *et al.*, 2016; Costa *et al.*, 2018; Pivato & Ballotari, 2021), and 150 μmol m^{-3} in the chloroplast stroma in the light and rather higher in the dark (Sai & Johnson, 2002; Williams, 2006; Costa *et al.*, 2018; Pottosin & Shabala, 2018; Pivato & Ballotari, 2021). The low concentration of free Ca^{2+} in these N compartments permits the use of Ca^{2+} in signalling (White & Broadley, 2003; Case *et al.*, 2007; Brunet & Arendt, 2016; Pivato & Ballotari, 2021).

However, phloem sieve tube sap, which is modified cytosol N compartment, has a higher concentration of free Ca^{2+}; using three different methods, Brauer *et al.* (1998) found free Ca^{2+} concentrations of 13–63 mmol m^{-3} and total Ca^{2+} concentration of 80–150 mmol m^{-3}, in the sieve tube sap of *Ricinus communis*. However, using two methods on sap collected by stylectomy found much lower values of free Ca^{2+}, that is 40–70 μmol m^{-3} (Furch *et al.*, 2009). The total Ca^{2+} concentration in sieve tube cited in Table 1 is significantly higher than the free Ca^{2+}. The low total Ca^{2+} concentration in phloem sap relative to that of sugars, organic N, and K^+, Mg^{2+}, and Cl^- results in the very low phloem mobility of Ca^{2+} (Van Goor & Wiersma, 1974; Wiersum, 1974; Raven, 1977; White & Broadley, 2003). What the higher free Ca^{2+} concentrations in phloem sap than in the cytosol of other plant cells means for the symplasmically connected companion cell is not clear (Brauer *et al.*, 1998), although free Ca^{2+} in sieve tubes of *Vicia faba* is close to that expected for companion cell cytosol (Furch *et al.*, 2009; see Sections Inorganic phosphate concentration in sieve tube sap, Co-precipitation of Ca^{2+} and P in N compartments and intracellular P compartments). Despite the relatively high free Ca^{2+} concentration in sieve tubes, the electrochemical potential difference between the apoplasm and sieve tube sap permits downhill Ca^{2+} entry in signalling, including propagated action potentials, as well as response to damage (Knoblauch *et al.*, 2001; van Bel *et al.*, 2004; Raven, 2022; see Sections Inorganic phosphate concentration in sieve tube sap, Co-precipitation of Ca^{2+} and P in N compartments and intracellular P compartments).

Concentration of inorganic phosphate in N compartments

Inorganic phosphate concentration in cytosol

Despite the importance of the inorganic phosphate concentration in the cytosol (N compartment) in determining the Gibbs energy (IUPAC, 1997) of hydrolysis of ATP and hence important bioenergetics consequences, there are very few measurements of cytosolic inorganic phosphate. Methods depending on the extraction of cytosol contents rapidly after killing cells are applicable to cyanobacteria where the only intracellular compartment other than the cytosol is the thylakoid lumen. Ritchie *et al.* (1997) used 10 min of $CHCl_3$ extraction on the freshwater cyanobacterium *Synechococcus* PCC 7942 and found the values of cytosol inorganic phosphate concentrations under a variety of conditions of 3–10.3 mol m^{-3}. Using the same cyanobacterium under a different range of conditions, and 1 min $CHCl_3$ extraction time, Ritchie *et al.* (2001) found inorganic phosphate concentrations in the cytosol of 4–17 mol m^{-3} in the light and 13–38 mol m^{-3} in the dark. There is the possibility of changes in inorganic phosphate during extraction by hydrolysis of organic or polyphosphate.

Inorganic phosphate concentrations in the cytosol (N compartment) of chemoorganotrophically grown *Acer pseudoplatanus* cells and photoorganotrophically grown *Arabidopsis thaliana* cells using *in vivo* ^{31}P nuclear magnetic resonance (NMR) under P sufficiency and P deficiency, using the ^{31}P inorganic phosphate chemical shift and the chemical shift of ^{31}P-methylphosphonate as a tracer of inorganic phosphate corresponding to pH 7.4, gave values of 55–80 mmol m^{-3} (Pratt *et al.*, 2009). This concentration is significantly lower than previous suggestions of *c.* 1–10 mol m^{-3} for the cytoplasm (i.e. cytosol plus non-vacuolar organelles) of flowering plant cells (Pratt *et al.*, 2009). Pratt *et al.* (2009) did not cite the earlier (Roberts *et al.*, 1985; Lee *et al.*, 1990) ^{31}P NMR-based estimates of ‘cytoplasmic’ inorganic phosphate in *Zea mays* root tips and mature roots and *Pisum sativum* root tips yielding values of 1–6 mol m^{-3}, with no separation of cytosol from mitochondria and leucoplasts. This same lack of separation of cytoplasmic components for apical and sub-apical zones of *Pinus serotina* gave inorganic phosphate concentrations of 1.9–5.4 mol m^{-3} (Ayling & Topa, 1998). Gout *et al.* (2014) used ^{31}P NMR to estimate the ATP : ADP ratio in the cytosol of chemoorganotrophically grown *Acer* cells; ^{31}P NMR was used to estimate ATP and ADP. Perchloric acid extracts of cells yielded the cytoplasm, that is cytosol plus mitochondria plus plastids; the vacuole (a P compartment) lacks ATP and ADP. Mitochondria plus plastids were obtained by cell extracts separated on a percoll gradient and ATP and ADP obtained by ^{31}P NMR (Gout *et al.*, 2014). The cytosol concentration was obtained by subtraction of the independently determined mitochondria plus plastid ATP plus ADP values from the cytoplasmic values; inorganic phosphate was not measured (Gout *et al.*, 2014). Stitt *et al.* (1982) used membrane filtration to measure ATP and ADP in the cytosol, mitochondria, and plastids of *Triticum* leaf protoplasts; again, inorganic phosphate was not measured.

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Table 1 Total Ca^{2+}, $H_2PO_4^-/HPO_4^{2-}$, and pH in phloem sap of flowering plants.

Species	Growth conditions	Ca^{2+}	$H_2PO_4^-/HPO_4^{2-}$	pH	Sap method	References
Ricinus communis	Not stated	0.5–2.5	3.7–5.7	8.0–8.2	Stem incision	Hall & Baker (1972)
Quercus rubra	Soil	1.23	1.68*	7.5	Stem incision	van Die & Willemse (1975)
Ricinus communis	Not stated	0.5–2.25	3.6–5.8	8.0–8.2	Stem incision	Baker *et al*. (1978)
Ricinus communis	Hydroponic, NO_3^-	0.59	4.9	nd	Stem incision	Mengel & Haeder (1977)
Oryza sativa	Not statd	4.8	23.3–28.7	8	Stylectomy	Fukumorita *et al*. (1983)
Ricinus communis	Hydroponic, NH_4^+	0.32	4.35	7.38	Stem incision	Allen & Raven (1987)
Ricinus communis	Hydroponic, NO_3^-	0.04	9.13	7.24	Stem incision	Allen & Raven (1987)
Ricinus communis	Sand with NO_3^-nutrient solution	1.21	6.36	nd	Stem incision	Peuke (2010)
Eucalyptus globulus	Soil	0.25	1.88*	nd	Stem incision	Pate *et al*. (1998)

Concentrations in mol m^{-3}.
nd, not determined.
*Inorganic + organic P.

For mammalian cytosol, Akerboom *et al.* (1978) measured whole cell and isolated mitochondrial inorganic phosphate concentration in foetal *Rattus* hepatocytes; the 'cytosol' value of 3.34 mol m^{-3} obtained by subtraction of the mitochondrial from the whole cell values includes P compartments of lysosomes and endoplasmic reticulum. Iles *et al.* (1985) had compared ^{31}P NMR spectroscopy *in vivo* measurements of inorganic phosphate in *Rattus* liver with analysis by ^{31}P NMR spectroscopy after freeze-clamping; the whole tissue inorganic phosphate was 4 mol m^{-3} after freeze-clamping and 1 mol m^{-3} by *in vivo* ^{31}P NMR, with no attempt to determine compartmentation of the *in vivo* ^{31}P signal. Freeze-fractionation has also been used to measure ATP and ADP, but not inorganic phosphate, in the cytosol and mitochondria of *Rattus* liver (Soboll *et al.*, 1978). Kanno *et al.* (2016) provide a critical review of the methods of determining the inorganic phosphate concentrations in the cytosol.

Since the review of Kanno *et al.* (2016), a further method of measuring phosphate in the cytosol *in vivo* has been developed; this uses genetically encoded fluorescence resonant energy transfer (FRET; Sahu *et al.*, 2020). Application of this technique to the cytosol of epidermal, cortical, and (where differentiated) endodermal cells from five zones along each of seedling roots, and primary and lateral mature roots of *Arabidopsis* yielded inorganic phosphate concentrations from 3.5 to 10 mol m^{-3} for phosphorus-replete plants. These concentrations are much higher than the ^{31}P NMR values of Pratt *et al.* (2009).

The inorganic phosphate concentrations in the plastids and mitochondria, with no differentiation of the two organelles, were also determined for *A. pseudoplatanus* and *A. thaliana* cells using the ^{31}P NMR chemical shift corresponding to pH 7.55 (Pratt *et al.*, 2009). For the chemoorganotrophically grown *A. pseudoplatanus* cells, the organelle inorganic phosphate concentration was 4.0–4.4 mol m^{-3} and for the photoorganotrophically grown *A. thaliana* cells the organelle inorganic phosphate concentration was 6.8–7.2 mol m^{-3} (Pratt *et al.*, 2009).

Earlier attempts to measure cytosol inorganic phosphate concentration in giant intermodal cells of *Chara corallina* by isolating cytoplasm (cells, including cell walls, other than vacuole) and correcting for the measured inorganic phosphate concentration of chloroplasts (Takeshiga & Tazawa, 1989) to produce a value for inorganic phosphate in 'cytosol' of 12 mol m^{-3} (see also Takeshiga *et al.*, 1992; Mimura *et al.*, 1998). However, this 'cytosol' contains mitochondria and a variety of membrane-bounded P compartments (Pickett-Heaps, 1966; Beilby & Shepherd, 1989; Beilby & Casanova, 2014), which could contain high concentrations of inorganic phosphate.

The cytosol inorganic phosphate concentrations of the cyanobacterium *Synechococcus* PCC 7942 from Ritchie *et al.* (1997, 2001) cited earlier using $CHCl_3$ extraction are about two orders of magnitude higher than the values for *Acer* and *Arabidopsis* using *in vivo* ^{31}P NMR (Pratt *et al.*, 2009). There is a possibility, discussed earlier, that the *Synechococcus* PCC 7942 values are overestimated as a result of the extraction technique used. There is also the possibility of contribution to the cytosol estimates for the freshwater *Synechococcus* PCC 7942 from inorganic phosphate accumulated in the periplasm, a phenomenon only reported so far in cyanobacteria from the oligotrophic ocean (Zubkov *et al.*, 2015; Kamennaya *et al.*, 2020; Rees & Raven, 2021). Taking the cytosol inorganic phosphate concentrations in *Synechococcus* PCC 7942 as accurate, it must be considered that the cytosol of cyanobacteria has not only the functions of eukaryotic cytosol, but also those of the chloroplast stroma and also the mitochondrial matrix. Accordingly, a comparison of inorganic phosphate concentration in the *Synechococcus* cytosol should be made with the values with the eukaryotic chloroplast stroma, considered in Section Inorganic phosphate concentration in chloroplasts.

Inorganic phosphate concentration in sieve tube sap

The phloem sieve tube sap is also a cytosolic (N) compartment. The total Ca^{2+} concentration is low relative to inorganic, and total, phosphate in sieve tube sap (Table 1; Wiersum, 1974; van Die & Willemse, 1975; Fukumorita *et al.*, 1983; Raven, 1987; White & Broadley, 2003; Peuke, 2010). Fukumorita *et al.* (1983) carried out ^{31}P NMR of the stylet exudate of *Oryza sativa*, and found no evidence of phosphorus compounds other than inorganic phosphate, despite evidence of organic phosphorus compounds such as ATP in the sieve tube sap of *O. sativa* (Hayashi & Chino, 1990, using HPLC) and other plants (Kluge & Ziegler, 1964; Gardner &

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Peel, 1969; Ohshima *et al.*, 1990). At the pH of cytosol N phases, typically 7–8, the free Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ are generally below the concentrations that allow precipitation of ACP or $Ca_5(PO_4)_3OH$ (hydroxylapatite; Van Goor & Wiersma, 1974; Wiersum, 1974; Williams, 2006). A possible exception is *O. sativa* where the absence of precipitation of ACP or apatite may be a result of organic anions associating with Ca^{2+} (Fukumorita *et al.*, 1983; see Sections Concentration of free Ca^{2+} in N compartments, Co-precipitation of Ca^{2+} and P in N compartments and intracellular P compartments).

Inorganic phosphate concentration in chloroplasts

Specifically for chloroplasts (N compartment stroma plus P compartment thylakoid lumen), Schröppel-Meier & Kaiser (1988) used aqueously extracted chloroplasts of *Spinacia oleracea* and found inorganic phosphate concentrations of 30 ± 10 mol m^{-3}. Dietz (2017) points out that the aqueous extraction method takes a minute in which metabolism can continue and the extracted chloroplasts could exchange solutes with the medium, possibly compromising the inorganic phosphate concentration measurements. A check on the integrity of the extracted chloroplasts is to measure the photosynthetic rate of the chloroplasts compared to that of the source leaf (summarised by Raven, 2020); similarities of the rates means that any phosphate loss has not compromised photosynthesis. The photosynthetic rate test of chloroplast integrity was not performed by Schröppel-Meier & Kaiser (1988). Takeshiga & Tazawa (1989) used aqueously extracted chloroplasts from giant intermodal cells of *C. corallina* and measured inorganic phosphate concentrations of 16 mol m^{-3}; chloroplast integrity was assayed by the absence of ferricyanide-dependent O_2 evolution by illuminated chloroplasts. Sharkey & Vanderveer (1989) used non-aqueously extracted chloroplasts of *Phaseolus vulgaris*; the inorganic phosphate concentration of 7 mol m^{-3} in chloroplasts from the control plants (exposed to normal atmospheric O_2 and CO_2) and suggested that 1–2 mol m^{-3} of the inorganic phosphate is metabolically inactive. This non-aqueous methodology is compared to aqueously extracted chloroplasts, and critiqued by Dietz (2017). The values of 3–38 mmol m^{-3} inorganic phosphate in the cytosol (also functioning as stroma and matrix) of *Synechococcus* (Ritchie *et al.*, 1997, 2001; Section Inorganic phosphate concentration in cytosol) agree with the 5–30 mol m^{-3} for inorganic phosphate in the chloroplast, mainly representing the stroma (Schröppel-Meier & Kaiser, 1988; Sharkey & Vanderveer, 1989: Takeshiga & Tazawa, 1989), and the plastid (stroma) plus mitochondrial (matrix) value of 4–7.2 mol m^{-3} using *in vivo* ^{31}P NMR (Pratt *et al.*, 2009).

Organic phosphate esters in N phases may also be relevant to forming precipitates with Ca^{2+}. However, at a given pH, phosphate esters have less negative charge per P and there are no reports of precipitation of phosphate esters with Ca^{2+} even at relatively high pH, for example, 8.0–8.2 for ribulose-1,5-bisphosphate^{2-} in the assay of ribulose-1,5-bisphosphate carboxylase-oxygenase (Karkehabadi *et al.*, 2003).

Inorganic phosphate concentration in mitochondria

There are no data for mitochondrial inorganic phosphate concentrations in photosynthetic organisms without contamination by plastids (Pratt *et al.*, 2009). For mammalian mitochondria, Soboll *et al.* (1978) used non-aqueous fractionation on perfused rat liver and found matrix and cytosol inorganic phosphate concentrations of 7.4–10.4 and 7.1–9.3 mol m^{-3}, respectively. These values are contributors, with ATP, ADP, AMP, and Mg concentrations, and pH, for determining the Gibbs energy of ATP hydrolysis in the matrix and in the cytosol, with higher Gibbs energy values in the cytosol than in the matrix as a result of the activity of the ATP export: ADP import using an antiporter, and inorganic phosphate influx by H^+: phosphate symporter, in the inner mitochondrial membrane (Soboll *et al.*, 1978; Nicholls & Ferguson, 2013; Gout *et al.*, 2014). Gout *et al.* (2014) used ^{31}P NMR on chemo-organotrophic cell cultures of *A. pseudoplatanus* and found ATP : ADP ratios of 2.25 in mitochondria and 16 in the cytosol; however, these values are based on a thick slurry of mitochondria of unknown metabolic state. If the inorganic phosphate concentration in organelle (mitochondria plus non-photosynthetic plastids) of chemo-organotrophically grown cell cultures of *A. pseudoplatanus* (4.0–4.4 mol m^{-3}) applies to the mitochondria, the inorganic phosphate concentration in the mitochondria is 50–73 times that in the cytosol using *in vivo* ^{32}P NMR (Pratt *et al.*, 2009; Section Inorganic phosphate concentration in chloroplasts). This contrasts with ratio of 0.4 : 1.26 comparing the ^{31}P NMR value from mitochondria and non-photosynthetic plastid value of *A. pseudoplatanus* (Pratt *et al.*, 2009; Section Inorganic phosphate concentration in chloroplasts) with the FRET value for *A. thaliana* cytosol (Sahu *et al.*, 2020; Section Inorganic phosphate concentration in cytosol), and the ratio of 0.6 : 1.4 in *Rattus* liver using freeze-fixed tissue in non-aqueous solvents (Soboll *et al.*, 1978).

With Ca^{2+} overload in the mitochondrial matrix where ACP, and ultimately crystalline calcium phosphate can occur in metazoan mitochondria (Wagner *et al.*, 2016; Jasielec *et al.*, 2020); the only evidence for mitochondria of photosynthetic organisms is ultrastructural data of Peverly *et al.* (1974) on isolated *Zea mays* mitochondria. More direct evidence on the occurrence of apatite precipitation in photoautotroph mitochondria is needed.

Co-precipitation of Ca^{2+} and P in N compartments and intracellular P compartments

As well as the precipitation of Ca^{2+} with orthophosphate in the matrix (N compartment) of mitochondria, there is co-precipitation of Ca^{2+} and P in an N phase as polyphosphate granules in the N compartment cytosol of cyanobacteria (Sans-Luque *et al.*, 2020), although there may also be soluble polyphosphate in the cytosol of cyanobacteria (Lawrence *et al.*, 1998). In eukaryotic photosynthetic organisms that produce polyphosphate the granules are in P compartments, that is intracellular acidicalcisomes in many algae (Zhu *et al.*, 2019; Sans-Luque *et al.*, 2020; see Section Free Ca^{2+} and inorganic phosphate concentrations, and possible supersaturation with respect to apatite in P compartments within and on terrestrial photosynthetic organisms) and in cell walls of some algae

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(Wermer *et al.*, 2007; see Section Free Ca^{2+} and inorganic phosphate concentrations, and possible supersaturation with respect to apatite in P compartments within and on terrestrial photosynthetic organisms).

Van Goor & Wiersma (1974) estimated how close Ca^{2+} in the sieve tube N compartment (Section Inorganic phosphate concentration in sieve tube sap) is to precipitation by measuring turbidity at 400 nm after adding successive quantities of $CaCl_2$ solution to isolated sieve tube sap of *Yucca* and *Ricinus*. In *Yucca*, any addition of Ca^{2+} caused precipitation, while for *Ricinus* five times the normal Ca^{2+} concentration caused precipitation; the nature of the precipitate was not determined (Van Goor & Wiersma, 1974). Fukumorita *et al.* (1983) calculated that calcium phosphate is supersaturated in the sieve tube sap of *O. sativa*, based on the total Ca^{2+} concentration, but point out that association of Ca^{2+} with organic anions could decrease the free Ca^{2+} available for association with phosphate (see Sections Concentration of free Ca^{2+} in N compartments, Inorganic phosphate concentration in sieve tube sap; Brauer *et al.*, 1998; Furch *et al.*, 2009).

Free Ca^{2+} and inorganic phosphate concentrations, and possible supersaturation with respect to apatite in P compartments within and on terrestrial photosynthetic organisms

Vacuoles

Where present, the large (relative to the cytoplasm) vacuole of photoautotrophs is involved in Ca^{2+} and inorganic phosphate storage (Raven, 1987, 1997). Cyanobacteria, including terrestrial representatives, lack aqueous vacuoles, and store inorganic phosphate as polyphosphate in the cytosol, with Ca^{2+} as a major counter-ion (Sans-Luque *et al.*, 2020). Many eukaryotic algae, including terrestrial representatives store Ca^{2+} and inorganic phosphate in acidicalcisomes, acidic P compartments occupying a small fraction of the cytoplasmic volume; acidicalcisomes occur in some algae that also have a large vacuole (Sans-Luque *et al.*, 2020). Using molecular genetic evidence, it was shown that among green algae the Chlorophyta examined, and the basal charophyte alga Mesostigma (Streptophyta), store P as polyphosphate in an acidic P compartment, the acidicalcisomes (Wang *et al.*, 2021). The rest of the charophyte algae examined by molecular genetic methods, including those closest (Zygnematophyceae) to the ancestors of the embryophytic 'higher plants', and the embryophytes, lack polyphosphate production (Wang *et al.*, 2021). However, chemical analysis has shown polyphosphate in two species of the Zygnematophyceae (Elgavish & Elgavish, 1980; Barcyté *et al.*, 2020). In the charophytes examined (Charophyceae) and in the embryophytes, both Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ accumulated in the vacuole, an acidic P compartment (Okihara & Kiyosawa, 1988; Yang *et al.*, 2017; Costa *et al.*, 2018; Wang *et al.*, 2021). In seeds, pollen grains and some vegetative structures of seed plants, inorganic phosphate is accumulated as phytate (inositol hexakisphosphate) salts (e.g. with Ca^{2+}; Campbell *et al.*, 2008; Yang *et al.*, 2017). As for polyphosphate salts in many algae (and cyanobacteria), phytate salts are osmotically inactive. Importantly in the present context, storage of Ca^{2+} and inorganic phosphate as Ca^{2+} polyphosphate, or Ca^{2+} phytate, limits the possibility of apatite precipitation from Ca^{2+} and inorganic phosphate.

Macklon *et al.* (1996) examined the inorganic and organic ion content of vacuoles in the roots of *Agrostris capillaris*, and calculated that apatite supersaturation would not occur at pH values below 5.2. However, supersaturation of apatite (hydroxylapatite) was predicted for a vacuolar pH 6, even allowing for Ca^{2+} associated with organic anions such as citrate that Macklon *et al.* (1996) measured. No values of vacuolar pH were given in Macklon *et al.* (1996), and no apatite crystals were reported. More complete data sets are needed to determine if supersaturation of apatite in vacuoles is likely.

Xylem sap

In endohydric homoiohydric embryophytes on land, there is very little access to external Ca^{2+} and inorganic phosphate by the aerial parts of the plants, and free Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ obtained from the soil is transported to the aerial parts in the xylem conduits; at maturity, these are apoplasmic P compartments (Greenway & Klepper, 1968; Smith *et al.*, 2003; Gillihan *et al.*, 2011). Especially during root pressure xylem flow, and growth with very low transpiratory water loss per dry matter gain, for example, in Crassulacean Acid Metabolism, there is the possibility of supersaturation of free Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$.

Table 2 gives the pH, and the concentrations of Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ in xylem sap obtained by various methods from a range of plants. The only non-invasive method is the collection of guttation fluid from hydathodes; this method yields the lowest concentrations of Ca^{2+} and of $H_2PO_4^-/HPO_4^{2-}$ in Table 2 (Goatley & Lewis, 1966). Nagai *et al.* (2013) using *Hordeum vulgare*, one of the species used by Goatley & Lewis (1966), applied ^{32}P-labelled $H_2PO_4^-/HPO_4^{2-}$ to guttation fluid and found that ^{32}P label is absorbed by hydathode cells and distributed to other parts of the plant, so the $H_2PO_4^-/HPO_4^{2-}$concentration measured by Goatley & Lewis (1966) is probably less than that in the xylem sap lower in the plants. The highest concentrations of Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$ in Table 2 are for the fluid exuded from cut stems of *Actinidia deliciosa* sampled between budbreak and leafburst, that is 'spring sap' (Clark *et al.*, 1986). From their extensive measurements of other solutes in the sap that can influence the free concentrations of Ca^{2+} and $H_2PO_4^-/HPO_4^{2-}$, and the pH (5.45), Clark *et al.* (1986; see also Bradfield, 1976) calculated that the xylem sap was supersaturated with respect to apatite, but no apatite crystals were observed; this metastable state can be compared to that of the flow of an aqueous solution up the xylem under tension (Venturas *et al.*, 2017). Again, more complete data sets are needed to determine if apatite supersaturation in xylem 'spring sap' is widespread.

Nectaries

Baker *et al.* (1978) report the composition of the P compartment nectar fluid of the extrafloral nectaries and of N compartment sieve tube exudate of *R. communis*. The sieve tube exudate has a pH 8.0–8.2, and concentrations of sucrose, Ca^{2+}, and inorganic phosphate

Table 2 Total Ca^{2+}, $H_2PO_4^-/HPO_4^{2-}$, and pH in xylem sap of flowering plants.

Species	Growth conditions	Ca^{2+}	$H_2PO_4^-/HPO_4^{2-}$	pH	Sap method	References
Secale cereale	Seedling on damp paper	0.0375	0.035	5.0	Guttation	Goatley & Lewis (1966)
Triticum aestivum	Seedling on damp paper	0.075	0.023	5.5	Guttation	Goatley & Lewis (1966)
Hordeum vulgare	Seedling on damp paper	0.12	0.074	6.7	Guttation	Goatley & Lewis (1966)
Quercus rubra	Soil	0.44	0.16–0.35	4.9–5.0	Double cut	van Die & Willemse (1975)
Glycine max	Hydroponic NO_3^- : $NH_4^+ = 28$	2.61	0.375	6.1	Root pressure	White *et al*. (1981)
Solanum lycopersum	Hydroponic NO_3^- : $NH_4^+ = 28$	2.64	0.16	6.45	Root pressure	White *et al*. (1981)
Actinidia delicisiosa	Soil	13.8	6.2	5.45	Root pressure	Clark *et al*. (1986)
Ricinus communis	Hydroponic, NH_4^+	3.84	3.46	5.03	Root pressure	Allen & Raven (1987)
Ricinus communis	Hydroponic, NO_3^-	3.20	2.14	5.37	Root pressure	Allen & Raven (1987)
Ricinus communis	Sand with NO_3^- nutrient solution	1.75	0.70	nd	Root pressure; root pressurised	Peuke (2010)
Zea mays	Soil, well-watered	2.1	5.5	5.3	Root pressure	Goodger *et al*. (2005)
Zea mays	Soil, well-watered	1.6	4.0	5.2	Mesocotyl pressurised	Goodger *et al*. (2005)
Zea mays	Soil, well-watered	0.89	2.6	5.0	Leaf midrib pressurised	Goodger *et al*. (2005)
Zea mays	Soil, water limited	3.5	6.2	5.2	Root pressure	Goodger *et al*. (2005)
Zea mays	Soil, water limited	1.8	3.9	5.3	Mesocotyl pressurised	Goodger *et al*. (2005)
Zea mays	Soil, water limited	1.2	2.2	3.2	Leaf midrib pressurised	Goodger *et al*. (2005)
Populus nigra	Soil, sap sampled October–April at 36° N	0.195–3.09	0.0007–0.13	nd	Stump of excised branch under negative pressure	Furukuwa *et al*. (2011)

Concentrations in mol m^{-3}.
nd, not determined.

of 234–298, 0.5–2 25, and 3.6–5.8 mol m^{-3}, respectively (Baker *et al.*, 1978). The extrafloral nectary fluid has a pH 6.8–7.2, and concentrations of sucrose, Ca^{2+}, and inorganic phosphate of 819, 12–20, and 1.8 mol m^{-3}, respectively (Baker *et al.*, 1978). The nectaries are supplied with xylem as well as phloem (Baker *et al.*, 1978), but how the enrichment of nectary fluid in sucrose and Ca^{2+}, but depletion in inorganic phosphate, comes about is not clear. Evaporative water loss from the secreted fluid could account for the increased concentration of sucrose and Ca^{2+}; further evaporative water loss would be required if the increase in Ca^{2+} relative to sucrose in the secreted fluid involves xylem supply of Ca^{2+}. The low concentration of phosphate in the nectary fluid requires phosphate removal by nectary cells from sieve tube sap and, perhaps, addition of xylem sap. The nectar of *Persea americana* sampled from male and female plants of two cultivars has < 3.8–6.2 mol m^{-3} Ca^{2+} and 9.7–20.8 mol m^{-3} phosphorus (Afik *et al.*, 2014). The highest concentrations of both elements are in plants of the Antillano cultivar; males have 5.4 mol m^{-3} Ca^{2+} and 18.3 mol m^{-3} phosphorus, and females have 4.1 mol m^{-3} Ca^{2+} and 20.8 mol m^{-3} phosphorus (Afik *et al.*, 2014). The chemical form of phosphorus is not clear, and there are no pH values cited (Afik *et al.*, 2014). In summary, apatite supersaturation in nectar is unlikely, but more data are needed.

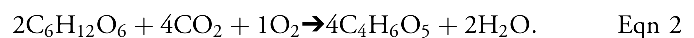
Calcium phosphate precipitation in the P compartment cells walls of shoot trichomes of terrestrial flowering plants

$Ca_5(PO_4)_3OH$ occurs in the cells walls of trichomes, including stinging hairs, on the shoots of some species of the Boraginaceae, Brassicaeae, and Loasaceae, sometimes alone, and in other cases with precipitates of $CaCO_3$ and SiO_2 (Ensikat *et al.*, 2016, 2017; Mustafa *et al.*, 2017, 2018a,b; Weigend *et al.*, 2018; Hopewell *et al.*, 2021).

For $Ca_5(PO_4)_3OH$ precipitation in the apoplasm of shoot trichome cells (Ensikat *et al.*, 2016, 2017; Mustafa *et al.*, 2017, 2018a,b; Weigend *et al.*, 2018; Hopewell *et al.*, 2021), the substrates are $5Ca^{2+}$ and $3H_2PO_4^-$ taken up from the soil solution (Sentenac & Grignon, 1985). The pH of xylem sap is < 7, so phosphate moves up the xylem as $H_2PO_4^-$; for charge balance $7Cl^-$ is assumed to move up the xylem with $5Ca^{2+}$ and $3H_2PO_4^-$. Precipitation of $Ca_5(PO_4)_3OH$ in the shoot would then proceed according to Eqn 1:

$$5Ca^{2+} + 3H_2PO_4^- + H_2O \rightarrow Ca_5(PO_4)_3OH + 7H^+. \qquad \text{Eqn 1}$$

However, there is no long-term sink for H^+ in shoots other that generation of OH^- by assimilation of NO_3^- into organic reduced N and SO_4^{2-} into organic reduced S (Raven & Smith, 1976; Raven, 1986), Eqn 1 does not represent $Ca_5(PO_4)_3OH$ precipitation in trichome cells in the shoot. A stoichiometrically suitable solution would be transport of $2C_6H_{12}O_6$ (as sucrose) with conversion to 4 malic acid, ignoring ionisation at cytosol pH, according to Eqn 2:



$$2C_6H_{12}O_6 + 4CO_2 + 1O_2 \rightarrow 4C_4H_6O_5 + 2H_2O. \qquad \text{Eqn 2}$$

Ionisation of 3 malic acid to form $3C_4H_4O_5^{2-}$ and 1 malic acid to form $1C_4H_5O_5^-$ in the cytosol, efflux of the $7H^+$ to the soil solution with influx of five Ca^{2+} and $3H_2PO_4^-$ gives charge balance

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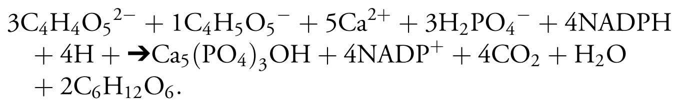
with cytosol acid–base regulation. Transfer to the xylem of $3C_4H_4O_5^{2-}$, $1C_4H_5O_5^-$, $5Ca^{2+}$, and $3H_2PO_4^-$, and movement up the xylem to the shoot trichome apoplasm allows precipitation of $Ca_5(PO_4)_3OH$ according to Eqn 3:



$$3C_4H_4O_5^{2-} + 1C_4H_5O_5^- + 5Ca^{2+} + 3H_2PO_4^- + 4NADPH + 4H + \rightarrow Ca_5(PO_4)_3OH + 4NADP^+ + 4CO_2 + H_2O + 2C_6H_{12}O_6.$$

Eqn 3

Calcium phosphate incorporation in extracellular calcium carbonate deposits of aquatic photosynthetic organisms

This has been shown for the freshwater benthic rhizophyte *Chara* spp. (Murphy *et al.*, 1983; Kiyosawa, 2001; Siong & Asada, 2006; Kufel *et al.*, 2013, 2015; Sand-Jensen *et al.*, 2018, 2021), and also in $CaCO_3$ incrustation on the upper leaf surface of the submerged flowering plant *Potamogeton crispus* (Liu *et al.*, 2016). The investigation by Sand-Jensen *et al.* (2021) showed that P in the *Chara* spp. biomass was up to 0.019 mol P m^{-2} sediment area, that in the sediment was 0.14 mol P m^{-2} sediment area, and that in the water body was only 0.00016 mol P m^{-2} sediment area. One-third of the phosphorus in the light-exposed part of the alga was in the $CaCO_3$ crust, and two-thirds associated with organic matter, in a dense charophyte stand (Sand-Jensen *et al.*, 2021).

The very low phosphate concentrations in the water round the *Chara* shoots means that precipitation of phosphate from this source is unlikely even at the high pH in the carbonate encrustation (Bisson & Walker, 1980; Beilby & Casanova, 2014; Sand-Jensen *et al.*, 2021). Sand-Jensen *et al.* (2021) suggested that the P in the *Chara* carbonate encrustation came from efflux of phosphate from the cells in shoot. Wustenberg *et al.* (2011) showed that *Chara hispida* could grow rapidly with P only supplied as apatite in the silica sand around the rhizoids, that is no P supplied around the shoots. Uptake of dissolved inorganic $H_2PO_4^-/HPO_4^{2-}$ by *C. hispida* rhizoids, and translocation to the shoot, had been shown earlier (Box *et al.*, 1984; Andrews, 1987). The conversion of the organic P and apatite in the sediment into inorganic soluble $H_2PO_4^-/HPO_4^{2-}$ taken up by *Chara* rhizoids presumably involves, respectively, secretion of extracellular phosphatases and of organic anions such as citrate by the rhizoids. These possible secretion processes need further investigation along the lines carried out for vascular plants, with or without ectomycorrhizal fungi (Raven *et al.*, 2018).

Calcium phosphate precipitation in freshwaters has been proposed as a negative feedback on eutrophication (Hamilton *et al.*, 2009). Photolithotrophic algal growth stimulated by input of phosphate and combined nitrogen and involving CO_2 removal increases the pH of the water body, resulting in $CaCO_3$ precipitation and associated calcium phosphate precipitation, thus removing the phosphate from the environment of the phytoplankton (Hamilton *et al.*, 2009). Ingalls *et al.* (2020) showed that the Ca : P ratio in $CaCO_3$ sedimented, with characteristic tufa morphology, from lakes of low Ca/alkalinity indicated the phosphate concentrations in the lake water.

For marine habitats, McGlathery *et al.* (1994) demonstrated phosphate adsorption on shallow water marine calcareous sediments in multiple sequential steps; this phosphate adsorption is not known to be associated with phototrophs. McGlathery *et al.* (1994) also showed that the relative phosphate uptake is greater with oligotrophic than with eutrophic overlying water. Sallstedt *et al.* (2018) report 1.6 Ga old stromatolites with evidence (gas bubbles) of oxygenic photosynthesis with alternating layers of carbonate and phosphate, consistent with phosphate accumulation by cyanobacteria (and possibly rhodophytes that probably occurred at this time: Bengtson *et al.*, 2017) and post mortem release of phosphate with precipitation of calcium phosphate resulting from high pH generated in photosynthesis by living cells. Büttner *et al.* (2020) discovered a living cyanobacterial phosphatic stromatolite on the coast of South Africa, with sharp boundaries between calcium carbonate and calcium phosphate. How the calcium phosphate is precipitated is not clear, since the ambient phosphate concentration is only 0.28 mmol m^{-3}. Whatever the mechanism of phosphate incorporation into the precipitated calcium carbonate, the finding (Büttner *et al.*, 2020) undermines the use (Shiraichi *et al.*, 2019) of fossil phosphatic stromatolites as a proxy from phosphate concentrations in surface seawater of at least 5 mmol m^{-3}. Phosphate has also been found in cell wall of rhodoliths, that is unattached coralline red algae, with a P : Ca ratio of 1.6 : 8.7 mmol mol^{-1}, the ratio increasing with growth rate (Sletten *et al.*, 2017). The P : Ca in these in the $CaCO_3$ of rhodoliths is higher than that of photosynthetically symbiotic corals (Jiang *et al.*, 2020).

These studies discussed in the previous four paragraphs refer to phosphate in $CaCO_3$ deposited in the extracellular P compartment. Many marine unicellular algae of the Haptophyta precipitate calcite intracellularly in the coccolith forming vesicle P compartment, with subsequent exocytosis to form an extracellular layer of coccoliths. Coccolith calcite is 'virtually free' of P (Sviben *et al.*, 2016). The possibility that Ca^{2+} associated with polyphosphate in acidocalcisomes was part of the pathway of extracellular Ca^{2+} to the coccolith forming vesicle (Sviben *et al.*, 2016; Gal *et al.*, 2017, 2018) has been disproved (Peled-Zehari & Gal, 2021).

Phosphate accumulation in cell walls with and without association with calcium

As indicated earlier, polyphosphate occurs in the cell walls of some green (Chlorophyta and Streptophyta) algae (Wermer *et al.*, 2007). The high degrees of polymerisation of polyphosphate in granules prevent diffusion out of the cell walls; the negative charge on polyphosphate is countered by association predominantly with the divalent cations Ca^{2+} and Mg^{2+} (Briggs *et al.*, 1961). There are also reports of accumulation of monomeric inorganic phosphate in the periplasm of marine cyanobacteria where accumulation and retention of phosphate depending on a proton motive force across the cell membrane in some marine cyanobacteria (Zubkov *et al.*, 2015; Kamennaya *et al.*, 2020; Rees & Raven, 2021). Ca^{2+} is also accumulated in the cyanobacterial periplasm by the Donnan equilibrium (Briggs *et al.*, 1961) related to the net negative charge on the periplasmic

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peptidoglycan (Raven & Sánchez-Baracaldo, 2021). It is not clear what the free phosphate concentration is in the periplasm; the phosphate is not free to diffuse out through pores in the outer membrane, since the net flux is into the periplasm (Raven & Sánchez-Baracaldo, 2021; Rees & Raven, 2021). Even less well characterised is the accumulation of phosphate in the organic matrix of colonial phytoplankton organisms such as the marine member of the Haptophyta *Phaeocystis pouchetti* (Veldhuis *et al.*, 1991; see also Ghyoot *et al.*, 2015 for work on *Phaeocystis globosa*). The surface layer of the matrix limits diffusion of inorganic phosphate (Veldhuis *et al.*, 1991), and it is not clear how phosphate is accumulated in the matrix. There is no evidence of calcium phosphate precipitates in the cell walls and matrices of *Phaeocystis* spp.

Evolution

One hypothesis for the origin of low free Ca^{2+} in the cytosol is that it reflects the free Ca^{2+} concentration in the environment at the origin of life (Kazmierczak *et al.*, 2013). Of course, the site of the origin of life (assuming, as is the case in this paper, it was on Earth!) is not clear (e.g. Raven & Sánchez-Baracaldo, 2021). While the free Ca^{2+} concentration of Hadean (4.54–4 Ga) and Archean (4–2.5 Ga) aquatic environments is not known, the suggestion of Toner & Catling (2020) on how the 'phosphate problem' of the origin of life could be solved has important implications for the external free Ca^{2+} concentration. The 'phosphate problem' is how the up to 1 kmol m^{-3} concentration of inorganic orthophosphate needed for some hypotheses of the origin of life was achieved when geochemistry suggests much lower concentrations. Toner & Catling (2020) considered the closed basins of carbonate-rich Searles Lake in the Mojave Basin, CA, with inorganic phosphate concentrations of 5–17 mol m^{-3}, and inorganic plus organic phosphate concentrations up to 50 mol m^{-3} in Goodenough and Last Chance Lakes in British Columbia, after periods of desiccation in summer and autumn. The high carbonate concentrations means the Ca^{2+} is removed as insoluble carbonates gaylussite ($Na_2CO_3{\cdot}CaCO_3{\cdot}5H_2O$) and calcite (a crystalline form of insoluble $CaCO_3$); there is no precipitation of apatite. The Supplementary Information of Toner & Catling (2020) shows free Ca^{2+} concentrations in these environments of 10–100 mol m^{-3}; these are > 0.1–1 mmol m^{-3} in N compartments, but less than the free Ca^{2+} concentrations in most present-day photolithotroph habitats, that is total Ca^{2+} in excess of 2.5 mmol m^{-3} in inland waters (Weyhenmeyer *et al.*, 2019), and the 10 mol m^{-3} total Ca^{2+} in seawater has a free Ca^{2+} ion concentration of 8.4 mol m^{-3} (Thompson & Ross Jr., 1996).

Another suggestion for the origin of low intracellular free Ca^{2+} concerns regulation of the electrical component the proton motive force (PMF; = H^+ electrochemical potential gradient) of transformation of chemical oxidation–reduction or light energy into energy used to phosphorylate ADP (Blackstone, 2015). The relative values of the membrane area based H^+ buffer capacity of the N compartment and of the capacitance of the membrane means that an excess of energised H^+ flux from the N to the P compartment over downhill H^+ flux back to the N phase through the ATP synthase can rapidly (*c.* 1 s) decrease the electrical potential of the N compartment relative to the P compartment to a value that causes electrical breakdown of the membrane. This possibility can be mitigated by influx of a cation other than H^+ from the P to the N compartment (or anion from the N to the P compartment). Blackstone (2015) suggests that the cation is Ca^{2+}, requiring that there is an electrochemical potential difference for downhill Ca^{2+} to the N compartment. However, this does not necessarily involve a lower N compartment than P compartment Ca^{2+} concentration in view of the capacitance of the membrane and energetic equivalence of a 30 mV electrical potential difference and a 10-fold free Ca^{2+} concentration difference (Nicholls & Ferguson, 2013). Even the low free Ca^{2+} concentration in the cytosol, chloroplast stroma, and mitochondrial matrix of eukaryotes (Logan & Knight, 2003; Logan, 2006; Williams, 2006; Wagner *et al.*, 2016; Pottosin & Shabala, 2018; Section Concentration of free Ca^{2+} in N compartments) would not prevent this role of poising the PDF (cf. Blackstone, 2015). It is known that the major roles in poising electrical and H^+ gradient components of the PMF are K^+ and, in thylakoids, Cl^-, and not Ca^{2+} (Pottosin & Shabala, 2018; Raven, 2020, 2021a,b; Korolev, 2021).

Conclusions

Granted the essentiality of Ca and P, taken up as Ca^{2+} and $H_2PO_4{}^-/HPO_4{}^{2-}$ respectively, for photosynthetic organisms, there could precipitation of one or more of the insoluble forms of calcium phosphate. The probability of precipitation depends on the free Ca^{2+} and phosphate anion concentration and on the pH. In N compartments (cytosol, chloroplast stroma, mitochondrial matrix) the very low free Ca^{2+} concentration avoids calcium phosphate precipitation, apart from occasionally in the mitochondrial matrix. While the low free Ca^{2+} concentration in N compartments is today related to the role of Ca^{2+} in signalling as well as avoiding calcium phosphate precipitation, the evolution of low free Ca^{2+} in N compartments at or just after the origin of life is most likely related to avoiding calcium phosphate precipitation. In P compartments such as cell walls, vacuoles, and xylem conduits there can be relatively high concentrations of Ca^{2+} and phosphate anion but pH and/or other ligands for Ca^{2+} means calcium phosphate precipitates are rare. Exceptions in terrestrial flowering plants are in evaporative water loss in hydathodes, and in certain leaf trichomes where precipitation is by unknown mechanisms. For aquatic macrophytes that deposit $CaCO_3$ on their cell walls or in their environment there are cases of phosphate incorporation in the $CaCO_3$; there is also calcium phosphate precipitation in some stromatolites. Conclusions about whether the general absence of calcium phosphate precipitation in within plants depends on lack of supersaturation, or on factors that limit precipitation (e.g. inhibitors of nucleation or crystal growth) await further experimentation and modelling, for example, estimation of *in vivo* saturation values for the range of precipitated calcium phosphate forms. In particular, consistency of the techniques (e.g. FRET) used for estimating inorganic phosphate concentration among N compartments is required.

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Competing interests

None declared.

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