Modulation of glycerophosphorylcholine and glycerophosphorylethanolamine in rice shoots by the environment oxygen level

A ^{31}P NMR study

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Glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine, recently hypothesized to play a central role in phospholipid metabolism, are shown for the first time to be present in higher plants, together with two other unknown phosphodiesters. The GPC concentration is fairly high in young rice (*Oryza sativa* L.) shoots (0.21 mmol·(kg fresh wt tissue)$^{-1}$). It is progressively reduced by growth in air but considerably increased under anoxia, (0.72 mmol·(kg fresh wt tissue)$^{-1}$), concomitantly with the anoxia-stimulated elongation of the rice coleoptile. The involvment of GPC concentration in membrane functionality is discussed in the light of a recent hypothesis.

Glycerophosphorylcholine *Glycerophosphorylethanolamine* *Rice coleoptile* *^{31}P NMR* *Stress adaptation* *Membrane expansion*

1. INTRODUCTION

Rice (*Oryza sativa* L.) at the seedling stage is known to be an organism able to survive prolonged periods of anoxia [1], by means of metabolic, structural, and developmental modifications [1–3]. However, the ability of rice and a few other plants to resist long periods of anoxia remains largely unexplained [4,5].

Oxygen removal blocks growth of roots and leaves in rice seedlings while stimulating elongation and tapering of the coleoptile [1,3]. It has been shown that rice coleoptiles steadily accumulate soluble organic phosphates throughout a 48 h period of anoxia [3]. Following the ^{31}P NMR study of extracts of these compounds from rice shoots, we can report that anoxia induces significant changes in the phosphodiester region of the ^{31}P NMR spectrum, with a marked increase of the GPC level.

To the authors' knowledge, the presence of this compound in higher plants has never been reported, but it has been found in a variety of organisms [6] and particularly in various mammalian tissues [7]. Interesting metabolic [8] and physiological [6] roles for GPC and a few related phosphodiesters have recently been proposed.

Abbreviations: GPC, glycerophosphorylcholine; GPE, glycerophosphorylethanolamine; SEP, L-serine ethanolamine phosphate; PC, phosphorylcholine; U_1 and U_2, unknown substances; FW, fresh weight

2. MATERIALS AND METHODS

2.1. *Rice germination and seedling treatments*

Rice (*O. sativa* L., var. Arborio) was germinated at 30°C in air in the dark for 3 days (reference aerobic seedling) and then for a further 48 h in air (aerobic treatment) or under nitrogen (anoxic

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treatments) for the specified times. Operations were as reported in [3] with the following differences: seeds were germinated in large (∅ = 22 cm) petri dishes with 100 seeds and 40 ml water per petri dish; anoxic treatments were carried out in an anoxic chamber and shoot dissection was performed under anoxia in the same chamber; dissected shoots were immediately frozen in liquid nitrogen and kept at −80°C until extracted.

2.2. *Preparation of $HClO_4$ extracts*

Perchloric acid extracts, after removal of $HClO_4$ [7] were treated with a chelex column, lyophilized, dissolved in H_2O plus D_2O (10%, v/v, final concentration) approximately in a proportion of 1 ml/g fresh wt material extracted, and then brought to pH 7.2.

2.3. *^{31}P NMR spectra*

^{31}P NMR spectra of $HClO_4$ extracts were recorded at 121.49 MHz on a Bruker CXP 300 instrument in a 10 mm tube. The sample temperature was maintained at 10°C. Quantitative analyses were performed using the following spectrometer conditions: pulse width, 15 μs (flip angle 45°); number of data poins, 16 K; acquisition time, 1.64 s; sweep width, 5000 Hz. A recycling delay of 13 s was used to allow for the nuclear relaxation. Proton decoupling was performed by using the gated technique, in which the decoupler is on during the acquisition period and off during the recycling period. The chemical shifts were measured in ppm with respect to 85% phosphoric acid (external) at pH 7.2.

2.4. *Peak identification and quantification*

Resonance identification was obtained by: (i) reference to literature data [9–11], (ii) determination of the pH dependence of the chemical shift of the particular resonance, (iii) addition of authentic compounds to the extract. The standard for quantitative analysis was a capillary (∅ = 1 mm) containing 0.1 M methylenediphosphonic acid. The capillary was calibrated against 0.002 M Na_2HPO_4 and the area of the phosphonic acid served as the reference for the quantification of single peaks [12]. The chemicals used were purchased from Sigma. Reported data refer to a single experiment using 300 shoots for each determination. A first experiment gave comparable results.

3. RESULTS

3.1. *Spectra of $HClO_4$ extracts and identification of resonances*

With reference to the extract from the 3-day-old aerobic shoot (fig.1), oxygen withdrawal for a 48 h period enhanced resonances in the phosphodiester region of the spectrum, $\delta = -2$ to 0 ppm, particularly those of peaks IV and VI. In contrast, a 48 h period of growth in air caused lowering of the same resonances. Variations in the phosphomonoester region of the spectrum, $\delta = -5$ to -3 ppm, will be treated elsewhere.

Peak I, $\delta = -3.85$ ppm [10], is PC and was controlled by addition of the authentic compound. Peak II, $\delta = -2.80$ ppm is P_i. Peak III, $\delta = 1.58$ ppm, is an unknown substance, U_1, which does not titrate with pH. A substance with a

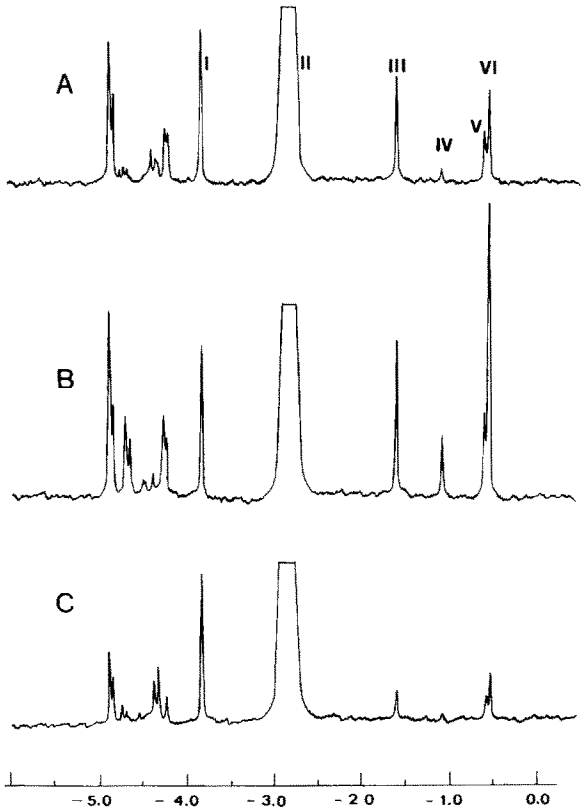

Fig.1. ^{31}P NMR spectra of $HClO_4$ extracts from rice shoots. (A) 3-day-old aerobic shoot; (B) the same after 48 h under nitrogen and (C) after 48 h in air. Peaks I–VI are, respectively: PC, P_i, U_1, GPE, U_2 and GPC.

chemical shift corresponding to U_1, 1.05 ppm downfield from GPC, was reported in brain extracts [7], but was acid labile while U_1 is not. Peak IV, δ = 1.07 ppm, is GPE. It exhibited a slight titration at pH above 7.5 [9] and was checked by the addition of authentic GPE. Peak V, δ = −0.58 ppm, is an unknown substance, U_2, which showed titration similar to that of GPE. Peak VI, δ = −0.53 ppm, is attributed to GPC owing to its non-titratability with pH, in the region of pH 4.5–8.3 [9]. This was true both before and after the addition of authentic GPC which, when added, produced enhancement of the peak VI resonance. The GPC chemical shift reported here is very close to that reported for yeast extracts [9], δ = −0.49 ppm, but differs more from that reported in [11], δ = +0.20 ppm, for Friend erythroleukemia cells. This difference is attributable to the different kind of the instrument magnet [11]. Minor variations in the chemical shift of this phosphodiester may also be caused by ionic strength and medium composition differences [13,14].

3.2. *Changes in GPC and other phosphodiester concentrations under air and anoxic conditions*

Fig.2B shows that GPC, U_1, and U_2 concentrations were fairly high in the starting young aerobic tissues. Under anoxia, there was a practically linear increase of GPC concentration throughout the 48 h treatment. As the concomitant fresh wt increase was also linear (fig.2A), the ratio GPC accumulated/fresh wt gained, remained constant throughout the anoxic treatment. GPE, U_1 and U_2 revealed similar trends, but at a reduced rate, and seemed to level off during a second 24 h period. Growth in air instead reduced the concentrations of all phosphodiesters (fig.1).

It has been shown that growth, in the anoxic rice shoot, is confined to the coleoptile and is due to enlargement of existing cells along the shoot axis [3,15]; in that situation cells may be considered as oblate spheroids. For solids of this type the increase in surface area, after a major axis increase, is linearly correlated with the volume increase and is maximal for a given increase in volume, as compared to the case of spherical solids [16]. Accordingly, the increase in GPC level is linearly correlated not only with fresh weight, i.e. volume increase, but also with the cell surface area increase,

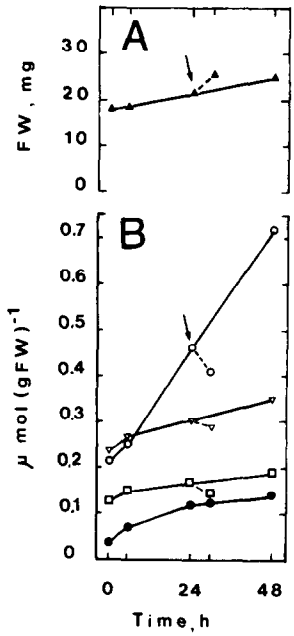

Fig.2. (A) Growth of rice shoots under anoxia, as the fresh weight increase of a single shoot. Time 0 corresponds to 3-day-old aerobic shoot. Dashed line, arrow, corresponds to oxygen readmission for 6 h. (B) Variations of (○) GPC, (▽) U_1, (□) U_2, and (●) GPC. Other explanations as in (A).

with the result that the ratio, GPC increase/plasma and tonoplast membrane surface increase, is maintained at a constant value during anoxic growth.

Oxygen readmission to the anaerobic seedling (fig.2B) inverted the anaerobic trend, leading to a reduction of the GPC level in the shoots.

4. DISCUSSION

Our findings may be summarized as follows: (i) The GPC (and companion phosphodiesters) level is fairly high in young aerobic tissues but is depressed with tissue maturation in air. (ii) Rice shoots respond to the anoxic stress of the environment, by accumulating GPC. (iii) Accumulation is linear throughout the progress of anoxia, with both fresh weight and cell surface increase; oxygen readmission inverts the anoxic trend.

It has been shown that anoxia causes significant changes in the lipid endowment of plant cells: net losses of phospholipids have been reported for rice

roots [17] and *Iris pseudacorus* rhizomes [18] subjected to long-term anoxia; the percentage of total lipids represented by phospholipids was lower in rice coleoptiles growing under anoxia than in coleoptiles growing in air, and marked differences have been reported between the acyl moieties of phospholipids and neutral lipids extracted from aerobic and anoxic rice coleoptiles [19].

Thus, the existence of pools of GPC and other phosphodiesters in rice shoots and the variations in these pools caused by anoxia here reported are in accordance with the changes in phospholipid endowment caused by anoxia in the plant tissues mentioned above. This is particularly clear if our findings are considered in the light of two recent paper [8,20]. In [8] a central role is assigned to GPC and similar phosphodiesters in new pathways for phospholipid synthesis and salvage of the polar moieties of phospholipids, postulated to explain the synthesis of acyl-specific phospholipids. The classical pathways of phospholipid biosynthesis do not require the specific existence of GPC and similar intermediates [21]. In [20] the authors show that the retailoring of phospholipid molecular species is important in maintaining cell membrane functionality under environmental stress, and what applies there for changes in ambient temperature and salinity may be extended to air-anoxia changes. The possibility that GPC, besides being of catabolic origin, may also be synthesized in a process analogous to that found in avians for SEP [22] is not to be discarded since the precursor PC is available (fig.1).

Recently GPC has been hypothesized as a regulator of membrane functionality [6]: the level of lysolecithin in membranes [23] is modulated by cytoplasmic GPC acting as an inhibitor of lysolecithinase. The prerequisite for this hypothesis is that the concentration of GPC varies according to different physiological states. In our system (fig.2B) the requirement is satisfied. To determine the precise role(s) and origin of GPC, however, a specific investigation would be required and this system promises to be useful for this purpose.

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REFERENCES

[1] Vartapetian, B.B., Andreeva, I.N. and Nuritdinov, N. (1978) in: Plant Life in Anaerobic Environments (Hook, D.D. et al. eds) pp.13–88, Ann Arbor Science, MI.
[2] Taylor, D.L. (1942) Am. J. Bot. 29, 721–738.
[3] Menegus, F., Brambilla, I. and Bertani, A. (1984) Physiol. Plant 61, 203–208.
[4] Alpi, A. and Beevers, H. (1983) Plant Physiol. 71, 30–34.
[5] Roberts, J.K.M., Callis, J., Wemmer, D., Wolbot, V. and Jardetzky, O. (1984) Proc. Natl. Acad. Sci. USA 81, 3379–3383.
[6] Burt, C.T. and Ribolow, H.J. (1984) Biochem. Med. 31, 21–30.
[7] Glonek, T., Kopp, S.J., Kot, E., Pettegrew, J.W., Harrison, W.H. and Cohen, M.M. (1982) J. Neurochem. 39, 1210–1219.
[8] Infante, J.P. (1984) FEBS Lett. 170, 1–14.
[9] Navon, G., Shulman, R.G., Yamane, T., Eccleshall, T.R., Keng-Bon Lam, Baronofoky, J.J. and Marmur, J. (1979) Biochemistry 18, 4487–4499.
[10] Evans, F.E. and Kaplan, N.O. (1977) Proc. Natl. Acad. Sci. USA 74, 4909–4113.
[11] Carpinelli, G., Podo, F., Di Vito, M., Proietti, E., Cessani, S. and Belardelli, F. (1984) FEBS Lett. 176, 88–92.
[12] Burt, C.T., Glonek, T. and Barany, M. (1976) J. Biol. Chem. 251, 2584–2591.
[13] Roberts, J.K.M., Wade-Jardetzky, N. and Jardetzky, O. (1981) Biochemistry 20, 5389–5394.
[14] Burt, C.T., Glonek, T. and Barany, M. (1976) Biochemistry 15, 4850–4853.
[15] Atwell, B.J., Waters, J. and Greenway, H. (1982) J. Exp. Bot. 33, 1030–1044.
[16] Handbook of Chemistry and Physics, 49th edn (1968–1969) Cleveland, A-258–A-259.
[17] Khoang, K.L., Sinyutina, N.F. and Chirkova, T.V. (1979) Sov. Plant Physiol. 26, 486–491.
[18] Hetherington, A.M., Hunter, M.I.S. and Crowford, R.M.M. (1982) Phytochem. 21, 1275–1278.
[19] Vartapetian, B.B., Mazliak, P. and Lance, C. (1978) Plant Sci. Lett. 13, 371.
[20] Lynch, D.V. and Thompson, G.A. jr (1984) Trends Biochem. Sci., 442–445.
[21] Lehninger, A. (1975) in: Biochemistry (Lehninger, A. ed.) 2nd edn, pp.659–691, Worth, New York.
[22] Rosemberg, H. and Ennov, A.H. (1966) Biochim. Biophys. Acta 115, 23–32.
[23] Weltzein, H.U. (1979) Biochim. Biophys. Acta 559, 259–287.