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Evidence for the Cytoplasmic Localization of Betaine in Leaf Cells of *Suaeda maritima*

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Abstract. An attempt has been made to localize glycinebetaine in shoots of *Suaeda maritima* L. Dum. using a technique based on the formation of an iodoplatinate precipitate. Deposits were largely restricted to the cytoplasm of salt-grown plants and were analysed by transmission analytical electron microscopy. The results are considered to support the hypothesis that glycinebetaine acts as a cytoplasmic osmoticum to balance high vacuolar salt levels in certain halophytes.

Key words: Betaine – Lophytes – Salt tolerance – *Suaeda*.

Introduction

Terrestrial halophytes are able to tolerate high levels of sodium chloride in the soil and to accumulate high concentrations of these ions in their shoots. There is considerable evidence that these ions are largely sequestered in the vacuole and therefore excluded from the cytoplasm (Flowers, Troke and Yeo, 1977). Recent evidence suggests that this salt resistance may be correlated with the accumulation of quaternary ammonium compounds, particularly glycinebetaine, in a number of plant species (Storey and Wyn Jones, 1977; Storey, Ahmad and Wyn Jones, 1977). Accumulation of betaine has also been reported in the halophyte *Suaeda maritima* when plants grown in the presence and absence of sodium chloride were compared (Flowers and Hall, 1978). Betaine is considered to act as a non-toxic cytoplasmic osmoticum which maintains the intracellular osmotic balance between the cytoplasm and the sodium chloride in the vacuole (Storey and Wyn Jones, 1977; Wyn Jones, Storey and Pollard, 1977; Flowers and Hall, 1978). Certainly high concentrations of betaine show no significant inhibition of certain cytoplasmic enzyme systems in *Suaeda maritima* (Flowers, Hall and Ward, 1978), although direct evidence for a cytoplasmic location of betaine is lacking.

In this paper, we have investigated the sites of betaine accumulation in *Suaeda maritima* cells using a procedure which is considered to identify quaternary ammonium compounds by the formation of complexes with iodoplatinate (Dierichs and Inczédy-Marcsek, 1976). The validity of the staining observed and its significance in relation to the osmoregulatory role of betaine are discussed.

Materials and Methods

Plants of *Suaeda maritima* L. Dum. were grown either in tap water or in culture solution in the presence of 1% or 3% NaCl as previously described (Flowers, 1972). Rice (*Oryza sativa* L. breeding line IR 2153-26-3-5-2 and cv IR 28) plants were grown in unairated culture solution (Yoshida et al., 1972) with a 12 h light period (c. 20 k flux) at a temperature of 30° C. The night temperature was 25° C and the humidity averaged 70 to 80%.

For conventional fixation, 1 mm thick slices of leaves of salt grown *Suaeda maritima* were incubated in 2.5% glutaraldehyde buffered in 50 mmol l^{-1} cacodylate buffer (pH 7.4) with 3% NaCl for 1 h at 0° C. The slices were washed thoroughly in buffer and incubated in the iodoplatinate staining medium as described by Dierichs and Inczédy-Marcsek (1976) at pH 6.8 for 2 h at room temperature. The staining medium was similar to that described below for freeze-substitution. The slices were finally rinsed, dehydrated and embedded in Spurr's (1969) resin.

For freeze-substitution, segments of *Suaeda maritima* leaves and rice leaves and roots were frozen and substituted in ethanol in the dark for 1 day at −72° C and 2 days at −40° C as described by Harvey, Hall and Flowers (1976) except that the substitution medium contained the reagents for iodoplatinate staining, consist-

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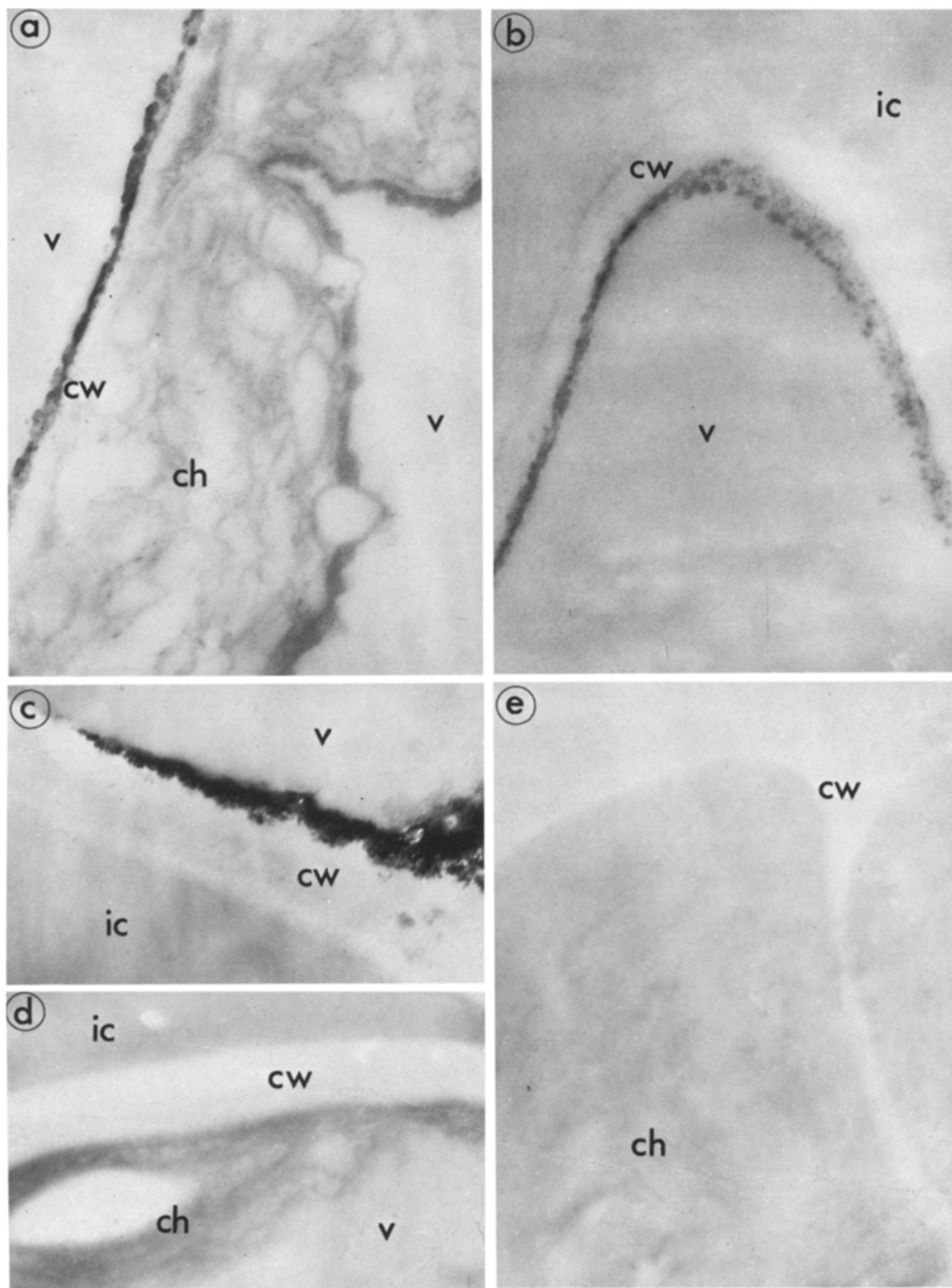

Fig. 1a–e. Electron micrographs of cells of *Suaeda* and rice freeze-substituted in the presence of the iodoplatinate stain: **a, b.** *Suaeda* grown in the presence of 1% NaCl showing dense deposits in the cytoplasm and no staining in the vacuole. a, ×25,500, b, ×38,000. **c** *Suaeda* grown in the presence of 3% NaCl showing dense cytoplasmic deposits ×30,500. **d** *Suaeda* grown on tap water showing no staining in cytoplasm or vacuole ×25,500. **e** Rice showing no electron dense staining ×21,000. cw, cell wall; ch, chloroplast; ic, intercellular space; v, vacuole

ing of 0.042 g of chloroplatinic acid and 0.7508 g sodium iodide per 25 ml. All solutions were prepared fresh, precooled, and kept in the dark. After substitution for 3 days, tissue was either washed with ethanol at −40° C for 3 periods of 1 h or treated in turn with saturated sodium metabisulphite (−40° C, 2 h) and saturated sodium sulphate (−40° C, 4 periods of 30 min) (Dierichs and Inczédy-Marcsek, 1976), and finally washed with ethanol at −40° C for 3 periods of 30 min. The segments were finally embedded in resin as described by Harvey et al. (1976). Sections were cut on water and examined without further staining.

Sections were cut onto formvar-coated grids, carbon-coated and examined using transmission analytical electron microscopy. The conditions of analysis using EMMA 4 were: operating voltage 60 kV, current 40 nA, live time of counting 50 s, magnification 25,000 x, spot size 200–400 nm.

Glycinebetaine content of the *Suaeda* used for electron micro-

scopy and of separate samples of rice were analysed as previously described (Flowers and Hall, 1978).

Results

When sections of glutaraldehyde-fixed leaf material were examined, no significant difference was observed between untreated material and segments incubated in the iodoplatinate stain.

In contrast, freeze-substituted tissues of *Suaeda maritima* grown with 1 and 3% NaCl showed distinct electron dense deposits in the cytoplasm (Fig. 1a–c); occasionally deposits were also observed in the cell wall, particularly in the region close to the plasma membrane, and in chloroplasts but not in the vacuole. Very sparse staining was observed in *Suaeda* grown on tap water or culture medium only but no deposits were observed in rice (Fig. 1d, e). The sections were not post-stained for ease both of identification of the precipitates and of X-ray analysis.

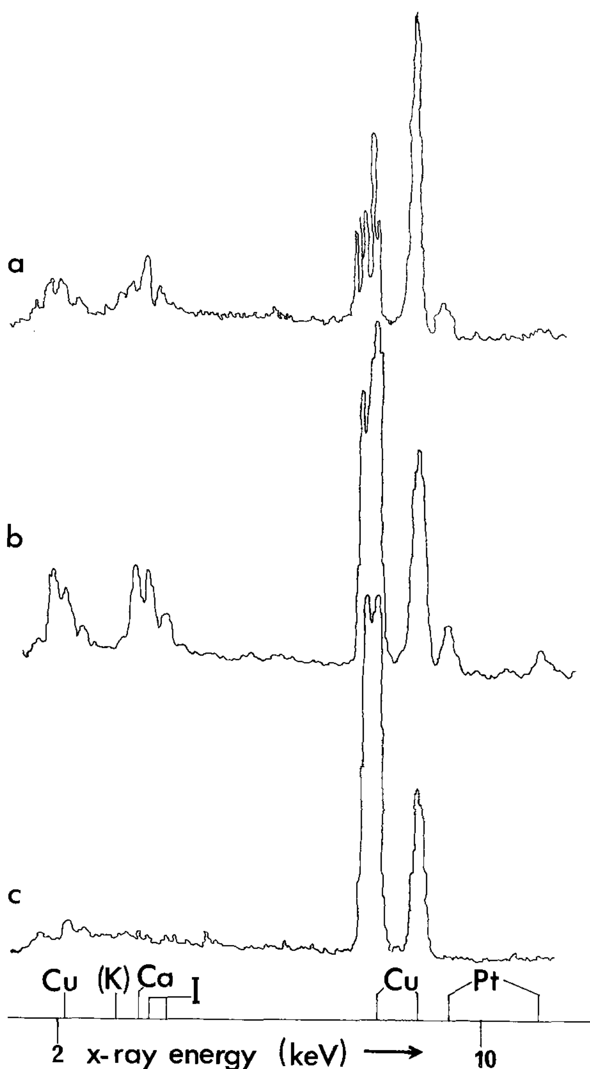

Fig. 2a–c. Microprobe analysis showing the x-ray spectra for elements present in areas of freeze-substituted, iodoplatinate-treated *Suaeda* leaf cells. **a** Analysis of cytoplasmic deposits; **b** analysis of cytoplasmic deposits close to the cell wall; **c** analysis of vacuolar contents. The copper peaks are from the grids

Table 1. Content of Quarternary Ammonium Compounds in *Oryza sativa* (cv. IR20) and *Suaeda maritima* shoots

Suaeda			*Oryza*
Growth	mg g^{-1} dry wt.	mmol g^{-1} dry wt.	mg g^{-1} dry wt.
0	78	0.67	2.5
1%	185	1.58	–
3%	258	2.20	–

When the deposits found in the salt-grown plants were examined by X-ray microprobe analysis, peaks were observed for platinum and iodine and sometimes for calcium (when close to the cell wall), but not for potassium (Fig. 2a, b). Analysis of the vacuole showed no peaks for iodine or platinum (Fig. 2c).

The glycinebetaine content of the three samples of *Suaeda maritima* analysed ranged from 78 to 258 mg g^{-1} dry wt. (Table 1). In contrast, rice contained only some 2.5 mg g^{-1} dry wt. of quaternary ammonium compound (Table 1) and this was shown by TLC to co-chromatograph with choline.

Discussion

A staining procedure based on the formation of complexes between quaternary ammonium compounds and platinum halogenates has been applied to leaf tissue of *Suaeda maritima* grown at different levels of salinity, and to rice. No staining was observed in tissues treated after glutaraldehyde fixation although some staining of mammalian surface membranes has been demonstrated by this procedure (Dierichs and Inczédy-Marcsek, 1976). The lack of intracellular deposits is not surprising since conventional fixation and embedding procedures lead to the loss of a high proportion of diffusible ions from this tissue (Harvey, Flowers and Hall, 1976). Furthermore, in vitro tests showed that no precipitate was formed when aqueous solutions of betaine were mixed with the staining medium although intracellular conditions may be somewhat different.

After freeze substitution, however, which allows greater ion retention (Harvey et al., 1976; Harvey, 1978), distinct cytoplasmic deposits were observed in salt-grown plants but few in plants grown without salt or on tap water. The deposits were very largely restricted to the cytoplasm and were not observed

in the vacuole. The cell wall deposits may well represent leakage from the cytoplasm since substitution in ethanol allows a somewhat greater loss of ions from *Suaeda* tissue than substitution in acetone or ether (Harvey, 1978); ethanol was used since the reagent was more soluble in this than in the other solvents. These observations correlate well with chemical determinations of betaine levels in these tissues and no deposits were observed in rice, a plant which contains no significant amounts of betaine.

The nature of the deposits was confirmed by transmission analytical electron microscopy which showed the presence of both platinum and iodide. No indication of potassium was observed. This is important since platinum halogenates can also precipitate potassium (Vogel, 1954). Since betaine is the only quaternary ammonium compund detectable in these *Suaeda* plants by chromatography (Flowers and Hall, 1978), it seems reasonable to conclude that the deposits observed indicate the sites of glycinebetaine. The clear cytoplasmic localization strongly supports the proposed role of this compound as a non-toxic cytoplasmic osmoticum in halophytes (Storey and Wyn Jones, 1977).

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