X-Ray Microanalysis of Barium and Calcium in Plant Material: Significance for the Analysis of Statoliths

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The identification of Ca^{2+} by X-ray microanalysis in statoliths could be masked by Ba^{2+}. Using Ba^{2+} and Ca^{2+} containing standards prepared from algal cell walls and cellulose acetate films it is shown that there is no interference of Ba^{2+} with Ca^{2+}. Hence Ba^{2+} is the main cationic constituent in the statoliths of *Chara* rhizoids.

In general, barium ions are considered to be toxic for living plants although this element has been found in many higher and lower plants and indeed shown to be beneficial in some cases (for literature see refs [1, 2]). Schröter *et al.* [1] studied the chemical nature of the statolith vacuoles of *Chara* rhizoids and revealed that they contain a considerable amount of barium sulphate biocrystallites embedded in an organic matrix. This finding strongly supports the hypothesis that $BaSO_4$ crystallites mediate the perception of gravity in the rhizoids of *Chara*.

Because of its chemical inertness (low solubility; solubility product: 1.08×10^{-10} at 25 °C [3]) and its high density ($\delta = 4.5$ g/cm³), $BaSO_4$ would be ideally suited for this function, if graviperception is mainly dependent on the mass (weight) of the statolith. However, if this hypothesis is correct, a high enrichment of barium in the statolith compartment is required, as the concentration of barium in the environment is usually extremely low (*e.g.* for pond water $< 10^{-7}$ M [1]).

As regards animal cells, Hubert *et al.* [4] also found an enrichment of barium in the Müller's bodies of Loxodidae which are assumed to function as statocysts. Kreger and Boeré [5] detected $BaSO_4$ microcrystals (length about $0.3 - 0.75$ µm) in the green alga *Spirogyra*. They estimated about 5000 freely movable crystallites in each *Spirogyra* cell, but did not give any explanation, why $BaSO_4$ is accumulated at a high rate in the cells.

In *Chara* and also in the Müller's bodies, $BaSO_4$ has been identified by X-ray microanalysis. It might be argued that by using this method interference effects (atomic number, absorption, and fluorescence effects [6]) could play a role. If this were true, inorganic components other than $BaSO_4$ might be underestimated, and the conclusion could be questioned that $BaSO_4$ is the main inorganic component in statoliths. The most probable candidate to be underestimated would be calcium (as $CaSO_4$). To exclude this possible source of error we studied the interaction of calcium and barium in X-ray microanalysis using standards prepared from cellulose acetate filters and from cell walls of Characean internodes.

To prepare standards from cellulose acetate filters (membrane filter SM 111 06 from Sartorius; thickness: ≅ 100 µm) a small droplet (10 µl) of a $CaCl_2$ and $BaCl_2$ containing solution was placed in the centre of a filter where it was absorbed rapidly. From the wet circular area thus formed the concentration of calcium and barium per unit area could be easily calculated. The filters were dried, placed on an aluminium disc, and wetted with dioxane to make them homogeneous. They were then dried again and fixed onto the disc with an electrically conducting resin. Standards from cell walls of *Chara corallina* were prepared from wall tubes free of alkali and alkali earth elements. The tubes were incubated for 1 to 2 days in neutral solutions containing $CaCl_2$ and $BaCl_2$ to perform a cation exchange of H^+ for Ca^{2+} and Ba^{2+} in the cell wall. The calcium content of the walls was determined radiochemically by using ^{45}Ca. The wall strips (double wall; thickness: ≅ 20 µm) were blotted, dried and fixed in the same way as the acetate films. All standards were coated with gold in the vacuum. The analysis of the standards was carried out using an ARL electron probe analyser with a wavelength dispersive detector (accelerating voltage: 10 kV; sample current: 100 namp; beam diameter: 1 µm).

Fig. 1 shows the calcium content of the cell walls determined by X-ray microanalysis as a function of the radiochemically determined calcium content. There is a linear relationship between the intensity

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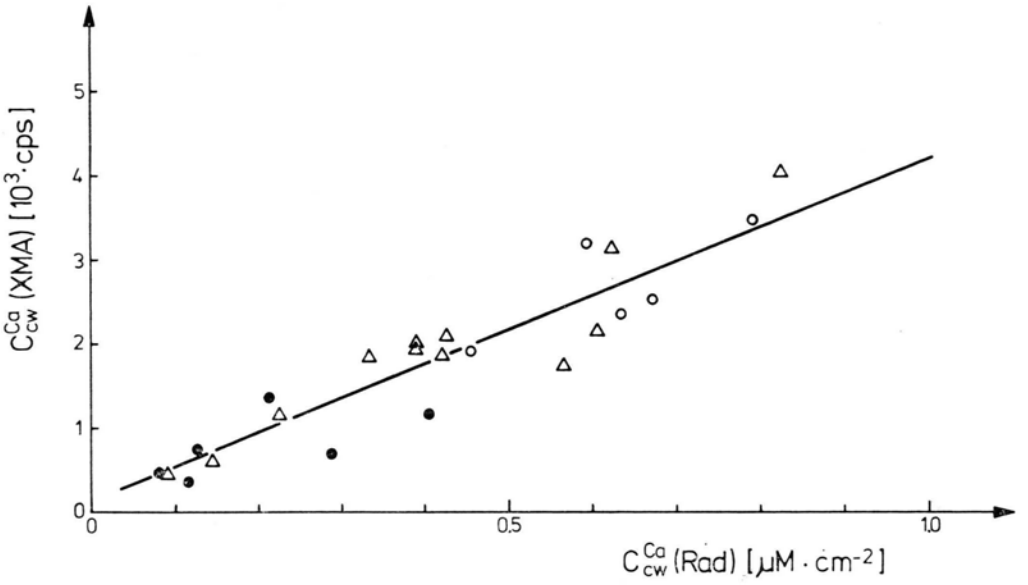

Fig. 1. Calcium standards for X-ray microanalysis prepared from cell walls of *Chara corallina*. The calcium concentration in the wall measured by the intensity of K_α-X-ray radiation, C^{Ca}_{cw} (XMA) in counts per second, is plotted against the radiochemically determined Ca-concentration, C^{Ca}_{cw} (RAD) (symbol ○). Barium at concentrations of 10 (△) or 100% (●) does not influence the determination of calcium. Note that each point in the figure was obtained from a cell wall of a different cell.

of the characteristic X-ray radiation for calcium in counts per second and the calcium content of the cell wall which is independent of the amount of barium absorbed by the walls (10% or 100% of the calcium content).

The ratio between the intensities of the X-ray radiation of both elements (K_α for Ca and L_α for Ba) found in cell walls as well as in acetate films is the same as in the bathing solutions (Table I).

Table I. Ca-standards from *Chara corallina* cell walls and cellulose acetate films. The concentration ratio between calcium and barium in the medium is also found in the standards by X-ray microanalysis independent of its barium content.

Standard	Ca concentration in medium [mM]	Ca : Ba concentration ratio in medium	Ca : Ba concentration ratio in the standard from X-ray microanalysis
Chara cell wall	10–200	10 : 1	9.81 ± 1.15 (5)
	1–200	1 : 1	1.01 ± 0.08 (7)
cellulose acetate film	100	10 : 1	9.59
	10–100	1 : 1	1.04 ± 0.12 (2)

Furthermore, it should be noted that both types of standards were homogeneous in the range of 1 µm and may, therefore, be used as standards for quantitative X-ray microanalysis.

We conclude from our results that the inorganic component of the statoliths of *Chara* rhizoids is indeed mainly $BaSO_4$, as any interference can be excluded between calcium and barium. This result is further supported by the finding of Schröter *et al.* [1] that the ratio of barium to sulphur found by X-ray microanalysis of both pure $BaSO_4$ and statoliths was very similar. We may postulate that $BaSO_4$ crystallites play a more general role in graviperception processes than considered hitherto, at least in algae.

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[1] K. Schröter, A. Läuchli, and A. Sievers, Planta **122,** 213 (1975).
[2] W. O. Robinson, R. R. Whetstone, and G. Edgington, Technical Bull. 1013, p. 1, U.S. Dept. Agriculture, 1950.
[3] Handbook of Chemistry and Physics, 56th Edition, p. B-236, CRC Press Inc., Cleveland, Ohio 1975.
[4] G. Hubert, N. Rieder, G. Schmitt, and W. Send, Z. Naturforsch. **30 c,** 422 (1975).
[5] D. R. Kreger and H. Boeré, Acta Bot. Neerl. **18,** 143 (1969).
[6] L. S. Birks, Electron Probe Microanalysis, 2nd edition, Wiley-Interscience, New York 1971.