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Plant Vacuole Degrades Exogenous Proteins

Rapid Communication

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It is known that most cellular proteins turn over. In animal cells, lysosomes are thought to play an important role in protein degradation (GOLDBERG and DICE 1974). In plant cells, the vacuole is assumed to be a lytic compartment (MATILE 1975). But there is no direct evidence that cellular proteins are really degraded in the vacuole. In the present study, we investigated whether or not the plant vacuole really functions as a compartment for protein degradation, using the giant alga *Chara australis*. Using the technique of vacuolar perfusion, the central vacuole of internodal cells was loaded with an exogenous protein, bovine serum albumin (BSA) to find out whether this protein was degraded or not. Internodal cells, 40–60 µl in volume, were vacuole-perfused with 10 µl of a solution containing 80 mM KCl, 30 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$ and 10 µg of BSA and ligated with threads (TAZAWA 1964). After incubating cells in 0.1 mM KCl, 0.1 mM NaCl and 0.1 mM $CaCl_2$, cellular proteins were analyzed using SDS-polyacrylamide gel electrophoresis. The band corresponding to BSA (MW 68,000) was detectable 30 minutes after loading but disappeared after

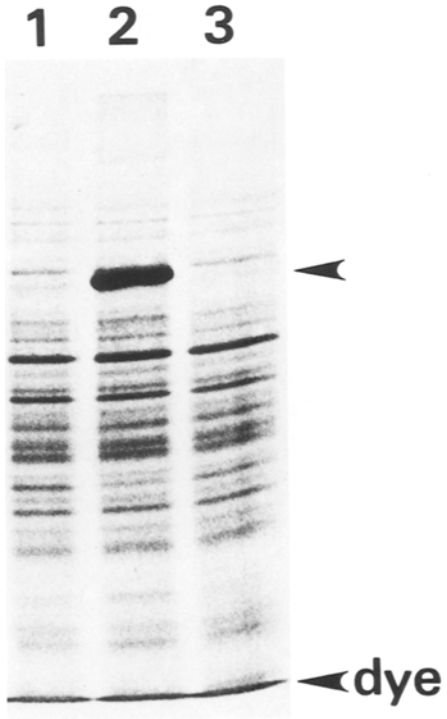

Fig. 1. The central vacuole of *Chara* internodal cells was loaded with 10 µg of bovine serum albumin and the proteins were analyzed using polyacrylamide gel electrophoresis. Lane *1* before protein loading, lane *2* 0.5 hour after loading, lane *3* 16 hours after loading. Proteins were silver-stained. The arrow shows the band corresponding to bovine serum albumin

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16 hours, whereas bands corresponding to native cellular proteins remained. (Fig. 1).

When the proteolytic activities of the vacuolar sap was removed by replacing the natural vacuolar sap with an artificial sap (80 mM KCl, 30 mM NaCl, 10 mM $CaCl_2$ and 10 mM $MgCl_2$), loaded BSA did not disappear (data not shown). When internodal cells were fractionated into vacuolar sap and a residual fraction, intermediate products in BSA breakdown could be detected in the vacuolar fraction but not in the residual cytoplasmic fraction. Also, when casein was introduced into the vacuole instead of BSA, similar results were obtained. The conclusion is that the vacuole is active in digesting the exogenous proteins, BSA and casein. This is the first report which directly demonstrates, that the plant vacuole behaves as a lytic compartment in relation to exogenous proteins.

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