

# AUTOLYSIS OF POTATO TUBER CELL WALLS

## INTRODUCTION

Cell wall autolysis has been studied in growing tissues of some monocots and dicots (2, 5, 6, 7, 9). Enzymes appear to be responsible for the cell wall autolysis in corn coleoptiles (3, 4) and pea epicotyls (1). Cell wall autolysis has also been studied in ripening tomato fruits (10). Studies of cell wall autolysis will not only help us to understand the physiology of cell walls, but also provide a tool for probing the chemical structure of cell walls.

We studied the autolysis of potato tuber cell walls to determine if the cell wall autolysis occurs in non-growing storage tissues. The optimum conditions for the autolysis were also investigated.

## MATERIALS AND METHODS

Potato tubers (cv Russet) were purchased locally and the cell walls were prepared in HEPES-MES buffer (100 mM, pH 7.8) with or without 10 mM Na<sub>2</sub>-EDTA, sodium acetate buffer (10 mM, pH 4.5), potassium phosphate buffer (10 mM, pH 6.7) or water using the Parr nitrogen bomb as the major cell disruption step. Cell walls were subsequently purified as described (8). The fresh cell wall preparations (approximately 10 mg dry weight) were incubated at 35°C in 2 ml of water, sodium acetate buffer (50 mM, pH 4.5 and 5), sodium citrate buffer (50 mM, pH 2.5 to 7) or potassium phosphate buffer (50 mM, pH 6 and 7). After centrifugation, the supernatant solutions were assayed for the total sugars by the phenol-sulfuric acid method. All incubation media contained 0.05% NaN<sub>3</sub>.

## RESULTS AND DISCUSSION

Pure cell walls prepared in HEPES-MES buffer containing EDTA were incubated in water at 35°C. After 20 h of incubation, the cell walls released as much as 10% of the cell wall dry weight as sugars (Fig. 1). Gel filtration chromatography using Sepharose 4B showed that the released sugars were polysaccharides with large molecular weights. Nearly half of the total sugars released was galacturonic acid, suggesting that large fragments of pectic polysaccharides were solubilized from the cell wall matrix during autolysis.

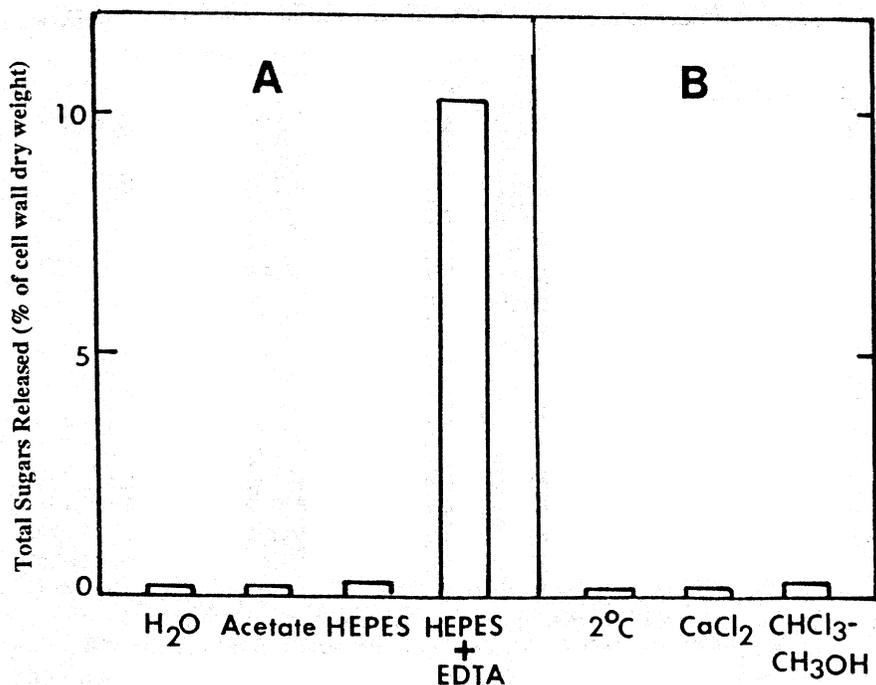


FIG. 1 (A) Effects of cell wall isolation media on the autolysis, and (B) effects of incubation conditions on the autolysis of cell walls isolated in HEPES-MES buffer containing EDTA.

Various incubation conditions prevented the autolytic process. At 2°C, only 0.2% of the cell wall dry weight was released after 24 h. Presence of 50 mM CaCl<sub>2</sub> in the incubation medium inhibited the cell wall autolysis and cell walls washed with CHCl<sub>3</sub>-CH<sub>3</sub>OH mixed solution (1:2) also showed very low autolysis (Fig. 1). At pH between 5 and 7 (50 mM sodium citrate buffer), autolysis was less than half of that in water. Water was a better incubation medium for the cell wall autolysis than the sodium citrate buffer (pH 2.5 to 7) and potassium phosphate buffer (pH 6 and 7), though the amount of sugars released in the buffer increased at lower pH than pH 5.

When the cell walls were prepared in water, sodium acetate buffer or HEPES-MES buffer without EDTA, virtually no autolysis was observed (Fig. 1). Incubation of those inactive cell walls in sodium acetate buffer (pH 4.5 and 5), sodium citrate buffer (pH 2.5 to 7) or potassium phosphate buffer (pH 6 and 7) did not greatly increase the autolysis. However, addition of 5 mM EDTA to the incubation buffers (sodium citrate and potassium phosphate) remarkably increased the sugar release, though the largest amount of the released sugars was only 40% of that in water from the cell walls prepared in HEPES-MES buffer with EDTA.

Results suggest that the potato cell wall autolysis may have been caused by EDTA chelation of Ca ions from the cell wall pectic polymers. The degree to which cell wall enzymes are involved in the autolytic process is yet to be determined.

## LITERATURE CITED

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