Short Communication

Changes in Sucrose Synthetase Activities in Aging Potato Tuber Slices

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Received September 28, 1967.

The mechanism by which the reversible conversion of starch to sucrose takes place in potato tubers exposed to cold is as yet essentially unknown. Early studies on the subject tried to correlate this conversion with variations in the activity of related enzymes (10). Those responsible for the synthesis of sucrose and sucrose-6-phosphate (1) are undoubtedly highly relevant. In addition to its role in sucrose synthesis, the sucrose synthetase lead to the formation of UDP-glucose and ADP-glucose (2), the glucosyl donors for starch synthesis catalyzed by the particulate (6) and the soluble (5) synthetase, respectively. As in sweet corn (4), the sugar nucleotides would thus be the link between starch and sucrose synthetase in potato tubers.

With the aim of obtaining some insight into the regulatory mechanism of these enzymes we have carried out experiments with aging potato tuber slices, in view of the special properties of the tissue in these conditions (3). Potato tubers (Solanum tuberosum var. Huincul) were used. Tissue plugs, 1.5 cm in diameter, were cut from the central parenchima with a cork borer. The plugs were sliced with a microtome into discs, 1 mm in thickness, which were aged according to Click and Hackett (3). The liquid of aging was either water or the solution indicated in each case. In preliminary experiments, similar results had been obtained by addition of calcium sulfate or antibiotics to the aging medium, or using aeration techniques (11).

For the assay of sucrose and sucrose-6-P synthetases, fresh (control) or aged slices were washed with water, blotted with filter paper and ground in a mortar with 0.01 M mercaptoethanol. The extract was strained through cheese-cloth and then centrifuged at 3000 × g in order to separate starch.

The supernatant was dialyzed overnight against 0.05 M tris-HCl buffer, pH 7.3, and used as a source of the enzyme. The assay was performed as follows: 0.5 µmole of UDP-glucose, 0.5 µmole of fructose or fructose-6-P, 5 µmole of tris-HCl buffer, pH 7.3, and 5 to 10 µl of enzyme (equivalent to 0.05-0.1 mg of protein), in a final volume of 25 µl, were incubated at 37° for 30 minutes. The reaction was stopped by adding NaOH to a final concentration of 0.25 M and heating for 15 minutes at 100°. This treatment destroyed free fructose or fructose-6-P, and sucrose or sucrose-6-P could be estimated with the thioarbitruric acid reagent (8)2. One unit of activity is the amount of enzyme producing the formation of 0.01 µmole of sucrose or sucrose-6-P under the described assay conditions, which were selected according to the results of Slabnik, Frydman, and Cardini (unpublished). The reaction was linear both with respect to enzyme concentration and time.

Assay of possible interfering phosphatases and invertase in these experimental conditions was performed in both enzymatic extracts with negative results.

The data of a typical experiment are shown in figure 1. Aging in water at room temperature produced an approximately 2-fold rise in sucrose synthetase activity. This increase was enhanced to 5-fold when 2,4-dichlorophenoxyacetic acid (2,4-D) was present in the medium. Activity was hardly affected when the experiments were repeated at 4° (fig 1A). Sucrose-6-P synthetase was not significantly modified by aging at room temperature or at 4° nor was affected by addition of 2,4-dichlorophenoxyacetic acid to the aging medium (fig 1B).

On the other hand, plant growth substances such as gibberellic acid (1 × 10⁻⁵ M), 3-indolacetic acid (2.2 × 10⁻⁴ M), kinetin (4.6 × 10⁻⁵ M) added to the aging liquid had the same effect on both sucrose synthetase than water alone.

1 This investigation was supported in part by a research grant (No. GM-03442) from the National Institutes of Health, United States Public Health Service, by the Rockefeller Foundation and by the Consejo Nacional de Investigaciones Científicas y Técnicas (R. Argentina).

2 Fructose-6-P, titrated enzymatically, and fructose gave the same color with the thioarbitruric acid reagent.
The rate of sucrose synthetase increase and its inhibition by chloramphenicol was studied. The results presented in figure 3 show a rise of about 2-fold within 2 days of aging in water, without any further variation between the second and the third day. When 2,4-dichlorophenoxyacetic acid was present in the aging medium, the activity increased exponentially at a higher rate and attained 5-fold the control value after 3 days. Chloramphenicol prevented the effect of 2,4-dichlorophenoxyacetic acid but did not affect the rise due to the aging in water.

The data presented in this paper indicate that sucrose and sucrose-6-phosphate synthetases are not regulated by the same mechanism, since the first enzyme increases considerably by aging, alone or with 2,4-D, whilst the second one is not modified. This would be of great interest if sucrose synthetase were responsible for the formation of UDP-glucose and ADP-glucose and sucrose-6-phosphate synthetase for that of sucrose.

**Figure 1.** Effect of aging on A) sucrose synthetase activity, and B) sucrose-6-P synthetase activity. Enzyme extracts were prepared from fresh tissues (control) and from discs previously aged in the dark for 3 days in water or in 2,4-dichlorophenoxyacetic acid (2,4-D) (1.1×10⁻⁴ M) at 20 to 22°C (////) or at 4°C (:::). Chloramphenicol (1 mg/ml) and actinomycin D (50 µg/ml) did not interfere with the enhancement of sucrose synthetase activity. However, the action of 2,4-dichlorophenoxyacetic acid was suppressed by the presence of these substances.

**Figure 2.** Action of protein synthesis inhibitors on the increase of sucrose synthetase activity in aged slices. Enzyme extracts were prepared from fresh tissues (control) and from slices previously aged in the dark for 3 days in water, chloramphenicol (CPH) (1 mg/ml), actinomycin D (Act D) (50 µg/ml), 2,4-D (1.1×10⁻⁴ M), chloramphenicol (1 mg/ml) plus 2,4-D (1.1×10⁻⁴ M) or actinomycin D (50 µg/ml) plus 2,4-D (1.1×10⁻⁴ M).
The results obtained with inhibitors of protein synthesis, like actinomycin D and chloramphenicol, suggest that the increase of sucrose synthetase in aged slices is the result of an activation of pre-existing enzyme. This is similar to the case described by Marré (7) for the activation of metabolism in the germinating endosperm of castor bean seeds. However, when 2,4-D is present in the aging medium, there seems to be a synthesis de novo of protein which can be prevented by the specific inhibitors already mentioned. This second mechanism would resemble that described by Click and Hackett in aging potato tuber slices (3).

We have not been able to find any evidence of variations of sucrose synthetases activities in potato tubers stored at low temperature. These results agree with those reported by Schwimmer and Rorem (9).

**Acknowledgments**

The authors express their gratitude to Dr. Eugenio Vonesch (Facultad de Agronomía y Veterinaria, Universidad de Buenos Aires) for the supply of potatoes var. Huineul (INTA, Estación Agrónomica Balcarce). They are also grateful to the members of the Instituto de Investigaciones Bioquímicas for helpful discussion and criticisms.

**Literature Cited**