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SHORT COMMUNICATION

# EFFECT OF ISOPROPANOL ON THE ACTIVITY OF PARTICULATE STARCH SYNTHETASE

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**Key Word Index**—*Solanum tuberosum*; Solanaceae; *Zea mays*; Graminiceae; starch synthetase; isopropanol activation.

**Abstract**—The effect of several alcohols on particulate starch synthetases from potato tuber and sweet corn endosperm has been studied. High concentrations of isopropanol in the incubation mixture produced a great increase of the enzyme activity. The action of this alcohol on kinetic constants and on the distribution of incorporated glucose between amylose and amylopectin has been studied.

## INTRODUCTION

STARCH grains contain an enzymatic system that transfers glucose from UDP-glucose (UDPG), ADP-glucose (ADPG) or other sugar nucleotides to starch (UDP-glucose: $\alpha$1,4 glucan $\alpha$-4 glucosyl transferase or starch synthetase, E.C.2.4.1.21). Many properties of this system have been studied in different varieties of starch grains,[1–4] but the relationship between the enzyme and the polysaccharide and the one between the particulate and the soluble enzyme[5,6] are still unknown.

The regular occurrence of lipids strongly associated with starch[7] suggests that these compounds could participate in some way in the formation of the grain. In intact amyloplasts from immature sweet corn endosperm an enzymatic system has been found which transfers glucose from UDPG to sterols,[8] thereby originating sterol-glucosides. This glucosylation diminishes the activity of ADPG-starch synthetase[9] and this reaction could well be involved in the regulation of this enzyme activity.

In an attempt to find out whether grain lipids could influence the activity of starch synthetase, we investigated this activity after having extracted the grains with lipid solvents at room temperature. Using aqueous butanol,[8] methanol, isopropanol or methanol–chloroform (1 : 1), one usually finds a decreased enzyme activity. After trying to reactivate the enzyme by adding the evaporated extracts previously suspended in water by sonication or dissolved in various solvents, we have found incidentally that isopropanol in high concentrations increases remarkably the enzyme activity with both substrates, ADPG or UDPG.

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TABLE 1. EFFECT OF ALCOHOLS ON THE ACTIVITY OF UDPG-STARCH SYNTHETASE

| Incubation medium | Radioactivity incorporated into the starch grains (cpm) |
|---|---|
| Water | 944 |
| Isopropanol 15% (v/v) | 777 |
| Isopropanol 35% | 612 |
| Isopropanol 70% | 1270 |
| Isopropanol 85% | 4640 |
| *n*-Propanol 15% | 105 |
| *n*-Propanol 85% | 2380 |
| Ethanol 50% | 528 |
| Ethanol 85% | 834 |
| Methanol 80% | 396 |

Potato starch grains (3 mg) were incubated at 37° for 60 min with 5 μmol of glycine buffer pH 8·4 and 3·6 nmol of UDPG-$^{14}$C (21 000 cpm) in a total volume of 35 μl.

## RESULTS AND DISCUSSION

Table 1 shows the results of an experiment utilizing UDPG and starch from potato tubers. At low concentrations, isopropanol inhibits, but above 70% (v/v) a clear activation occurs. With pure isopropanol an activation also takes place, but the grains are not readily suspended and results are erratic, By adding extracts of grain lipids, sitosterol or lecithin to the isopropanol, results are not modified significantly. *n*-Propanol has less activity and both ethanol and methanol are inhibitory. Similar results are obtained with ADPG as well as with grains of milky endosperm from different corn varieties. Activity is of the same order with or without the addition of buffer under optimal conditions of pH (Tables 2 and 3).

TABLE 2. TIME COURSE OF THE REACTION

| Starch | Sugar nucleotide | Incubation medium | Radioactivity incorporated 15 min | 30 min | 60 min |
|---|---|---|---|---|---|
| A | ADPG-$^{14}$C 10 nmol (7500 cpm) | Water | — | 178 | 386 |
| | | Isop. | — | 1814 | 2226 |
| | UDPG-$^{14}$C 2 nmol (10 000 cpm) | Water | — | 28 | 110 |
| | | Isop. | — | 989 | 1757 |
| | UDPG-$^{14}$C 3·6 nmol (21 000 cpm) | Buffer | — | 564 | 1052 |
| | | Isop.buffer | — | 2612 | 4170 |
| B | UDPG-$^{14}$C 9 nmol (65 000 cpm) | Buffer | 1064 | 1270 | 1868 |
| | | Isop.buffer | 14 800 | 18 400 | 19 190 |

Potato starch grains (5 mg, A) or sweet corn grains (5 mg, B) were incubated at 37° and at different time intervals with UDPG-$^{14}$C or ADPG-$^{14}$C in a total volume of 0·035 ml. Incubation medium: water, isopropanol 85% (v/v) (isop.), 5 μmol glycine buffer pH 8·4 in water (buffer), and 5 μmol glycine buffer pH 8·4 in isopropanol 85% (isop.buffer).

Glucose incorporated from ADPG and UDPG into both kinds of starch grains can be recovered as maltose by treatment with β-amylase, and its distribution between amylose and amylopectin is similar whether isopropanol is present or not: 10–15% in the amylose

TABLE 3. RELATION BETWEEN ACTIVITY AND STARCH CONCENTRATION

| Starch | Sugar nucleotide | Incubation medium | Radioactivity incorporated (cpm) 1 mg | 3 mg | 5 mg |
|---|---|---|---|---|---|
| A | ADPG-$^{14}$C 10 nmol (7500 cpm) | Water | 121 | 315 | 400 |
| | | Isop. | 500 | 1371 | 1600 |
| | UDPG-$^{14}$C 2 nmol (10 000 cpm) | Water | 28 | 70 | 110 |
| | | Buffer | — | 420 | 612 |
| | | Isop.buffer | 660 | 1600 | 1850 |
| B | UDPG-$^{14}$C 3·6 nmol (21 000 cpm) | Buffer | 302 | 791 | 1188 |
| | | Isop.buffer | 1457 | 3310 | 4331 |

Potato (A) or sweet corn (B) starch grains (1,3 or 5 mg) were incubated 60 min at 37° in the same conditions as Table 2.

fraction and 85–90% in the amylopectin fraction. Apparent affinity constants of incubations with water or isopropanol (85%), were determined for ADPG with potato starch grains, both values being around 2·5 mM. Contrarily, $V_{max}$ values (expressed in mμ/mol of glucose incorporated per hr, per mg starch) increases from 1·8 to almost 10 for isopropanol. The type of activation obtained with isopropanol is very similar to the one described for the cation potassium.[10–12] For the latter Nitsos and Evans[12] have suggested an alteration of the relation between the enzyme and the polysaccharide. This statement might be partially confirmed by the fact that an alteration of grain structure through grinding[3] or 7 M urea[8] provokes an increase of ADPG-synthetase activity, while the activity with UDPG disappears. A more probable hypothesis is that isopropanol would produce an increased accessibility of the enzyme's active site which may be located in a lipidic zone. In any case, it seems of importance that the transglycosidation reaction could occur in a practically anhydrous medium. Amylopectin, a strongly hydrophilic polysaccharide, is present in the starch grain in an insoluble and anhydrous state, suggesting that it could be formed in a non-aqueous medium. This seems to be one of the most obscure problems concerning the formation of the grain in the amyloplasts.

## EXPERIMENTAL

The preparation of the grains, as well as the reagents and methods utilized, have been previously described.[4] They were employed with some modifications. A small amount of nucleotide remains in the grain after incubations containing isoPrOH were washed with 50% EtOH. This can be avoided by washing with $H_2O$. Therefore, after incubation, the grains were washed 5× with 1 ml of $H_2O$, suspending the grains each time by means of a vortex. Amylose and amylopectin were separated by the method of Montgomery and Senti.[13]

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