Effect of Cetyltrimethylammonium Bromide on the Activity of Particulate Starch Synthetase from Potato Tuber

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ABSTRACT

The action of some detergents on the incorporation of glucose from uridine diphosphate glucose or adenosine diphosphate glucose into the potato tuber starch grain was studied. It was found that the cationic detergent, cetyltrimethylammonium bromide, produces a rapid binding of both sugar nucleotides to the grain and a great increase in the incorporation of glucose into the polysaccharide. Kinetic constants of starch synthetase are also modified, there being an affinity increase for both sugar nucleotides. Neutral detergents are without effect and anionic detergents are inhibitors.

In a previous communication (6) on the synthesis of steryl-glucosides from UDP-glucose in amyloplasts of sweet corn endosperm, we have found that the cationic detergent, cetyltrimethylammonium bromide, inhibited that synthesis but increased considerably the incorporation of radioactive into the grains. We have now tried to investigate the latter effect to see if it was related to an increase in the activity of starch synthetase (UDP-glucose: α-1,4-glucan α-4-glucosyltransferase, EC 2.4.1.21) included inside the grain (4, 8, 12) or to some modification of the permeability of the granule.

MATERIALS AND METHODS

The starch granules were obtained as described previously (2). UDP-14C-glucose was prepared as described by Thomas et al. (15) and UDP-3P-glucose by the method of Dankert et al. (3). ADP-3C-glucose, 3C-ATP, and 3C-UTP were purchased from New England Nuclear Corp., CTAB1 and Tween 60 from Amend and lauryl sodium sulfate from British Drug House.

Paper chromatography of nucleotides was performed as described by Paladini and Leloir (10). Paper electrophoresis was carried out according to Recondo et al. (11) or with acetic acid-pyridine buffer, pH 6.5, at 80 v/cm for 2 hr (4 ml of acetic acid, 100 ml of pyridine, and 900 ml of water). Amylose and amyllopectin were separated by the method of Montgomery and Senti (9).

The incorporation of radioactive glucose into starch granules was measured according to Leloir et al. (8) with slight modifications (2, 6). After incubation with the labeled sugar nucleotide, the starch granules were washed by suspending them in a Vortex with 1 ml of water. This operation was repeated four times more. When indicated, two additional washings with 1 ml of 50% boiling methanol were performed.

Other methods were the same as already described (2).

RESULTS

The action of CTAB was assayed with starch grains from different varieties of corn and potato tubers. In all cases CTAB produced a striking and reproducible increase on the radioactivity incorporated from UDP-14C-glucose into the starch granules. The nonionic detergents Triton X-100 and Tween 60 showed no action, and the anionic detergent, lauryl sodium sulfate, had a great inhibitory effect.

The assays described below were performed with potato tuber starch grains due to their easier preparation and handling. The grains obtained as previously described (2), including the treatment with acetone, cannot form lipid sugar (6) but the same results were obtained with grains in which the acetone washing step was omitted. Otherwise the potato starch grains have very little lipid sugar formation activity (7).

It was found that the optimum concentration of CTAB was about 10 mM. Higher concentrations were less effective or actually inhibitory. The same relative activation was obtained whether or not glycine-NaOH buffer, pH 8.6 (2), was included in the incubation mixture. In the latter case, the pH of the mixture was that of the CTAB solution, that is about 7.6. When the distribution of radioactivity between amylose and amyllopectin was studied, it was found that only a part of the radioactivity incorporated could be recovered into these two polysaccharides or as maltose after treatment with β-amylase. An important fraction of the radioactivity was consistently recovered as UDP-14C-glucose, which could be identified by paper chromatography and electrophoresis. This fraction cannot be extracted by the usual washing techniques (2), even by repeated washings with 50% ethanol or with water. Otherwise it can be recovered with boiling 50% methanol. This retention of UDP-14C-glucose is not observed if the incubation of the grains is carried out in water or in buffer alone.

As can be seen in Figure 1, A and B, the radioactivity that remains incorporated in the grain after the washings with water is several times higher in the presence of CTAB, but near one-half of it belongs to the UDP-14C-glucose included.

In trying to investigate this retention of the nucleotide, we have found that in the presence of detergent a very rapid fixation of UDP-14C-glucose occurs somewhere in the grain, which cannot be washed out with water or alcohol. This can be deduced from the following experiments in which UDP-14C-glucose is added after a preincubation of the grain with CTAB.

The starch grains were preincubated at 37° C for 1 hr with

1 Abbreviation: CTAB: cetyltrimethylammonium bromide.
the easy sedimentability of the potato grains, the washings do not last more than 10 to 14 min. It was observed that under these conditions a large and constant part of the radioactivity remains incorporated and can be extracted almost completely by washing with boiling 50% methanol (Fig. 2A). The radioactivity was identified as UDP-\(^{14}\)C-glucose or its decomposition product, \(\alpha\)-d-glucopyranosyl 1,2-cyclic phosphate (10). Precipitation with water or buffer alone did not produce a similar action.

When the radioactive wet grains obtained in the former experiments, after five washings with water, were reincubated at 37°C, it was observed that the amount that could be extracted with boiling methanol diminishes with time and appears in starch (Fig. 2B). This seems to indicate that in the presence of detergent UDP-\(^{14}\)C-glucose is fixed in an enzymatically active site.

When the experiments were performed with UDP-\(^{32}\)P-glucose, the same results were obtained; also the \(^{32}\)P-UDP formed during the reaction is retained by the grain just as is the UDP-\(^{32}\)P-glucose.

Similar results were found when ADP-\(^{14}\)C-glucose was used instead of UDP-\(^{14}\)C-glucose, with the only difference that the extractable fraction with boiling 50% methanol was very low, nearly all the radioactivity being recovered as amylase and amylpectin (Fig. 1, C and D; Fig. 2, C and D). This difference could be related to the higher velocity of incorporation of glucose into starch from ADP-glucose than from UDP-glucose (12). Ten to 15 min, viz. the washing time, were enough to allow glucose to be incorporated into starch.

As can be seen in Table I, uridine and adenosine mono- and diphosphates prevented this rapid fixation of UDP-glucose and ADP-glucose when added after the preincubation with CTAB, just before the addition of sugar nucleotides.

The action of CTAB on the kinetic constants of starch synthetase was determined for both sugar nucleotides. In the case of UDP-glucose the determination was made in the presence of glycine-NaOH buffer (2), owing to the slow activity of the enzyme with water alone. After the incubation period, the grains were washed with water and then extracted two times with 1 ml of boiling 50% methanol.

In both cases there is a clear increase of activity by the action of CTAB: the \(K_m\) for UDP-glucose decreases from 6.3 to

<table>
<thead>
<tr>
<th>Additions</th>
<th>Radioactivity Incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-(^{32})P-glucose</td>
<td>ADP-(^{32})P-glucose</td>
</tr>
<tr>
<td>None</td>
<td>13,198</td>
</tr>
<tr>
<td>UMP</td>
<td>1,546</td>
</tr>
<tr>
<td>UDP</td>
<td>1,684</td>
</tr>
<tr>
<td>UDP-glucose</td>
<td>475</td>
</tr>
<tr>
<td>AMP</td>
<td>703</td>
</tr>
<tr>
<td>ADP</td>
<td>58</td>
</tr>
</tbody>
</table>
3.7 mm and that for ADP-glucose from 2.7 to 0.8 mm. The $V_{max}$ in the case of UDP-glucose is not modified and is about 10. With ADP-glucose there is an increase from 4 to 18 (values are expressed in nmoles/hr·mg of starch grain).

CTAB also modified the distribution of radioactivity incorporated from ADP-glucose or UDP-glucose between amylose and amylodextrin. Thus, 80% of the total radioactivity incorporated in grains incubated with water or buffer was in the amylopectin fraction, and this amount was increased 90 to 95% when CTAB was added to the incubation mixture.

It has been found that besides sugar nucleotides CTAB has also the ability to bind $^{14}$C-ATP and $^{14}$C-UTP to the starch granules. In both cases all the radioactivity incorporated can be recovered as uncharged nucleotides, boiling the grains with 50% methanol. $^{14}$C-Glucose-1-P, $^{14}$C-sucrose, or $^{14}$C-glucose are not fixed to the grain under these conditions.

**DISCUSSION**

The results presented in this paper show that the cationic detergent, CTAB, produces a great increase in the incorporation of glucose from UDP-glucose and ADP-glucose into the starch grain. Kinetic constants of starch synthetase are also modified, there being an affinity increase for both sugar nucleotides.

The experiments indicate that in the presence of detergent a rapid binding of sugar nucleotides occurs at some enzymatically active site of the grain. Neutral detergents are without effect and anionic detergents are inhibitors. Mono- or diphospho-nucleotides, if present in the incubation medium, inhibited binding.

It is difficult to give a clear interpretation of the results presented here due to the fact that the nature of the bond between the polysaccharide and the enzyme starch synthetase inside the grain is still unknown. Results of Geddes and Greenwood (5) on the peridate oxidation of intact starch grains indicate that low molecular weight metabolites can diffuse freely through the grain. This probably also applies to sugar nucleotides. Thus, an increased binding at some specific site in the grain seems to be a more reasonable explanation than an increased penetration into the grain.

One possible explanation could be that the cationic detergent becomes attached to a zone where the enzyme is located, thereby conferring on it a positive charge. This would produce an increase of the local concentration of negatively charged sugar nucleotides and hence an increase of the enzyme activity. In this way the region of the grain bound to CTAB could function as an anion-exchange resin, binding not only UDP-glucose or ADP-glucose, but also other nucleotides with similar charge as ATP or UTP. Also CTAB could produce changes in the enzyme conformation or in its relation to the polysaccharide increasing the affinity of the enzyme for the sugar nucleotides.

A similar effect of CTAB on the kidney microsomal glucose 6-phosphatase (d-glucose-6-P phosphohydrolase, EC 3.1.3.9) has been reported by Soos and Nordlie (14). This enzyme occurs in the phospholipid-rich hydrophobic membrane of the endoplasmic reticulum and CTAB favors the binding of the anionic substrate, as well as increases the enzyme affinity for the substrate.

There are several references in the literature to the regular occurrence of phospholipids in the starch grains (1, 13). If the starch synthetase would be located in some lipid-rich zone of the grain, the detergent could have a similar action to that reported for the microsomal glucose-6-phosphatase.

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**LITERATURE CITED**


