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# ACTIVATION OF PARTICULATE STARCH SYNTHETASE FROM *ZEA MAYS* EMBRYO

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**Key Word Index**—*Zea mays*; Gramineae; maize; starch synthetase; spermine, citrate, activation.

**Abstract**—The effect of spermine on particulate ADP-glucose:starch synthetase from the developing embryo of sweet corn has been studied. Spermine induces a considerable increase of glucose incorporation from ADP-glucose into the starch granules. The change in kinetic constants, the distribution of incorporated glucose between amylose and amylopectin and the pattern of incorporation into starch granules or malto-oligosaccharides has been studied. The data were compared with those obtained with citrate ions.

## INTRODUCTION

An enzyme (ADP-glucose: $\alpha$-1,4-glucan $\alpha$-4-glucosyltransferase, E.C.2.4.1.2.1., particulate starch synthetase) which catalyzes the transfer of glucose moieties from ADP-glucose and other sugar nucleotides to the granule itself or to malto-oligosaccharides [1,2] has been detected in starch granules. This particulate starch synthetase isolated from reserve starch granules can utilize different sugar nucleotides as glucosyl donors [2]. However, in the case of the synthetase bound to chloroplasts or germinated soya seed starch grains, the enzyme has a specific requirement for ADP-glucose [3,4]. Activation of this particulate enzyme by potasium ($K^+$ ions) might be of physiological significance, since this requirement could explain the defective starch synthesis observed in plants cultivated in K-deficient soils [5,6]. On the other hand, the reaction is not affected by different inorganic anions [7].

It is known that organic cations like spermine, agmatine and other polyamines are normally present in plants and play an important role in macromolecular synthesis (proteins, nucleic acids). It is also known that the increase observed in polyamine content in the plant parallels a decrease in its K-level [8].

We have already shown that the cationic detergent cetyltrimethylammonium bromide (Cetavlon) increases the incorporation of glucose from ADP-glucose into starch grains, and that this seems to be partially due to the cationic nature of the detergent [9].

All these facts led us to investigate whether natural occurring organic cations, like polyamines, could be related to the biosynthesis of starch. For the present study we have chosen the embryo of germinated sweet corn seeds, a plant tissue rich in polyamines. The results obtained with starch granules from this tissue (sporophytic generation) were compared to those from endosperm, (of gametophytic origin) which is relatively poor in polyamines [10].

Since we have previously shown that citrate ions activate starch synthetase [11], their effect was compared with that of the polyamines.

## RESULTS

Figures 1 and 2 show the effect of polyamines and citrate on particulate starch synthetase activity from embryo and endosperm of germinating seeds. Starch granules from both tissues incorporated glucose from ADP-glucose into starch, without any addition to the standard reaction mixture. This incorporation is pH independent between 7 and 8·5. The addition of spermine or agmatine produced a great increase in the enzymatic activity of the embryo starch granules (Fig. 1a and b). Particulate starch synthetase from endosperm was activated to a minor extent by these polyamines (Fig. 2a and b). As already described [11], citrate

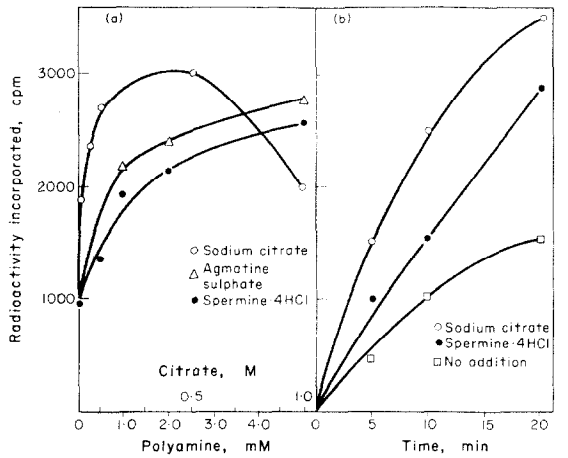


Fig. 1. Activation of particulate starch synthetase activity from the embryo of germinated seed. (a) Effect of spermine, agmatine and sodium citrate as a function of concentration. The standard reaction mixture containing starch granules from the embryo of germinating seed was incubated at 37° for 10 min with the addition of spermine, agmatine or sodium citrate (pH 6·4) at the concentration indicated. (b) Effect of spermine and sodium citrate as a function of time. The standard reaction mixture containing starch granules from the embryo of germinating seed was incubated at 37° without any addition or with the addition of 5 mM spermine or 0·1 M sodium citrate (pH 6·4) for the times indicated. At the end of the reaction radioactivity into the starch granules was measured as already described [1].

ions stimulated the reaction in a concentration range between 0·02 M through 0·5 M (Fig. 1a, 2a). Also in this case the effect on the embryo enzyme was more pronounced than on the endosperm one.

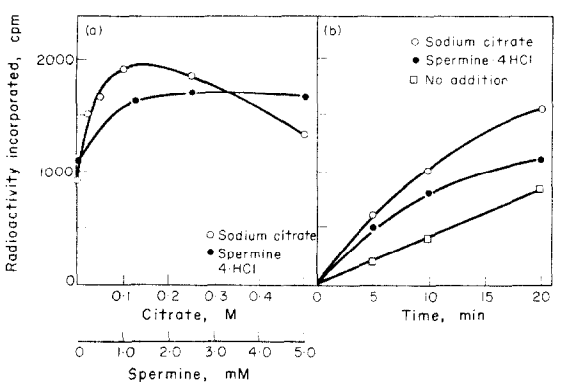


Fig. 2. Activation of particulate starch synthetase activity from the endosperm of germinated seed. (a) Effect of spermine and sodium citrate as a function of concentration. (b) Effect of spermine and sodium citrate as a function of time. Conditions, assay and symbols as in Fig. 1, except that starch granules from endosperm of germinating seed were used.

The pH optimum for citrate activation was determined. The reaction has a broad optimum (5 to 7); however, a maximal effect could be attained at pH 6·5.

To establish whether the glucose transferred from ADP-glucose, in the presence or absence of activators, becomes joined in α-1,4 linkage, β-amylolysis assays were carried out. In all cases, the radioactivity incorporated into starch granules was totally released as maltose by the action of β-amylase. The distribution of the glucose-[$^{14}$C] incorporated into starch between amylose and amylopectin was modified by the action of both activators. As can be seen in Fig. 3, 25% of the total radioactivity incorporated into starch, in the absence of activators, was found in the amylose fraction. This value rose to 40% in the presence of spermine or citrate. The addition of malto-oligosaccharides (maltose, maltotriose or maltotetraose) did not alter these results.

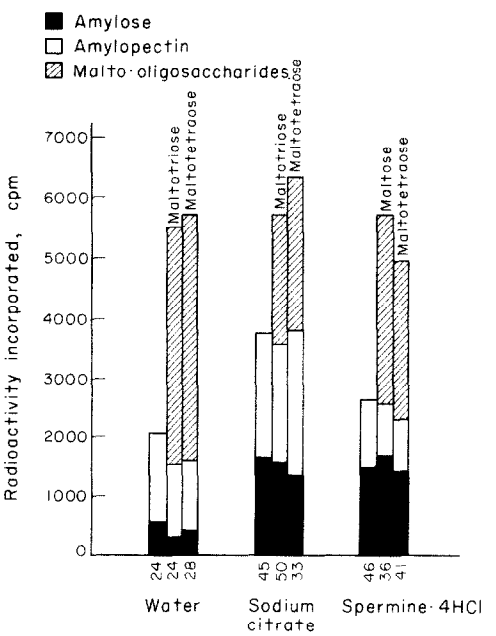


Fig. 3. Action of spermine and sodium citrate on the radioactivity incorporated into starch components and malto-oligosaccharides. The standard reaction mixture, containing starch granules from embryo of germinating seed, was incubated for 20 min with water or for 10 min with 0·1 M sodium citrate (pH 6·4) or 5 mM spermine. Malto-oligosaccharides (3·3 mM) were added when indicated. Radioactivity incorporated into amylose, amylopectin and malto-oligosaccharides was determined as described before [1]. Numbers under the bars indicate the percentage of total radioactivity incorporated into starch that was found in amylose in each case.

Table 1. Effect of activators on the kinetic constants for ADP-glucose of particulate starch synthetase from embryo of germinating seeds

| Additions | $K_m$ (mM) | $V_{max}$* |
|---|---|---|
| None | 2·5 | 45 |
| Spermine, 5 mM | 0·8 | 45 |
| $NH_4^+$, 11 mM | 0·8 | 31 |
| Sodium citrate, 100 mM | 1·4 | 68 |

* Expressed in nmol of glucose-[$^{14}$C] incorporated into starch granules/hr/mg of starch.

In order to study the effect of the activators on the mechanism of action of the particulate enzyme, the radioactivity incorporated into the end groups of starch chains, was determined according to the technique of Anderson *et al.* [12]. While this method does not distinguish between the glucose-[$^{14}$C] moieties incorporated into amylose or into amylopectin, it does indicate the pattern of incorporation presumably modified by the action of spermine and citrate. Without any addition to the assay mixture, 17·3% of the total radioactivity incorporated into starch was found in the end groups of the external chains. This percentage decreased to 11·7 and 8·2% by the presence of 100 mM sodium citrate and 2·5 mM spermine, respectively. This suggested that citrate as well as spermine induced a tight binding of the sugar donor to the enzyme–polysaccharide complex which resulted in a preferential enlargement of some chains.

The incorporation into oligosaccharides, when maltotetraose was present in the incubation mixture, was also studied. As previously reported [1], a multi-chain pattern of incorporation of glucosyl residues into oligosaccharides was obtained when no activator was present. In the presence of citrate or spermine the main products were oligosaccharides longer than maltoheptaose. Also in this case it can be assumed that both activators cause a stronger binding of the sugar nucleotide to the enzyme–substrate complex. The effect of the activators on the kinetic constants of the particulate starch synthetase is shown in Table 1. Spermine produced an increase of the starch enzyme affinity for ADP-glucose, similar to that caused by ammonium ions, without modifying the $V_{max}$ value. Citrate produced a less pronounced decrease of the $K_m$ value for ADP-glucose and caused a net rise in $V_{max}$.

## DISCUSSION

The data presented in this paper show the activating effect of the polyamine spermine on the glucose incorporation from ADP-glucose into starch granules of embryos and endosperms isolated from germinating sweet corn seeds. In the case of the enzyme bound to embryo starch granules the activating effect was more pronounced than in endosperm.

The influence of spermine on the kinetic constants of particulate starch synthetase appears to be different from that reported for other activator substances. We have shown a clear increase of the affinity for ADP-glucose produced by spermine without modifications of $V_{max}$. As can be deduced from the results in Table 1, the amino groups of spermine could be related to its activating effect. On the other hand, $K^+$ ions as well as isopropanol enhance the $V_{max}$ value without changing the $K_m$ value for ADP-glucose [6,13] and Cetavlon changes both the $V_{max}$ and the $K_m$ values for the sugar nucleotide [9].

The activating effect of spermine results in an increase of the amount of labelled glucose found in amylose. In the case of Cetavlon the enhancement produced is found mainly in amylopectin [9]. Isopropanol does not vary the distribution of glucose-[$^{14}$C] residues between the two components of starch [13]. There are no available data for the $K^+$ activation in this respect. Also the mechanism by which the glucosyl moieties were incorporated into malto-oligosaccharides was affected by spermine action, changing from a multi-chain to a single-chain pattern.

The results reported in this paper were obtained with physiological concentrations of polyamines (0·5–1·0 mM) [10], suggesting an involvement of these organic cations in the starch biosynthetic pathway. From the different polyamine content in embryo and endosperm, it is tempting to speculate that the enzymatic activity of the starch synthesizing system is related either to the availability of polyamines in plant tissues and/or to the sensitivity of the corresponding enzymes to those organic cations.

Citrate also activates the particulate starch synthetase from germinating sweet corn seeds, and modifies the apparent affinity constant for ADP-glucose, the $V_{max}$ and the enlargement mechanism of the enzyme. Activation assays were performed with citrate concentrations between 20 and 500 mM. Soluble starch synthetases were also found to be activated by 10–500 mM sodium citrate [11,14]. Although the effect of this organic anion was attained at concentrations higher than the cellular level (5–10 mM) [15], its participation in the biosynthesis of starch cannot be discounted since the environment is not known for starch synthetase *in vivo*.

## EXPERIMENTAL

The isolation of starch granules, incorporation of radioactive glucose from ADP-glucose-[$^{14}$C] into starch granules and oligosaccharides, and all other reagents and analytical methods used were previously described [1,2]. The starch granules were isolated from embryos and endosperms obtained from sweet corn seeds after 3 days germination [11]. Spermine (4HCl) and agmatine sulphate soln were used at pH 7·5. Glucose-[$^{14}$C] incorporated into the non-reducing terminal groups of starch was determined by the periodate method of Anderson *et al.* [12] and the formic acid released was measured according to Verhue and Hers [16]. The standard assay mixture contained, in a final vol. of 30 $\mu$l, 2–4 mg of starch granules and 10 nmol of ADP-glucose-[$^{14}$C] (8000 cpm).

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