The Control of Food Mobilization in Seeds of *Cucumis sativus* L. VI. The Production of Starch

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**Abstract**

During germination and early seedling growth of cucumber, cotyledonary lipid and protein reserve material mobilization is controlled through a cotyledon/axis, source/sink relationship. Earlier work has shown that some cotyledonary lipid breakdown is able to occur even in the absence of the embryonic axis. This study shows that such breakdown is probably a consequence of the formation of a transitory starch store. Excised cucumber cotyledons accumulate large amounts of starch following the accumulation of reserve breakdown products which can no longer be utilized in the absence of the axis and represents a mechanism in the tissue to regulate the concentration of osmotically active substances such as sucrose and glucose. The starch content of both attached and excised cotyledons increased during the experimental period. In the former case, however, the increase was very small and starch levels fell subsequently, while in the latter instance the increase continued, reaching a maximum at day 8 after which it stabilized at this elevated level. The increase in starch content is most closely correlated with an increase in the activity of bound ADPG-starch synthase although soluble ADPG-starch synthase activity also increases during the experimental period.

Key words: Cucumber, *Cucumis sativus*, starch, food mobilization, germination, ADPG-starch synthase.

**Introduction**

The control of lipid and protein mobilization in seeds of cucumber (*Cucumis sativus* L.) has been intensively studied (Slack, Black and Chapman, 1977; Davies and Chapman, 1979a, 1980, 1981; Davies *et al.* 1981), particularly with regard to the influence of the embryonic axis on reserve mobilization in the cotyledons. The overall conclusion of this work was that control of cotyledonary reserve mobilization in cucumber is mediated through the presence of an axial sink and that mobilization enzymes are able to develop fully in isolated cotyledons in the absence of the axis (Chapman and Davies, 1983).

A criticism of the idea of source/sink control has been the observation that some lipid material in cucumber is still mobilized even in the absence of the axis (e.g. Fig. 1a and b in Davies and Chapman, 1979a). The development of a secondary sink has been suggested as a possible explanation of this (e.g. Davies and Slack, 1981). In cucumber one potential candidate for this is starch since this has been observed to accumulate in the cotyledons in the absence of the embryonic axis (Davies and Chapman, 1981). As cucumber seed reserve material is normally lipid and protein this suggests that the enzymes for starch synthesis are under regulatory control and are only synthesized under certain conditions.

In the work presented, starch content, soluble and bound ADPG-starch synthase activities (ADPG-α-glucan 4-α-glucosyltransferase; E.C.2.4.1.21) have been studied and possible correlations with starch appearance in isolated cucumber cotyledons made.

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Abbreviations: ADP, adenosine diphosphate; ADPG, adenosine diphosphoglucose; EDTA, ethylene diamine tetra acetic acid.

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MATERIALS AND METHODS

Seed germination

Seeds of *Cucumis sativus* L., cv. Ridge Long Green, were washed in distilled water and then allowed to imbibe for 3 h at room temperature. Batches of each experimental treatment used (i.e. with or without axis and testa) were placed in seed trays (21.5 x 35.5 cm) lined with aluminium foil and four layers of absorbent tissue moistened with water to saturation (200 cm³). These trays were covered with another inverted tray and both wrapped in aluminium foil. Seeds were then incubated in the dark at 25 ± 1 °C. for the length of time specified.

Enzyme separation

**Soluble ADPG-starch synthase.** Samples of 10 cotyledons were homogenized in an ice-cold mortar with 10 cm³ of 0.35 mol dm⁻³ Tris-acetate buffer (pH 8.4) containing 10 mmol dm⁻³ EDTA, 5 mmol dm⁻³ glutathione, 11 mmol dm⁻³ sodium diethyldithiocarbamate and 6 per cent polyethylene glycol (Carbowax) 4000. The homogenate was centrifuged at 10000 g for 20 min and the supernatant collected. Aliquots of the supernatant (2 cm³) were de-salted using Biogel-P2 or Sephadex G25 columns (22 x 1.0 cm) with 0.35 mol dm⁻³ Tris-acetate buffer (pH 8.4) as the eluting buffer. Fractions (5 cm³) were collected and the enzyme was assayed immediately. All operations were carried out at 2–4 °C.

**Bound ADPG-starch synthase.** This was prepared using the method of Leloir, De Fekete and Cardini (1961) using samples of 250 cotyledon pairs for each imbibition time.

Enzyme assay

The standard mixture for each assay was as follows: (a) Soluble starch synthase: 100 µmol Tris-HCl buffer (pH 8.4), 0.2 µmol cold ADPG, 1.42 x 10⁻⁴ µmol ADP-¹⁴C-G (1110 Bq), 5.0 µmol sodium fluoride, 100 µl of soluble starch (25 mg cm⁻³) and 200 µl of enzyme preparation in a total volume of 400 µl. (b) Bound starch synthase: 100 µmol Tris-HCl buffer (pH 8.4), 0.2 µmol cold ADPG, 1.42 x 10⁻⁴ µmol ADP-¹⁴C-G (1110 Bq), 0.02 µmol EDTA and 50 mg of isolated starch granules in a total volume of 13 µl.

Incubations were carried out at 35 °C in plastic tubes with a routine incubation time of 10 min (the reaction was linear for up to 20 min with the most active preparation). Reactions were terminated by the addition of 5 cm³ of a 70 per cent methanol/1 per cent aqueous KCl mixture, left for 1 h on ice and then centrifuged in a bench centrifuge. The residue was resuspended in 1 cm³ of distilled water, reprecipitated by addition of 5 cm³ of methanol/KCl and, after a further period of standing on ice for 1 h the mixture was centrifuged. This procedure was repeated twice and finally the white precipitate was resuspended in 300 µl of water and transferred to a scintillation vial. Each sample was then solubilized by the addition of 1 cm³ of Soluene-350 (Packard Instrument Co. Inc.) and incubation for 12 h at room temperature. Finally, 10 cm³ of Instagel (Packard Instrument Co. Inc.) was added and after mixing, the samples were counted in a Packard Tricarb 460 CD scintillation counter. Total recovery of activity in this procedure (i.e. total of incorporated and non-incorporated counts) was 95 per cent. The maximum incorporation of activity was high at 40 per cent (10–20 per cent average) but the reaction was linear up to this point.
Starch determination

Samples (10 mg) of starch containing granules were solubilized in 3 cm³ of distilled water by boiling for 10 min which also removed contaminating protein (about 50 per cent of the granules). After centrifugation 0.2 cm³ aliquots of the supernatants were taken for starch determination. Starch was assayed by the addition of 30 cm³ of I₂-KI reagent (0.65 g I₂ + 1.95 g KI in 1 l diluted to 25 fold before use). Readings were taken at 610 nm and compared with a calibration curve produced using BDH soluble starch.

RESULTS AND DISCUSSION

The starch content of cucumber cotyledons during germination and early seedling growth is illustrated in Fig. 1. In the absence of the embryonic axis the amount of starch increases up to the 8th day of incubation when it reaches a plateau at about 175 μg starch per cotyledon pair. There is also a very small but reproducible increase in starch in the cotyledons of intact seeds reaching a peak of 10 μg per cotyledon pair at 2 d.

As early as 1889 Nadelmann noticed the formation of transitory starch granules in cotyledons of fenugreek (Trigonella foenum-graecum) as the endosperm was dissolved following the germination process: this starch is formed from galactomannans as they are mobilized in the endosperm (e.g. Reid, 1971). Many others instances are known. For example, starch is produced following germination of Douglas fir (Ching, 1966), groundnuts (Wankhede, Saroja and Rao, 1977), lupins (Matheson and Saini, 1977) and soybean (Adams, Rinne and Fjerstad, 1980).

Our results confirm the cytochemical observations of starch production in isolated cucumber cotyledons first reported by Davies and Chapman (1981). Additionally, the observations provide another example of the formation of transitory starch production during the mobilization of food reserves following germination and early seedling growth of seeds not normally containing starch as part of their reserve material.

Starch production depends upon the concerted activity of several enzymes, the type and amount depending upon species and developmental age of the material (e.g. Hawker, Marschner and Krauss, 1979; Matters and Boyer, 1981). In all cases, however, the activity of ADPG–starch synthase is of prime importance in starch synthesis (Recondo and Leloir, 1961; Murata, Sugiyama and Akazawa, 1964). Fig. 2 shows the activity of
Fig. 2. Soluble ADPG-starch synthase activity in intact and excised cotyledons. Enzyme was extracted from samples of 10 cotyledons and activity measured as described in materials and methods. Each point represents an average of 3 determinations. Maximum deviation ± 5 per cent. • Attached cotyledons; ▲ excised cotyledons.

Fig. 3. Bound ADPG-starch synthase activity in intact and excised cotyledons. Bound ADPG-starch synthase activity was determined in samples of starch granules isolated from 250 cotyledon pairs for each imbibition time. Activity was determined as described in Materials and Methods. Each point represents an average of 3 determinations. Maximum deviation ± 5 per cent. • Attached cotyledons; ▲ excised cotyledons.

soluble ADPG-starch synthase in intact and excised cotyledons. The enzyme is present at low levels in dry unimbibed cotyledons and following imbibition immediately increases in amount for the duration of the experiment in both treatments, showing a similar pattern in each case.

In contrast, bound ADPG-starch synthase appears to be absent from unimbibed cotyledons (Fig. 3) and remains at a low level for the first 4 d when there is a considerable
increase in activity within excised cotyledons reaching a peak at 12 d. The activity remains low in intact cotyledons and finally disappears at about 10 d.

Murata and Akazawa (1966) demonstrated differences between the occurrence of soluble and bound enzymes in rice: the soluble form occurs almost exclusively in waxy (or glutinous) rice whereas the bound form occurs predominantly in non-waxy (non-glutinous) varieties. These differences have been connected with the different concentrations of amylose and amyllopectin found within plants and it has been suggested that the formation of bound enzyme depends upon the presence of amylose to which it can adsorb (e.g. Preiss and Levi, 1980). In many tissues more activity is found in the soluble starch synthase than in the bound enzyme reaction (e.g. in maize and potato, Ozbun et al., 1973; Hawker and Downton, 1974; Downton and Hawker, 1975). This appears to be the situation in cucumber cotyledons where the level of activity of the soluble enzyme is always greater than that of the bound synthase. (c.f. Fig. 2 and 3). However, in our case the level of the soluble enzyme does not seem to have any connection with the presence of starch. On the contrary as might be expected starch amounts seem to be directly correlated with the amount of bound starch synthase activity, a low amount of starch in the intact cotyledons being mirrored by a low activity of the enzyme. It is of interest that the activity of the bound enzyme in excised cotyledons continues to increase until the 12th day whereas the amount of extractable starch reaches a maximum at the 8th day. Starch accumulation is a function of both biosynthetic and degradative reactions and these results clearly suggest that the activity of enzymes promoting degradation of starch reserves are also of importance in controlling the amount of starch produced in excised cotyledons. In this regard it should be noted that starch degrading enzymes such as amylases have been identified in seeds normally storing lipid and protein (e.g. Scots Pine, Nyman, 1971; Squash, Penner, 1968).

It has been suggested that starch biosynthesis is regulated by the D-glycerate-3-P/Pi ratio acting on starch synthases in photosynthetic tissues (Heldt et al., 1977) and by D-glycerate-3-P levels acting on starch synthases and ADP-glucose pyrophosphorylase in non-photosynthetic tissues (Preiss and Levi, 1980). In our experiments the synthesis of starch may be a reflection of the need to produce an internal metabolic sink for soluble sugars in the cotyledons (albeit only a temporary one in the case of the intact system). During the incubation of excised cotyledons, lipid breakdown is reduced and sugar levels are increased. A similar effect is found in the intact system if growth is suppressed by the presence of polyethylene glycol during imbibition, (Davies and Chapman, 1979). The synthesis of starch could thus represent the formation of a secondary pool of carbon and could prevent the increase of sugar concentrations to inhibitory levels (Davies and Slack, 1981).

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