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SHORT COMMUNICATION

STARCH SYNTHETASE IN DEVELOPING BARLEY AMYLOPLASTS

E. D. BAXTER and C. M. DUFFUS

Department of Agricultural Biochemistry, School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh EH9, Scotland

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Abstract—The activity of starch synthetase in amyloplasts from barley endosperm was measured. The relative effectiveness of UDPG and ADPG as glucosyl donors was determined, and correlated with amyloplast age. Only UDPG linked synthesis could be detected up to about 12 days after anthesis. After this time ADPG was the main glucosyl donor.

INTRODUCTION

THE INVOLVEMENT of sugar nucleotides in starch synthesis was first reported by De Fekete *et al.*[1] Since then both UDP and ADP[2, 3] linked mechanisms have been observed in many plant tissues. There is some evidence that the rate of glucose transfer from ADPG may exceed that from UDPG.[3] This paper describes an investigation into the relative importance in the developing amyloplasts of UDP glucose α1–4 glucan α4 glucosyl transferase (E.C. 2.4.111 starch synthetase) and the corresponding activity using ADPG as glucosyl donor.

RESULTS

Starch was visible in the plastids from 2 to 3 days after anthesis, although starch synthetase activity was detected only from 6 days after anthesis. Plastid size increased until 25 days. From 21 days onward small plastids could be seen inside the larger ones.[4] No starch was detected in the soluble fraction.

Both UDPG and ADPG linked starch synthetase increased in activity with plastid size (Fig. 1). However, the relative activity of the UDPG enzyme decreased with increasing plastid size (Fig. 2) whilst the ADPG enzyme became increasingly important. When starch was added to the assay system there was an increase in activity in the plastids. The percentage increase was greatest with very young plastids (about 6–7 days after anthesis) indicating that at this stage of development primer concentration is limiting.

The starch synthetase activity of the soluble fraction at first appeared to be greater than that of plastids of the same age. The activity of the soluble fraction of 14 day grains exceeded that of plastids of the same age by a factor of 10^3 (using UDPG) or 10^2 (using ADPG), when assayed by UDP (or ADP) production. There was, however, no stimulation of activity when starch was added as a primer, although the original soluble fraction did not contain any

[1] M. A. R. De FEKETE, L. F. LELOIR and C. E. CARDINI, *Nature* **187**, 918 (1960).
[2] L. F. LELOIR, M. A. R. De FEKETE, and C. E. CARDINI, *J. Biol. Chem.* **236**, 636 (1961).
[3] E. RECONDO and L. F. LELOIR, *Biochem. Biophys. Res. Commun.* **6**, 85 (1961).
[4] M. S. BUTTROSE, *J. Ultrastruct. Res.* **4**, 231 (1960).

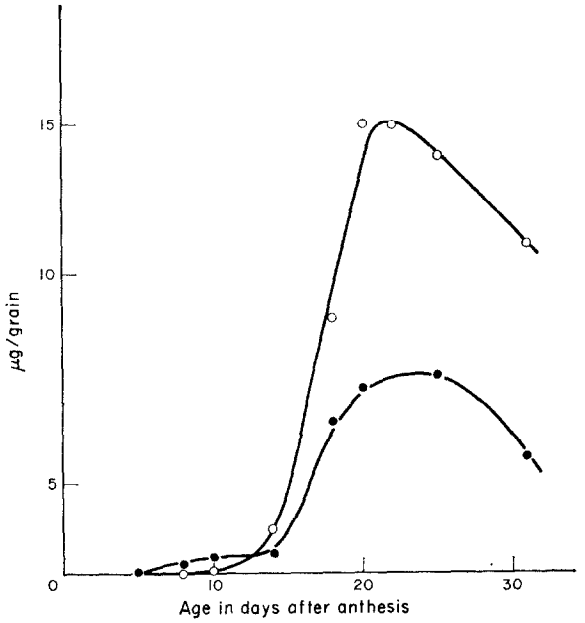

FIG. 1. LEVELS OF NUCLEOSIDE PYROPHOSPHATES SYNTHESIZED BY PLASTIDS DURING 2 hr INCUBATION IN μgm/grain.

ADP and UDP values corrected for endogenous levels (see text) ● UDP; ○ ADP.

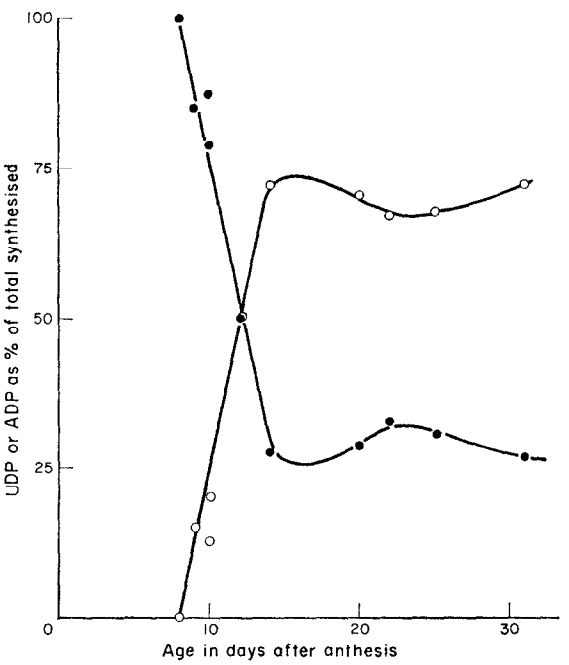

FIG. 2. AMOUNT OF UDP (OR ADP) SYNTHESIZED BY PLASTIDS EXPRESSED AS A PERCENTAGE OF TOTAL UDP + ADP SYNTHESIZED.

● UDP; ○ ADP.

detectable starch. Enzyme fractions were therefore incubated with UDP (^{14}C) G and starch as a carrier. Incorporation of (^{14}C) glucose into an insoluble product associated with the starch pellet occurred only with the plastid fraction. This suggests that the high concentrations of UDP and ADP detected in the previous assays of the soluble fraction are formed by the breakdown of the UDPG and ADPG added without the glucosyl moiety being incorporated into a high molecular weight polymer. These high levels of UDP and ADP may be due to the activity of sucrose synthetase, an enzyme which is generally reversible,[5] although synthesis of sucrose may be somewhat slower than the reverse reaction at physiological pH.[6] This enzyme is highly reactive in the soluble fraction of barley grain homogenates. The specific activity (in nmoles sucrose/min/grain) increased from 1·4 at 7 days after anthesis to a peak of 624 at 28 days, afterwards levelling off to a value of around 500 in the mature grain. Similar, or slightly lower, levels of activity in the direction of sucrose synthesis, could account for all the UDP and ADP produced by the soluble fraction during the assay for starch synthetase by the pyruvate kinase method. No sucrose synthetase activity could be detected in the plastids.

DISCUSSION

These results agree with those of previous workers[3, 7, 8] which suggest that proliferation of starch in developing seeds is mainly brought about by ADPG linked starch synthetase associated with the amyloplasts. Starch is, however, visible in the plastids before starch synthetase activity can be detected. Also, this enzyme has been shown to require an oligosaccharide primer.[2] These observations suggest that a different enzyme is involved in the early synthesis of starch from low molecular weight primers. Tsai and Nelson[9] have isolated four phosphorylase isoenzymes from maize, three of which are present in the developing endosperm and one in the embryo. Their work suggests that two of the endosperm isoenzymes may be capable of synthesizing starch without the addition of primer. Slabnik and Frydman[10] have detected a similar phosphorylase enzyme in potatoes. Classical phosphorylase activity can be detected in the soluble fraction of young barley endosperm, using soluble starch as a primer. This activity increased from 195 ng starch produced/grain/min at 12 days after anthesis to 334 ng at 19 days. So far attempts to obtain synthesis of starch from glucose-1-phosphate by phosphorylase without added primer have been unsuccessful. However, there is some evidence[9] that an inhibitor of certain phosphorylase isoenzymes may be present in crude cereal grain homogenates. Bird[11] has shown that in destarched chloroplasts supplied with glucose-1-phosphate, phosphorylase will bring about the synthesis of an insoluble glucose polymer which is sufficient to prime the starch synthetase reaction. In young barley endosperm, such a primer would be slowly converted into starch by UDPG linked starch synthetase. By 12–15 days after anthesis, when ADPG linked starch synthetase has become more active, the starch content of the plastids increases rapidly and starch is no longer limiting. At this later stage UDP and UDPG may be involved in the utilisation, via sucrose synthetase, of the sucrose supplied to the endosperm by the leaves.[5, 6]

[5] D. P. Delmar and P. Albersheim, *Plant Physiol.* **45**, 782 (1970).
[6] R. Pressey, *Plant Physiol.* **44**, 759 (1969).
[7] C. Y. Tsai, F. Salamini and O. E. Nelson, *Plant Physiol.* **46**, 299 (1970).
[8] L. C. Baun, E. P. Palmiano, C. M. Perez and B. Juliano, *Plant Physiol.* **46**, 429 (1970).
[9] C. Y. Tsai, O. E. Nelson, *Plant Physiol.* **44**, 159 (1969).
[10] E. Slabnik and R. Frydman, *Biochem. Biophys. Res. Commun.* **38**, 709 (1969).
[11] I. F. Bird, Ph.D. Thesis, University of London, 1969.

EXPERIMENTAL

Plant material. Two row barley plants (*Hordeum distichium* (L.). Lam. v. Maris Baldric) were used. Conditions of growth and methods used to determine the date of anthesis were as described by Merritt and Walker.[12] Grains could be stored at −15° for 3 months without loss of activity.

Preparation of amyloplasts. Endosperms were separated from embryo and aleurone by hand, homogenised in an all-glass Potter type homogenizer in 2 vol. of H_2O and filtered through double muslin to remove cell debris. Amyloplasts were separated from the soluble components by centrifugation for 10 min at 4° and 2500 *g*. They were resuspended in H_2O.

Enzyme assays. Starch synthetase was assayed by the method of Leloir,[2] but in a total volume of 0·4 ml using 0·1 ml of the suspended amyloplasts as the source of enzyme. The supernatant solution from the centrifugation, containing the soluble components of the endosperm, was also assayed for synthetic activity. All assays were corrected for endogenous nucleotides using controls incubated without UDPG or ADPG.

ADP and UDP were measured by the pyruvate kinase method[13] and compared with standards run simultaneously. Assays carried out without amyloplasts showed that neither UDPG nor ADPG dissociated under the conditions of the assays. Starch synthetase assays were also carried out using ^{14}C labelled UDPG (see Leloir).[2] UDP-(^{14}C) G (ammonium salt, 237 mc/mM) was obtained from the Radiochemical Centre, Amersham. The soluble fraction was assayed for phosphorylase by the method of Slabnik and Frydmann.[10] Sucrose synthetase was assayed by the method of Avigad and Milner.[14]

Light microscopy. Each amyloplast preparation was examined using a light microscope equipped with phase contrast lenses (magnification ×500). An age approximating to the number of days after anthesis was assigned to each preparation according to the average plastid size. The presence of starch was detected by staining with a 2% KI–0·2% I_2 solution.

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[12] N. R. MERRITT and J. T. WALKER, *J. Inst. Brewing* **75**, 156 (1969).
[13] L. F. LELOIR and S. H. GOLDEMBERG, *Methods in Enzymology*, Vol. V, p. 145, Academic Press, London (1962).
[14] G. AVIGDAD and Y. MILNER, *Methods in Enzymology*, Vol. VIII, p. 341, Academic Press, London.