PARTICULATE UDP-GLUCOSE:PROTEIN TRANSGLUCOSYLASE FROM POTATO TUBER

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1. Introduction

We have previously shown [1] that a particulate fraction constituted mainly of proplastids could be isolated from potato tuber juice by differential centrifugation. This fraction contained besides phosphorylase, a starch synthetase activity with similar specificity for the glucosyl donor to that demonstrated for the starch synthetase attached to the starch grain [2]. These transglucosylase activities could be demonstrated without primer addition, since primer was already present in the preparation [1].

This paper reports the results of further studies on the proplastid particulate fraction and presents information about its capacity to transfer glucose, specifically from UDP-glucose, to a trichloroacetic acid (TCA)-insoluble product which shows the properties of a glucoprotein. This glucoprotein can act as acceptor for the synthesis of a 1,4-α-glucan from ADP-glucose or glucose 1-phosphate with the same enzymatic system.

2. Materials and methods

Potato tubers were obtained from the local market. About 100 g of tubers were peeled and grated with a glass grater in the presence of 0.25 ml of β-mercaptoethanol (final concentration in the juice, about 5 mM). The suspension was strained through gauze and the filtrate was centrifuged at 2000 *g* for 10 min, in order to separate starch. The supernatant solution was centrifuged again at 25,000 *g* for 10 min. After centrifugation of the supernatant fluid at 140,000 *g* for 2 hr, the pellet was suspended in 2 ml of 0.1 M Tris-HCl buffer (pH 7.2), containing 0.05% β-mercaptoethanol and stored at 4°. This suspension is refered to as the "particulate preparation".

2.1. *Enzyme assay*

The incubation mixture contained, in a final volume of 40 μl, 0.3 μmole of $MnCl_2$, 20 μl of particulate preparation (0.4–0.5 mg of protein) and the glucosyl donor indicated in each case. After incubation at 37° for 15 min, the radioactive material precipitated with 5% TCA or methanol was measured as follows:

i) TCA precipitate: the reaction was stopped with 1 ml of 5% TCA. The precipitate was collected on nitrocellulose (Millipore) filters, washed several times with 5% TCA, and counted in a scintillation spectrometer using a toluene-2,5-diphenyloxazole-1,4-*bis*-[2-(4-methyl-5-phenyl)oxazolyl]-benzene mixture.

ii) Total methanol precipitate: after enzyme action, 1 ml of 70% methanol containing 1% KCl was added and the suspension was centrifuged. The precipitate was washed twice with 1 ml of methanol–KCl, suspended in 0.5 ml of water, plated on aluminium planchets, dried and counted in a gas-flow counter.

iii) Methanol precipitate after precipitation with TCA: the reaction was stopped with 1 ml of 5% TCA and the suspension was centrifuged. Phytoglycogen (0.6 mg) as carrier and 1.5 vol of methanol were added to the supernatant fluid. The precipitate was collected by centrifugation, washed and counted as indicated for the total methanol precipitate assay.

Protein was measured by the method of Lowry et al. [3]. Enzyme digestions were performed under toluene vapors. Pronase (Calbiochem) was used at pH 7.5 in the presence of 7 mM $CaCl_2$ [4]. β-Amylase

Table 1
Radioactivity incorporation from UDP-[^{14}C]glucose into TCA- or methanol-insoluble products.

Additions	Radioactivity incorporation (cpm)		
	TCA precipitate	Total methanol precipitate	Methanol precipitate after TCA treatment
None	4,410	4,120	50
$MnCl_2$ (1 μmole)	5,820	5,890	0
Glucose 1-phosphate (1 μmole)	3,520	4,300	90
Glucose 1-phosphate (1 μmole) + $MnCl_2$ (1 μmole)	19,880	19,900	700
Phytoglycogen (0.3 mg)	3,540	8,150	5,870
Phytoglycogen (0.3 mg) + $MnCl_2$ (1 μmole)	4,530	9,920	5,000

The reaction mixture contained 0.16 nmole of UDP[^{14}C]-glucose (55,000 cpm), 20 μl of particulate preparation (0.4 mg of protein) and additions indicated in the table, in a total volume of 50 μl. Conditions as described under Materials and methods.

Table 2
Effect of several treatments on the TCA precipitate.

Treatment	Conditions	Radioactivity precipitated by TCA (%)
A. NaOH (0.5 N)	4°, 24 hr	17
Urea (8 M)	4°, 20 hr	66
		Radioactivity in the phenol phase (%)
B. Phenol (80%)	Room temp., 5 min	97
		Remaining radioactivity in the TCA precipitate (%)
C. HCl (1 N)	100°, 1 hr	0
Pronase	37°, 24 hr	8
β-Amylase	37°, 24 hr	95
α-Amylase	37°, 24 hr	68

Conditions as described under Materials and methods, including 0.16 nmole of UDP[^{14}C]glucose (68,000 cpm) in the reaction mixture. The TCA precipitate was collected by centrifugation, washed with methanol and dried before the treatments indicated in each case. After treatments A, the solubilized material was reprecipitated with 10% TCA and the pellet was counted. The phenol phase obtained in treatment B was washed with phenol-saturated water and radioactivity was measured. After treatments indicated in C the suspensions obtained were centrifuged and the pellet was counted for remaining radioactivity.

(Sigma) was assayed at pH 5 and α-amylase (saliva source) treatment was carried out in the presence of 0.01 M NaCl. Hydrolysis products were identified by paper chromatography with butanol–pyridine–water (6:4:3) as solvent [5] and paper electrophoresis in pyridine acetate buffer, pH 6.4, for 2 hr at 1000 V. All other materials were as described elsewhere [6].

3. Results

As can be seen in table 1, incubation of the particulate preparation with UDP[^{14}C]glucose resulted in an incorporation of radioactivity into a product insoluble in TCA and methanol. Table 1 also shows that this activity was enhanced in the presence of $MnCl_2$ or, $MnCl_2$ plus glucose 1-phosphate. This increase is commented on later. The addition of phytoglycogen to the incubation mixture did not alter the incorporation into the TCA-insoluble product, but increased the radioactivity of the total methanol precipitate. Radioactivity precipitated with methanol was approximately equivalent to the sum of radioactivities in the TCA precipitate and in the methanol precipitate after treatment with TCA (see table 1).

Fig. 1 shows the time course of the formation of radioactive TCA precipitate from different glucosyl donors. Under the experimental conditions described in Materials and methods, UDP[^{14}C]glucose was the specific sugar donor. The reaction was very rapid and reached 80% of its completion in 3 min. The apparent

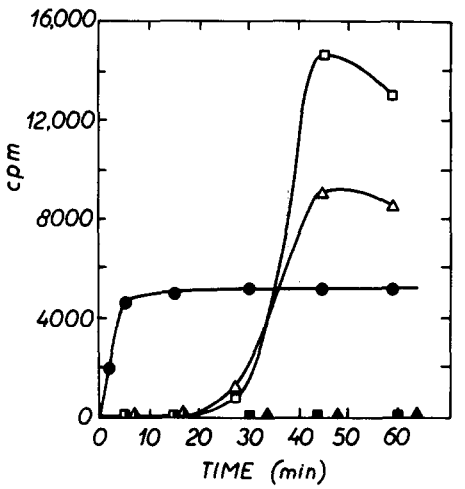

Fig. 1. Time course of the formation of TCA-insoluble precipitate from different glucosyl donors. Conditions as under Materials and methods. The glucosyl donors included in the reaction mixture were as follows: (●—●—●) 0.16 nmole of UDP[^{14}C]glucose (68,000 cpm), or (■—■—■) 0.19 nmole of ADP[^{14}C]glucose (60,000 cpm), or (▲—▲—▲) 0.15 nmole of [^{14}C]glucose 1-phosphate (51,000 cpm), or (□—□—□) 0.19 nmole of ADP[^{14}C]glucose (60,000 cpm) plus 0.25 μmole of unlabeled UDP-glucose, or (△—△—△) 0.15 nmole of [^{14}C]glucose 1-phosphate (51,000 cpm) plus 0.25 μmole of unlabeled UDP-glucose. At the times indicated in the figure, radioactivity in the TCA precipitate was measured as described in the text.

Michaelis constant for UDP-glucose was 1×10^{-5} M. ADP[^{14}C]glucose or [^{14}C]glucose 1-phosphate could not replace UDP[^{14}C]glucose, but in the presence of unlabeled UDP-glucose, both were capable to transfer glucose to the TCA precipitate, after a lag period of 20 min. Probably this lag period reflects the time required for the other transglucosylases present in the enzymatic preparation to enlarge the product formed from UDP-glucose to act as a more efficient primer for themselves.

The radioactive TCA precipitate obtained with labeled UDP-glucose was insoluble in water or methanol at room temp. The results of different treatments on this product are shown in table 2. It can be seen that it was soluble in phenol at room temp. After solubilization in 8 M urea, the radioactivity could be reprecipitated by 5% TCA. Mild alkaline treatment, which could split *O*-glycosidic linkages, gave a radioactive product soluble in TCA. Acid hydrolysis was complete in 1 hr and yielded glucose as the sole sugar. Radioactivity was almost completely released into solution by pronase, giving products that behaved like glucopeptides in chromatography and electrophoresis. After α-amylolysis only part of the radioactivity was released mainly as maltotriose. β-Amylase had no significant effect (see table 2). When the TCA precipitate was chromatographed on Sephadex G-75, using 0.1 M Tris-HCl buffer (pH 7.2) as eluting agent, all the radioactivity was excluded. Pronase digests were not excluded in the same conditions and radioactivity was eluted in the salt zone. Mild alkaline treatment product was recovered as a single peak after chromatrography on a Sephadex G-10 column. According to the elution position the molecular weight was about 650. From the data presented above, the [^{14}C]glucose-labeled TCA precipitate obtained from UDP[^{14}C]-glucose appeared to be a glucoprotein, containing glucosidic chains of four glucose units which were attached to the peptide portion by *O*-glycosidic linkages.

The radioactive TCA precipitate obtained from ADP[^{14}C]glucose or [^{14}C]glucose 1-phosphate, both in the presence of unlabeled UDP-glucose, after 45 min of incubation (see fig. 1) showed similar properties to those described in table 2 for the TCA precipitate obtained from UDP[^{14}C]glucose, except for the effect of amylases. As expected, under the same conditions as described in table 2, α-amylolysis as well as β-amylolysis was almost complete and resulted in the release of radioactivity, mainly as maltotriose and only as maltose, respectively, indicating larger glucosidic chains than were obtained with UDP-glucose alone. A similar action of α- and β-amylase was observed on the radioactive TCA precipitate obtained from UDP[^{14}C]glucose in the presence of glucose 1-phosphate and Mn^{2+}(fig. 1), except that part of the radioactivity was unattacked by amylases. These results could be explained assuming a double mechanism of incorporation of glucose from labelled UDP-glucose. First, UDP-glucose acts as a donor for the formation of the glucoprotein which is not attacked by β-amylase (exp. C of table 2). Second, an excess of cold glucose 1-phosphate enlarges some glucoprotein chains, which in turn become efficient primers for the UDP-glucose-starch synthetase present in the preparation [1]. The radioactive product thus formed would be sensitive to α- and β-amylase.

4. Discussion

Green and Stumpf [7] and Illingworth et al. [8] found unprimed synthesis by plant and animal phosphorylases, respectively, and suggested that the enzyme itself could act as a primer, forming a sort of glucoprotein. More recently, it has been shown that certain forms of phosphorylase [9, 10] and soluble starch synthetase [11] were able to synthesize polysaccharides in the absence of added primer. The question arose whether an integral part of these enzyme forms, probably a glycoprotein, could initiate the synthesis of glucan chains. On the other hand, although an exogenous primer is not required for the activity of starch synthetase attached to the starch grain [2], we do not know whether the polysaccharide itself or an endogenous glycoprotein can act as a primer. In a recent paper, Krisman [12] reported the formation, from UDP-glucose, of a possible glucoprotein intermediate in the initiation of liver glycogen synthesis. A similar process for starch biosynthesis is suggested by the data obtained with the potato particulate preparation. This fraction, consisting mainly of starch-free proplastids, contains phosphorylase besides starch synthetase activity. The latter resembles starch-bound synthetase activity in its glucosyl donor specificity [2] and the former could be related to a potato phosphorylase previously described [9]. Both activities can be demonstrated without added primer [1]. Moreover, the system is capable of forming a glucoproteic acceptor for those transglucosylases from UDP-glucose. Therefore, the potato particulate fraction would contain the complete system for the formation of the starch granule. According to the data obtained with mature starch grains, the enzymes would be located in a lipid zone [6] and would be able to act in an anhydrous medium [13]. This fact could explain the formation of such an anhydrous and insoluble structure with two markedly hydrophilic polysaccharides.

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References

[1] N. Lavintman and C.E. Cardini, in: Biochemistry of the Glycosidic Linkage, eds. R. Piras and H.G. Pontis (Academic Press, New York, 1972) p. 503.
[2] C.E. Cardini and R.B. Frydman, in: Methods in Enzymology, Vol. VIII, eds. E.F. Neufeld and V. Ginsburg (Academic Press, New York, 1966) p. 387.
[3] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[4] R.G. Spiro, in: Methods in Enzymology, Vol. VIII, eds. E.F. Neufeld and V. Ginsburg (Academic Press, New York, 1966) p. 26.
[5] A. Jeanes, C.S. Wise and R.J. Dimler, Anal. Chem. 23 (1951) 415.
[6] N. Lavintman and C.E. Cardini, Plant. Physiol. 50 (1972) in press.
[7] D.E. Green and P.K. Stumpf, J. Biol. Chem. 142 (1942) 355.
[8] B. Illingworth, D.H. Brown and C.F. Cori, Proc. Natl. Acad. Sci. U.S. 47 (1961) 469.
[9] E. Slabnik and R.B. Frydman, Biochem. Biophys. Res. Commun. 38 (1970) 709.
[10] C.Y. Tsai and D.E. Nelson, Plant Physiol. 44 (1970) 159.
[11] J.L. Ozbun, J.S. Hawker and J. Preiss, Biochem. Biophys. Res. Commun. 43 (1971) 631.
[12] C.R. Krisman, Biochem. Biophys. Res. Commun. 46 (1972) 1206.
[13] N.D. Judewicz, N. Lavintman and C.E. Cardini, Phytochemistry 11 (1972) 2213.