GALACTOSE 1-PHOSPHATE URIDYL TRANSFERASE ACTIVITY IN SOYBEAN EXTRACTS *

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The pathways for the biosynthesis and degradation of galactose-containing oligosaccharides in plants have not yet been fully elucidated. On the basis of the occurrence of UDP-galactose in plant tissues (Ginsburg, et al., 1956) it is reasonable to expect that uridine nucleotides are involved in the metabolism of galactose. Three enzymic reactions by which UDP-galactose may be synthesized in biological systems have been discovered and these are shown diagrammatically in equations 1, 2, and 3 (Kalckar, 1958):

$$\text{UTP} + \text{Gal-1-P} \longrightarrow \text{UDPGal} + \text{PP}_i \qquad (1)$$

$$\text{UTP} + \text{G-1-P} \longrightarrow \text{UDPG} + \text{PP}_i \qquad (2)$$
$$\text{UDPG} \longrightarrow \text{UDPGal}$$

$$\text{UDPG} + \text{Gal-1-P} \longrightarrow \text{UDPGal} + \text{G-1-P} \qquad (3)$$

Reaction 1 is catalyzed by the UDP-galactose pyrophosphorylase present in plant extracts (Neufeld, et al., 1957). Reaction 2 is catalyzed by UDP-glucose pyrophosphorylase and UDP-galactose 4-epimerase first discovered in yeast (Leloir, 1951) and also present in plant tissues (Neufeld, et al., 1957). Reaction 3 is catalyzed by galactose 1-phosphate uridyl transferase which has been found in some strains of microorganisms and in animal tissues (Kalckar, 1957; Kurahashi, et al., 1960) but not previously detected in plants. The reversibility of the steps in these reactions has been established by several

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methods (Kalckar, 1958).

In this communication evidence is presented for the presence of an enzyme with galactose 1-phosphate uridyl transferase activity in soybean extracts. Such an enzyme has an important role in the metabolism of galactose in yeasts and most probably has an important function in the metabolism of galactose-containing oligosaccharides in the plant. The transferase activity was detected by a new procedure based on the measurement of production of labeled UDP-glucose from non-labeled UDP-galactose and labeled glucose 1-phosphate.

The enzyme extract was prepared from sprouts of soybeans (Glycine max) which had been germinated for 24 to 36 hours. The sprouts, together with a small amount of cotyledonary tissue, from 50 beans were crushed in 10 ml. of 0.1 M potassium phosphate buffer of pH 7.2 and containing 0.05 M magnesium chloride. The supernatant obtained by centrifugation of the mixture at moderate speed in a Servall centrifuge consituted the enzyme extract. UDP-galactose was prepared by use of a plant UDP-galactose pyrophosphorylase and P^{32} labeled glucose 1-phosphate by use of potato phosphorylase. The details of these procedures will be published elsewhere. A sample of 3 micromoles of UDP-galactose and 6 micromoles of glucose-1-P^{32} was dissolved in 0.1 ml. of the enzyme extract. Aliquots of 10 microliters were removed from the digest after incubation at room temperature for 0.05, 0.5, 1, and 2 hours and placed on a paper chromatogram. The chromatogram was developed in a solvent system of isobutyric acid, concentrated ammonium hydroxide and water (66:1:33 by volume) and the positions of the radioactive components located by radioautography. The nucleotides were also detected on the chromatogram by means of UV light. Control experiments included the incubation of glucose-1-P^{32} alone with the enzyme preparation followed by analysis for radioactive products and incubation of UDP-galactose alone with the enzyme extract followed by analysis for UTP synthesis.

The chromatographic procedure used in this study resulted in a

separation of the glucose-1-P^{32} and the uridine diphosphate hexoses into distinct spots with Rf values of 0.17 and 0.09, respectively. The radioactivity values for the glucose-1-P and UDP-hexose areas on the chromatogram were determined directly on the paper with a G. M. counting assembly. Values from a typical experiment are recorded in Table 1.

Table 1

Radioactivities of products in a digest of glucose-1-P^{32} and UDP-galactose with a soybean extract

Time (hrs.)	Rf value, 0.17 * c.p.m.	Rf value, 0.09 * c.p.m.
UDP-Gal and G-1-P^{32}		
0.05	26,400	465
0.5	25,100	1,320
1.0	24,700	2.240
2.0	23,900	2,850
Control of G-1-P^{32}		
0.05	25,900	120
2.0	25,800	240

* The Rf value of G-1-P is 0.17 and of UDP-glucose or UDP-galactose 0.09 in the solvent system of isobutyric acid, ammonium hydroxide and water (66:1:33 by volume).

The data in the table show that a radioactive compound which migrated at the rate of a uridine diphosphate hexose was produced rapidly on incubation of UDP-galactose and glucose-1-P^{32} with the enzyme extract. Further it is noted that this radioactive compound was not produced when only glucose-1-P^{32} was incubated with the enzyme preparation. The UV absorption behavior of the compound at Rf value of 0.09 was that typical of a uridine-carbohydrate compound. This UV absorbing material was isolated from the two hour digest with particular care to free it from unreacted glucose-1-P^{32}. The sample was

hydrolyzed in 0.1 N hydrochloric acid for 1 hour and neutralized with sodium bicarbonate. In the hydrolysate uridine 5-monophosphate was identified by its characteristic Rf value in several solvent systems and UV absorption spectrum, galactose was identified by its Rf value and glucose was identified by the glucose-oxidase reaction. On the basis of the color intensities in the glucose-oxidase reaction, it was estimated that the isolated nucleotide fraction consisted of 30% UDP-glucose and 70% UDP-galactose. The original UDP-galactose preparation used in the experiment contained less than 2% UDP-glucose as measured by the glucose-oxidase reaction.

A considerable synthesis of UDP-glucose was obtained under the conditions of our experiment and several possible routes for the synthesis of UDP-glucose have been considered. First, a reversal of equation 1 to yield UTP from UDP-galactose and trace amounts of inorganic pyrophosphate which may be present in the enzyme preparation, followed by reaction 1 of equation 2. However, this route is not likely since UTP was not synthesized in measurable amounts in a reaction mixture of UDP-galactose with the enzyme preparation. Second, a reversal of the two reactions in equation 2 followed by the synthesis of labeled UDP-glucose from UTP and glucose-1-P^{32}. This route is also not likely since under the conditions of our experiments, no significant epimerization of UDP-galactose to UDP-glucose was observed. Third and the most probable route of synthesis of the P^{32} labeled UDP-glucose is via a reversal of reaction 3 involving the transfer of the uridyl moiety of UDP-galactose to glucose-1-P^{32}. Such a reaction is catalyzed by a galactose 1-phosphate uridyl transferase. This type of enzymic activity has not been previously detected in plant tissues and studies to assess the importance of this enzyme in the metabolism of galactose in soybeans are in progress in our laboratory.

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