THE ENZYMIC SYNTHESIS OF URIDINE DIPHOSPHATE L-RHAMNOSE*

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It has been reported that an enzyme preparation from mung bean leaves (Phaseolus aureus) catalyzes the transfer of L-rhamnose from thymidine diphosphate (dTDP) L-rhamnose to 3-quercetin β-D-glucoside to form rutin (3-quercetin O-α-L-rhamnosyl-(1→6)-β-D-glucoside) (Barber and Neufeld, 1961; Barber, 1962). The physiologic importance of this glycosylation is uncertain, however, since neither the occurrence nor the synthesis of dTDP-L-rhamnose has been demonstrated in plants. A recent observation in this laboratory that chemically synthesized uridine diphosphate (UDP) L-rhamnose functions more effectively than dTDP-L-rhamnose in the rhamnosylation of 3-quercetin β-D-glucoside suggested that the natural donor of L-rhamnose in the mung bean might be the UDP rather than the dTDP derivative of that sugar. Experiments were designed to test this hypothesis in which the system forming rutin was used to trap L-rhamnose produced in intermediate reactions. As a result of this investigation it has been found that a preparation from mung bean leaves catalyzes the conversion of UDP-D-glucose to UDP-L-rhamnose.

Methods. Methods used to prepare the enzyme and radioactive substrates and to estimate the incorporation of radioactivity into rutin were given in a previous publication (Barber, 1962). UDP-L-rhamnose labeled with C^{14} in the

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rhamnose moiety was synthesized by a microadaptation of the morpholidate procedure (Moffatt, 1961). About 0.15 μmole (5μC) of L-rhamnose-C^{14} 1-phosphate (obtained from dTDP-L-rhamnose by hydrolysis with snake venom) was converted to the pyridinium salt by electrophoresis on paper in 0.1 M pyridinium formate buffer, pH 3.7. Residual buffer was removed from the eluted material by chromatography in propanol-ethyl acetate-water (7:1:2). The published procedure was then followed except that a 4 fold excess of uridine phosphoromorpholidate was used, the products of the reaction were separated by electrophoresis on paper in 0.2 M ammonium formate buffer, pH 3.7, and the sugar nucleotide was further purified by chromatography in ethanol-1 M ammonium acetate (7:3). Yields of the product were low, but since unreacted sugar phosphate was readily recovered, the process could be repeated.

Rhamnosyl transfer from UDP-L-rhamnose. dTDP-L-rhamnose and UDP-L-rhamnose labeled with C^{14} in the L-rhamnose residues were each supplied as donors of L-rhamnose to the system which synthesizes rutin from 3-quercetin β-D-glucoside. Mixtures were incubated for 2 hr at 37°. 97% of the radioactivity of UDP-L-rhamnose and 65% of that of TDP-L-rhamnose was incorporated into a compound chromatographically indistinguishable from rutin.

Formation of rhamnosyl labeled rutin from UDP-D-glucose. When a 25-50% ammonium sulfate fraction from young mung bean leaves was incubated with C^{14}-labeled UDP-D-glucose, TPNH, $MgCl_2$, and 3-quercetin β-D-glucoside, radioactive rutin was formed. Conditions for this reaction are given in Table I. Rutin was eluted from the chromatogram and hydrolyzed with rhamnodiastase (Barber, 1962). The labeled rutinose produced was isolated by chromatography with propanol-ethyl acetate-water (7:1:2). Upon hydrolysis of the rutinose in 1 N HCl for 30 min at 100° and chromatography of the hydrolysate with the same solvent system, a radioactive band appeared in the position of L-rhamnose. The compound was eluted with water, mixed with 10 mg of authentic L-rhamnose, and the phenylosazone was prepared. The specific activity of the osazone did not decrease through three crystallizations from 50% ethanol.

C^{14}-labeled glucose and galactose nucleoside diphosphates of uridine, thymidine and deoxyuridine (Neufeld, 1962) were compared for their effectiveness as precursors of rhamnose in rutin. Only UDP-D-glucose and UDP-D-galactose produced significant labeling (Table I). Since UDP-D-galactose 4-epimerase is present in this preparation, however, it is not known whether UDP-D-glucose or UDP-D-galactose is the immediate progenitor of the rhamnosyl compound.

TABLE I

THE FORMATION OF RUTIN LABELED WITH C^{14} IN THE RHAMNOSYL MOIETY FROM VARIOUS SUGAR NUCLEOTIDES

Reaction mixtures contained 20 µl mung bean leaf enzyme (ca 1.1 mg protein) in 0.025 M Tris.HCl/0.01 M mercaptoethanol buffer, pH 7.5, 0.3 µmole ATP, 0.2 µmole TPNH, 0.01 µmole DPN, 0.2 µmole $MgCl_2$, 1 µl of a 1% aqueous suspension of 3-quercetin β-D-glucoside, sugar nucleotide labeled with C^{14} in the glucose or galactose moiety (about 0.008 µmole (0.06 µC) of the galactose compound and 0.0016 µmole (0.06 µC) of the glucose compound) in a total volume of 35 µl. The mixtures were incubated in sealed capillary tubes for 2 hr at 37°. Incorporation of radioactivity into rutin was estimated as described previously (Barber, 1962).

Sugar nucleotide	% of total recovered radioactivity incorporated into rutin
UDP-D-glucose	25
UDP-D-galactose	22
dTDP-D-glucose	< 1
dTDP-D-galactose	< 1
dUDP-D-glucose	2
dUDP-D-galactose	1

Requirements for the synthesis of C^{14}-labeled rhamnose from radioactive UDP-D-glucose were also determined by the incorporation of radioactivity into rutin (Table II). TPNH was required in this reaction as it is in the synthesis of dTDP-L-rhamnose from dTDP-D-glucose (Glaser and Kornfeld, 1961). ATP is thought to stimulate the transfer of rhamnose from dTDP-L-rhamnose by protecting the compound against hydrolysis by endogenous enzymes. In this reaction it had no effect, presumably due to the presence of TPNH which may similarly protect the substrate.

TABLE II

REQUIREMENTS FOR THE SYNTHESIS OF RHAMNOSE-LABELED RUTIN FROM UDP-D-GLUCOSE-C^{14}

The complete reaction mixture contained UDP-D-glucose-C^{14} and the components described in Table I. Mixtures were incubated in capillary tubes for 2 hr at 37°. Incorporation of radioactivity into rutin was estimated as described previously (Barber, 1962).

	System	% total recovered radioactivity incorporated into rutin
Exp 1.	Complete	25
	- TPNH	4
	- TPNH, + DPNH	2
Exp 2.	Complete	17
	- ATP	25
	- $MgCl_2$	14
	- DPN	17

Isolation of UDP-L-rhamnose. A sugar nucleotide with the properties of UDP-L-rhamnose was isolated directly from reaction mixtures incubated without the rhamnosyl acceptor, 3-quercetin β-D-glucoside. The mixture contained the components given in Table I with 0.008 μmole (0.33 μC) of radioactive UDP-D-glucose. After incubation for 2 hr at 37°, UDP-hexoses were separated by paper electrophoresis in 0.2 M ammonium formate buffer, pH 3.7, and eluted with water. Evidence for the presence of UDP-L-rhamnose in the eluate was obtained as follows:

1. A portion was treated with venom of Crotalus adamanteus, and radioactive hexose phosphate was separated from the mixture by paper electrophoresis. It was eluted and hydrolyzed in 1 N HCl for 15 mir at 100°. The hydrolysate was found to contain a radioactive compound, the position of which coincided with authentic L-rhamnose upon chromatography in two dimensions (water-saturated phenol and butanol-acetic acid-water (52:13:35)).

2. Another portion was chromatographed on paper in ethanol-1 M ammonium acetate (7:3). In this solvent the authentic compounds moved as follows in 18 hr at room temperature: UDP-D-glucose 16.5 cm; UDP-L-rhamnose 18.0 cm; TDP-D-glucose 20 cm; and TDP-L-rhamnose 20 cm. The chromatogram

indicated that the enzymically produced material was a mixture of UDP-D-glucose and UDP-L-rhamnose.

3. The above mixture was eluted from the chromatogram, hydrolyzed in 0.01 N HCl for 15 min at 100°, and chromatographed with propanol-ethyl acetate-water. A radioactive substance in the rhamnose area of the chromatogram was eluted with water, mixed with 10 mg of authentic L-rhamnose, and the phenylosazone was prepared. Specific activity did not decrease through three crystallizations from 50% ethanol.

Discussion. These data strongly suggest that in the mung bean leaf L-rhamnose is synthesized as the UDP derivative by the reduction and epimerization of the glucosyl residue of UDP-D-glucose. This is in contrast to those bacterial systems in which the glucosyl moiety of UDP-D-glucose is completely inactive as a precursor of L-rhamnose; this sugar is formed instead as the dTDP compound (Baddiley et al., 1961; Glaser and Kornfeld, 1961; Okazaki et al., 1961; Pazur and Shuey, 1961). However, UDP-L-rhamnose has been reported to occur in pneumococcus Type II (Smith et al., 1959), suggesting that a pathway of L-rhamnose formation similar to that described here may also exist in some microorganisms.

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