The 4-Epimerization and Decarboxylation of Uridine Diphosphate D-Glucuronic Acid by Extracts from Phaseolus aureus Seedlings*

DAVID S. FEINGOLD, ELIZABETH F. NEUFELD, AND W. Z. HASSID

From the Department of Biochemistry, University of California, Berkeley, California

(Received for publication, September 30, 1959)

It has been previously demonstrated (1) that particulate preparations from Phaseolus aureus (mung bean) seedlings are capable of catalyzing the formation of uridine diphosphate-D-galacturonic acid and a mixture of UDP-n-xylose and UDP-L-arabinose from UDP-n-glucuronic acid. The results indicated that the preparations contained UDP-uronic acid 4-epimerase and UDP uronic acid decarboxylase activities. However, the individual reactions involved were not investigated.

The work reported in this paper shows that three enzyme systems are operative in the conversions: a UDP-D-galacturonic acid-4-epimerase, a UDP-n-glucuronic acid decarboxylase, and a UDP-L-arabinose-4-epimerase. The enzyme systems catalyzing these reactions have been partially separated and the equilibrium constants have been determined for the two epimerization reactions.

**EXPERIMENTAL**

Substrates—UDP-n-glucuronic acid labeled with C\(^14\) in the n-glucuronic acid moiety was prepared by the enzymatic oxidation of radioactive UDP-n-glucose (7). In a typical preparation 3 \(\mu\)moles (110 \(\mu\)c) of radioactive UDP-n-glucose were dissolved in 0.4 ml of 0.1 m glycine buffer, pH 8.7, to which 0.1 ml of 0.2 m cysteine and 0.2 ml of 0.07 m diphosphopyridine nucleotide were added. The pH of the mixture was brought to 8.5 and 500 to 1,000 units of UDP-n-glucose liver dehydrogenase (8) were added. The reaction mixture was incubated at 37\(^\circ\) for 5 hours, and the UDP-n-glucuronic acid produced was separated and purified by subjecting the mixture to paper electrophoresis in 0.2 m ammonium formate buffer, pH 3.6. Since it was found that the ammonium formate buffer acted as an enzyme inhibitor, in all preparations of UDP-uronic acids and of UDP-pentose the residual ammonium formate was removed by drying the paper strips in a vacuum desiccator before elution. Once eluted, the UDP-uronic acid solutions were never taken to dryness, since such treatment often caused decomposition. The C\(^14\)-labeled UDP-n-glucuronic acid as well as the n-glucuronic acid obtained from it by hydrolysis was found to be homogeneous and free from radioactive impurities when examined electrophoretically at pH 3.0 and 5.8. Furthermore, radioactive n-glucuronic acid 1-phosphate was the only substance formed when the UDP-n-glucuronic acid was hydrolysed by nucleotide pyrophosphatase (9), whereas n-glucuronic acid was the only radioactive product obtained upon hydrolysis with a mixture of nucleotide pyrophosphatase and seminal phosphomonoesterase.

UDP-n-galacturonic acid labeled with C\(^14\) in the n-galacturonic acid moiety was prepared from radioactive UDP-n-glucuronic acid using a particulate preparation from radish root, and was purified by paper electrophoresis at pH 3.6. It was shown to be homogeneous by the criteria used for determining the purity of UDP-n-glucuronic acid.

UDP-pentose labeled with C\(^14\) in the pentosyl moiety was prepared as previously described (10) and its purity shown by the criteria described above. It consisted of a mixture of 65% UDP-n-xylose and 35% UDP-L-arabinose.

Enzymes—All operations were performed at 0-4\(^\circ\). Mung bean and radish particulate preparations which formed UDP-n-galacturonic acid and UDP-pentoses from UDP-n-glucuronic acid were prepared as follows: Radish roots (100 g, purchased from a local grocer) were cut into small pieces and homogenized in a chilled Waring Blendor for 1 minute with 70 ml of cold 0.01 m sodium-potassium phosphate buffer, pH 7.0. The slurry was filtered through two thicknesses of cheesecloth. Coarse debris and starch were removed by centrifugation at 2000 \(\times g\) for 5 minutes. The supernatant liquid was centrifuged at 18,000 \(\times g\) for 30 minutes and the particulate material which sedimented was reuspended in 0.5 ml of 0.1 m Tris buffer, pH 7.5. Particulate preparations from 4 to 5 day old mung bean seedlings (purchased from a local grocer) were made by an identical procedure, except that the mung bean seedlings were not cut into pieces before homogenization. The mung bean particulate preparation in 0.1 m Tris buffer, pH 7.5, was vigorous.

* This investigation was supported in part by a research grant (No. A-1418) from the United States Public Health Service, National Institutes of Health, and by a research contract with the United States Atomic Energy Commission.

The configuration of the glycosyl moieties of the nucleotides UDP-galacturonic acid and UDP-xylose as the D- or L-forms has not yet been rigorously established. However, it was shown (2) that at least 88% of the arabinosyl moiety of UDP-arabinose exists in the L-form. Physiological investigations of the metabolism of D-glucuronic acid in plants showed that it is converted to D-xylosyl residues of pectin and to the D-galakuronosyl residues of pectin and to the L-arabinosyl moieties of hemicellulose (3-5). Enzymatic experiments with plant extracts show that UDP-L-arabinose and UDP-n-xylose are interconvertible (6). In view of these facts and considering, on a theoretical basis, that the structural relationship of the D-glucuronic acid to the monosaccharides in question is compatible with these configurations, it is reasonable to assume that the assignment of the particular configurations to the sugar moieties in the nucleotides is correct. They will be so designated throughout the present paper.
ously shaken by hand for about 30 seconds with an equal volume of a 1% solution of digitonin, 0.1 M with respect to Tris buffer, pH 7.5. The supernatant liquid ("digitonin extract") obtained after centrifugation at 18,000 × g for 30 minutes proved to be devoid of UDP-n-galacturonic acid 4-epimerase activity, but was capable of deacetylating UDP-n-galacturonic acid.

A soluble UDP-n-galacturonic acid 4-epimerase preparation was obtained in the following manner: Mung bean seedlings (100 g) were ground to a slurry in a chilled mortar with 70 ml of 0.01 M sodium-potassium buffer, pH 7.0, 0.1 M with respect to mercaptoethanol. The homogenate obtained after filtering the slurry through cheesecloth was centrifuged at 18,000 × g for 30 minutes.

To 55 ml of the supernatant liquid, 37 g of solid ammonium sulfate were added, and the resulting precipitate was dissolved in 8 ml of 0.01 M ammonium sulfate. Each of these precipitates was dissolved in 0.25 ml of 0.1 M Tris buffer, pH 7.5, 0.1 M with respect to mercaptoethanol, and dialyzed overnight against 2 liters of the same buffer. All these fractions were then tested for the presence of UDP-n-glucuronic acid decarboxylase activity; the fraction which proved to be free of decarboxylase activity was used for studies of the UDP-n-galacturonic acid 4-epimerase. The second fraction usually contained the desired activity.

UDP-n-glucose dehydrogenase (8) was purchased from the Sigma Chemical Company.

Analytical Methods—Paper electrophoresis was carried out in 0.1 M ammonium formate buffer, pH 3.6, or in 0.2 M ammonium acetate buffer, pH 5.8, as previously described (7). The width of the bands was such that the various components in the mixtures could be unequivocally separated from each other. Pentoses were chromatographed one-dimensionally on Whatman No. 1 paper, with water-saturated phenol, or two-dimensionally with water-saturated phenol in the first dimension and butanolic acid-water (52:13:35) in the second. Sugars were detected with p-aminobenzoic acid spray reagent (1 g p-aminobenzoic acid in 140 ml of 70% ethanol). Radioactive substances were located on paper as previously described (7). They were eluted from the paper into ether or steel planchet, dried in a vacuum desiccator, and counted with a thin window Geiger-Müller tube coupled to a conventional scaler.

All enzymatic reactions and acid hydrolyses were carried out in capillary tubes of 1.2 to 1.5 mm diameter. Enzymatic reactions were stopped by immersing the tubes in boiling water for 1 minute.

Protein was estimated by the method of Lowry et al. (11).

RESULTS

Enzyme Activities in Particulate and Soluble Fractions—As previously reported (1), particulate preparations from mung bean seedlings catalyze the formation of UDP-n-galacturonic acid, UDP-D-xylene, and UDP-L-arabinose from UDP-D-glucuronic acid. It was subsequently found that "digitonin extracts" catalyze the formation of UDP-pentose from UDP-D-glucuronic acid, but did not catalyze the conversion of UDP-D-glucuronic acid to UDP-D-galacturonic acid. This indicated the presence in the particulate preparations of a UDP-D-galacturonic acid 4-epimerase system which was responsible for the interconversion of the UDP-D-glucuronic and UDP-D-galacturonic acids. After extraction with digitonin the particulate preparation still contained UDP-D-galacturonic acid 4-epimerase activity.

The soluble fraction of the mung bean seedling homogenate was also found to contain UDP-D-glucuronic acid decarboxylase and UDP-D-galacturonic acid 4-epimerase activity, as well as the UDP-L-arabinose 4-epimerase activity which had been previously reported (6). These soluble enzymes were relatively unstable, losing their activity after 3 days at 4°C. The particulate enzyme systems, on the other hand, were much more stable, retaining essentially full activity for at least 1 year when stored at -10°C. However, since the soluble UDP-D-galacturonic acid 4-epimerase proved to be easier to purify than the particulate enzyme, the soluble enzyme was used for the determination of the equilibrium constant of the 4-epimerization of UDP-D-galacturonic acid.

Determination of Equilibrium Constant for 4-Epimerization of UDP-D-glucuronic Acid—Radioactive UDP-D-glucuronic acid (2.7 × 10^-4 mmole), radioactive UDP-D-galacturonic acid (0.7 × 10^-4 mmole), or mixtures of the two in the molar ratios of 1:1, 7:3, and 3:7 were incubated at 37°C with the soluble UDP-D-galacturonic acid 4-epimerase preparation (0.37 mg of protein) in 0.08 M Tris buffer, pH 7.5, in a total volume of 60 μl. At specified times (see Fig. 1), 5 to 15 μl samples of reaction mixture were inactivated by heating, the products (mixtures of UDP-D-glucuronic and UDP-D-galacturonic acids) separated by electrophoresis, and their relative quantities determined by radioactivity measurements.
4-Epimerization and Decarboxylation of UDP-<i>d</i>-glucuronic Acid

Vol. 235, No. 4

... counting of radioactivity. These results are presented in Fig. 1. It is evident from the figure that pure UDP-<i>d</i>-glucuronic acid and pure UDP-<i>l</i>-galacturonic acid are enzymatically converted to a mixture containing approximately equimolar quantities of each substance. The same relative quantities are also obtained starting from mixtures of the two UDP-uronic acids. The fact that the same ratio of UDP-<i>d</i>-glucuronic acid to UDP-<i>l</i>-galacturonic acid is obtained regardless of the composition of the original mixture shows that, within the limits of experimental error, equilibrium has been reached. The value of the equilibrium constant \( K \) for the reaction:

\[
\text{UDP-}d\text{-glucuronic acid} \rightleftharpoons \text{UDP-}l\text{-galacturonic acid}
\]
calculated from the average of the ratios at 8 hours is 1.1.

**Decarboxylation of UDP-<i>d</i>-glucuronic Acid**—Since particulate preparations convert UDP-<i>d</i>-glucuronic acid to a mixture of UDP-<i>d</i>-xylose and UDP-<i>l</i>-arabinose, it was of interest to examine the variation of the relative proportions of these reaction products with time.

Labeled UDP-<i>d</i>-glucuronic acid (6.8 \( \times \) 10\(^{-3} \) pmole) was incubated at 37\(^\circ\) with a particulate preparation from radish root, representing 1 g of fresh plant material, in 0.03 M Tris buffer, pH 7.5, in a total volume of 35 \( \mu\)l. At specified times, 5 \( \mu\)l samples were removed, inactivated by heating, and the reaction products, which included UDP-<i>d</i>-galacturonic acid and UDP-pentose, were separated by electrophoresis, and the ratio of UDP-<i>d</i>-xylose to UDP-<i>l</i>-arabinose was determined as follows: the UDP-pentose area was eluted and hydrolyzed in 1 N HCl at 100\(^\circ\) for 30 minutes, and the pentoses were separated chromatographically in water-saturated phenol. The relative amount of <i>d</i>-xylose and <i>l</i>-arabinose obtained was determined by counting. The results are plotted in Fig. 2 (upper curve).

The "digitonin extract" was subsequently tested in a similar manner. UDP-<i>d</i>-glucuronic acid (4.1 \( \times \) 10\(^{-3} \) pmole) was incubated at 25\(^\circ\) in the presence of the mung bean digitonin extract, containing 0.96 mg of protein, in 0.08 M Tris buffer, pH 7.5, in a total volume of 65 \( \mu\)l. Samples of 10 \( \mu\)l were removed, inactivated at specified times, and the ratio of UDP-<i>l</i>-arabinose to UDP-<i>d</i>-xylose was determined as described above. These results are plotted in Fig. 2 (lower curve).

The preponderance of UDP-<i>d</i>-xylose during the early stages of the reaction indicates that the decarboxylation of UDP-<i>d</i>-glucuronic acid yields UDP-<i>d</i>-xylose, the latter then being converted to UDP-<i>l</i>-arabinose. Extrapolation of the lower curve of Fig. 2 gives a value for UDP-<i>d</i>-xylose which approaches 100% at zero time.

Whereas the digitonin extract catalyzed the decarboxylation of UDP-<i>d</i>-glucuronic acid, no reaction occurred upon incubation of UDP-<i>l</i>-galacturonic acid with this enzyme preparation. However, UDP-<i>l</i>-galacturonic acid was converted to a mixture of UDP-<i>d</i>-glucuronic acid and UDP-pentose by a mung bean particulate preparation. In this case, the UDP-pentose presumably originated from the UDP-<i>d</i>-glucuronic acid formed by 4-epimerization of the <i>l</i>-galacturonic acid.

The equilibrium constant for the decarboxylation of UDP-<i>d</i>-glucuronic acid has not yet been determined. In several instances, however, an essentially quantitative conversion of UDP-<i>d</i>-glucuronic to UDP-pentose was observed, indicating that the equilibrium must be far in the direction of decarboxylation.

**Determination of Equilibrium Constant for 4-Epimerization of**
UDP-α-xylose—The ratio of UDP-α-xylose to UDP-α-arabinose was observed to change when mixtures of these two substances were incubated with digitonin extract, indicating the presence of UDP-α-arabinose-4-epimerase. The equilibrium constant for this interconversion was determined as follows:

A mixture of radioactive UDP-α-xylose and UDP-α-arabinose (ratio, 65:35) was used in the determination. The mixed labeled nucleotides (1.2 × 10⁻⁴ μmole) were incubated at 37° with “digitonin extract” containing 0.19 mg of protein and 0.08 μl Tris buffer, pH 7.5, in a total volume of 30 μl. At specified times, 5-μl samples of reaction mixture were inactivated, and the ratio of UDP-α-xylose to UDP-α-arabinose was determined as described above. These results are presented in Fig. 3. It is evident that the reaction has stopped after 12 hours of incubation, indicating that equilibrium has been attained. At this time the ratio of UDP-α-xylose to UDP-α-arabinose is 49:51; hence the equilibrium constant for the reaction UDP-α-xylose ☞ UDP-α-arabinose is 1.0.

**DISCUSSION**

Separation of the enzyme systems responsible for the deacetylation and the 4-epimerization of UDP-α-glucuronic acid made it possible to investigate these reactions in greater detail. Although the UDP-α-glucuronic acid-deacetylase and UDP-α-arabinose-4-epimerase activities were not separated, their activities in the preparations were such that the deacetylation occurred at a considerably faster rate than the subsequent epimerization, resulting in the accumulation of UDP-α-xylose during the early stages of the reaction (see Fig. 2). It can therefore be concluded that UDP-α-glucuronic acid is deacetylated with retention of the α-xylose configuration. The digitonin-solubilized deacetylating system is specific for UDP-α-glucuronic acid; it does not catalyze the deacetylation of UDP-α-galacturonic acid. However, one cannot rule out the possibility that the mung bean seedlings also contain a specific UDP-α-galacturonic acid deacetylase, undetected in this study.

The subsequent formation of UDP-α-arabinose from UDP-α-glucuronic acid by the digitonin extract is accounted for by the presence of a UDP-α-arabinose-4-epimerase system. Since the separation of the activities of the UDP-α-galacturonic acid-4-epimerase and the UDP-α-arabinose-4-epimerase systems has been achieved, it is evident that these two enzyme systems are not identical.

The equilibrium constant K for the 4-epimerization of UDP-α-glucuronic acid was determined to be 1.1, whereas that for the 4-epimerization of UDP-α-xylose was found to be 1.0. Considering the structural similarity of the glycosyl moieties of the compounds involved in the two reactions, it is not surprising that their equilibrium constants differ so little. A value of the same order of magnitude, K = 0.33, has been found by Leloir (12) for the analogous interconversion:

$$\text{UDP-α-glucose} \rightleftharpoons \text{UDP-α-galactose}$$

It is noteworthy that the UDP-α-glucuronic acid decarboxylase, the UDP-α-galacturonic acid 4-epimerase, and the UDP-α-arabinose 4-epimerase are present both in the particulate and soluble fractions of mung bean extracts. Possibly these enzymes are particle-bound in intact cells, but are liberated to varying degrees during the preparation of homogenates.

**SUMMARY**

1. The transformation of UDP-α-glucuronic acid to UDP-α-galacturonic acid and UDP-pentose catalyzed by mung bean seedlings has been shown to involve three separate reactions:

$$\text{UDP-α-glucuronic acid} \rightleftharpoons \text{UDP-α-galacturonic acid} \quad (1)$$
$$\text{UDP-α-glucuronic acid} \rightarrow \text{UDP-α-xylose} + \text{CO}_2 \quad (2)$$
$$\text{UDP-α-xylose} \rightleftharpoons \text{UDP-α-arabinose} \quad (3)$$

2. The equilibrium constants for reactions (1) and (3) were found to be 1.1 and 1.0, respectively.

**REFERENCES**

The 4-Epimerization and Decarboxylation of Uridine Diphosphate d-Glucuronic Acid by Extracts from Phaseolus aureus Seedlings
David S. Feingold, Elizabeth F. Neufeld and W. Z. Hassid


Access the most updated version of this article at http://www.jbc.org/content/235/4/910.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/235/4/910.citation.full.html#ref-list-1