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PREPARATION OF 2-AMINO-2-DEOXY-α-D-GLUCOPYRANOSYL PHOSPHATE FROM URIDINE 5′-(2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSYL PYROPHOSPHATE)*

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ABSTRACT

Hydrazine treatment of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) for 1 h resulted in *N*-deacetylation and cleavage of the pyrophosphate bond to give 2-amino-2-deoxy-α-D-glucopyranosyl phosphate as the main compound. It was separated from other degradation products by paper electrophoresis and isolated in a yield of 50–60%.

INTRODUCTION

Hydrazinolysis was originally introduced in protein chemistry by Akabori *et al.*[1] for cleaving peptide linkages. This method has been applied to the removal of ester- and amide-linked fatty acids from aminodeoxy sugars. During investigations[2] on the structure of lipid A, the effect of hydrazine on phosphate and pyrophosphate bonds was studied. In experiments with such model substances as ribonucleic acids, cephalin, and uridine diphosphate-bound sugars, it was shown that anhydrous hydrazine cleaved the phosphate–phosphate bond of the pyrophosphate diester bridge, whereas phosphate diester bridges were found to be much more resistant[3]. We now report the action of hydrazine on uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) (UDP-GlcNAc) for preparing 2-amino-2-deoxy-α-D-glucopyranosyl phosphate.

RESULTS AND DISCUSSION

Two methods have been described in the literature for the preparation of 2-amino-2-deoxy-α-D-glucopyranosyl phosphate, a chemical synthesis by Maley *et al.*[4], and a biosynthesis by Kornfeld and Glaser[5] based on the partial conversion by a mutase reaction of 2-amino-2-deoxy-D-glucose 6-phosphate into 2-amino-2-deoxy-α-D-glucopyranosyl phosphate to yield a mixture of the two phosphates that could be

*Dedicated to Professor Michael Heidelberger in honor of his 87th birthday.

separated only with difficulty. The method described in this paper is based on the degradation of UDP-GlcNAc by treatment with hydrazine, whereby the phosphate–phosphate linkage is cleaved and the amino group is deacetylated. Under the conditions used, 2-amino-2-deoxy-α-D-glucopyranosyl phosphate was obtained in 50–60% yield. The method is simple and fast, and, probably, can be applied to the preparation of other glycosyl phosphates from the corresponding nucleotide diphosphate sugar.

EXPERIMENTAL

Materials. — UDP-GlcNAc was obtained from Boehringer (Mannheim), UDP-[^{14}C-U]GlcNAc from the Radiochemical Centre (Amersham), and hydrazine from Roth OHG (Karlsruhe).

Analytical methods. — 2-Amino-2-deoxy-D-glucose was determined by the Morgan–Elson method after *N*-acetylation, as described by Strominger *et al.*[6], 2-acetamido-2-deoxy-D-glucose according to Reissig *et al.*[7], and total and phosphatase-released phosphate groups by the method of Lowry *et al.*[8]. Uridine was estimated by measuring the extinction coefficient at 260 nm.

Paper electrophoresis and paper chromatography. — Paper electrophoresis was performed according to Kickhöfen and Warth[9] in pyridine–acetic acid–water (v/v, pH 5.3), and paper chromatography in ethanol–M ammonium acetate (7.5:3, v/v, pH 7.5). Radioactivity on the paper was located with a Packard Radiochromatogram Scanner. For quantitative determination of the radioactivity, the paper electrograms (3 cm wide) were cut into 1-cm strips that were submerged in scintillation fluid [25% Triton X-114, 0.3% diphenyloxazole, and 4% water in xylene, plus additional water (0.5 ml) per sample] and counted on a Packard Scintillation Counter (counting efficiency, 60–70%).

Treatment with hydrazine. — Samples of UDP-[^{14}C-U]GlcNAc (1–5 μmoles 0.05 Ci/mole) were dried *in vacuo* for 4 h at 70°, suspended in anhydrous hydrazine (0.2 ml), and heated in sealed ampoules at 100° in a water-bath. After suitable time intervals, the samples were cooled and hydrazine was removed *in vacuo* in the presence of sulfuric acid. The residues were dissolved in water, and the products separated by paper electrophoresis. In large-scale preparations starting from 50–100 μmoles of UDP-[^{14}C-U]GlcNAc, hydrazine and hydrazides were removed by washing the samples 3 times with acetone (3 ml). The precipitates were dissolved in water and treated as just indicated.

Analysis of the products of reaction with hydrazine. — Paper electrophoresis of a sample of ^{14}C-labelled UDP-GlcNAc treated for 1 h as described in the preceding paragraph gave three defined peaks (see Fig. 1). The corresponding fractions (A–C) were eluted from the paper and purified a second time by paper electrophoresis.

Fraction A ($M_{Glc-6-P}$ 1.08)* was electrophoretically and chromatographically indistinguishable from UDP-GlcNAc and represents unreacted starting material.

*Abbreviation: Glc-6-P, D-glucose 6-phosphate.

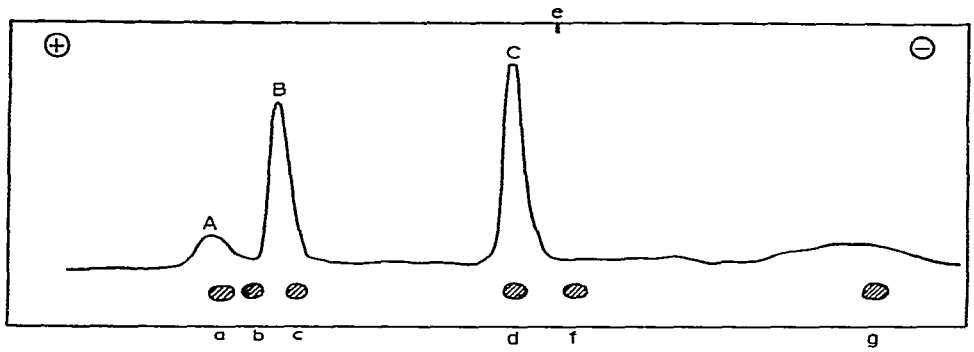

Fig. 1. Radioscan of a fractionation by paper electrophoresis, showing the products obtained after treatment of UDP-[^{14}C-U]GlcNAc with hydrazine for 1 h and standards: (a) UDP-GlcNAc, (b) D-glucose 6-phosphate, (c) 2-acetamido-2-deoxy-D-glucose 6-phosphate, (d) 2-amino-2-deoxy-D-glucose 6-phosphate, (e) origin, (f) D-glucose, and (g) 2-amino-2-deoxy-D-glucose.

Fraction B ($M_{Glc-6-P}$ 0.86) contained a 2-amino-2-deoxy-D-glucose residue and a phosphate group in the ratio of 1:1, and had the mobility of 2-acetamido-2-deoxy-D-glucose 6-phosphate. However, it was nonreducing, and did not react with ninhydrin and in the direct Morgan–Elson test. Mild acid hydrolysis (0.1M hydrochloric acid, 15 min, 100°) or phosphatase treatment yielded 2-acetamido-2-deoxy-D-glucose. These data indicate the presence of 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate.

Fraction C was nearly neutral, contained the bulk of radioactivity, and was stained by ninhydrin but did not react with the alkaline silver reagent, nor with the Morgan–Elson reaction, even after *N*-acetylation. Hydrolysis with phosphomonoesterase or with M hydrochloric acid for 15 min at 100° gave 2-amino-2-deoxy-D-glucose and phosphoric acid in the ratio 1:1. These data indicate the presence of 2-amino-2-deoxy-α-D-glucopyranosyl phosphate.

The radioactive compounds that moved toward the cathode were not studied further.

Time course of degradation of UDP-GlcNAc during treatment with hydrazine. — The formation of 2-amino-2-deoxy-α-D-glucopyranosyl phosphate or other degradation products from UDP-[^{14}C-U]GlcNAc during hydrazine treatment for various lengths of time was studied by paper electrophoretic separation, and the relative amount of the products was determined in a scintillation counter (see Table I and Fig. 2). After 1 h, about 95% of the UDP-GlcNAc was degraded. About 55% of the radioactivity was recovered as 2-amino-2-deoxy-D-glucopyranosyl phosphate. Prolonged treatment with hydrazine led to progressive decrease in the amount of 2-amino-2-deoxy-D-glucopyranosyl phosphate formed, and after 10 h, only 10% of this compound was detectable. At this time, almost all the radioactivity was found to be distributed in the cathodic region of the paper electrophoretogram. The observation that, after 15 min of hydrazinolysis, about 65% of 2-acetamido-2-deoxy-D-gluco-

pyranosyl phosphate and only 13% of 2-amino-2-deoxy-D-glucopyranosyl phosphate were formed indicates that the pyrophosphate bridge of UDP-GlcNAc is more sensitive to hydrazine than is the linkage of the *N*-acetyl group.

TABLE I

PROPORTIONS OF DEGRADATION PRODUCTS OBTAINED FROM UDP-GlcNAc BY TREATMENT WITH HYDRAZINE FOR VARIOUS LENGTHS OF TIME[a]

Components	*Time of treatment (h)*						
	0.25	*0.5*	*1*	*2*	*4*	*6*	*10*
UDP-GlcNAc	13.8	9.4	4.9	3.8	2.9	1.5	
2-Acetamido-2-deoxy-α-D-glucopyranosyl phosphate	65.9	56.0	27.9	17.3	5.1	1.5	0.9
2-Amino-2-deoxy-α-D-glucopyranosyl phosphate	12.9	24.3	56.3	53.8	32.9	18.4	9.6
Unidentified degradation products	7.4	10.3	11.9	25.1	59.1	78.6	89.5

[a]Relative percentage of radioactivity.

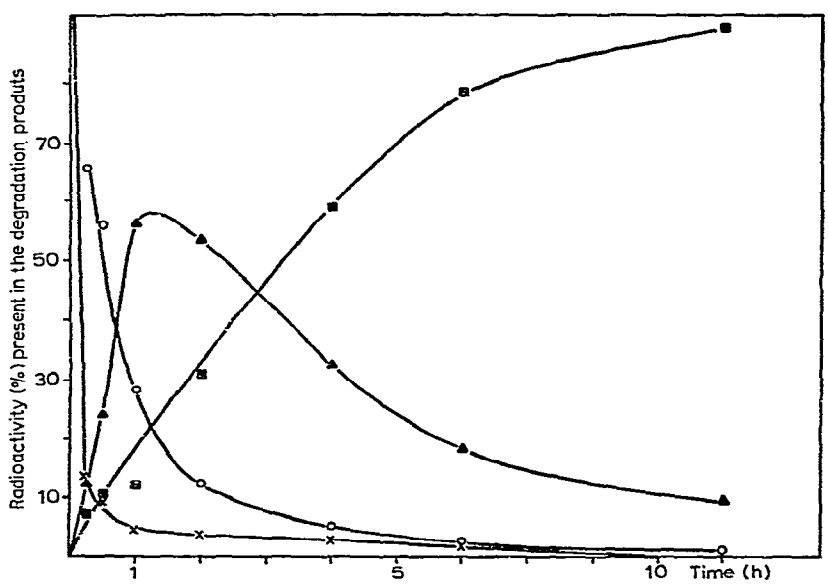

Fig. 2. Formation and degradation of the products obtained by treatment of UDP-GlcNAc with hydrazine: UDP-GlcNAc, ×——×; 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate, O——O; 2-amino-2-deoxy-α-D-glucopyranosyl phosphate, ▲——▲; and other degradation products, ■——■.

Large-scale preparation of 2-amino-2-deoxy-α-D-glucopyranosyl phosphate and determination of the anomeric configuration. — In a large-scale experiment, UDP-[^{14}C-U]GlcNAc (115 mg, ~175 μmoles, 0.01 Ci/mole) was treated with hydrazine for

2 h. After removal of hydrazides and excess of hydrazine with acetone, the degradation products were separated by paper electrophoresis. The almost neutral fraction corresponding to 2-amino-2-deoxy-D-glucopyranosyl phosphate was eluted with water, and the eluate was treated with Dowex 50 (H^+) ion-exchange resin. After centrifugation, the supernatant was lyophilized to give 2-amino-2-deoxy-α-D-glucopyranosyl phosphate in 60% yield (30 mg).

Anal. Calc. for 2-amino-2-deoxy-α-D-glucopyranosyl phosphate: 2-amino-2-deoxy-D-glucose, 69; P, 11.9. Found: 2-amino-2-deoxy-D-glucose, 64; P, 10.9.

The α configuration of the isolated 2-amino-2-deoxy-α-D-glucopyranosyl phosphate was established by *N*-acetylation of a sample and then conversion into UDP-GlcNAc with specific pyrophosphorylase from yeast[10] in the presence of UTP. After purification by paper electrophoresis, the compound was identified by paper chromatography (R_{UMP} 1.44). About 80% of the radioactivity of the original 2-amino-2-deoxy-α-D-glucopyranosyl phosphate was obtained as UDP-GlcNAc.

Anal. Ratio of uridine: GlcNAc: total phosphate: labile phosphate. Calc.: 1:1:2:1. Found: 1.0:0.98:2.0:0.89.

This result established that the α configuration is not modified during treatment with hydrazine.

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