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ANALOGS OF COENZYMES OF CARBOHYDRATE METABOLISM

IX. Synthesis of N_1-Methyladenosine Diphosphate Glucose*

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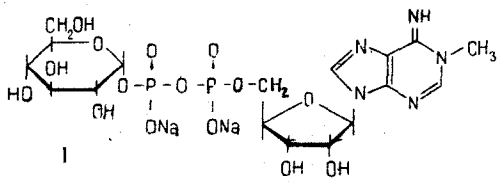
We have previously put forward the hypothesis that the features of the structure of the biochemical properties of natural nucleoside diphosphate sugars are due to the existence of a definite secondary structure of these compounds [2]. The behavior of some synthetic analogs of UDPG** studied in this connection have shown that their biological activity depends directly on the structure of the heterocyclic nucleus. A systematic investigation of the biochemical [3] and chemical [4] properties of analogs of UDPG [5] with a modified uracil nucleus has enabled certain conclusions to be drawn concerning the elements of the structure that are necessary for biological activity and for the existence of a secondary structure.

The elucidation of such laws for the nucleoside diphosphate sugars of the adenosine series is of considerable interest. As is well known, ADGP is capable of participating in the biosynthesis of starch [6]. It was later found that ADGP also participates in other glycosylation reactions [7], and recently Dryuzhinina and Gabrielyan have observed the participation of this coenzyme in a completely different process of carbohydrate metabolism—in the epimerization of a glucose residue at C_4 [8].

In order to study the connection between the structure and biological function of derivatives of ADGP, it is necessary to synthesize a number of analogs of ADGP differing from the natural coenzyme in the structure of the heterocyclic nucleus. The present work is the first report on the synthesis of analogs of ADGP, and gives the synthesis of N_1-methyl-ADGP (I).



I

The behavior of this substance in biological reactions is of particular interest, since analogous substitution at N_3 in the uracil nucleus of UDPG [5] leads to the complete loss of the biological activity of the natural nucleoside diphosphate sugar [3].

The methylation of adenosine derivatives by means of dimethyl sulfate [9, 10] and diazomethane [11] has been reported in the literature.

In order to obtain compound I we first studied the direct methylation of adenosine-5' phosphoromorpholidate. A publication has recently appeared reporting the successful conversion of thymidine-5'phosphoromorpholidate into

Table 1

Chromatographic and Electrophoretic Properties of the Substances Obtained

Substance	R_f in systems		Electrophoretic mobility R_{AMP} at	
	1	2	pH 7.5	pH 4.0
AMP	0.30	0.23	1.00	1.00
ADPG	0.35	0.31	0.75	1.28
N_1-methyl-ADGP	0.80	—	0.45	0.54
Adenosine-5'-phosphoromorpholidate	0.14	0.62	0.50	1.00

*For communication VIII, see [1].

**The following abbreviations are used in the paper: ADGP—adenosine-5' diphosphate α-D-glucopyranose, AMP—adenosine-5' phosphate, and UDPG—uridine-5' diphosphate α-D-glucopyranose.

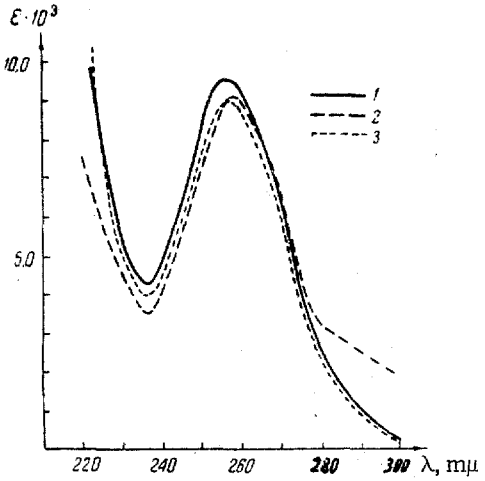
N_3-methylthymidine-5' phosphoromorpholidate [12]. In our case, however, attempts at the analogous reaction were unsuccessful. In the methylation of adenosine-5' phosphoromorpholidate with dimethyl sulfate in neutral aqueous solution, a complex mixture of substances was formed which did not contain products of methylation at the adenine nucleus in appreciable amounts, while on methylation with diazomethane in methanol the main product was apparently adenosine-5' methyl phosphoromorpholidate. This follows from spectral data, the electrophoretic mobility of the reaction product, and the possibility of the conversion of the substance under mild acid hydrolysis (0.1 N HCl, 100°C, 20 min) into adenosine-5' methyl phosphate, which was identified by paper chromatography.

Table 2

UV spectrum of N_1-methyl-ADGP

pH	ε_{260}	λmax	εmax	λmin	εmin	$\varepsilon_{250}/\varepsilon_{260}$	$\varepsilon_{280}/\varepsilon_{260}$	$\varepsilon_{300}/\varepsilon_{260}$
7	9370	257	9570	235	4400	0.85	0.28	0.05
1	8850	257	9000	236	4100	0.84	0.29	0.05
11	9000	258	9100	235	3700	0.78	0.36	0.21

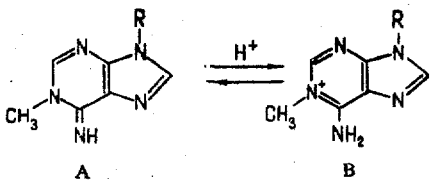
The lack of success of attempts at the methylation of adenosine-5' phosphoromorpholidate at the N_1 of the heterocyclic nucleus forced us to study the direct methylation of ADGP, which could be the shortest route to the synthesis of the desired substance. The synthesis of the ADGP required for this was carried out by condensing adenosine-5' phosphoromorpholidate with α-D-glucose-1 phosphate in anhydrous pyridine by Khorana's method [13] as modified by us [14]. The ADGP obtained was separated from the excess of glucose-1 phosphate and byproducts (AMP and diadenosine pyrophosphate) by ion exchange chromatography on DEAE cellulose (in the Cl^- form). The inorganic salt was separated by gel filtration [15] on Sephadex G-10. The resulting sample of the sodium salt of ADGP was homogeneous on paper chromatography and paper electrophoresis.



UV spectrum of N_1-methyl-ADGP at pH 7 (1), pH 11 (2), and pH 1 (3).

We have studied the methylation of ADGP with dimethyl sulfate in aqueous solution at pH 4.5–5.0. An analysis of the reaction mixture by paper chromatography and electrophoresis showed that under these conditions only one reaction product is formed, and this was isolated by ion-exchange chromatography on Dowex 1 × 2 (Cl^- form) and separated from inorganic salts by gel filtration on Sephadex G-10. The elementary analysis and the results of a determination of the ratio of acid-labile phosphorus to total phosphorus to glucose (1.9 : 2.06 : 1.00) in the material correspond to the structure of the disodium salt of methyl-ADGP.

The electrophoretic mobility (Table 1) shows that the substance has one positive charge more than ADGP at pH 4.0 and 7.5. It possesses a UV spectrum (Figure and Table 2) closely with those of N_1-methyladenosine-5' phosphate [9] and N_1-methyladenosine-5' diphosphate [10]. Extremely characteristic is the change in the UV spectrum on passing from pH 7 to pH 11, which consists in the appearance of considerable absorption in the long-wave region. From these data it may be concluded that even at pH 7.5 the compound is present in the protonated form (B) and derivatives of N_1-methyladenosine are therefore fairly strong bases. The change in the UV spectrum on increasing the pH from 7 to 11 is apparently due to the B→A transition, which sharply distinguishes it from the spectrum of adenosine and its derivatives and directly shows substitution at the N_1 nitrogen of the adenosine nucleus, and, consequently, confirms the structure of the substance obtained as N_1-methyladenosine diphosphate glucose.



A B

Experimental

The amounts of material were determined from the optical density at 260 mμ (for ADGP, ε_{260} = 15000 [19]). The chromatography was carried out on Leningrad (medium) paper with the following systems of solvents 1) (ascending)

saturated solution of $(NH_4)_2SO_4$–isopropanol–1M phosphate buffer with pH 7.2 (79 : 2 : 19), 2) (descending) ethanol-1 M solution of ammonium acetate, pH 7.5 (5 : 2). The paper electrophoresis was carried out in a EFA-1 instrument in the following buffer solutions: 1) 0.05 M triethylammonium bicarbonate (pH 7.5), 2) 0.02 M triethylammonium acetate (pH 4.0) at a gradient of 23 V/cm. The position of the spots absorbing in the ultraviolet was established by means of an "Ultrakhemiskop." The phosphorus was determined colorimetrically [16] and the glucose by the method of Park and Johnson [17] after hydrolysis with 0.01 N hydrochloric acid at 100°C for 15 min.

The R_f values and electrophoretic mobilities relative to AMP (R_{AMP}) for the substances obtained are given in Table 1 on p. 105.

Adenosine diphosphate glucose. Fifty milligrams (2250 O.U., 0.15 mmole) of adenosine-5' phosphoromorpholidate [18] ($E^{1\%}_{1\,cm}$ = 192, which corresponds to 81% of adenosine-5' phosphoromorpholidate, the substance being chromatographically homogeneous) was dissolved in 10 ml of absolute pyridine. To the resulting solution was added 4.5 ml (0.45 mmole) of a 0.1 M solution of the morpholine dicyclohexylcarboxamidinium salt of α-D-glucose-1 phosphate in absolute pyridine. The reaction mixture was dried by the distillation off of the pyridine (1 × 20 ml), the residue was dissolved in 1.5 ml of absolute pyridine, and the solution was placed in a thermostat at 60°C for 4 hr, being carefully protected from the access of moisture (the course of the reaction was monitored by paper electrophoresis in buffer II). The reaction solution was evaporated to dryness and traces of pyridine were distilled off with water. The residue was dissolved in a mixture of water and ether (10 : 5 ml), and extracted with ether (3 × 5 ml). The ethereal layer was washed with water (2 × 5 ml), and the combined aqueous phases were passed through a column of the cation exchanger Dowex 50 (Na^+ form, 2 × 10 cm). The column was washed with water until there was no longer any UV absorption in the eluate. The resulting solution was then passed through a column of DEAE-cellulose (Cl^- form, 1.0 × 15.5 cm), and the column was washed with water until there was no longer any UV absorption in the eluate, after which it was eluted with 0.003 N hydrochloric acid (350 ml) (530 O.U. of AMP and the excess of glucose 1-phosphate were eluted), after which the resin was washed with water to neutrality, and then 0.03 M aqueous sodium chloride (300 ml) eluted the diadenosine pyrophosphate (70 O.U.). The ADGP was eluted from the resin with 0.08 M salt solution (350 ml). The fractions containing the ADGP (1650 O.U., 73.5%) were evaporated to dryness, the residue was dissolved in 1 ml of water and the solution was freed from salt in a column of Sephadex G-10 (3 × 83 cm). The ADGP began to appear after the passage of 90 ml of water and salt appeared in the eluate after the passage of 175 ml. The fractions containing the ADGP and not containing salts were combined (1480 O.U.) and freeze-dried. The substance was chromatographically and electrophoretically homogeneous (see Table 1).

N_1-Methyladenosine diphosphate glucose. In small portions, with stirring, dimethyl sulfate (189 mg, 1.5 mmole) was added to a solution of 1500 O.U. (0.1 mmole) of the sodium salt of ADGP in 5 ml of water. The addition took 1 hr, the pH of the reaction solution being kept at 4.5–5.0 (pH-meter) by the addition of a 1 M aqueous solution of sodium carbonate. After this, the reaction mixture was stirred until the pH of the solution ceased to change (another 3–4 hr). The methylation process was checked by paper chromatography in system 1 and by electrophoresis at pH 7.5 and 4.0 (see Table 1). When the reaction did not go to completion, methylation was repeated under the same conditions with the addition of a further 1.5 mmole of dimethyl sulfate. When the initial ADGP had reacted sufficiently, the reaction solution was diluted ten-fold and passed through a column of Dowex 1 × 2 (Cl^- form, 2 × 10 cm). The column was washed with water and a 0.01 M aqueous solution of sodium chloride (100 ml each). The N_1-methyl-ADGP was eluted with 0.03 salt solution (740 O.U., 80%). The fractions containing the substance were evaporated to dryness and desalted on a column of Sephadex G-10 (3 × 83 cm). The column was washed with water. The substance appeared in the eluate after 90 ml of water had passed through, and after 135 ml the salt began to issue. Freeze-drying of the desalted product yielded the disodium salt of N_1-methyl-ADGP (42.5 mg, 68%).

Found, %: P 9.4. Calculated for $C_{17}H_{26}O_{15}N_5P_2Na_2$, %: P 9.52.

Ratio of acid-stable phosphorus to total phosphorus to glucose. Found: 1.09 : 2.06 : 1.00. Calculated 1 : 2 : 1.

The spectral characteristics of N_1-methyl-ADGP (see figure) are given in Table 2. The substance was chromatographically and electrophoretically homogeneous.

Summary

1. The synthesis of N_1-methyl-ADGP, an analog of the natural coenzyme adenosine diphosphate glucose, has been synthesized.

2. On the basis of an analysis of the UV spectra and the electrophoretic mobility of derivatives of N_1-methyladenosine, the hypothesis has been put forward that at pH 7.5 it exists in solutions in the protonated form.

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