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Abstract

Full Text

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CHEMISTRY

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SECONDARY STRUCTURE OF NUCLEOSIDE DIPHOSPHATE SUGARS

HYDROGENATION OF URIDINE DIPHOSPHATE GLUCOSE

AND ITS SYNTHETIC ANALOGS

We recently proposed that the regularities in the structure and biochemical properties of natural nucleoside diphosphate sugars (NDP-sugars) are determined by the existence of a definite secondary structure of these compounds, fixed by hydrogen bonds ([1]). A systematic biochemical study of synthetic analogs of uridine diphosphate glucose (UDPG) ([2]), being carried out in the Laboratory of Biological Tests of the Institute of Chemistry of Natural Compounds of the Academy of Sciences of the USSR ([3,4]), as well as individual data obtained in other laboratories ([5,6]), agree well with the consequences following from the hypothesis of the secondary structure of NDP-sugars. However, biochemical data of this kind cannot in principle have an unambiguous interpretation: the identical specificity of different enzymes acting on UDPG may be associated either with the necessity of a secondary

Fig. 1. Kinetics of hydrogenation of nucleoside-5′-phosphates and nucleoside diphosphate sugars (**1** and **2**, respectively) in lithium acetate buffer and in a 7 M urea solution (dashed curves). For hydrogenation conditions see the experimental section. Nucleoside residues: **a** and **b**—uridyl (I); **c**—azauridyl (III); **d**—cytidyl (V); **e**—N_3-methyluridyl (IV); **f**—2′-deoxyuridyl (II)

reaction scheme

Figure 2: reaction scheme

structure of NDPS for the formation of an enzyme–substrate complex, or with a center, identical for different enzymes, that binds the substrate. In other words, the grouping necessary for participation in the enzymatic reaction, $N_{(3)}H—C_{(4)} = X$ $(X = O, S)$, in UDPG analogs [1] serves to bind the nucleus either to the monosaccharide residue of NDPS, or to the enzyme.

In the first case there must exist a fixed secondary structure; in the second, a definite group binding the coenzyme must be identical in the enzymes studied (sucrose synthetase, arbutin synthetase, UDPG hydrogenase).

In this connection, for an independent and unambiguous proof of the existence of a secondary structure in NDPS, it is necessary to study the chemical and physical properties of these compounds. The present communication gives data on the kinetics of the catalytic hydrogenation of UDPG and its synthetic analogs.

In connection with the need to obtain one of the UDPG analogs—its 5,6-dihydro derivative*, which is of interest for testing the hypothesis mentioned above [1], we studied the catalytic hydrogenation of UDPG and of several related compounds. Hydrogenation of the double bond in uridine and some of its derivatives over rhodium on alumina is well known [7,8].

I $(X = OH)$; II $(X = H)$

(III) (IV) (V)

a) $R = P(O)(OH)OP(O)OC_6H_{11}O_5$

b) $R = P(O)(OH)_2$

However, it turned out that hydrogenation of UDPG (Ia) proceeds very slowly and is completed only after 6–8 h. Under the same conditions, uridine 5′-phosphate (Ib) is hydrogenated rapidly and quantitatively in 15–30 min. This fact indicated a substantial difference in the steric accessibility of the double bond in I and II for addition of hydrogen or for fixation on the surface of the catalyst, which must have been a consequence of conformational differences between the two compounds and confirmed the hypothesis of the existence of a "fixed" secondary structure in UDPG. If this is indeed so, then it should be expected that, for all analogs possessing a secondary structure and exhibiting biological activity [3,6], hydrogenation will be hindered, in contrast to inactive analogs lacking a fixed secondary structure.

* The synthesis of this compound will be described separately.

In connection with this, we investigated more carefully the hydrogenation of UDPG and its synthetic analogs, studying the kinetics of this process. The experiments were carried out under conditions ensuring a constant rate of stirring

of the reaction mixture (see the experimental part). The hydrogenation process was monitored by the change in absorption of the reaction mixture at 260 mμ. At this wavelength the absorption of dihydrouridine and analogous compounds is very small in comparison with the absorption of the starting substances, which contain an aromatic heterocyclic ring (cf. [9]).

The difference in the rate of hydrogenation of UMP and UDPG is seen in Fig. 1,a. A similar picture is observed in the case of 6-azauridine diphosphate glucose (IIIa), which is hydrogenated considerably more slowly than 6-azauridine 5′-phosphate (IIIb) (see Fig. 1,b). By contrast, in the case of derivatives of 3-*N*-methyluridine (IVa, b), cytidine (Va, b), and 2′-deoxyuridine (IIa, b), no difference is observed in the rate of hydrogenation of the nucleoside 5′-phosphates and the corresponding nucleoside diphosphate glucoses (see Fig. 1,c–e). The rate of hydrogenation differs somewhat for different batches of catalyst; however, the ratio of the hydrogenation rates for the nucleoside monophosphate and the nucleoside diphosphate glucose remains constant.

The results obtained indicate the existence of a quite definite specificity for the heterogeneous-catalytic hydrogenation reaction of uridine derivatives and its analogs. This specificity is opposite to the specificity observed for enzymatic reactions of UDPG analogs and is in complete agreement with the conclusions arising from the concept of the secondary structure of NDPS. Thus, Ia and IIa, which are capable of forming a secondary structure and of participating in enzymatic reactions, are hydrogenated considerably more slowly than their corresponding monophosphates, whereas IVa and Va, in which formation of a "twisted" secondary structure of the analogous type is impossible, do not participate in enzymatic reactions and are hydrogenated at a rate equal to the rate of hydrogenation of the corresponding monophosphates. The reason for such differences with respect to the hydrogenation process remains not entirely clear. Most likely, the existence of the secondary structure of NDPS hinders adsorption of the compound on the catalyst or the approach of hydrogen to the intermediate complex being formed, which inhibits the hydrogenation process.

Thus, the data obtained indicate a direct correspondence between heterogeneous-catalytic and enzymatic reactions in the series of nucleoside diphosphate sugars and serve as direct experimental proof of the existence of a secondary structure in these compounds. In this connection, it is of interest to study the biochemical properties of 2′-deoxyuridine diphosphate glucose (IIa), recently synthesized in our laboratory. On the basis of the results of catalytic hydrogenation, it may be expected that this compound has no fixed secondary structure and should not enter into enzymatic reactions.

The nature of the bonds participating in the formation of the secondary structure of NDPS can be judged from certain data obtained in the study of the hydrogenation of UDPG and UMP Ia and Ib in 7 M urea solution (see Fig. 1,b). It turned out that the rate of hydrogenation of uridine 5′-phosphate under these conditions is noticeably reduced in comparison with the rate of hydrogenation in water, whereas the rate of hydrogenation of UDPG increases somewhat. This

result should evidently be explained by weakening of the secondary structure of UDPG through rupture of intramolecular hydrogen bonds under the action of urea. Although this effect is not reproduced in strictly quantitative fashion and varies somewhat from experiment to experiment, which is evidently associated with the nonstandard nature of individual batches of catalyst and the complication of the hydrogenation process in concentrated urea solutions, nevertheless qualitatively this result clearly indicates that fixation of the secondary structure of UDPG is effected through intramolecular hydrogen bonds, which, as is well known, are weakened in concentrated—

solutions of urea. The detailed data presented in this work thus confirm that UDPG and its analogs, which exhibit biological activity as substrates in enzymatic reactions, possess in solution a secondary structure fixed by intramolecular hydrogen bonds.

Experimental Section

Preparation of the rhodium catalyst $Rh(Al_2O_3)$. To a saturated solution of 1 g of $RhCl_3 \cdot 4H_2O$ in water were added 7.4 g of neutral aluminum oxide and 7 ml of a 40% formaldehyde solution. The mixture was thoroughly stirred, heated to 60°, and at this temperature, with constant stirring, 2 ml of a 50% solution of caustic potassium were added dropwise. The resulting mixture was boiled for 30 min, then washed by decantation with water to a neutral reaction and until Cl ions were absent from the wash waters. The catalyst was filtered off and dried in vacuo over ignited silica gel.

Hydrogenation of nucleoside 5′-phosphates and nucleoside diphosphate sugars (see Fig. 1, *a, b, c, d,* and *e*). 0.1 mmol of the substance was dissolved in 1 ml of lithium acetate buffer (0.005 M, pH 4.0), 5.2–6.0 mg of catalyst were added, and the mixture was hydrogenated (1 atm, 20°), with stirring by means of a vibromixer at constant frequency and amplitude of oscillation.

At specified time intervals aliquots were withdrawn and, after appropriate dilution with water, the optical density at 260 mμ was measured (Fig. 1).

To determine the effect of urea on the rate of hydrogenation of UMP and UDPG, the substances were dissolved in a 7 M urea solution and hydrogenated as described above (see Fig. 1, *b*).

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Note: Figure translations are in progress. See original paper for figures.