PRELIMINARY NOTE

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The interaction of adenosine diphosphate glucose and its synthetic analogs with uridine diphosphate glucose–fructose glucosyltransferase from pea seedlings

After the recent discovery that some enzymes of oligo- and polysaccharide biosynthesis can use uridine diphosphate glucose (UDPG) as well as adenosine diphosphate glucose (ADPG)[1,2], the question arose about the mechanism of interaction of such different compounds with the same enzyme It has been shown[3] that several enzymes of UDPG metabolism have rather strict specificity to the structure of nucleoside moiety, the grouping $-C^2(X)-N^3(H)-C^4(X)-$ in heterocyclic nucleus seems to be necessary for the ability of UDPG analogs to participate in enzymic reactions

Unfortunately, similar information is still lacking for ADPG We have tried to clarify this point with the use of synthetic ADPG analogs with modified nucleoside moeity

UDPG–fructose glusocyltransferase (UDPG D-fructose 2-glucosyltransferase, EC 2 4 1 13) from pea seedlings was chosen for the investigation, its purification has recently been described[4] Similar enzymes from wheat germ[5] and maize grains[6] were reported to interact with UDPG and ADPG

We have found that ADPG is also capable of interacting with the enzyme from pea seedlings The results obtained demonstrate that initial velocity (v_0) of reaction

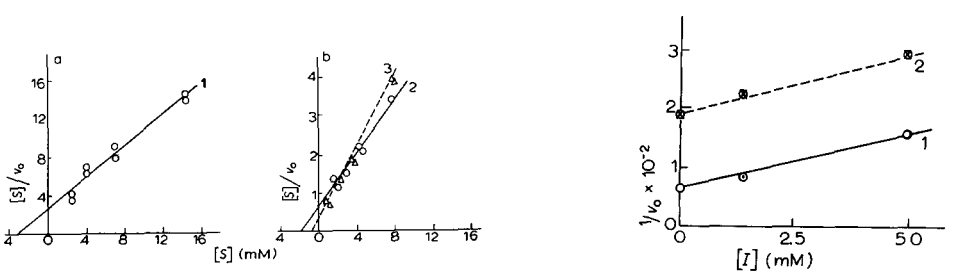


Fig 1 (a) Effect of ADPG concentration on the rate of sucrose formation with sucrose synthetase (b) Effect of UDPG concentration (2) and UDPG with ADPG (3) on the rate of sucrose formation The ratio of the substrate concentration to the rate is plotted *versus* substrate concentration The lines are fitted by least square method For conditions of assay see Table I The content of the nucleotides in the assay mixture were (1) ADPG (0 4–3 μmoles), (2) UDPG (0 3–1 6 μmoles), (3) UDPG (0 2–2 μmoles) + ADPG (3 μmoles) Concentration of substrate ([S]) is expressed in μmoles/ml, and velocity (v_0) is expressed as μmoles of sucrose after 30-min incubation

Fig 2 The inhibitory effect of 1-*N*-methyl-A DPG on the sucrose formation in presence of UDPG (Curve 1) and ADPG (Curve 2) The dependence of v_0 from inhibitor concentration For conditions of assays, see Table I The amount of enzyme is 900 μg, the time of incubation, 30 min Nucleoside contents in incubation mixture (1) UDPG (1 2 μmoles) + 1-*N*-methyl-ADPG (0 30 and 0 98 μmole), (2) ADPG (0 8 μmole) + 1-*N*-methyl-ADPG (0 30 and 0 98 μmole)

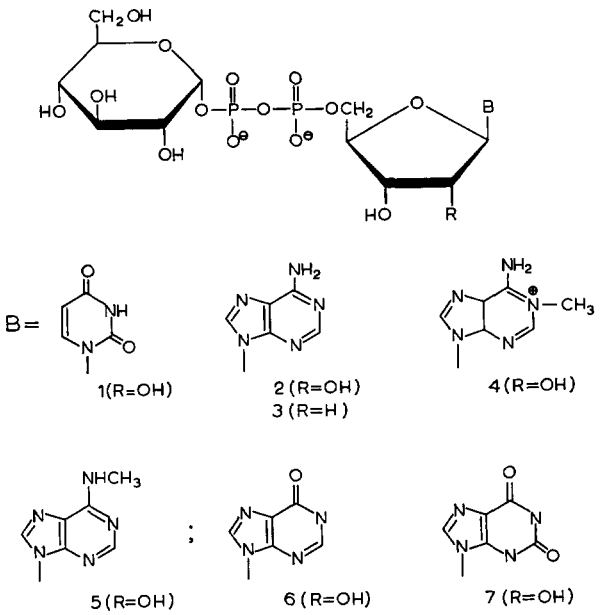
Abbreviations UDPG, uridine diphosphate glucose, ADPG, adenosine diphosphate glucose

with ADPG as substrate under conditions of assay is about 25% of that with UDPG

It was found that the Michaelis constant for ADPG is slightly higher than that for UDPG (3 2 mM and 1 9 mM, respectively), while maximum velocity of reaction is considerably slower (about 0 25 of that for UDPG) (see Fig. 1a)

The rate of sucrose formation from UDPG is slightly lowered in the presence of high concentration of ADPG (see Fig 1b) The inhibition observed seems not to be competitive.

Some synthetic analogs of ADPG were prepared for elucidation of structural elements of ADPG essential for its interaction with UDPG–fructose glucosyltransferase These included nucleoside diphosphate glucoses containing 2-deoxyadenosine (3), 1-*N*-methyladenosine (4), 6-*N*-methyladenosine (5), inosine (6) and xanthosine (7) moieties



Analog 4 was obtained by methylation of ADPG with dimethyl sulphate[7], Analogs 3, 5, 6 and 7 were prepared from the corresponding nucleoside 5′-phosphates *via* nucleoside 5′-phosphomorpholidates[8,9] 6-*N*-Methyladenosine 5-phosphate, the starting compound for the synthesis of Analog 5 was obtained by Dimroth rearrangement of 1-*N*-methyladenosine 5′-phosphate[10], for the preparation of xanthosine 5′-phosphate deamination of guanosine 5′-phosphate was used[11] All nucleoside diphosphate sugars were purified by ion-exchange chromatography and were desalted by gel filtration through the Sephadex G-10 column[12]

The interaction of ADPG analogs with enzyme were studied in this work using kinetic methods. The enzyme preparation was highly purified and free from interfering enzymes[4] To compare the initial rates of enzyme-catalyzed reaction of ADPG and its synthetic analogs with the initial rates observed with UDPG, their concentrations

TABLE I

THE INTERACTION OF ADPG AND ITS SYNTHETIC ANALOGS WITH UDPG–FRUCTOSE GLUCOSYLTRANSFERASE FROM PEA SEEDLINGS

v_0 is assumed to be 1 00 for UDPG The incubation mixture contained (except for nucleotides) fructose (3 μmoles), Tris–HCl buffer (pH 7 3) (75 mmoles) and 750 mg enzyme preparation[4] in a total volume of 0 2 ml After incubation for 30 min at 37° 0 2 ml 1 M NaOH and water (to 0 5 ml) were added and sucrose was determined colorimetrically[13]

No	*Substance*	*Contents in incubation mixture (mmoles)*	*v_0 (mmoles sucrose in incubation mixture after 30 min)*
1	UDPG	0 60	1 00
2	ADPG	0 60	0 25*
3	2′-Deoxy-ADPG	0 48	0 10*
4	1-*N*-Methyl-ADPG	1 1	<0 05**
5	6-*N*-Methyl-ADPG	1 2	<0 05
6	IDPG	2 0	<0 05
7	XDPG	1 5	<0 05

* The sucrose formation was confirmed by paper chromatography in *n*-butanol–acetic acid–water (4 1 2, by vol) system

** The slight differences of absorbance between tests and blanks were observed, but these were found to be independent of enzyme concentration and time of incubation

were adjusted to be equal to or larger than the concentration of UDPG, with provided saturation of the enzyme

The data of Table I demonstrate that only 2′-deoxy-ADPG may be used as substrate for sucrose formation Thus the substitution of hydroxyl at $C^{2'}$ of ribose moiety of ADPG does not deprive the substance of its biological activity Analogs 4 and 5 cannot participate in the enzymic reaction, this result shows that the grouping $-N^1-C^6(NH_2)-$ of adenine moiety is essential for ADPG interaction with the enzyme The incorporation of grouping CO–NH–CO in purine nucleus leads to Analogs 6 and 7 which are unable to substitute UDPG or ADPG in the enzymic reactions investigated

It seems remarkable that 1-*N*-methyl-ADPG is a strong inhibitor of the enzymic reaction both with ADPG and UDPG although it is unable to serve as substrate for the enzyme

Further investigation of the behavior of ADPG analogs seems necessary to obtain more definitive information about the role of different elements of ADPG structure in enzyme–substrate interaction

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