ADENOSINE DIPHOSPHATE MANNOSE, ADENOSINE DIPHOSPHATE GALACTOSE AND ADENOSINE DIPHOSPHATE ACETYLGLUCOSAMINE FROM CORN GRAINS

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Received December 3, 1963

In a recent communication (Recondo, Dankert and Leloir, 1963) the isolation of ADP-glucose from corn grains was reported. The compound was contaminated with other substances of similar structure which have now been identified as ADP-mannose and ADP-galactose. In addition, ADP-acetylglucosamine has been found as a minor component of the corn extract.

The isolation was carried out as described before with some modifications. After the elution from charcoal the extract was passed through a Dowex-1 X4 column in the chloride form, and the adsorbed nucleotides were eluted with a linear gradient of ammonium chloride (0.10 to 0.24 M). Five peaks of material absorbing at 260 mμ were detected. The uridine diphosphate sugars appeared in the second peak and those of adenosine in the fourth. The latter fraction (24 μmoles) was purified by paper chromatography in ethanol-ammonium acetate of pH 7 and ethanol-ammonium acetate of pH 3.8 (Paladini and Leloir, 1952). This treatment gave two fractions with the

This investigation was supported in part by a research grant (No. GM 03442) from the National Institutes of Health, U. S. Public Health Service, by the Rockefeller Foundation and by the Consejo Nacional de Investigaciones Científicas y Técnicas (República Argentina). Two of the authors (M.D. and S.P.) are fellows and one (E.R.) is a career investigator of the latter institution.

mobilities of UDP-glucose (peak 4a) and ADP-glucose (peak 4b) respectively, in the two solvents.

ADP-acetylglucosamine.-The band running like UDP-glucose in the neutral and acid ethanol-ammonium acetate solvents (peak 4a) had an ultraviolet spectrum similar to that of adenosine. After acid hydrolysis at pH 2 and 100° C for 10 minutes the substance decomposed into a nucleotide and a sugar. The nucleotide moiety was identified as ADP and traces of AMP by paper electrophoresis in phosphate buffer (0.1 M) of pH 7.5 and paper chromatography in the neutral ethanol-ammonium acetate solvent. A more drastic acid hydrolysis in 3 N HCl at 100° C for 1 hour and chromatography in isopropanol-HCl-water (170:41:39) (Wyatt, 1951), showed that adenine was the only base present. The sugar moiety resulting from the mild acid hydrolysis had the same mobility as acetylglucosamine in butanol-pyridine-water (6:4:3). It reduced the alkaline silver reagent (Trevelyan, Procter and Harrison, 1950), and gave a positive Morgan and Elson reaction (Cardini and Leloir, 1957) and a negative ninhydrin test. After running with the same solvent but with borate treated paper (Cabib, Leloir and Cardini, 1953) only a single spot in the acetylglucosamine zone was detectable. This technique clearly distinguishes the latter from acetylgalactosamine and acetylmannosamine. The results of analysis for phosphate, reducing power after hydrolysis (Park and Johnson, 1949) and for acetylhexosamine (Reissig, Strominger and Leloir, 1955) are shown in Table I and correspond to ADP-acetylglucosamine. The non-hydrolyzed nucleotide gave a negative acetylhexosamine reaction which suggests that the sugar is linked to the phosphate through the carbon one. As in the case of UDP-acetylglucosamine (Paladini and Leloir, 1952) the compound was found to be stable during paper chromatography with an alkaline solvent.

ADP-mannose, ADP-galactose and ADP-glucose.-Many tests were carried out in order to find a satisfactory solvent system for

the separation of nucleoside diphosphate sugars differing only in the sugar moiety. Ethyleneglycol dimethylether-methyl ethyl ketone-morpholinium tetraborate(*) (7:2:3) (Solvent A), or alternatively ethanol-methyl ethyl ketone-morpholinium tetraborate (7:2:3) (solvent B) gave a good separation between ADP-glucose, ADP-galactose and ADP-mannose as is shown in Table I (Carminatti, Passeron, Recondo and Dankert, in preparation).

TABLE I

Composition of the Nucleotides

Compound identified	$R_{ADP\text{-}glucose}$ in solvent B	µmoles obtained	Total P[1]	Adenosine content[2]	Sugar content[3]
ADP-acetyl-glucosamine	1.42	1.9	1.98	1.00	1.03[4] 1.11[5]
ADP-glucose	1.00	9.00	2.04	1.00	1.00[6]
ADP-mannose	0.89	4.1	1.90	1.00	1.00[5]
ADP-galactose	0.74	3.7	1.80	1.00	1.14[5]

[1] Fiske and SubbaRow (1925).

[2] Calculated from absorbancy at 260 mµ and taken as 1.00.

[3] The corresponding sugars were used as standards.

[4] As acetylglucosamine (Reissig, Strominger and Leloir, 1955).

[5] Park and Johnson (1949).

[6] Glucose oxidase (Huggett and Nixon, 1957).

Resolution was improved by adding EDTA to the solvent (0.01 M in the aqueous phase); the paper was impregnated with EDTA 0.01 M and dried before use.

(*) Prepared by adding solid boric acid to a 0.5 M aqueous morpholine solution until the pH was 8.6.

Fraction 4b was submitted to paper chromatography in solvent B for five days. The bands which had the same mobilities as synthetic samples of ADP-glucose, ADP-mannose and ADP-galactose were eluted. The nucleotides were then adsorbed on charcoal at pH 3 and eluted with ethanol-ammonia-water (25:0.5:75). The relative amounts (as measured by absorbancy at 260 mμ) were: ADP-glucose (100), ADP-mannose (47), ADP-galactose (42). The nucleotides were pure except for minor contaminations from each other. Paper chromatography in butanol-pyridine-water (6:4:3) after acid hydrolysis at pH 2 for 10 minutes showed that the fractions with $R_{ADP\text{-}glucose}$ of 1.00, 0.89 and 0.74 contained glucose, mannose and galactose as the main sugar respectively. The nucleotide residue remaining after the acid hydrolysis was shown to be ADP by paper electrophoresis in sodium carbonate-sodium bicarbonate buffer of pH 9.2. This system separates this nucleotide from UDP, GDP and CDP. The results of analysis are shown in Table I.

ADP-mannose was further purified by chromatography in ethanol-ammonia (70:30). This treatment decomposes ADP-glucose and ADP-galactose in AMP and a cyclic hexose phosphate but does not act on the mannose nucleotide which has the hydroxyl groups at position 1 and 2 in trans relationship. A final purification was carried out by chromatography in ethanol-ammonium acetate of pH 3.8. After mild acid hydrolysis, mannose was identified by paper chromatography in butanol-pyridine-morpholinium tetraborate (0.05 M) (7:5:2) or paper electrophoresis in potassium tetraborate (0.05 M). Both techniques clearly distinguishes mannose from fructose.

ADP-galactose was not purified further. After mild acid hydrolysis the identity of the sugar moiety was confirmed by paper chromatography in butanol-pyridine-morpholinium tetraborate (0.05 M) (7:5:2) or ethyl acetate-acetic acid-boric acid (saturated solution in water) (9:1:1). These solvents will separate compounds such as galactose and sorbitol which run together in other systems.

Full reports on the isolation of nucleotides from corn grains and on paper chromatography with morpholine borate solvents will be published elsewhere.

Acknowledgments.-The authors wish to express their gratitude to Noel & Co. Ltd. for collaboration in the large scale step.

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