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ISOLATION OF ADENOSINE DIPHOSPHATE D-GLUCOSE FROM CORN GRAINS

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Adenosine diphosphate D-glucose (ADPG) has been found to react about ten-fold faster than uridine diphosphate D-glucose (UDPG) in the synthesis of starch (Recondo and Leloir, 1961). Subsequently some evidence was obtained indicating that ADPG is a natural compound. Thus a specific enzyme which catalyzes ADPG formation from adenosine triphosphate and glucose 1-phosphate was isolated from wheat flour (Espada, 1962). Furthermore Kauss and Kandler (1962) observed that after administering $^{14}CO_2$ to Chlorella a radioactive compound which cochromatographed with synthetic ADPG could be detected. Recently, a phosphorylase which acts preferentially on ADPG and catalyzes its phosphorolysis to adenosine diphosphate and glucose 1-phosphate has been found in wheat germ (Dankert, Gonçalves and Recondo, 1963). The present communication reports the isolation of ADPG from an alcoholic extract of corn grains.

Five kilograms of sweet corn grains in the milky stage were disintegrated in 10 liters of ethanol (95 %) with a blendor and filtered. The extract was precipitated with mercuric acetate as described by Caputto, Leloir, Cardini and Paladini (1950). After decomposing the mercury salts with H_2S the nucleotides were adsorbed on charcoal and eluted with ethanol-ammonia-water (25:0.50:75). Aliquots were then submitted to paper chromatography in ethanol-ammonium acetate of pH 7.5 (Paladini and Leloir, 1952) (Whatman 17 paper). A band with the same mobility of a synthetic ADPG was eluted and rechromatographed in ethanol-ammonium acetate of pH 3.8 (Paladini and Leloir, 1952). After a final chromatography in the pH 7.5 solvent, a single band with the mobility of ADPG was isolated. About 8 μmoles of ADPG were obtained per kilogram of corn grain.

The ultraviolet spectrum of the compound was identical to that of adenosine in acid, neutral or alkaline medium. The substance moved like ADPG during paper electrophoresis in sodium carbonate-sodium bicarbonate buffer of pH 9.2 and sodium phosphate buffer of pH 7.5. Chromatography in ethanol-ammonia-water (Paladini and Leloir, 1952) produced adenosine monophosphate and a cyclic sugar phosphate as does synthetic ADPG.

After acid hydrolysis at pH 2 and 100º for 10 minutes, the substance gave adenosine diphosphate, some adenosine monophosphate and traces of adenine, as was shown by chromatography in the neutral ethanol-ammonium acetate solvent. Paper chromatography of the hydrolysis products in butanol-pyridine-water (Jeanes, Wise and Dimler, 1951) and paper electrophoresis in potassium tetraborate followed by treatment with the silver nitrate-sodium hydroxide reagent (Trevelyan, Procter and Harrison, 1950) revealed a large glucose spot, and smaller ones in the zones corresponding to galactose and mannose. Adenine was the only ultraviolet absorbing product produced by hydrolysis of the ADPG fraction in 3 N HCl at 100º during 1 hour, as judged by chromatography in isopropanol-HCl-water (170:41:39) (Wyatt, 1951).

The ratio adenosine-total phosphate-reducing sugars expressed as glucose was 1:1.9:1.04. Total phosphate was determined by the Fiske and Subbarow method (1925) and reducing power by the Somogyi (1945) and Nelson (1944) methods.

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An enzymatic assay with glucose oxidase (Huggett and Nixon, 1957) after acid hydrolysis showed that 60 % to 70 % of the reducing sugar was glucose. The nucleoside diphosphate sugar was tested with the starch synthesizing enzyme from beans (Leloir, Fekete and Cardini, 1961) and the orthophosphate adenylyl transferase (Dankert, Gonçalves and Recondo, 1963) from wheat germ. The results are shown in Table I.

Apparently the substance isolated is mainly ADPG contaminated with other nucleotides of similar structure. Further studies are being carried out to clarify the nature of the contaminating nucleotides.

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TABLE I

Enzyme	Product measured	ADPG from corn (100 mμmoles)[1]	Synthetic ADPG (100 mμmoles)[1]
		mμmoles	mμmoles
Starch synthetase	Adenosine diphosphate[2]	45	90
Orthophosphate adenylyl transferase	Adenosine diphosphate[2]	50	66
	Glucose 1-phosphate[3]	40	57
Glucose oxidase	Glucose	70	100

1 Calculated from absorbancy at 260 mμ.

2 Determined with pyruvate kinase (Cabib and Leloir, 1958).

3 Measured with phosphoglucomutase-glucose 6-phosphate dehydrogenase (Munch-Petersen, 1955).

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