Free Nucleotides in the Primary Root of Maize

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Dedicated to Academician S. Prát on the occasion of his 80th birthday

Abstract. Free nucleotides of the primary root of maize were extracted with 5% HClO₄ and separated on a column of Dowex 1 × 8 ion exchanger in HCOO⁻ cycle. A two-step elution gradient (HCOOH, HCOOHNH₄) was used for the elution of the nucleotides. The incorporation of ³²P into the nucleotides was followed at different time intervals and also in young and more mature root tissues. The nucleotides AMP, GMP, ADP, GDP, and ATP were identified. Labelled phosphorus was found in ATP after 30 s, in ADP after 3 min, and in AMP after 5 min incubation of the roots. More mature roots (18 days) contained higher amounts of AMP than the young ones (3 days).

The velocity of incorporation of inorganic phosphate into organic compounds in the roots represents the measure of metabolism taking place in this organ of uptake and transport of substances. ATP is the first organic compound in which an inorganic phosphate ion appears. The velocity of phosphate incorporation can be expressed in seconds (LOUGHMAN and SCOTT-RUSSEL 1957, KURSANOV and VYSKRIBENTSEVA 1960). When following the velocity of incorporation of inorganic phosphate it is also possible to characterize the differences in the metabolism of plant tissues differing in maturity. In this paper we summarize some methodical findings obtained during the investigation of free nucleotides in maize roots as well as observed differences found when comparing tissues at different stages of maturity.

Material and Methods

Preparation of Plant Material

The primary root of maize (Zea mays L., cv. 'TA 45 S') served as the experimental object in our experiments. Maize seeds were soaked for 24 h in running tap water and then germinated on wet filtration paper in a thermostat at 25 °C. The three days old seedlings obtained were either analyzed or transferred to a 1/2 Knop nutrient solution and grown in a controlled environment plant growth chamber (at 20 °C and 12 h photoperiod). For the determination of the incorporation velocity of phosphate, labelled phosphorus was applied in Knop nutrient solution (KH₂³²PO₄, 2.4 μCi ml⁻¹) without carrier. The more mature plants were grown before

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transferring them to the nutrient solution containing labelled phosphorus for one day in 1/2 Knop nutrient solution from which PO₄³⁻ was omitted. The roots of the seedlings were then submerged into the radioactive solution. Both the 3 days old and the 18 days old roots were incubated in the radioactive solution for a time ranging from 30 s to 2 h.

Extractions of Free Nucleotides

10 g f.w. samples of 1.5 cm long segments of seminal root were taken from the 3 days old maize seedlings and 10 g f.w. samples of 6.5 cm long segments of seminal root were taken from the older maize seedlings. The roots were frozen in liquid nitrogen and extracted with 5% HClO₄ (2 ml g⁻¹ f.w.) for 30 min at 5 °C. The homogenate was centrifuged for 20 min at 10 000 g at 0 °C. The supernatant was decanted, lipophile substances were removed by means of ether extraction, and at 0 °C carefully adjusted to pH 6.2 with 10% KOH. KClO₄ precipitate was removed by means of centrifugation at 5000 g for 10 min at 0 °C and the supernatant obtained was applied to the column of ion exchanger.

Preparation of Ion Exchange Column and the Separation of Free Nucleotides

The standards of the nucleotides and the extracts of plant material were separated on anionic ion exchanger. Dowex 1 × 8, 200---400 mesh was floated after HAMILTON (1958); the fraction with particle size from 20 to 34 μm was used. The exchanger was then transferred to the HCOO⁻ cycle. A concave two-step elution gradient described by INGLE (1962) (H₂O --- 4M HCOOH, 4M MCOOH + 0.8 M HCOOHNH₂) was used for the elution of the nucleotides from the column. Eluate absorbance was recorded automatically at 260 nm and besides that it was further measured in 1 cm quartz cuvettes on the “Spektromom 203” spectrophotometer. When working with the radioactively labelled material, 1 ml samples were taken from each fraction, dried and their radioactivity was determined using an end-window counter (mass of the window 3.1 mg cm⁻²).

Identification of the Nucleotides

The fractions were purified by means of absorption onto and consequent elution from activated charcoal after FRIČ and HORVATOVICOVÁ (1967). The elution system consisted of 96% ethanol, pyridin, 25% ammonium solution and water (50 : 5 : 4 : 1, v/v). The eluates were chromatographed on Whatman 1 paper using the solvent mixtures proposed by Pabst Laboratories (Circular No 10). Chromatographic spots were detected under an UV lamp (UV Lampe Cargame) at 264 nm, eluted with 0.1 N HCl and absorption spectra of the eluates were then recorded in the range from 200 to 300 nm on a UNICAM SP-800 spectrophotometer.

Results and Discussion

The determination of free nucleotides in green plant parts is complicated because of the low content of free nucleotides and because pigments are present in these tissues. During the extraction procedure, when different methods are used, considerable losses occur. This is also the reason why the number of papers which describe methods trying to overcome these difficulties is so high.

For the determination of free nucleotides either the extraction with diluted ethanol or the extraction with diluted perchloric or trichloroacetic acids, or a combination of both, proved satisfactory. The use of diluted acids (0.2—0.6 N) proved to be better for tissues containing pigments, because pigments are extracted simultaneously with ethanol. Acid extracts from plant tissues containing nucleotides also contain other compounds which must be removed from the extracts. The nucleotides can be precipitated

Abbreviations used: CMP... cytidine monophosphate, AMP... adenosine monophosphate, GMP... guanosine monophosphate, ADP... adenosine diphosphate, GDP... guanosine diphosphate, ATP... adenosine triphosphate.