Soluble Nucleotides from the Immature Fruit of Tomato

James Brown and Philip Nordin

The soluble nucleotides from immature tomato fruit were extracted by blending with chloroform-methanol (2 to 1). They were isolated from the aqueous phase employing chromatography on Dowex 1 × 8 (Cl^- form), concentration on Norit, two-dimensional chromatography in ethanol–ammonium acetate, pH 7.5 and 3.8, and paper electrophoresis. Identification was by R_f, ultraviolet spectra, and identification of the products of partial acid hydrolysis. The following compounds were identified: NAD^+, ATP, ADP, UDP, GDP, AMP, GMP, UDP-glucose, UDP-galactose, UDP-*N*-acetylglucosamine, UDP-glucuronic acid, GDP-mannose, GDP-galactose, and GDP-glucose.

The soluble nucleotides present in tomato leaves have been reported (Roux, 1963). While several nucleotides unsubstituted by sugar residues were reported, only UDP-hexose (glucose or galactose) was found. The tomato fruit contains pectin, cellulose, and other polysaccharides (Williams and Bevenue, 1954) in an easily extractable form. It was of interest, therefore, to identify possible precursors of these polysaccharides.

Sugar nucleotides have been thoroughly identified as the donors of sugar residue in polysaccharides (Hassid, 1967; Kelleher, 1965; Leloir, 1964; Nordin and Kirkwood, 1965). The number of such sugar nucleotides identified in higher plants is small compared to those from animal tissue. This is particularly true of guanosine-containing nucleotides. Only recently GDP-glucose, GDP-galactose, GDP-mannose, and GDP-xylose were identified in strawberry leaves (Selvendran and Isherwood, 1967). In the present report guanosine-containing nucleotides were positively identified in the fruit of tomatoes, along with several other nucleotides. Conventional methods of isolation and purification with only slight modifications were employed.

EXPERIMENTAL

Tomatoes. Young, green tomatoes were obtained from plants grown in a greenhouse. The varieties used primarily were Jubilee and Glamour; no differences were observed among varieties used.

Chemicals. Nucleotides and monosaccharides generally of the highest purity available were obtained from various commercial sources. All chemicals used for extraction, chromatography, etc., were of reagent grade.

Extraction of Nucleotides. The extraction procedure was a modification of that of Bieleski (1964). The method used involved no acid or heat treatment and is generally used to extract lipids. In a preliminary trial it gave more consistent results than the ethanol-extraction method, yielded additional compounds, and was extremely easy to carry out. Significant hydrolysis by phosphatases did not occur as reported for other plant tissues (Bieleski, 1964). The method, therefore, was used in all extractions. Young, green tomatoes were homogenized in a Waring blender for 3 minutes in 6 volumes (w./v.) of chloroform-methanol (2 to 1). The homogenate was filtered under vacuum, and the resulting biphasic filtrate was separated. The dark green organic layer was discarded, and the light yellow aqueous phase was washed with 2 volumes of ether. The resulting nearly colorless solution was adjusted to pH 7 with NH_4OH and concentrated under vacuum at 30° to 35° C. to a volume approximately equal to the weight of the original tomatoes. At higher pH values a gelatinous colloid developed that interfered with chromatography. The extract was filtered through a pad of Celite and was then ready for Dowex chromatography.

Separation and Identification of Nucleotides. With some minor changes, purification and identification of the nucleotides were the same as for seedlings and spores (Elnaghy and Nordin, 1965, 1966). The tomato extract was passed through a column of Dowex 1 × 8, Cl^- form (200- to 400-mesh). The column was washed with water and the nucleotides were eluted in total with a solution 0.01*N* in HCl and 0.5*N* in NaCl. Following neutralization and purification on Norit, the nucleotides were separated by two-dimensional paper chromatography (Paladini and Leloir, 1952) in 95% ethanol–1*M* ammonium acetate, pH 7.5 (5 to 2), and 95% ethanol–1*M* ammonium acetate, pH 3.8 (5 to 2). Following chromatography, ultraviolet-absorbing areas were eluted and subjected to electrophoresis (Markham and Smith, 1951) in 0.01*M* sodium tetraborate, pH 9.1.

Identification was made through R_{AMP} values and R_G values against authentic compounds. Ultraviolet spectra were obtained from compounds after electrophoresis. The compounds obtained through partial acid hydrolysis, 0.01*N* HCl for 15 minutes, were identified by comparing their R_f's with knowns. Sugars were identified by paper chromatography in butanol-pyridine-water (10 to 3 to 3). They were located by the silver nitrate dip technique (Elnaghy and Nordin, 1966).

Sugars and nucleotide fragments were also identified simultaneously by electrophoresis (0.01*M* sodium tetraborate). To obtain reliable results it was necessary to neutralize the sample with ammonia before electrophoresis. Nucleotide fragments could be identified by observation under ultraviolet light. Sugars were re-

Department of Biochemistry, Kansas State University, Manhattan, Kan. 66502

Table I. Yields of Nucleotides from Green Tomatoes

Nucleotide	μmoles/100 G. Wet Weight[a]
ATP	0.22, 0.16
ADP	0.32
UDP	0.05, 0.02
GDP	0.08, 0.05
AMP	0.27
UMP	0.02
GMP	0.09, 0.02
UDP-glucose and UDP-galactose	0.59, 0.76
UDP-*N*-acetylglucosamine	0.05
UDP-glucuronic acid, GDP-glucose, GDP-mannose, and GDP-galactose	0.08, 0.04[b]

[a] Calculated from molar extinction coefficients of bases, measured after paper chromatography.
Adenosine. 15×10^3 (259 mμ).
Uridine. 10.0×10^3 (260 mμ).
Guanosine. 13.7×10^3 (252 mμ).
[b] Data available only on mixture of nucleotides. Molar absorbancy of mixture assumed to have a value of 12.0×10^3.

vealed by silver nitrate, but more concentrated solutions were required than for paper chromatography to overcome the inhibition due to borate. The solutions were 1% silver nitrate in 95% acetone and 2% sodium hydroxide in methanol. The sodium thiosulfate was 5%.

RESULTS AND DISCUSSION

Quantitative results of the study are given in Table I. In each case reliable spectra were obtained on the compounds at pH 7.0 and 2.0 following electrophoresis. Reliable spectra could be obtained from Whatman No. 1 paper if the nucleotide concentration was high enough to be seen under the ultraviolet light, and if the resolution had been good. The best criterion is identification of the products of acid hydrolysis. If it is performed by two different techniques, chromatography and paper electrophoresis, this could suffice in most instances. The results for chromatography and electrophoresis of GDP-mannose hydrolyzate are shown in Figures 1 and 2. The main sugar component was mannose, but low levels of glucose and galactose could be detected by both methods. Presumably these sugars came from GDP-glucose and GDP-mannose that were not separated.

In all cases except UDP-glucuronic acid, the components were completely separated by chromatography. In the latter it was incompletely separated from NAD^+ and GDP-mannose by chromatography but was resolved by paper electrophoresis.

In tomatoes, as in many other plant tissues, UDP-glucose is the main sugar nucleotide found (Table I).

Electrophoresis has been employed by many workers to separate and identify nucleotides (Bergkvist, 1956, 1957; Crestfield and Allen, 1955; Lin and Hassid, 1966; Markham and Smith, 1952). The authors observed that a sodium tetraborate system could be used to identify both sugars and nucleotides released by acid hydrolysis. Identifying both components in one experiment saves time and, more importantly, unknown material. This technique has not been used extensively by others. The electrophoresis apparatus of Markham and Smith (1951) employing a carbon tetrachloride cooling bath gave excellent results and was simple to construct. In 0.01*M* sodium tetraborate nucleoside diphosphate and monophosphate pairs could be separated–i.e., ADP and AMP, UDP and UMP, etc—but not in 0.1*M* borate. The latter concentration has been employed more frequently for nucleotides.

The authors' results with the tomato fruit vary from those with the leaves (Roux, 1963). Roux (1963) found several triphosphates (ATP, UTP, GTP, and CTP) and only one sugar nucleotide (UDP-hexose), whereas the authors found only one triphosphate (ATP) and several sugar nucleotides.

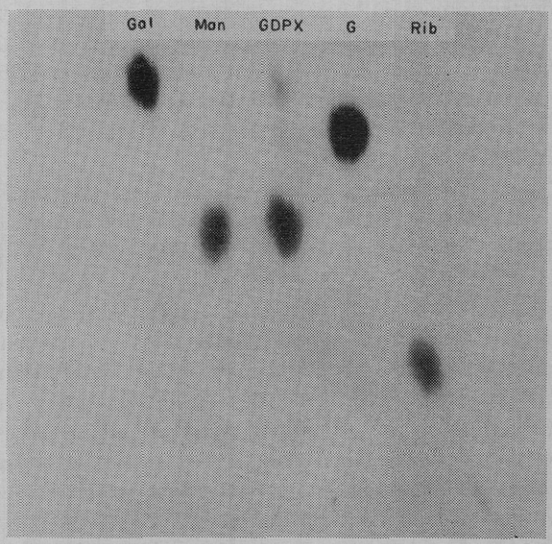

Figure 1. Chromatogram of acid hydrolyzate of GDPX, identified as GDP-mannose

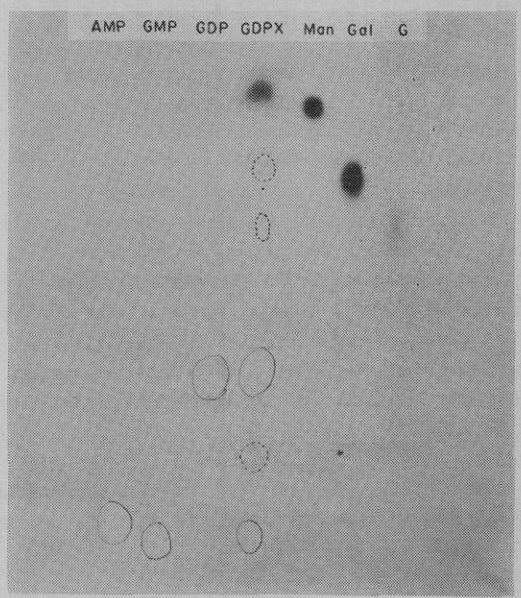

Figure 2. Electrophoresis of acid hydrolyzate of GDPX, identified as GDP-mannose

All the sugar nucleotides found are known precursors for cell wall material. They could serve as precursors for the known polysaccharides in ripe tomatoes (Williams and Bevenue, 1954). UDP-*N*-acetylglucosamine could be a precursor for the glycoprotein extensin, which has been reported in tomato cell wall (Lamport, 1967).

An examination in our laboratory revealed large amounts of starch in green tomatoes, apparently absent from ripe tomatoes (Williams and Bevenue, 1954). However, no evidence was found for ADP-glucose, its reported precursor (Leloir, 1964), despite careful examination. When ADP-glucose was added to the extraction medium it was readily recovered.

LITERATURE CITED

Bergkvist, R., *Acta Chem. Scand.* **10,** 1303 (1956).
Bergkvist, R., *Acta Chem. Scand.* **11,** 1457, 1465 (1957).
Bieleski, R. L., *Anal. Biochem.* **9,** 431 (1964).
Crestfield, A. M., Allen, F. W., *Anal. Chem.* **27,** 424 (1955).
Elnaghy, M. A., Nordin, P., *Arch. Biochem. Biophys.* **110,** 593 (1965).
Elnaghy, M. A., Nordin, P., *Arch. Biochem. Biophys.* **113,** 72 (1966).
Hassid, W. Z., *Ann. Rev. Plant Physiol.* **18,** 253 (1967).
Kelleher, W. J., *J. Pharm. Sci.* **54**(8), 1081 (1965).
Lamport, D. T. A., *Federation Proc.* **26**(2), 608 (1967).
Leloir, L. E., Proceedings of 6th International Congress on Biochemistry, New York, Vol. **33,** p. 15, 1964.
Lin, T. Y., Hassid, W. Z., *J. Biol. Chem.* **241,** 3283 (1966).
Markham, R., Smith, J. D., *Biochem. J.* **52,** 552 (1952).
Markham, R., Smith, J. D., *Nature* **168,** 406 (1951).
Nordin, J. H., Kirkwood, S., *Ann. Rev. Plant Physiol.* **16,** 393 (1965).
Paladini, A. C., Leloir, L. F., *Biochem. J.* **51,** 426 (1952).
Roux, L., *Ann. Physiol. Vegetale* **5,** 203 (1963).
Selvendran, R. A., Isherwood, R. A., *Biochem. J.* **105,** 723 (1967).
Williams, K. T., Bevenue, A., J. AGR. FOOD CHEM. **2,** 472 (1954).

Received for review August 22, 1968. Accepted January 13, 1969. Contribution 83. Department of Biochemistry, Kansas Agricultural Experiment Station, Manhattan, taken in part from a Ph.D. dissertation by James Brown.