Journal of Chromatography, 75 (1973) 133–136
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

Note

CHROM. 6376

Absence of pseudouridylic acid in the total ribonucleic acid extracts of wheat leaves

The base composition of the nucleic acids from the wheat leaves has been previously reported[1,2], and it indicated the presence of pseudouridylic acid in the chloroplastic and ribosomal nucleic acids.

During our recent studies on the nucleic acid metabolism of wheat plants (*Triticum vulgare* var. Talbot) during their cold adaptation, we failed to observe the presence of pseudouridylic acid in the extracts of total ribonucleic acids. In this paper, we describe the chromatographic method used and the results obtained by using an improved two-dimensional thin-layer chromatographic method to study the absence of pseudouridylic acid.

Experimental

We used only the aerial parts of the plants for our studies. The plants used were 11-day-old winter wheat (*Triticum vulgare* var. Talbot) grown at 20 ± 2° during the day and 17 ± 2° at night with a 15-h photoperiod. The extraction of ribonucleic acids was carried out as described by HADZIYEV *et al.*[2] with minor modifications. Commercial pseudouridine 2'- and 3'-monophosphoric acids were obtained from Sigma Chemical Co., U.S.A., ^{32}P-phosphoric acid, carrier free, was obtained from New England Nuclear Corp., U.S.A. ^{32}P (50 μCi/ml) was used for labelling the wheat leaves in distilled water and the labelling was carried out for 16 h. The counts were made on Beckman liquid scintillation counters using an external standard.

The purified RNA preparations were hydrolysed with 0.4 *N* KOH at 37° for 18 h as described previously[3]. After being transferred to an ice-bath, the hydrolysates were acidified with perchloric acid in order to precipitate the perchlorate salts. The supernatant liquid was then brought to pH 6–7 with potassium hydroxide solution. Generally, 10–15 μl of samples having approximately 0.5–1.0 O.D. units at 260 nm were used for chromatography.

Pre-coated TLC plates (layer 0.1 mm, Cellulose MN 300) purchased from Brinkmann Instruments (Canada) Ltd. were used for TLC separations. The spots were applied at one corner of the plate, 3 cm from the side and bottom, and the chromatography was carried out by upward displacement. The first solvent system was similar to that described by Pabst Laboratories[4] except that isobutyric acid was replaced with *n*-butyric acid. The *n*-butyric acid is mixed with ammonia and water in the proportions 57:4:39, respectively, without previous saturation. This solvent system takes about 5–6 h to run about 12–15 cm. The chromatograms thus obtained are left overnight under a hood to attain complete dryness.

In order to prevent uneven travel of the second solvent system, the area from 2 cm below the starting point immersed in the first solvent system and the area from 1 cm below the solvent front was scraped off.

The second solvent system consists of saturated ammonium sulphate–sodium

acetate (1 *M*)–isopropanol (79:19:2), according to the technique of MARKHAM AND SMITH[5]. This solvent system takes about 3–4 h to run to the edge of the plate.

After the chromatography, the spots were outlined with a pencil and traced on to transparent paper for reference. The chromatograms were then autoradiographed to confirm the purity of the hydrolysate. The spots delineated on the cellulose were transferred with a spatula into scintillator vials containing 5 ml of scintillator mixture. Radioactive counts were made in a Beckman liquid scintillation counter using an external standard. The delineated areas were eluted with 0.1 *M* HCl for UV identification.

Results and discussion

The separation of the mononucleotides by two-dimensional thin-layer chromatography is shown in Fig. 1 and the autoradiogram of the chromatogram is shown

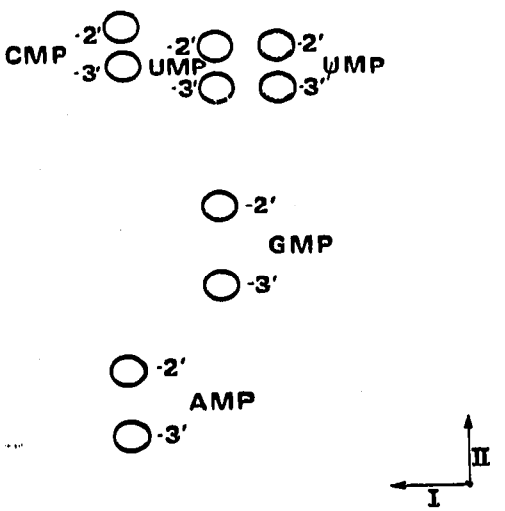

Fig. 1. Diagram showing the separation of all the major mononucleotides of wheat leaves together with the commercial pseudouridine monophosphates.

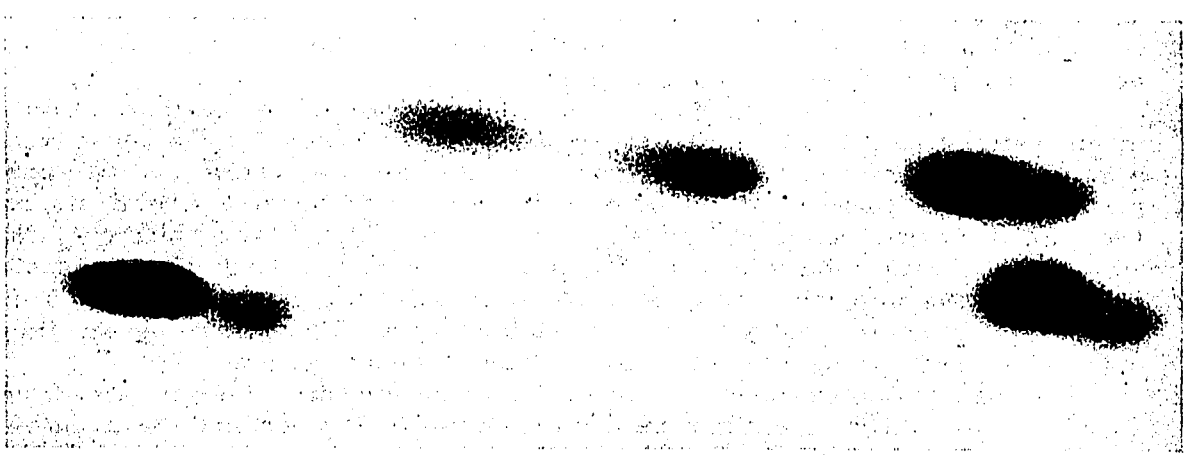

Fig. 2. Autoradiogram of the chromatogram obtained after chromatography of the alkaline hydrolysate of the total ribonucleic acid extracted from wheat leaves.

TABLE I

BASE RATIOS OF 2'- AND 3'-NUCLEOTIDE MONOPHOSPHATES OF CYTIDINE, ADENOSINE, GUANOSINE, URIDINE AND OTHER NUCLEOTIDES

Variety	*CMP*	*AMP*	*GMP*	*UMP*	*ψMP*	*Purines / Pyrimidines*	*Reference*
Ramsey	23.5	22.7	30.8	20.2	2.8	1.15	
Stewart	23.8	22.3	30.8	20.3	2.8	1.13	2[a]
Talbot	24.5	26.9	24.0	24.7	—	1.03	Present work[b]

[a] By UV absorption method.
[b] Average values of three determinations, expressed as percentage of radioactivity.

in Fig. 2. The results obtained from our experiments and the results of other workers are shown in Table I. The ratios of the nucleotides as determined by UV absorption agreed with the published data[6], which confirmed their identities.

Most of the minor nucleotide constituents of RNA have been reported[7,10] to occur mainly in the sRNA. Pseudouridylic acid was found as a normal constituent of wheat germ RNA together with other methylated nucleotides[8,9]. HADZIYEV *et al.*[2] reported an average of 12.6 moles of pseudouridylic acid in the extracts of total RNA from wheat leaves. If this value is correct, we should have observed at least trace amounts of pseudouridylic acid. However, we did not detect its presence, which was confirmed by the autoradiogram of the chromatograms obtained. The over-all characteristic pattern of the total wheat-leaf RNA (Fig. 3) is the same as that observed

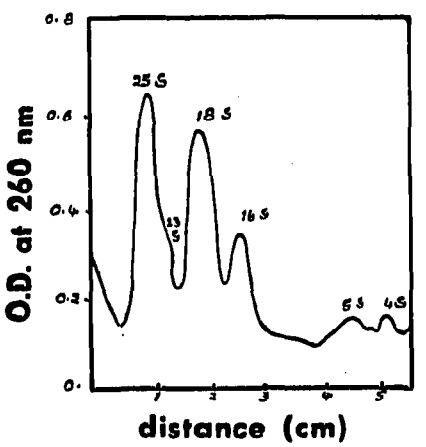

Fig. 3. Separation of total RNA extract of wheat leaves by polyacrylamide gel electrophoresis.

by other workers[2], and it comprises mainly 25S and 18S components. Our method of separation and detection of nucleotides is more sensitive compared with the methods used by other workers[2]. It is probable that the presence of pseudouridylic acid in the extracts of total RNA of wheat leaves might be an artifact produced during the stepwise elution of nucleotides from a Dowex column. Our experiments using ^{32}P confirmed the preliminary observations, indicating the absence of pseudouridylic acid. We did not

observe any other labelled material, except that of the major nucleotides in the autoradiograms, although a long exposure time was used. These results also confirm the absence of other methylated nucleotides in the extracts of total ribonucleic acids from wheat leaves.

Part of this work was supported from the Medical Research Council Grant No. MA-3974.

Department of Microbiology, University Hospital Centre, University of Sherbrooke, Sherbrooke, Quebec (Canada) — D. S. S. KUMAR

University of Montreal, Montreal, Quebec (Canada) — F. SARHAN, M. J. D'AOUST

1 M. LJ. MIHAILVOIC, S. A. GRUJIC AND D. HADZIYEV, *Biochim. Biophys. Acta*, 87 (1964) 499.
2 D. HADZIYEV, S. L. MEHTA AND S. ZALIK, *Plant Physiol.*, 13 (1968) 229.
3 T. SUGIYAMA AND H. FRAENKEL-CONRAT, *Proc. Nat. Acad. Sci. U.S.*, 47 (1961) 1393.
4 *Circular OR-10*, Pabst Brewing Co., Milwaukee, 1956.
5 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 69 (1957) 401.
6 K. BURTON, in R. M. C. DAWSON, D. C. ELLIOT, W. H. ELLIOT AND K. M. JONES (Editors), *Data for Biochemical Research*, Oxford University Press, New York and London, 2nd ed., 1969.
7 G. L. BROWN, in J. N. DAVIDSON AND W. E. COHN (Editors), *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 2, Academic Press, New York, 1963, p. 260.
8 D. G. GLITZ AND C. A. DEKKER, *Biochemistry*, 2 (1966) 1185.
9 J. W. LITTLEFIELD AND D. B. DUNN, *Biochem. J.*, 70 (1958) 642.
10 B. G. LANE AND F. W. ALLEN, *Biochim. Biophys. Acta*, 47 (1961) 36.

Received August 22nd, 1972