Occurrence of a Phosphodiesterase in the Insoluble Fractions from Wheat Seedlings

Sir:

Previous communications\(^1\)\(^-\)\(^3\) from this laboratory reported that the microsomes from young wheat roots and pea seedlings contained RNases which were inactivated by EDTA. A similar enzyme was found by Kessler and Engelberg\(^4\) in the cytoplasmic particles from apple leaves. These enzymes required magnesium ions for their activities. Generally, phosphodiesterases which need magnesium ions for their activities produce 5'-nucleotides from RNA. Therefore, we studied whether or not the hydrolysis products of RNA by the enzyme from wheat seedlings were 5'-nucleotides. The occurrence of a phosphodiesterase in plants which produces 5'-nucleotides has not yet been reported.

Five days old wheat seedlings were detached from endosperms, cut into small pieces, cooled to 5°C and ground in a mechanical mortar with the same weight of sea sand. The mixture was filtered through cotton cloth and centrifuged at 3,000×g for 20 minutes and ammonium sulfate was added to the supernatant to 40 per cent saturation. The precipitate which was considered to contain cell debris, nuclei and cytoplasmic particles was collected and dialyzed against water for 2 days. The precipitate was collected again by centrifugation and suspended in water. The suspension was used as the enzyme preparation.

Two hundred mg of yeast RNA was dissolved in 40 ml of a solution containing 2 mmoles of Tris and 400 μmoles of magnesium chloride, and pH was adjusted to 9.5 with potassium hydroxide solution. To this solution, 10 ml of the enzyme suspension described above was added, and the mixture was incubated at 60°C for 3 hours. Then 15 ml of the solution containing 0.2 n perchloric acid and 0.25 per cent uranium acetate was added, and the filtrate was neutralized. This neutralized solution was applied on a column (1×20 cm) filled with Dowex 1×2 (formate form). The column was washed with water to remove lightly adsorbed materials, and nucleotides were eluted by gradient method with formic acid\(^5\).

Aliquots, No. 22, 27 and 51 shown in Fig. 1, were fractionated by paper chromatography. From the results of Fig. 2, it was considered that No. 22 is 5'-adenylic acid and No. 51, 5'-guanylic acid. The chromatogram of No. 27 gave two spots. The upper spot seems to be 3'-adenylic acid; the lower spot has not yet identified. Besides, the reaction of these fractions with periodate-Schiff reagent\(^6\) was tested. No. 22 and 51 showed positive results but No. 27 did not. DNA was hydrolyzed by this enzyme preparation to a certain degree;

\(^2\) S. Matsushita, ibid., 18, 8 (1959).
\(^4\) B. Keasler and N. Engelberg, ibid., 55, 70 (1960).
FIG. 1. Column Chromatogram of the Nucleotides Prepared from the Hydrolysate of RNA by the Insoluble Fractions from Wheat Seedlings.

bis (p-nitrophenyl) phosphate, which was the specific substrate\(^7\) for phosphodiesterase, was also hydrolyzed.

It was not apparent that this enzyme was derived from the debris of nuclei or originated from microsomal preparation. Also, it was not decided whether or not this enzyme is the same as the enzymes\(^1\textsuperscript{3}\) which were found in the microsomal preparation from wheat roots and pea seedlings, and in the cytoplasmic particles from apple leaves\(^4\). However, from the above results, it may be concluded that a phosphodiesterase which produces 5'-nucleotides from RNA occurs in the insoluble fraction of wheat seedlings together with the RNases which produce 3'-nucleotides from


\(^8\) R. Markham and J.D. Smith, *Biochem. J.*, 46, 513 (1950).

\(^9\) R. Markham and J.D. Smith, *ibid.*, 45, 294 (1949).
The solvent used was (NH₄)₂SO₄-sodium acetate-isopropanol solvent system. The paper used was Toyo No. 51A. After developing, the paper was printed on a photographic printing paper by the modification of Markham's method.

No. 1, authentic sample of 3'-adenylic acid; No. 2, fraction No. 22; No. 3, authentic sample of 2'- and 3'-adenylic acid; No. 4, fraction No. 27; No. 5, fraction No. 31; No. 6, authentic sample of 5'-guanylic acid; No. 7, authentic sample of 3'-guanylic acid.

RNA.

Further studies including the cellular distribution of this enzyme are now in progress.

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