ANOMALOUS NUCLEOSIDES AND RELATED COMPOUNDS

XV.* USE OF DINITROPHENYLHYDRAZINE TO DETERMINE THE TERMINAL NUCLEOSIDES IN RIBONUCLEIC ACIDS

V. P. Chernetskii, É. A. Ponomareva, and V. V. Stavitskii

UDC 547.963.32'556.8.9:543.422.6'544

A method of determining the terminal nucleoside in ribonucleic acids by coupling 2,4-dinitrophenylhydrazine with periodate-oxidized t-RNA has been developed.

In order to increase the sensitivity of nonradioactive methods of determining terminal nucleosides in ribonucleic acids (RNA), the reaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with periodate-oxidized total t-RNA has been studied. The oxidation of t-RNA was carried out with 0.1 M sodium periodate, and its dialdehyde derivative was coupled with 2,4-DNPH in 2-methoxyethanol [2]. However, under these conditions we were unable to obtain dinitrophenylhydrazine derivatives of t-RNA in satisfactory yield. The yield of reaction products was determined by comparing the molar extinction coefficient of the 2,4-DNPH derivative of the t-RNA at 355 nm with the molar extinction coefficient at the same wavelength of 2,4-DNPH derivatives of nucleosides that we had synthesized previously [1]. In view of the low yield, in order to determine the optimum conditions for coupling 2,4-DNPH with periodate-oxidized t-RNA we performed a series of experiments at various pH values and temperatures in a three-component system of solvents (water–ethyl acetate–ethanol). The best yield was found at 37°C and pH 3.25 (Fig. 1). Raising or lowering the pH or the temperature led to a smaller yield. In order to determine the terminal nucleoside, the coupling of 2,4-DNPH with periodate-oxidized t-RNA was carried out under the optimum conditions.

The method makes it possible to determine the 3' end of a ribonucleic acid, and the amount of starting material required is 10-20 times less than in the determination of the same end by other nonradioactive methods [3,4]. It may also prove suitable for the determination of 3' end or the terminal sequence in higher molecular-weight RNAs and also for the automation of terminal analysis.

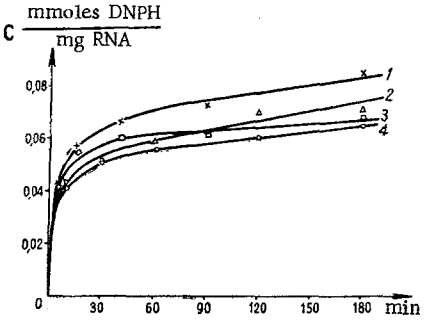

Fig. 1. Change in the reaction of 2,4-dinitrophenylhydrazine with periodate-oxidized t-RNA as functions of the pH, the temperature, and the time of the reaction. 1) pH 3.25, 37°C; 2) pH 3.25, 22°C; 3) pH 3.6, 37°C; 4) pH 3.6, 22°C.

EXPERIMENTAL

Performance of the Analysis. One mg of carefully purified t-RNA was dissolved in 0.05-0.1 ml of 0.05 M acetate buffer (pH 5), and 0.025 ml of 0.2 M sodium periodate was added. Oxidation was carried out in the dark at room temperature for 1 h. The excess of sodium periodate was precipitated by the addition of 1 mg of potassium chloride at 0°C. After centrifuging (3500 rpm), the supernatant liquid was poured into another centrifuge tube. The

*For Communication XIV, see [1].

Institute of Microbiology and Virology, Academy of Sciences of the Ukrainian SSR, Kiev. Translated from Khimiya Geterotsiklicheskikh Soedinenii, Vol. 6, No. 7, pp. 987-988, July, 1970. Original article submitted June 6, 1968.

dialdehyde derivative of t-RNA was precipitated with ethanol and centrifuged off. The residue obtained was dissolved in 0.2 ml of 0.1 M acetate buffer (pH 3.25), and 0.25 ml of a saturated ethyl acetate solution of 2,4-DNPH was added. The two-layer mixture was homogenized by the addition of ethanol. After having been thermostated under the conditions given previously for 2 h, the mixture was subjected to five treatments with ethyl acetate to remove the excess of 2,4-DNPH. The modified t-RNA was precipitated with ethanol, dried in vacuum over P_2O_5, and dissolved in 0.3 ml of borax-phosphate buffer (pH 7.7), and this solution was treated with 0.5 mg of pancreatic ribonuclease. After the solution had become turbid (2-3 min), 1 ml of ethanol was added, the mixture was centrifuged, the supernatant liquid was evaporated in vacuum, the resulting residue was dissolved in 2-3 drops of ethanol, and the 2,4-DNPH derivatives of the terminal nucleosides of the t-RNA that had been split off were identified chromatographically and spectrophotometrically with the aid of previously synthesized 2,4-DNPH derivatives of nucleosides [1]. The analysis showed that the terminal nucleoside of t-RNA is adenosine.

LITERATURE CITED

1. V. P. Chernetskii, D. V. Semenyuk, and N. K. Vatutina, KhGS, 6, 986 (1970).
2. R. Monier, M. L. Stephenson, and P. C. Zamecknik, Biochim. Biophys. Acta, 43, 1 (1960).
3. Chuan Tao Yu and P. C. Zamecknik, Biochim. Biophys. Acta., 45, 148 (1960).
4. B. G. Lahne, Biochim. Biophys. Acta, 47, 36 (1961).