Distribution of adenylic acid residues in nucleic acid of tobacco mosaic virus

Recent studies on the nature of arrangement of purine and pyrimidine nucleotides in TMV–NA showed that they both occur "singly" and also in "clusters" of two or more[1–3]. Additional information on the mode of distribution of adenylic acid residues obtained by simultaneously digesting TMV–NA with two ribonucleases possessing different specificities, is presented in this communication.

The mechanism of degradation of RNA by pancreatic RNAase has been well established[4]. When RNA is digested with this RNAase the end products are cytidylic acid, uridylic acid and several oligonucleotides of varying lengths terminated by cytidylic acid and uridylic acid (equation I). Another RNAase differing in specificity from the pancreatic has been purified from takadiastase and its properties have been studied. Unlike pancreatic RNAase it specifically cleaves the secondary phosphate esters of guanosine 3′-phosphate[5]. We have recently purified this enzyme in our laboratory employing a modified procedure and used its specific action to study the distribution of guanylic acid residues in TMV–NA[3]. This enzyme will be hereinafter referred to as takaribonuclease-I (taka-RNAase-I). When RNA is digested with this enzyme I the end products are guanylic acid and several oligonucleotides of varying lengths terminated by guanylic acid (equation II). On the other hand if RNA is digested simultaneously with both the RNAase the expected end products are those given in equation III.

$$\text{RNA} \xrightarrow{\text{(pancreatic RNAase)}} \text{Cp} + \text{Up} + \text{ApCp} + (\text{Rp})_n\text{ApCp} + \text{ApUp} + (\text{Rp})_n\text{ApUp} + \text{GpCp} + (\text{Rp})_n\text{GpCp} + \text{GpUp} + (\text{Rp})_n\text{GpUp} \quad \text{I}$$

(Rp = adenylic acid, guanylic acid; n = 1 or more)

$$\text{RNA} \xrightarrow{\text{(taka-RNAase)}} \text{Gp} + \text{ApGp} + (\text{Xp})_n\text{ApGp} + \text{CpGp} + (\text{Xp})_n\text{CpGp} + \text{UpGp} + (\text{Xp})_n\text{UpGp} \quad \text{II}$$

(Xp = adenylic acid, cytidylic acid, uridylic acid; n = 1 or more)

$$\text{RNA} \xrightarrow[\text{(taka-RNAase)}]{\text{(pancreatic RNAase)}} \text{Cp} + \text{Up} + (\text{Ap})_n\text{Cp} + (\text{Ap})_n\text{Up} + (\text{Ap})_n\text{Gp} \quad \text{III}$$

(n = 1 or more)

It is evident from the products in equation III that it is possible to determine the extent to which the adenylic acid residues in RNA occur "singly" and in "clusters" by using the combined actions of the two RNAases. TMV–NA was digested exhaustively at pH 7.4 with both enzymes and the digestion products were separated by a two-dimensional technique involving electrophoresis and chromatography, details of which will be reported at a later date. The separated products were identified and estimated using the methods described earlier[1,2] and a summary of the results is given below.

Since the fragments containing adenylic acid in the TMV–NA digests were obtained as a result of the combined actions of pancreatic and taka-RNAases, which specifically cleave the secondary phosphate esters of pyrimidine ribonucleoside 3′-phosphates and guanosine 3′-phosphate, respectively, it can be assumed that

Abbreviations: RNA, ribonucleic acid; TMV–NA, nucleic acid of tobacco mosaic virus; C, cytidine; A, adenosine; G, guanosine; U, uridine; p, phosphate.

these fragments occur in the intact TMV–NA preceded by any one of the three nucleotides, Cp, Up, and Gp. Of the total adenylic acid residues in TMV-NA 19.1 %, 19.6 % and 17.9 % occur "singly" as NpApCp, NpApUp and NpApGp, respectively; 12.1 %, 12.9 % and 12.8 % occur in "clusters" as NpAp(Ap)$_n$Cp, NpAp(Ap)$_n$Up and NpAp(Ap)$_n$Gp, respectively (where Np is is any one of the three nucleotides, Cp, Up, and Gp; $n = 1$ or more). The longest polyadenylic acid fragments so far isolated contained three adenylic acid residues. The possibility of the occurrence in TMV–NA of extremely small amounts of polyadenylic acid fragments containing more than three adenylic acid residues can not, however, be excluded.

Ful details of this investigation will be published elsewhere.

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Virus Laboratory, University of California, Berkeley, Calif. (U.S.A.) K. K. REDDI*

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* Present address: Department of Biochemistry, New York University of Medicine, New York 16, N.Y. (U.S.A.).

Radiosensitivity of nuclear RNA

Very little is known of the function of RNA in the cell nucleus, but it seems not improbable that it plays an important role in the synthesis of DNA. Since this process is highly radiosensitive, we undertook an investigation of the radiosensitivity of nuclear RNA. The latter was fractionated with 1 *M* NaCl, the heterogeneity of nuclear RNA now being well established by several authors[1–4]. Reports on the radiosensitivity of nuclear RNA have already been published[5–8] but in these studies no fractionation was attempted.

In this communication we shall briefly report the results of experiments concerning the metabolism and radiosensitivity of total and fractionated nuclear RNA from liver and thymus.

Groups of 5 young adult male albino rats, weighing 150 g, were irradiated in a perspex cage. Control groups consisted of 5 non-irradiated rats. All further procedures were carried out in exactly the same manner for the irradiated and the control group. The dose was 700 R in the experiments with liver tissue and 300 R with thymus tissue. In the experiments on liver nuclear RNA, the rats 4 h after irradiation were injected intravenously with 0.5 ml of neutralized 0.1 % Na_2HPO_4 containing 200 μC $Na_2H^{32}PO_4$, and killed 3.5 h later. In the studies on thymus nuclear RNA 200 μC

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UMP, uridine monophosphate.