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Degradation of Homopolyribonucleotides by Ribonuclease F_1

(ribonuclease F_1 / homopolyribonucleotide / base specificity)

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Ribonuclease F_1, a guanine specific endoribonuclease of *Fusarium moniliforme*, has been found to degrade rapidly poly(I). Other homopolyribonucleotides, poly(A), poly(C) and poly(U), are also degraded by the enzyme, though much more slowly than poly(I). The minimal substrates corresponding to these polymers, diribonucleoside monophosphates, are also degraded by the enzyme very slowly. These results show that the specificity of ribonuclease F_1 toward guanine base is strict but not absolute.

Introduction

Ribonuclease (RNase) F_1 is an endoribonuclease isolated from the culture filtrate of a phytopathogenic fungus *Fusarium moniliforme*. Omori *et al.* have shown that it is guanine specific using natural RNA as a substrate: it splits internucleotide bonds in RNA between 3'-guanylic acid and the 5'-hydroxyl group of the adjacent nucleotide with intermediary formation of guanosine 2':3'-cyclic phosphate (G>p), which is eventually hydrolyzed to guanosine 3'-phosphate (G3'p) (1). This enzymatic property and its molecular weight (*ca.* 11,000) suggested that RNase F_1 might be a homolog of well-known RNase T_1 from *Aspergillus oryzae*. This hypothesis was proven by the primary sequence study (2).

In the RNase T_1 family, RNase F_1 and other RNases of *Fusarium* genus form a subgroup which is rather distant from RNase T_1. Therefore, it will be of interest to compare RNase F_1 with RNase T_1 from structural and functional points of view.

Recently, the tertiary structure of RNase F_1 was elucidated by NMR spectroscopy (3) and X-ray crystallography (4). The results show that RNase F_1 and RNase T_1 are very similar in the secondary structure and the active site topology, but that they differ considerably in other parts. With such structural information available, comparative studies on function are awaited. In this paper, we reexamine strictness of the base specificity of RNase F_1 using homopolyribonucleotides and diribonucleoside monophosphates.

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Materials and Methods

Materials — RNase F_1 was prepared as described previously (5) and was determined on the basis of absorbance at 280 nm using a molecular absorbance of 13,900 $M^{-1}\,cm^{-1}$ (6). Poly(I), poly(A), poly(C) and diribonucleoside monophosphates were purchased from Sigma. Poly(U) was obtained from Miles. The commercial poly(C) preparation had a wide size distribution and was, therefore, fractionated on Sephadex G-100 to eliminate small size portions prior to enzymatic degradation.

Degradation of Homopolyribonucleotides — This was followed by high performance liquid chromatography (HPLC) using a Toso liquid chromatograph HLC-803A. A TSK gel column G2000 SW (7.5 × 600 mm, Toso) was used with a solvent, 66.7 mM potassium sodium phosphate buffer (pH 6.8) containing 0.1 M KCl. The flow rate was 1.0 ml/min and the detection wavelengths were 250, 260 and 270 nm for poly(I), poly(A) and poly(U), and poly(C), respectively. The reaction mixture (200 μl) contained a polymer substrate, 12 μmol of Tris-HCl buffer (pH 7.5) and RNase F_1. The amounts of the enzyme and the substrate used were: 111 pg of RNase F_1 for 41 μg of poly(I), 5.56 μg of RNase F_1 for 500 μg of

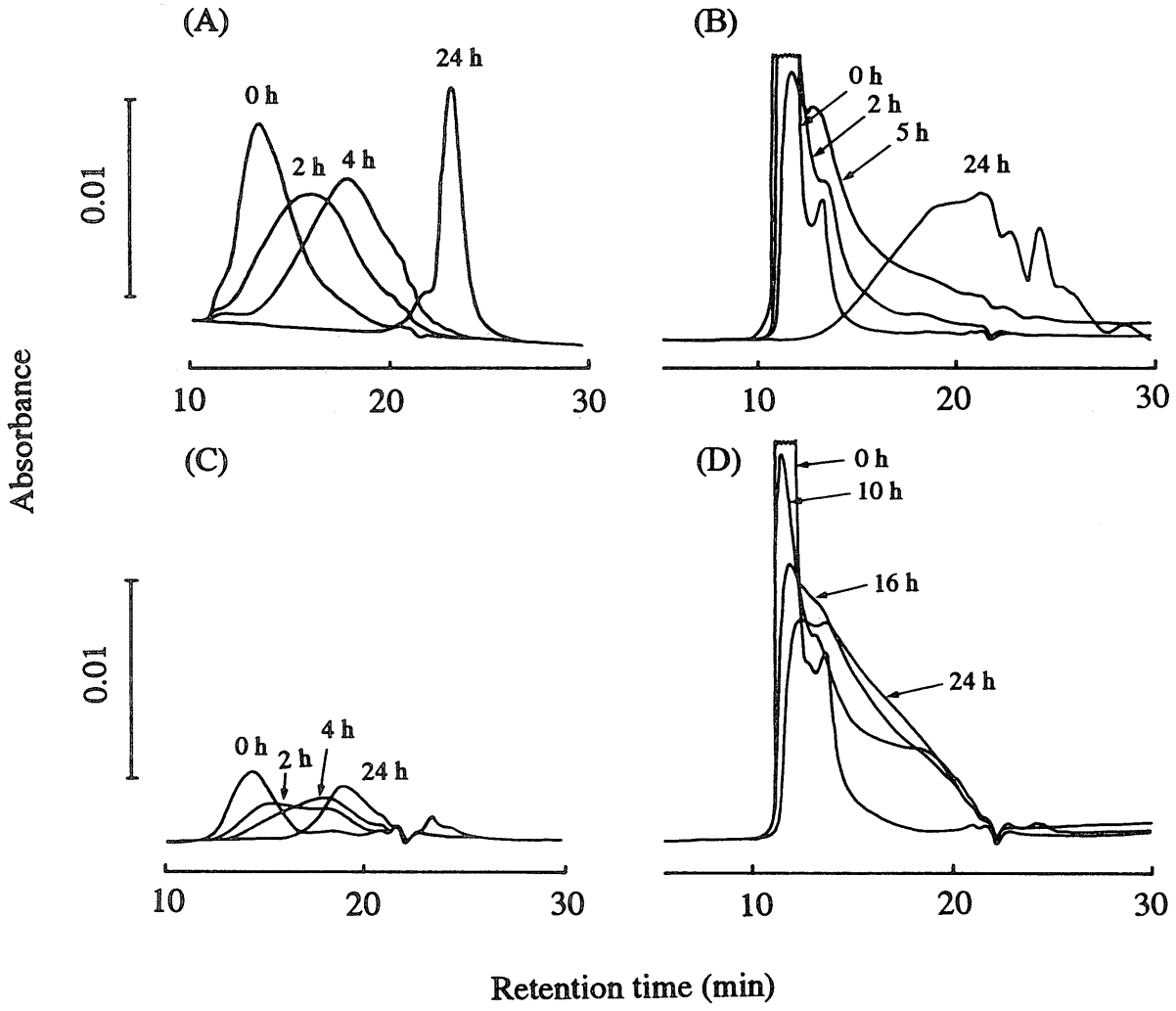

Fig. 1. HPLC pattern showing degradation of homopolyribonucleotides by RNase F_1. (A) poly(I), (B) poly(A), (C) poly(C) and (D) poly(U). Experimental details are described in "Materials and Methods." The reaction times are indicated in the figure.

poly(A) or poly(U), and 5.56 μg of RNase F_1 for 17 μg of poly(C). The mixture was incubated at 37 ℃. At various times, 20-μl portions of the mixture were withdrawn and analyzed by the HPLC.

Degradation of Diribonucleoside Monophosphates — This was followed by HPLC using a Shimadzu liquid chromatograph LC-6A. An IEX-540K column (4 × 300 mm, Toso) was used with a solvent, 0.25 M KH_2PO_4. The flow rate was 1.0 ml/min and the detection wavelength was 260 nm. The reaction mixture (80 μl) contained a diribonucleoside monophosphate, 4 μmol of Tris-HCl buffer (pH 7.5) and 18 μg of RNase F_1. The amounts of the substrates used were: 90 nmol of ApA, 85 nmol of CpC and 97 nmol of UpU. The mixture was incubated at 37 ℃ and 2-μl portions were withdrawn at appropriate times and analyzed by the HPLC.

Results and Discussion

Degradation of Homopolyribonucleotides — The base specificity of RNase F_1 was reexamined first with homopolyribonucleotides as substrates. Poly(G) could not be used, because it forms aggregates in solution and resists to the enzyme action. Therefore, poly(I) which has hypoxanthine base was employed as a quasi legitimate polymer substrate. As expected, poly(I) was degraded rapidly by the enzyme (Fig. 1A). This is consistent with the finding with RNase T_1. Hypoxanthine with its structural resemblance to

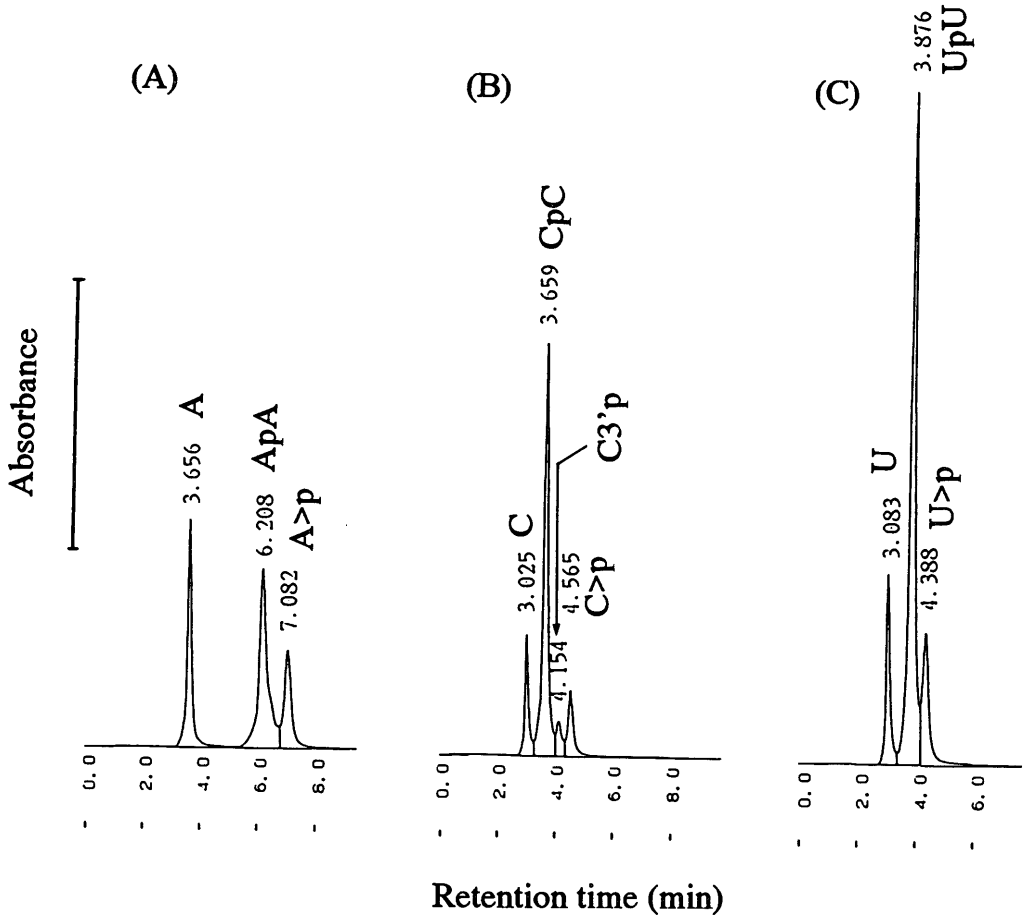

Fig. 2. HPLC pattern for diribonucleoside monophosphate digested by RNase F1 for 24 h. (A) ApA, (B) CpC and (C) UpU. Experimental details are described in "Materials and Methods." Identity of each peak as clarified by comparison of its retention time with that of an authentic compound is indicated in the figure. The scale bar for absorbance indicates 0.1 unit for (A) and (B) or 0.05 unit for (C).

guanine may be recognized by the enzyme as a nearly legitimate base. In addition, poly(A), poly(C) and poly(U) were also found to be degraded endonucleolytically by the enzyme, although much more slowly than poly(I) (Fig. 1B, C and D). The base specificity of RNase F1, therefore, is strict but not absolute. The same phenomenon was also reported by Irie on RNase T1 (7). However, as Irie admitted, the possibility of contamination with base nonspecific RNase T2 cannot be entirely excluded in the case of RNase T1 and thus some ambiguity remained. In contrast, the starting material of RNase F1 contains no base nonspecific RNase (1). Although the material contains another nucleolytic enzyme, PDM phosphatase, the enzyme degrades homopolyribonucleotides in an entirely different manner (8). Therefore we can conclude that the degrading activity toward poly(A), poly(C) and poly(U) is not due to any contaminant enzyme but an intrinsic property of RNase F1. Taking into account the amount of enzyme used and the degradation time course, RNase F1 is estimated to degrade poly(A) at least 10^4 times slower than poly(I). Poly(C) and poly(U) are degraded even more slowly than poly(A).

Degradation of Diribonucleoside Monophosphates — In order to confirm the degrading activity of RNase F1 toward the poor polymer substrates, we examined whether the enzyme could degrade the potential minimal substrates corresponding to these polymers, *i.e.* diribonucleoside monophosphate (NpN). Fig. 2 shows the HPLC patterns at 24-h incubation time. Clearly,

NpN's were degraded into the corresponding nucleoside 2':3'-cyclic phosphate (N>p) and the nucleoside (N). In accordance with the results for the polymer substrates, ApA was degraded faster than CpC and UpU. It was observed that the degradation proceeded almost linearly during the incubation (data not shown). The degradation rates under the specified conditions were calculated to be 2.0 nmol/h for ApA and 1.3 nmol/h for both CpC and UpU. Interestingly, a small peak at a retention time 4.154 min was observed for CpC (Fig. 2B). This retention time coincides with that of C3'p but not with that of C2'p (3.948 min). Closer inspection of the chromatogram for ApA shows a shoulder at the tail of the peak of ApA with an approximate retention time corresponding to that of A3'p (6.418 min). In this case, no peak was observed at the elution position of A2'p (5.623 min). These results show that RNase F_1 can catalyze not only the transesterification (NpN → N>p + N), but also the hydrolysis (N>p → N3'p) for ApA and CpC. Unfortunately, in the case of UpU, this could not be observed, because U2'p (4.338 min) and U3'p (4.334 min) were eluted at almost the same position which fell at a narrow space between UpU and U>p. However, in an independent experiment, we confirmed that U>p was also hydrolyzed to U3'p (data not shown).

All these results clearly show that the base specificity of RNase F_1 toward guanine base is strict but not absolute.

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References

(1) Omori, A., Sato, S. & Tamiya, N. (1972) *Biochim. Biophys. Acta* **268**, 125-131

(2) Hirabayashi, J. & Yoshida, H. (1983) *Biochem. Int.* **7**, 255-262

(3) Nakai, T., Yoshikawa, W., Nakamura, H. & Yoshida, H. (1992) *Eur. J. Biochem.* **208**, 41-51

(4) Vassylyev, D. G., Katayanagi, K., Ishikawa, K., Tsujimoto-Hirano, M., Danno, M., Pähler, A., Matsumoto, O., Matsushima, M., Yoshida, H. & Morikawa, K. (1993) *J. Mol. Biol.*. **230**, 979-996

(5) Yoshida, H., Fukuda, I. & Hashiguchi, M. (1980) *J. Biochem.* **88**, 1813-1818

(6) Yoshida, H. & Hanazawa, H. (1989) *Biochimie* **71**, 687-692

(7) Irie, M. (1965) *J. Biochem.* **58**, 599-603

(8) Yoshida, H. & Konno, O. (1988) *Chem. Lett.*, 1919-1922

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