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Mode of Degradation of Homopolyribonucleotides by PDM Phosphatase of *Fusarium moniliforme*

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PDM phosphatase degrades homopolyribonucleotides, poly(A), poly(I), poly(C), and poly(U). The former three are degraded exonucleolytically into nucleoside and inorganic phosphate through 5'-nucleotide. In the case of poly(U), the enzyme acts endonucleolytically and exonucleolytically at the same time.

PDM phosphatase[1] isolated from the culture filtrate of a phytopathogenic fungus, *Fusarium moniliforme*, is a unique phosphohydrolase acting on both phosphodiesters and phosphomonoesters.[2] Its natural substrates have been presumed to be nucleotides and polynucleotides because of its high activity and strong affinity toward nucleotide substrates.[3] We have already shown that the enzyme can cleave internucleotide linkage of diribonucleoside phosphates.[4] Thus, it is of interest to study if and how the enzyme degrades polynucleotides or nucleic acids. As the first step toward this goal, we have investigated the mode of action on the homopolyribonucleotides, poly(A), poly(I), poly(C), and poly(U).[5]

Susceptibility of the homopolymers to PDM phosphatase was first examined as follows. The reaction mixture contained in a total volume of 100 µl: 250 µg of polymer, 5 µmol of sodium acetate buffer (pH 5.3), and 0.27 unit of the enzyme purified as described previously.[6] After incubation at 37 °C for the indicated time, the reaction was stopped by the addition of 0.1 ml of uranyl reagent (0.75% (w/v) uranyl acetate $2H_2O$ in 25% (w/w) $HClO_4$). The mixture was stood at 0 °C for 15 min, added with 2.8 ml of distilled water, then centrifuged at 2500 rev./min for 10 min. The absorbance of the supernatant was measured at 260 nm against the sample at time 0, which was obtained by adding first the uranyl reagent then the enzyme in the above procedure. The results were expressed in mol% of the acid soluble nucleotide (and/or nucleoside) to the total nucleotide in the polymer, assuming that the acid soluble absorbance was due to mononucleotide and/or nucleoside (for the basis of this assumption, see below). As shown in Fig. 1, the rate of degradation decreased in the following order: poly(I) ≫ poly(C) > poly(A) > poly(U). This does not accord with the order of degradation rate for diribonucleoside phosphates, which is CpC > ApA ≃ GpG ≫ UpU in terms of k_{cat}.[4] However, conformation of the polymers should be taken into account in interpretation of the results. Poly(A) and poly(C) have been known to assume double stranded structure on protonation.[7] At pH 5.3 where the degradation reaction was carried out, these polymers must have, at least in part, such a secondary structure. Contrary,

poly(I) and poly(U) are devoid of secondary structure. This may be the reason for the rapid degradation of poly(I). The resistance of poly(U) to the enzyme action will be explained by its mode of degradation as described later.

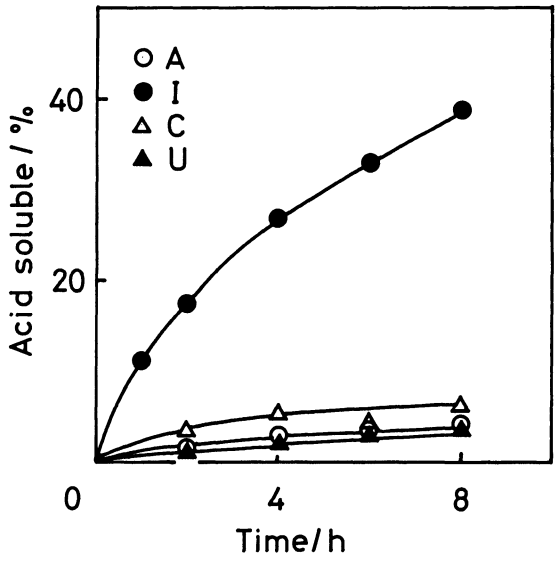

Fig. 1. Time course of the degradation of homopolyribonucleotides by PDM phosphatase.

The degradation process for the polymers was followed by means of high performance liquid chromatography (HPLC). The reaction mixture contained, in a total volume of 200 µl, 25 µmol of sodium acetate buffer (pH 5.3) and the following amount of the enzyme and substrate: 2.1 unit for 500 µg of poly(A), 0.85 unit for 500 µg of poly(I), 0.021 unit for 44 µg of poly(C), and 1.3 unit for 500 µg of poly(U). At appropriate times, a portion (20 µl for poly(C) or 10 µl for the others) of the mixture was withdrawn and analyzed by HPLC, the conditions for which were as follows. Chromatograph, Toso HLC-803A; column, TSKgel G3000PW 7.5 mm (i.d.) x 600 mm with a guard column (7.5 mm x 75 mm); eluent, 25 mmol dm^{-3} potassium sodium phosphate buffer (pH 6.8); flow rate, 1.0 ml/min; detection, absorbance at 260 nm. As shown in Fig. 2(a) and (c), poly(A) and poly(C) were degraded in the same way. Polymers of the original size remained throughout the degradation process and nucleoside was the sole product that could be detected clearly. These results show that PDM phosphatase acts on poly(A) or poly(C) as a processive exonuclease: once the enzyme attaches to one end of a polymer molecule and begins to excise the monomer unit, it will not dissociate from the molecule until complete degradation is attained. However, the processiveness is by no means rigorous, because slight but definitive amounts of smaller size polymers were observable. Poly(I) was degraded in much the same way (Fig. 2(b)) with the only difference that mononucleotide was detected in addition to nucleoside. The mononucleotide was identified as 5'-IMP using another HPLC system capable of separating 5'-IMP and 3'-IMP (data not shown). Probably, the first product of exonucleolytic action on poly(A) or poly(C) was also 5'-mononucleotide. However, since 5'-AMP and 5'-CMP were good substrates of the enzyme,[3] they must have been immediately hydrolyzed.

In contrast to these results, gradual depolymerization of poly(U) was clearly seen even in the early phase where little amount of 5'-UMP was detected (Fig. 2(d)). Therefore, poly(U) must have been degraded endonucleolytically, though exonucleolytic degradation also took place in view of the release of mononucleotide and nucleoside. This mode of degradation may explain the resistance of poly(U) described earlier. The products of endonucleolytic action must be acid insoluble,

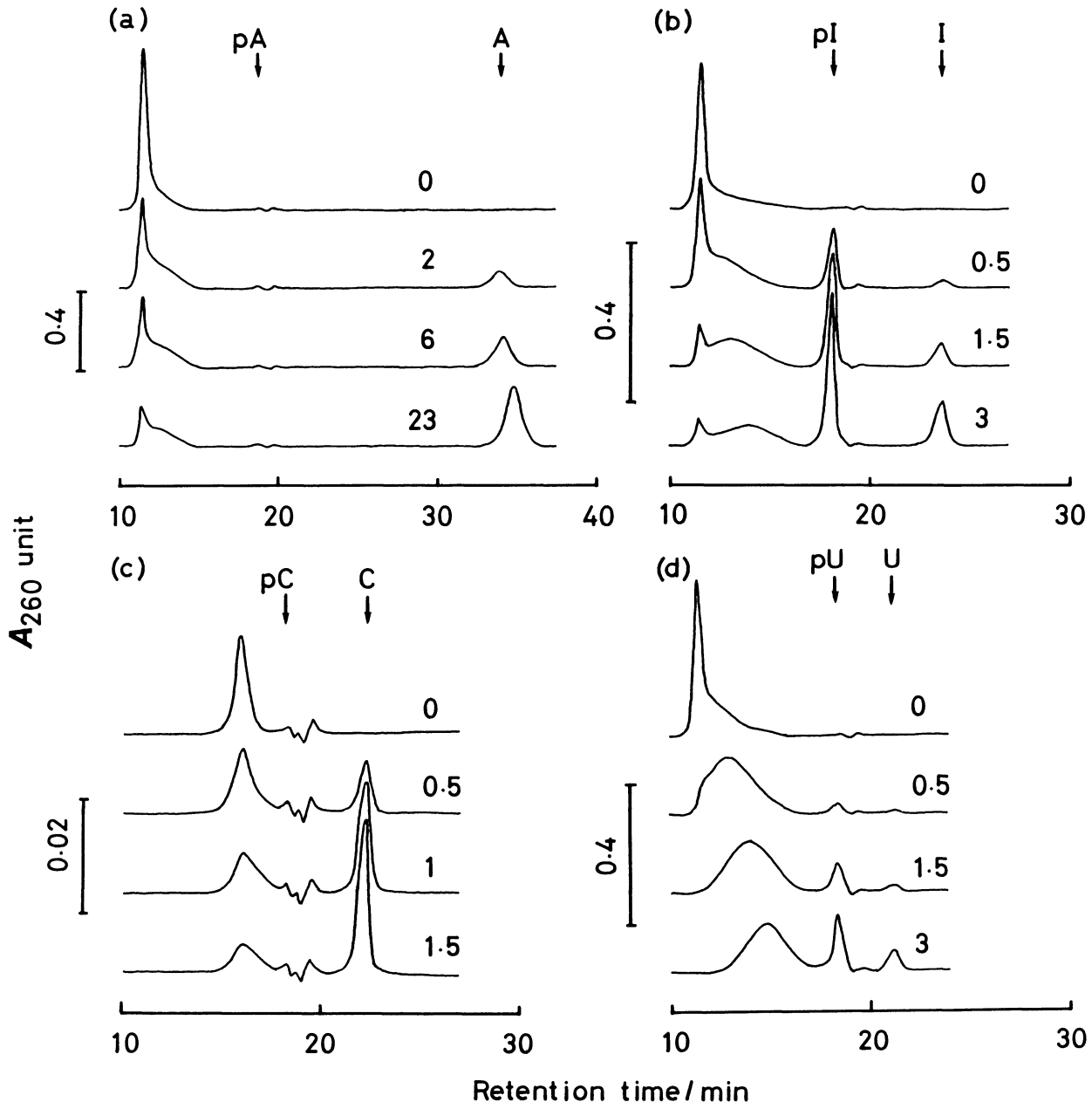

Fig. 2. HPLC analysis of the process of degradation of homopolyribonucleotides by PDM phosphatase. (a) poly(A), (b) poly(I), (c) poly(C), (d) poly(U). Each elution profile is labeled with the reaction time/h. The elution positions of 5'-mononucleotide (pN) and nucleoside (N) are indicated by arrows.

thus poly(U) degradation was not detected by the method employed. If the degradation rate is compared on the basis of the number of internucleotide linkages split, poly(U) will become a much better substrate than it appears in Fig. 1. Therefore, the secondary structure of polynucleotides is considered to affect their susceptibility to the enzyme to a great extent. Single stranded polynucleotides like poly(I) or poly(U) are presumed to be easily degradable, whereas double

stranded polynucleotides are hard or possibly unable to be hydrolyzed. The enzyme may exert its degradative action on poly(A) or poly(C) through the single stranded form which is present in equilibrium with the double stranded form.

In conclusion, PDM phosphatase degrades the homopolyribonucleotides except poly(U) as a processive exonuclease, yielding 5'-nucleotide as the first product, which is hydrolyzed eventually to nucleoside and inorganic phosphate. Only poly(U) is degraded endonucleolytically and exonucleolytically at the same time. To our knowledge, PDM phosphatase is the first nuclease that switches mode of action depending on the nature of base in polynucleotide substrates. The reason for this switch is not clear at present, but it should be recalled that diribonucleoside phosphates of the type UpN are poor substrates of the enzyme.[4] This fact may be pertinent to the bizarre action of the enzyme on poly(U). The terminal structure is so hard to be cleaved that it inhibits strongly the exonucleolytic action of the enzyme. Under the circumstances, the enzyme may be forced to attack internal phosphodiester bonds.

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References

1) The formerly used name, phosphodiesterase-phosphomonoesterase (PDMase in an abbreviated form), is replaced by a new name for the sake of simplicity.
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5) The commercial sources of homopolyribonucleotides were: poly(A), poly(I), and poly(C) all potassium salts (Sigma); and poly(U) ammonium salt (Miles). Since the poly(C) had a wide size distribution, it was fractionated on Sephadex G-100 prior to the experiment shown in Fig. 2.
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