Comparison of Nuclease P$_1$-Malonogalactan Complex with Nuclease P$_1$

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In a previous paper, it has been shown that nuclease P$_1$ of *Penicillium citrinum* is produced in a form of complex with malonogalactan in wheat bran culture.

In this paper, properties of nuclease P$_1$-malonogalactan complex (P$_1$-MG) are compared with those of the polysaccharide-free nuclease P$_1$.

**MATERIALS AND METHODS**

*Materials.* P$_1$-MG II$^9$ and its enzymatically demalonylated product were employed in this paper as the nuclease P$_1$-malonogalactan complex (P$_1$-MG) and nuclease P$_1$-galactan complex (P$_1$-G). Chymotrypsin, trypsin and pronase were purchased from Seikagaku Kogyo Co. Source of substrates was described previously.$^9$

*Enzyme assay.* Nucleolytic activity and phosphomonoesterase activity were assayed as described previously.$^{1,4}$

$^9$ Studies on a Nuclease from *Penicillium citrinum*. Part VIII. See References 1~8). This work was presented at the Annual Meeting of Agricultural Chemical Society of Japan, Tokyo, April 2, 1971.

**RESULTS**

*Effect of pH on enzyme activity*

pH-activity curves of P$_1$-MG for 3'-AMP, RNA and heat-denatured DNA were compared with those of nuclease P$_1$ (Fig. 1). No difference was observed between them in pH optimum for 3'-AMP. While pH optima of P$_1$-MG for RNA and heat-denatured DNA were lower than those of nuclease P$_1$.

*Effect of ionic strength on enzyme activity*

At lower than 0.001 of ionic strength, RNA and heat-denatured DNA were attacked hardly by P$_1$-MG, but attacked well by nuclease P$_1$ (Fig. 2). Relationship between RNA-degrading activity and ionic strength in P$_1$-G was almost the same as that in nuclease P$_1$, while the relationship between heat-denatured DNA-degrading activity and ionic strength in P$_1$-G was intermediate between the relationships in P$_1$-MG and nuclease P$_1$. On the other hand, ionic strength of the reaction mixture...
FIG. 1. pH-activity Curves of P1-MG and P1 for RNA, Heat-denatured DNA and 3'-AMP.

○—○, nuclease P1; ■—■, P1-MG.

FIG. 2. Effect of Ionic Strength on the Activity of P1-MG, P1-G, and Nuclease P1 toward RNA and Heat-denatured DNA.

A, RNA; B, heat-denatured DNA.
○—○, nuclease P1; ■—■, P1-MG; △—△, P1-G.

Effect of temperature on enzyme activity

Temperature optima of P1-MG for RNA, heat-denatured DNA and 3'-AMP were around 70°C like those of nuclease P1. However, the increase in the rate of hydrolysis of RNA or heat-denatured DNA with an elevation of temperature from 37°C to 70°C was not so marked in P1-MG as compared with P1-G or nuclease P1 (Table I). Such difference was not observed in hydrolysis of 3'-AMP.

**TABLE I. COMPARISON OF THE REACTION RATES OF P1-MG, P1-G AND NUCLEASE P1 TOWARD 3'-AMP, RNA AND HEAT-DENATURED DNA AT 37°C AND 70°C**

<table>
<thead>
<tr>
<th></th>
<th>3'-AMP</th>
<th>RNA</th>
<th>Heat-denatured DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-MG</td>
<td>6.0</td>
<td>12.2</td>
<td>11.3</td>
</tr>
<tr>
<td>P1-G</td>
<td>5.9</td>
<td>16.7</td>
<td>16.1</td>
</tr>
<tr>
<td>Nuclease P1</td>
<td>5.7</td>
<td>17.3</td>
<td>16.3</td>
</tr>
</tbody>
</table>

\( \kappa_0 \): initial reaction rate (The rates were calculated from the amounts of products formed during 15-min incubation.)

**Thermostability**

Aqueous solutions of P1-MG and nuclease P1 at the same concentration were heated at 70°C, then the remaining activities were determined. As shown in Fig. 3, P1-MG was more stable at 70°C than nuclease P1.

**Susceptibility to proteolysis**

Aqueous solutions of P1-MG and nuclease P1 were incubated with chymotrypsin, trypsin or pronase. After incubation, the remaining nuclease activities were determined. As shown
Properties of Nuclease P1-Malonogalactan Complex

TABLE II. SENSITIVITY OF P1-MG AND NUCLEASE P1 TO PROTEASESa)

<table>
<thead>
<tr>
<th>Residual activity (%) after treatment with</th>
<th>Chymotrypsin</th>
<th>Trypsin</th>
<th>Pronase</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-MG</td>
<td>106</td>
<td>81</td>
<td>33</td>
</tr>
<tr>
<td>Nuclease P1</td>
<td>63</td>
<td>50</td>
<td>8</td>
</tr>
</tbody>
</table>

a) The reaction mixture containing 150 units of nuclease P1 or P1-MG and 5 mg of each protease in 1 ml of 0.014 M veronal buffer, pH 6.0, was incubated at 37°C for 60 min. After the incubation, the remaining activity was assayed.

in Table II, P1-MG was less susceptible to proteolysis than nuclease P1.

Hydrolysis rates for various substrates

The hydrolysis rates of P1-MG toward various substrates at the optimal pH were compared with those of nuclease P1 (Table III). Generally the hydrolysis rate of P1-MG was lower than that of nuclease P1. The repressing effect of malonogalactan was especially marked for native DNA, and only slight for 3'-nucleotides. Time-courses of hydrolysis of heat-denatured DNA and native DNA with P1-MG were compared with those with nuclease P1 (Table IV). From this result, preference of P1-MG for heat-denatured DNA over native DNA was found to be much higher than that of nuclease P1.

TABLE III. HYDROLYSIS RATE OF P1-MG AND NUCLEASE P1 TOWARD VARIOUS SUBSTRATES AT THE OPTIMAL pH

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Rate of hydrolysisa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nuclease P1</td>
</tr>
<tr>
<td>RNA</td>
<td>406.8 (6.2)</td>
</tr>
<tr>
<td>Poly (A)</td>
<td>593.3 (6.0)</td>
</tr>
<tr>
<td>Poly (C)</td>
<td>370.2 (6.0)</td>
</tr>
<tr>
<td>Poly (U)</td>
<td>472.2 (4.0)</td>
</tr>
<tr>
<td>Poly (I)</td>
<td>655.0 (4.5)</td>
</tr>
<tr>
<td>Heat-denatured DNA</td>
<td>264.4 (5.3)</td>
</tr>
<tr>
<td>Native DNA</td>
<td>2.3 (5.3)</td>
</tr>
<tr>
<td>3'-AMP</td>
<td>1215.9 (7.2)</td>
</tr>
<tr>
<td>3'-GMP</td>
<td>1743.3 (8.5)</td>
</tr>
<tr>
<td>3'-CMP</td>
<td>1033.4 (6.0)</td>
</tr>
<tr>
<td>3'-UMP</td>
<td>847.8 (6.0)</td>
</tr>
</tbody>
</table>

a) μmoles of phosphodi- or monoester cleaved per 1 mg of the enzyme in 1 min at 37°C at the optimal pH indicated in parentheses except native DNA.

b) The nuclease P1 content of P1-MG was estimated on the basis of the enzyme activity toward 3'-AMP.

In addition it was found that after demalonylation of P1-MG with the carboxylesterase the activities toward 3'-AMP, RNA, heat-denatured DNA and native DNA increased by 1.01-, 1.34-, 2.36- and 2.70- times, respectively.

DISCUSSION

It has been demonstrated that properties
of P₁-MG are markedly different from those of nuclease P₁ in influence of ionic strength, pH, and temperature on the nucleolytic activity. The result of influence of pH and ionic strength on the nuclease activity of P₁-MG seems to support the previous suggestion⁹ that the interaction between the enzyme and the polysaccharide is electrostatic. The fact that pH optima of P₁-MG toward polynucleotides are lower than those of nuclease P₁ can be interpreted that the binding is more loose at lower pH. Dependence of nucleolytic activity of P₁-MG on ionic strength suggests that the binding in P₁-MG would be tight with decreasing ion strength. Formation of ovomucin-lysozyme complex, whose interaction is due to an electrostatic interaction, is reported to be affected markedly by pH and ionic strength.⁹

Elevation of temperature increases the reaction rates in a smaller extent for RNA and heat-denatured DNA in P₁-MG than in nuclease P₁, probably because association with the polysaccharide would make nuclease P₁ protein less flexible. It should be noted that relationship between temperature and activity in immobilized nuclease P₁ is similar to that of P₁-MG.⁹

The influence of ionic strength, pH, and temperature on the activity of nuclease P₁-galactan (P₁-G) is similar to that of nuclease P₁. However, the activity of P₁-G toward native DNA is about 1/50 to that of nuclease P₁ and the relationship between heat-denatured DNA degrading activity and ionic strength in P₁-G is intermediate between the relationships in P₁-MG and nuclease P₁. From these results it is conceivable that the binding of the enzyme to the polysaccharide becomes loose after the removal of the malonic acid.

It is interesting that the associated malonogalactan represses the nucleolytic activity, especially toward native DNA, but does not affect 3'-phosphomonoesterase activity. P₁-MG is expected to be much more useful for preferential hydrolysis of single-stranded regions of DNA than nuclease P₁.

P₁-MG is more thermostable than nuclease P₁. This finding would be consistent with a view observed in number of glycoproteins and carbohydrate-protein complexes that carbohydrate moiety plays a role in thermostability. Moreover, P₁-MG shows less susceptibility to protease than nuclease P₁. In addition, activity of nuclease P₁ is confirmed not to be affected by the presence of isolated malonogalactan.

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REFERENCES