Purification and Immunological Characterization of Human Pancreatic Ribonuclease

Minoru Kurihara,1 Michio Ogawa, Toshiyuki Ohta, Eiji Kurokawa, Takeshi Kitahara, Goro Kosaki, Takehiko Watanabe, and Hiroshi Wada

Second Department of Surgery, Osaka University Medical School, Fukushima-ku, Osaka 553 [M. O., T. O., E. K., T. K., G. K.], and Second Department of Pharmacology, Osaka University Medical School, Kita-ku, Osaka 530 [T. W., H. W.], Japan

ABSTRACT

Three alkaline RNases were purified from human pancreatic juice to near homogeneity as judged by sodium dodecyl sulfate and native polyacrylamide gel electrophoreses. The molecular weights of these RNases determined by sodium dodecyl sulfate electrophoresis were 27,000, 19,000, and 13,000. The activities of these three RNases were increased by addition of Mg2+, Ca2+, Co2+, K+, Na+, spermine, and spermidine and decreased by the addition of heparin, Cu2+, and Zn2+. These RNases showed higher hydrolytic activity toward polycytidylic acid than toward polyguanylic acid, polyuridylic acid, or polyadenylic acid.

The three human pancreatic RNases were immunologically identical but differed from human liver RNase, as shown by Ouchterlony double-diffusion test. Radioimmunoassay of human pancreatic RNase showed that immunologically similar RNases are present in human saliva, urine, and serum.

INTRODUCTION

RNase activity has been observed to be elevated in the serum of patients with malignant tumors (4, 12, 32). Recently, Reddi and Holland (21, 23) reported that pancreatic RNase activity in human serum could be measured in a specific way with poly(C) as substrate and could serve as a biochemical marker in the diagnosis of carcinoma of the pancreas. More recently, however, Peterson (20) found that patients with pancreatic cancer could not be distinguished in this way from those with pancreatitis or with nonpancreatic disease, although the RNase activity in all cases of pancreatic cancer was different from that in normal individuals. Moreover, he reported that poly(C)-specific RNase activity did not decrease in the serum of patients after total pancreatectomy.

These conflicting results could be because serum RNase with preference for poly(C) may originate from other organs beside the pancreas or could be due to variation in the stage of disease and secondary pathology of patients from whom sera were obtained.

In the present study, we purified RNase from human pancreatic juice, examined its properties, and developed a RIA for human pancreatic RNase. By this sensitive and specific method, we investigated the immunoreactivity of pancreatic RNase and compared it with those of RNases in various body fluids.

MATERIALS AND METHODS

Materials

Pancreatic juice was obtained by surgical cannulation of the pancreatic duct of patients undergoing resection of the head of the pancreas and stored at -20°C until use. These patients had a peripancreal neoplasm or gastric cancer with pancreatic infiltration. Human serum, urine, and saliva used for dilution curves were obtained from a healthy 33-year-old man. The following reagents were used: poly(C) (Yamasa Shōyu Co., Japan); yeast RNA, poly(G), poly(U), poly(A), SDS electrophoresis protein markers, and bovine RNase A (Sigma Chemical Co., St. Louis, Mo.); Sephadex G-75 superfine, Sephadex G-25, Sepharose 4B (Pharmacia, Uppsala, Sweden); phosphocellulose and ampholine (Brown Co., England); cellulose dialysis tubing (Nakarai Chemicals, Japan); Freund’s complete adjuvant (Wako Pure Chemical Industries, Ltd., Japan); Na131I (New England Nuclear); goat anti-guinea pig IgG serum and normal guinea pig serum (Elken Chemicals Co., Japan). Human liver RNase was purified in our laboratory (19). Poly(G) agar was prepared by the method of Schmukler et al. (25). Heparin Sepharose-CL-6B was purchased from Pharmacia. The gel was allowed to swell in distilled water and washed for about 15 min on a sintered glass filter with 0.05 M potassium phosphate buffer, pH 8.0, before being packed into a column for use.

Enzyme Assay

RNase activity was assayed by the method of Reddi (21). The standard assay system was as follows. The sample was diluted 500-fold with 0.1 M potassium phosphate buffer, pH 6.8. Then, 0.05 ml of diluted solution, 0.05 ml of poly(C) (4 mg/ml), and 0.15 ml of 0.1 M potassium phosphate buffer, pH 6.8, were mixed and incubated at 37°C for 15 min. The reaction was stopped by adding 0.25 ml of cold 12% perchloric acid containing 20 mM lanthanum nitrate. The solution stood in an ice bath for 15 min and was centrifuged in the cold at 12,000 × g for 15 min. Then, 200 μl of supernatant was diluted with 1 ml of distilled water, and its absorbance at 260 nm was measured. The RNase activity that caused an increase in absorbance of 1.0 under these assay conditions was defined as 1 unit.

Protein Determination

Protein concentration was determined by the method of Lowry et al. (13) or from the absorbance at 280 nm.

Purification of Human Pancreatic RNase

All procedures were carried out at 4°C unless otherwise stated.

Step 1. Acid Treatment. Frozen pancreatic juice (2,500 ml) was brought to room temperature before use. The pH was adjusted to 3.5 with 35% HCl, and the solution was kept in an ice bath for about 1 hr. The solution was centrifuged at 10,000 × g for 10 min, and the precipitate was discarded.

1 To whom requests for reprints should be addressed, at The Second Department of Surgery, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan.
Step 2. First Phosphocellulose Column Chromatography. The supernatant (2510 ml) was applied to a phosphocellulose column using a glass filter (10 cm in diameter x 5 cm in height) previously equilibrated with 0.05 M potassium phosphate-HCl buffer, pH 3.5. The column was washed with 1500 ml of 0.05 M potassium phosphate buffer, pH 6.8, and RNase was eluted with the same buffer containing 1.5 M NaCl. The solution was dialyzed twice against 5 liters of the same buffer.

Step 3. Second Phosphocellulose Column Chromatography. The sample (670 ml) was applied to a phosphocellulose column (2.5 x 52 cm) previously equilibrated with 0.05 M potassium phosphate buffer, pH 6.8. The column was washed with 300 ml of the buffer, and then protein was eluted with 2 liters of a linear gradient of NaCl (0 to 0.8 M) in the same buffer (Chart 1A). The flow rate was 40 ml/hr, and fractions of 20 ml were collected. Fractions with activity were combined (250 ml) and dialyzed twice against 5 liters of the above buffer.

Step 4. Third Phosphocellulose Column Chromatography. The sample was applied to a smaller phosphocellulose column (1.5 x 45 cm) under the same conditions as before, and the protein was eluted with 800 ml of a linear gradient of 0.1 to 0.7 M NaCl (Chart 1B). The flow rate was 20 ml/hr. Fractions of 10 ml were collected, and those with activity were combined (420 ml) and dialyzed against 4 liters of 0.05 M potassium phosphate buffer, pH 8.0.

Step 5. Poly(G) Affinity Chromatography. The sample was applied to a poly(G) affinity column (5.5 x 1.5 cm) equilibrated with 0.05 M potassium phosphate buffer, pH 8.0. The column was washed, and then RNase was eluted with the same buffer containing 1 M NaCl (Chart 1C). The sample (72 ml) was dialyzed against 1 liter of the same buffer and concentrated by lyophilization.

Step 6. Sephadex G-75 Gel Filtration. The concentrated preparation (2 ml) was applied to a Sephadex G-75 superfine column (1.8 x 113 cm) equilibrated with 0.05 M potassium phosphate buffer, pH 8.0, containing 0.3 M NaCl. The flow rate was 2 ml/hr, and fractions of 0.25 ml were collected. Three peaks of RNase activity were found (Chart 1D) and Peak 1 (RNase I), Peak 2 (RNase II), and Peak 3 (RNase III) were collected separately. RNase I was purified by an additional step.

Step 7. Isoelectric Focusing. The eluate of Peak 1 was dialyzed against 0.05 M potassium phosphate buffer, pH 8.0, and applied to an isoelectric focusing column (pH 9 to 11). Fractions of 1 ml were collected. The RNase activity was located at pH 10.0 (Chart 1E).

Heparin affinity chromatography could be used instead of isoelectric focusing. When heparin affinity chromatography was used, the sample from Step 6 was dialyzed extensively against 0.05 M potassium phosphate buffer, pH 8.0, and applied to a heparin affinity column (1.5 x 20 cm) equilibrated with the same buffer. The column was washed, and

![Chart 1. Purification of RNase from human pancreatic juice. A, second phosphocellulose column chromatography. The eluate from the first phosphocellulose column was applied to a second phosphocellulose column (2.5 x 52 cm) equilibrated with 0.05 M potassium phosphate buffer (pH 6.8). The protein was eluted with a linear gradient of NaCl (0 to 0.8 M). Fractions of 20 ml were collected, and those with activity (Tube Nos. 55 to 80) were pooled. Bars, fractions collected for further purification. B, third phosphocellulose column chromatography. The sample shown in A was applied to a third phosphocellulose column (1.5 x 45 cm) equilibrated with 0.05 M potassium phosphate buffer (pH 6.8). The protein was eluted with a linear gradient of NaCl (0.1 to 0.7 M). Fractions of 10 ml were collected and active fractions (Tube Nos. 25 to 65) were pooled. C, poly(G) affinity column chromatography. The sample shown in B was applied to a poly(G) affinity column (1.5 x 5.5 cm) equilibrated with 0.05 M potassium phosphate buffer (pH 8.0). The protein was eluted with the same buffer containing 1 M NaCl. Fractions of 5 ml were collected. D, Sephadex G-75 gel filtration. The eluate from the poly(G) affinity column was concentrated as described in the text and applied to a Sephadex G-75 superfine column (1.8 x 113 cm) previously equilibrated with 0.05 M potassium phosphate buffer (pH 8.0) containing 0.3 M NaCl. RNase was separated into 3 peaks of activity, which are referred to as RNases I, II, and III, in order of elution. E, isoelectric focusing. The RNase I fraction in D was applied to an LKB 8011 column. Electrophoresis was carried out for 48 hr at 0.3 ma and 600 V in 0.5% ampholine (pH 9 to 11) with a sucrose gradient. Fractions of 1 ml were collected.](chart1.png)
then material was eluted with a linear gradient of 0 to 0.8 M NaCl. RNase activity was eluted with 0.45 to 0.55 M NaCl.

**Electrophoresis**

Polyacrylamide gel electrophoresis was performed by the method of Shapiro et al. (26) using 10% polyacrylamide gel in the presence of 0.1% SDS. Polyacrylamide gel electrophoresis at pH 4.5 was performed by the method of Reisfeld et al. (24) using 10% polyacrylamide gel.

**Preparations of Antiserum**

Antiserum against human pancreatic RNase was produced in guinea pigs. The animals were given injections into multiple sites in the skin with a mixture of 0.25 ml of human RNase I (0.25 mg/ml) and an equal amount of complete Freund's adjuvant. The injection was repeated after 3 weeks and 6 weeks and, 10 days after the last injection, blood was collected by heart puncture, and the serum was separated and stored at -20°C.

**Ouchterlony Analysis**

Double diffusion in agar was performed by the procedure of Ouchterlony (19).

**RIA Procedure**

The purified human pancreatic RNase I was labeled with radioiodine following the method of Hunter and Greenwood (10). The procedure used for radioimmunoassay for human pancreatic RNase was a modification of the double-antibody technique of Morgan and Lazarow (14).

The reaction mixture consisted of 0.4 ml of 0.9% NaCl solution buffered with 0.01 M phosphate, pH 7.6, containing 0.2% bovine serum albumin and 0.1% sodium azide (NaN₃). 0.1 ml of standard solution or samples, 0.1 ml of labeled RNase I (about 3 x 10⁶ cpm) as tracer antigen, and 0.1 ml of diluted antiserum (1:10,000). The mixture was mixed well and stood overnight at 4°C. After this first incubation, 0.1 ml of diluted normal guinea pig serum (1:100) and 0.1 ml of diluted antiguinea pig IgG serum (1:20) were added with thorough mixing. The mixture stood overnight at 4°C and then was centrifuged at 3,000 rpm at 4°C for 30 min. The supernatant was decanted, and the radioactivity of the precipitate was counted in a Packard auto gamma counter. The percentage of binding of the tracer antigen was expressed as the ratio of the radioactivity of the bound fraction to the radioactivity of the same fraction in the absence of the unlabeled antigen. All samples were measured in duplicate.

**RESULTS**

**Purification**

RNase was purified from human pancreatic juice as described in "Materials and Methods." On Sephadex G-75 gel filtration, RNase was separated into 3 peaks (Chart 1D): RNase I, RNase II, and RNase III, in order of elution. RNase II and RNase III were essentially homogeneous at this step. RNase I was purified further by isoelectric focusing. Changes in specific activity and recovery during the purification procedure are summarized in Table 1. With this purification procedure, the yield of enzymic activity was 8.9% with about 1000-fold increase in specific activity.

**Purity**

The purities of the RNase preparations were examined by polyacrylamide gel electrophoresis. Each of the 3 human pancreatic RNases moved as a single, broad band on 10% polyacrylamide gel at pH 4.5, and the activity coincided in the position with the protein band (Fig. 1). On polyacrylamide gel

**Table 1**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Volume</th>
<th>Total activity (units)</th>
<th>Total protein (A₂₈₀)</th>
<th>Specific activity</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic juice</td>
<td>2500</td>
<td>772.5</td>
<td>13575</td>
<td>0.057</td>
<td>100</td>
</tr>
<tr>
<td>Acid treatment</td>
<td>2510</td>
<td>1096.87</td>
<td>14106.2</td>
<td>0.078</td>
<td>142</td>
</tr>
<tr>
<td>1st phosphocellulose</td>
<td>670</td>
<td>650.6</td>
<td>1742</td>
<td>0.37</td>
<td>84</td>
</tr>
<tr>
<td>2nd phosphocellulose</td>
<td>500</td>
<td>510</td>
<td>406.5</td>
<td>1.25</td>
<td>66</td>
</tr>
<tr>
<td>3rd phosphocellulose</td>
<td>420</td>
<td>394</td>
<td>157.8</td>
<td>2.54</td>
<td>51</td>
</tr>
<tr>
<td>Poly(G) affinity</td>
<td>72</td>
<td>172.8</td>
<td>53.3</td>
<td>3.24</td>
<td>22</td>
</tr>
<tr>
<td>Sephadex G-75 gel filtration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNase I</td>
<td>23.5</td>
<td>44.2</td>
<td>50.8</td>
<td>0.87</td>
<td>5.72</td>
</tr>
<tr>
<td>RNase II</td>
<td>14</td>
<td>44.16</td>
<td>0.62</td>
<td>71.68</td>
<td>5.72</td>
</tr>
<tr>
<td>RNase III</td>
<td>13.2</td>
<td>14.94</td>
<td>0.20</td>
<td>75.47</td>
<td>1.84</td>
</tr>
<tr>
<td>Isoelectric focusing RNase I</td>
<td>6.5</td>
<td>9.62</td>
<td>ND</td>
<td>ND</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*ND, not determined because the sample contained ampholine.
Table 2
Effects of ions and chemicals on enzyme activity
Assays were carried out in 0.1 M potassium phosphate buffer, pH 6.8, containing 5 mM ions or chemicals. Activity in the absence of ions or chemicals was taken as 100%.

<table>
<thead>
<tr>
<th>Ions and chemicals (5 mM)</th>
<th>RNase I</th>
<th>RNase II</th>
<th>RNase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Na⁺</td>
<td>114</td>
<td>115</td>
<td>112</td>
</tr>
<tr>
<td>K⁺</td>
<td>110</td>
<td>137</td>
<td>141</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>172</td>
<td>115</td>
<td>189</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>154</td>
<td>115</td>
<td>115</td>
</tr>
<tr>
<td>Spermine</td>
<td>141</td>
<td>126</td>
<td>265</td>
</tr>
<tr>
<td>Spermidine</td>
<td>204</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>40</td>
<td>33</td>
<td>82</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>38</td>
<td>33</td>
<td>82</td>
</tr>
<tr>
<td>Heparin</td>
<td>33</td>
<td>30</td>
<td>14</td>
</tr>
</tbody>
</table>

Chart 3. Cross-reactivities of bovine RNase A with antiserum with human pancreatic RNase and human liver RNase. The conditions for incubation and separation of bound antigens are described in "Materials and Methods." Data are shown on a semilog plot.

Table 3
Substrate specificity
Assays were carried out as described in the text. The concentration of substrate was 4 mg/ml. The activity with poly(C) as substrate was taken as 100%.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Poly(C)</th>
<th>Poly(A)</th>
<th>Poly(G)</th>
<th>Poly(U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase I</td>
<td>100</td>
<td>6</td>
<td>2.2</td>
<td>8.6</td>
</tr>
<tr>
<td>RNase II</td>
<td>100</td>
<td>5</td>
<td>4.3</td>
<td>0.5</td>
</tr>
<tr>
<td>RNase III</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RNase A</td>
<td>100</td>
<td>4.7</td>
<td>0.6</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Enzymological Properties

Effects of Ions and Chemicals on Enzymatic Activity. The enzymatic activities of the 3 RNases were increased in the presence of 5 mM of Na⁺, K⁺, Mg²⁺, Co²⁺, spermine, and spermidine and decreased by 5 mM of Cu²⁺, Zn²⁺, and heparin (Table 2). There were no remarkable differences in the effects of these ions and chemicals on the 3 RNases, although RNase II gave slightly different results from RNase I and RNase III with spermidine, Cu²⁺, and heparin.

Substrate Specificity. These RNases cleaved poly(C) better than poly(A), poly(G), or poly(U). Their substrate specificity was similar to that of bovine RNase A (Table 3).

Immunological Properties

Ouchterlony Test. Single precipitation lines were formed between anti-human pancreatic RNase serum and the 3 pancreatic RNases, and all the lines fused. No precipitation line was formed between anti-human pancreatic RNase I serum and liver RNase (Fig. 2).

Radioimmunoassay. The standard curve is shown in Chart 3. Using this standard curve, the concentration of human pancreatic RNase could be measured in the range 10 to 500 ng. As shown in Chart 3, slight cross-reactivity was detected between human pancreatic RNase and human liver RNase (about 10% at the point of 50% B/B₀), but there was no cross-reactivity between human pancreatic RNase and bovine RNase A. The dilution curves of human serum, urine, saliva, and pancreatic juice were parallel with the standard curve for human pancreatic RNase (Chart 4). The samples used for dilution curves were from the patients whose pancreatic juice was used for purification of RNase. The amounts of RNase, calculated from the dilution curves, were 480 ng/ml in serum, 200 ng/ml in saliva, 240 ng/ml in urine, and 6400 ng/ml in pancreatic juice.

DISCUSSION

RNases are widely distributed in various human tissues, and their purifications have been reported by several groups (6, 11, 16, 18, 22, 28, 29). Since the report of Reddi (21), pancreatic RNase has been of interest because of its possible diagnostic value as a marker of pancreatic cancer. At present, however, little is actually known about human pancreatic RNase in relation to the origin of human serum RNase. In the present study, we purified RNase from human pancreatic juice and examined its properties. Using this purified enzyme, we developed a RIA for determination of the immunoreactivities of various RNases in human biological fluids.

The pancreatic RNase that we have purified has 3 isozymes...
with different molecular weights. Weickmann et al. (31) purified an RNase from human pancreas. Their RNase gave a single band of protein on polyacrylamide gel electrophoresis in the presence of SDS, but on electrophoresis in 10% polyacrylamide at pH 4.5 it gave at least 3 protein bands. They purified their enzyme to the step of affinity chromatography, but we have purified RNase further by Sephadex G-75 gel filtration and isoelectric focusing. In the present study, pancreatic RNase activity was separated into 3 fractions by gel filtration. We also purified pancreatic RNase from human pancreas in a separate investigation, to be published, and found that it was also separated into at least 2 distinct fractions of activity with different molecular weights on gel filtration. Although we could not analyze the amino acid compositions or sugar compositions of these fractions because of the limited quantity of purified enzyme available, we tentatively conclude from the present results that human pancreatic RNase has at least 3 isozymes, consisting of the same polypeptide chain and differing only in their degree of glycosylation, as shown for bovine pancreatic RNase (2). However, it is still possible that some of these 3 RNases might be artifacts formed on acid treatment (29), that they are allelotypes, not isozymes, because pancreatic RNase was collected from several individuals, or that their difference might result from disease, although the pancreatic juice was collected from patients with periampullary neoplasm and gastric cancer.

Previous papers described slight cross-reactivity between human pancreatic RNase and bovine RNase A (31) and no cross-reactivity between pancreatic RNase and human liver RNase (17). However, our results were different; we found no cross-reactivity between human pancreatic RNase and bovine RNase A by RIA. However, the variation in results may simply be due to differences in the methods used. Moreover, slight cross-reactivity was demonstrated between human liver RNase and human pancreatic RNase by RIA, although no precipitation line was formed in an Ouchterlony double-diffusion test between human liver RNase and antisera to human pancreatic RNase.

Human serum contains many types of acidic RNases and alkaline RNases originating from various tissues (1, 3, 15). Alkaline RNase has been of interest because its activity is thought to be elevated in the serum of patients with malignant tumors. Houck and Berman (9) first reported the serum RNase levels in patients with various diseases, indicating that conjugative heart failure, myocardial infarction, and primary kidney disease with uremia were associated with an elevated serum RNase level. Zytko and Cantero (32) and Chretien et al. (4) reported that the serum RNase level was increased in patients with carcinoma. Serum RNase was also reported to increase in malnourished children (27). Akagi et al. (1) reported that there are at least 5 alkaline RNases in human serum and that 4 of them have properties similar to those of the bovine pancreatic enzyme.

Recently, Reddi (21) reported that human serum RNase was a good biochemical marker in diagnosis of carcinoma of the pancreas because of its unique specificity for poly(C). Warshaw et al. (30) also reported that increased poly(C)-specific serum RNase was of adjunctive value in the diagnosis of pancreatic carcinoma. On the contrary, Peterson (20) reported that the level of serum RNase activity toward poly(C) of patients with pancreatic cancer was not different from that of patients with nonpancreatic diseases. Doran et al. (5) and Höbling et al. (8) also reported that serum RNase with preference for poly(C) was not a specific marker of carcinoma of the pancreas. Furthermore, Peterson (20) reported that after total pancreatectomy, serum RNase activity remained in the normal range. Recently, we also experienced a case in which serum RNase activity with poly(C) as substrate was normal after total pancreatectomy.

In the present study, dilution curves of saliva were parallel with the standard curve of human pancreatic RNase in RIA, suggesting that salivary RNase is immunologically very similar to pancreatic RNase. Bovine RNase from the salivary gland has also been reported to be very similar to bovine pancreatic RNase (7). Human salivary gland may be a source of serum RNase in some patients. The previous reports and present results suggested that further study is needed to clarify the reasons for elevation of RNase in the serum of patients with malignant tumors.

ACKNOWLEDGMENTS

We thank Prof. Charles A. Dekker, University of California, and Prof. Masachika Irie, Hoshi College of Pharmacy, for valuable advice during preparation of the manuscript.

REFERENCES

Human Pancreatic RNase


Fig. 1. Gel electrophoresis of RNases I, II, and III on 10% acrylamide gels at pH 4.5. Approximately 3 to 5 µg of enzyme was applied per tube, and electrophoresis was carried out for 8 hr at 2 ma per tube. Electrophoresis was done in duplicate. One was stained with Coomassie brilliant blue. The other was cut into 2-mm sections with a gel slicer, and RNase activity was assayed. Migration was from left (anode) to right (cathode). Arrow, direction of electrophoretic migration.

Fig. 2. Immunodiffusion of RNases. The center well contained anti-RNase I serum. Peripheral wells contained RNases I, II, III, and human liver RNase. Well I, RNase I; Well II, RNase II; Well III, RNase III; Well IV, human liver RNase.
Purification and Immunological Characterization of Human Pancreatic Ribonuclease

Minoru Kurihara, Michio Ogawa, Toshiyuki Ohta, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/11/4836

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/42/11/4836. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.