The Immunological Characterization of Several Human Ribonucleases by using Primary Binding Tests

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RNAases (ribonucleases), purified from four human tissues, as well as bovine pancreatic RNAase (RNAase A), were studied by immunodiffusion methods and by two different primary binding tests. The enzymes fell into two groups immunologically, those purified from plasma and pancreas in one and those from spleen and liver in the other. No antigenic cross-reaction between the two groups was detected by any of the immunoassays used. There was a slight antigenic cross-reaction between the human and bovine pancreatic RNAases. The liver and spleen RNAases were immunologically identical by all criteria used, whereas a small but consistent antigenic difference between the human plasma and human pancreas enzymes was detected. The significance of this difference between the human plasma and pancreas RNAases is discussed in relation to similarities and differences in their properties.

Reports of patients with cystic fibrosis (Bardon *et al.*, 1976) and of patients with pancreatic carcinoma (Reddi & Holland, 1976) suggest that RNAase† activity in plasma may be a useful marker of certain disease states involving the pancreas. However, since there are two primary types of soluble human RNAases, the identification of the enzyme(s) responsible for abnormal RNAase activity in such pathological states remains to be determined. One major type of soluble human RNAase has been purified from plasma and tends to hydrolyse at cytidylic acid residues when reacting with yeast RNA (Schmukler *et al.*, 1975). The other major type of soluble human RNAase preferentially releases purine residues at the 5'-terminus of the phosphodiester bond and has been purified from human spleen (Neuwelt *et al.*, 1976) and from human liver (Frank & Levy, 1976). Although these two types of RNAases have different specificities, it is not always possible to identify unambiguously the presence of one and/or both of these enzymes in crude preparations, such as plasma, on the basis of hydrolysis of synthetic substrates. As a result, in the present work we attempted to compare and contrast the antigenicity of these two types of human RNAases in the hope of obtaining a more unambiguous means of differentiating between them.

The enzymes studied were RNAases purified from human spleen (Neuwelt *et al.*, 1976), human liver

(Frank & Levy, 1976) and human plasma (Schmukler *et al.*, 1975), as well as commercially purified RNAase A (bovine pancreas). Because of the recent interest in plasma RNAase activity in patients with pancreatic disorders, an additional RNAase, purified 916-fold from human pancreas, was also studied. Although preliminary immunological investigations were carried out by classical immunodiffusion techniques, these techniques can lack the necessary sensitivity for antigenic comparisons of enzymes (Neuwelt *et al.*, 1973). As a result, more sensitive and specific immunoassays which directly measure the interaction between antigen and antibody (i.e. primary binding tests) were also utilized. The primary binding tests chosen for the present studies, the blocking primary enzyme immunoassay and the $(\text{NH}_4)_2\text{SO}_4$ test, were specially designed so as not to require either purified antigen or monospecific antibody (Neuwelt *et al.*, 1973).

Experimental

Materials

Samples of human spleen, pancreas and liver were obtained from autopsy specimens kindly provided by the Baltimore City Medical Examiner's office. Human plasma was obtained from normal volunteers. RNAase A (chromatographically homogeneous) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

The methods used in the purification of yeast RNA and ribonucleotide polymers, as well as their com-

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† Abbreviation: RNAase, ribonuclease.

mercial sources, have been described elsewhere (Schmukler *et al.*, 1975).

The polyamine spermidine was obtained from Calbiochem, San Diego, CA, U.S.A.

CNBr-activated Sepharose 4B was purchased from Pharmacia, Piscataway, NJ, U.S.A.

The commercial sources and the molecular weights of the individual proteins used as standards in the estimation of the molecular weight of the human pancreatic ribonuclease were the same as those used in the determination of the human plasma RNAase as reported previously (Schmukler *et al.*, 1975).

Complete Freund's adjuvant was purchased from Difco Laboratories, Detroit, MI, U.S.A.

Assay of human ribonucleases

The standard assay system for the plasma and pancreas RNAases contained $1.5\mu\text{mol}$ of poly(C), $100\mu\text{mol}$ of Tris/HCl buffer, pH 7.5, 0.5 mg of bovine serum albumin and enzyme in 1 ml. After incubation for 15 min at 25°C , the reaction was stopped by the addition of 1 ml of 2M-HClO_4 , and the vessel containing the reaction mixture was placed in an ice bath for 10 min. The cloudy solution was then clarified by centrifugation ($30\,000g$ for 10 min) and the A_{260} of the acid-soluble nucleotides measured (Kalnitsky *et al.*, 1959). An enzyme unit is defined as the amount of enzyme needed to cause an increase in A_{260} of 1.0 under the conditions of the assay.

The standard assay system for the liver and spleen RNAases differed in that the reaction mixture contained 0.25 mg of yeast RNA, $100\mu\text{mol}$ of sodium phosphate buffer, pH 6.0, 0.5 mg of bovine serum albumin and enzyme in 1 ml. After incubation at 25°C for 15 min, enzyme activity was determined as above.

Other enzyme assays

Catalase and horse liver alcohol dehydrogenase, which were used as standards in the estimation of the molecular weight of the human spleen RNAase, were assayed as described by Beers & Sizer (1952) and by Vallee & Hoch (1955) respectively.

Enzyme purifications

RNAases from human plasma (Schmukler *et al.*, 1975), human liver (Frank & Levy, 1976) and human spleen (Neuwelt *et al.*, 1976) were purified as described previously. Because all of the purified human RNAases studied have been shown to exist in a variety of aggregated states, it has not been possible to demonstrate the homogeneity of any of the enzymes by polyacrylamide-gel electrophoresis (Neuwelt *et al.*, 1976; Schmukler *et al.*, 1975; Frank & Levy, 1976).

Protein determinations

Protein concentration was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard, or at low concentrations (less than 0.05mg/ml) by the fluorescence technique of Bohlen *et al.* (1973).

Estimation of molecular weight of the enzyme

The molecular weight of the human pancreatic RNAase was estimated by sucrose-density-gradient centrifugation (Martin & Ames, 1961). It was assumed that the enzyme protein was globular.

Preparation of antibody to human liver and pancreatic RNAase

The preparation of antibody to human liver RNAase and RNAase A was as described previously (Neuwelt *et al.*, 1971, 1976). Similarly, antibody to human pancreatic RNAase was prepared as the others, except that human pancreatic RNAase (specific activity 3018 units/g of protein; $8\mu\text{g/ml}$) was suspended in an equal volume of complete Freund's adjuvant and a portion (0.15 ml) of the suspension was injected directly into rabbit popliteal lymph nodes (Neuwelt *et al.*, 1971). The collection of antiserum was by cardiac procedure.

Detection of antibody to human ribonucleases

Human liver RNAase, human pancreatic RNAase or RNAase A (2.5 enzyme units in 0.3 ml of 0.85% NaCl) was added to a portion (0.1 ml) of pre- and post-immunization rabbit serum. In each case, the mixture was stirred mechanically at 4°C , and after 30 min a cold saturated solution of $(\text{NH}_4)_2\text{SO}_4$ (0.2 ml) was added and stirring was continued for a further 10 min (Neuwelt *et al.*, 1976). After centrifugation ($30\,000g$ for 10 min) to remove the precipitated enzyme-antibody complexes, RNAase activity in the supernatant solution was measured as described above.

Preparation of immobilized antisera

Unfractionated antiserum (3 ml) obtained as described above was dialysed for 2 h against 4 litres of 0.1M-NaHCO_3 containing 1M-NaCl . The antiserum to human liver RNAase, human pancreatic RNAase or RNAase A was coupled to 12 g of CNBr-activated Sepharose 4B essentially as described previously (Neuwelt *et al.*, 1973), except that the coupling reaction was carried out for 2 h at 25°C .

Immunodiffusion studies

Ouchterlony slides were prepared with 1% agarose, as described previously (Ouchterlony, 1949). Each well contained $10\mu\text{l}$ of either enzyme or whole serum.

Table 1. *Purification of human pancreatic ribonuclease*

Step	Volume (ml)	Protein (mg)	Activity (units)	Specific activity (units/mg)	Yield (%)
Crude extract	122	1248.0	1055056	845	100
(NH ₄) ₂ SO ₄ fractionation	9	474.8	798152	1680	76
Phosphocellulose chromatography	98	8.3	290570	34882	28
Affinity chromatography	19	0.3	247708	774087	24

Table 2. *Hydrolysis of synthetic polyribonucleotides by purified human pancreatic ribonuclease*

For the assay, 2 units of enzyme were added to a reaction mixture containing 1.5 μ mol of substrate and 100 μ mol of Tris/HCl buffer, pH 7.5. After incubation at 25°C for 7.5 min, the reaction was stopped by the addition of 1 ml of 20 mM-lanthanum nitrate in 2 M-HClO₄ (Schmukler *et al.*, 1972). Enzyme activity was measured as described in the Experimental section.

Substrate	Enzyme activity (A ₂₆₀)
Poly(C)	1.13
Poly(U)	0.05
Poly(G)	0
Poly(A)	0
Poly(A,C)	2.08
Poly(A,G)	0.03
Poly(A,U)	0.34
Poly(C,G)	0.58
Poly(G,I)	0.01
Poly(G,U)	0.01

Blocking primary enzyme immunoassay

This was carried out as described previously (Neuwelt *et al.*, 1973).

Results

Purification of human pancreatic RNAase (Table 1)

Human pancreas (24.0 g) was washed extensively and then homogenized in 120 ml of 1.0 mM-sodium phosphate buffer, pH 6.8, for 1 min in a Sorvall Omnimixer. Cellular debris was removed by centrifugation at 50000g for 15 min and the supernatant solution retained.

The remaining three purification steps (ammonium sulphate fractionation, cellulose phosphate chromatography and affinity chromatography) were carried out in a manner analogous to the methods reported previously for the plasma RNAase (Schmukler *et al.*, 1975).

Properties of human pancreatic RNAase

pH optima. When the production of acid-soluble nucleotides was used as an index of enzyme activity,

considerable variations in pH optima were found, not only between individual substrates, but also between different buffers used in the measurement of hydrolysis of the same substrate. Thus, at the optimum pH (7.5) in Tris/HCl buffer, hydrolysis of poly(C) was approx. 1.5 times that at the optimum pH (6.5) in phosphate buffer. The hydrolysis of yeast RNA showed the same optimum pH in either Tris/HCl or phosphate buffer (pH 8.0), but the enzyme activity in phosphate buffer was approximately twice that in Tris/HCl.

Hydrolysis of synthetic polyribonucleotides

The activity of the purified pancreatic enzyme towards synthetic polyribonucleotides paralleled closely that of the plasma RNAase (Schmukler *et al.*, 1975). Of the homopolymers studied, only poly(C) was significantly attacked. Similarly, only those copolymers containing poly(C) were hydrolysed, except for poly(A,U), which was hydrolysed to a small extent (Table 2).

Molecular weight. The molecular weight of the purified pancreatic RNAase was estimated by sucrose-density-gradient centrifugation (Martin & Ames, 1961) as 33500 in the presence of 0.05 M-sodium phosphate buffer (pH 7.6) and 1.0 mM-spermidine at 4°C.

Inhibition by poly(A). Since the inhibitory effects of poly(A) on RNAase from human plasma, liver and spleen and the reversal of that inhibition by spermidine have been investigated (Neuwelt *et al.*, 1976; Frank & Levy, 1976; Schmukler *et al.*, 1975; Heiter *et al.*, 1976), studies were carried out to determine if similar effects could be demonstrated with human pancreatic RNAase. The results are analogous to those for the other three human enzymes studied in that poly(A) inhibited human pancreatic RNAase activity (Table 3) and this inhibition was readily reversed by spermidine. It is noteworthy that compared with human plasma RNAase, the human pancreatic enzyme seemed to be somewhat more sensitive to the effects of spermidine (Table 3).

Immunodiffusion studies

Initially, immunodiffusion studies were used to examine each post-immunization serum for the presence of antibody against each type of RNAase used

Table 3. *Inhibition of RNAase activity by poly(A) and reversal of inhibition by spermidine*

RNAase activity was measured as described in the Experimental section, except that 1 unit of enzyme was used. When the effects of poly(A) were studied, 0.15 μ mol of poly(A) was added to the standard reaction mixture before the addition of substrate [0.15 μ mol of poly(C)]. Reversal of poly(A) inhibition by spermidine was studied in the standard reaction mixture, except that 0.15 μ mol of poly(A) and 2.0 μ mol of spermidine were added before the addition of 1 unit of enzyme. The values above are representative of three determinations, and the variation of replicate determinations was less than 7%.

Enzyme	RNAase activity (units)			
	Control	Poly(A)	Spermidine	Poly(A)+spermidine
Human pancreas	1.04	0.43	1.76	0.812
Human plasma	1.21	0.56	1.41	0.89

Fig. 1. Immunoprecipitation of RNAase A (A) and human pancreas RNAase (P) (a) with anti-RNAase A (AA) and (b) with anti-human pancreas RNAase (AP) rabbit sera

Ouchterlony slides were prepared with 1% agarose as described previously (Ouchterlony, 1949; Stumpf *et al.*, 1971), except that each well contained 10 μ l of either enzyme or whole serum. The concentrations of RNAase A and of human pancreas RNAase were 1 mg/ml (specific activity 1100 units/g of protein) and 10 μ g/ml (specific activity 775 units/g of protein) respectively. The antisera were prepared as described in the Experimental section.

as an antigen. Fig. 1 shows precipitin lines indicative of antibody production between RNAase A and its antiserum, and between human pancreas RNAase and its antiserum. Although RNAase A is derived from bovine pancreas, there was no demonstrable cross-reaction between the human and bovine enzymes, despite the use of a 100-fold greater concentration of RNAase A (Fig. 1b). Antigenic simi-

larity was seen, however, as a weak line of identity between human pancreatic and plasma RNAases when the antiserum to human pancreatic RNAase was used (result not shown). No precipitin lines were detected between the liver RNAase and the serum obtained from three rabbits immunized with this enzyme. Thus the immunodiffusion studies only demonstrated the presence of antibody in serum from the rabbits immunized with human pancreas RNAase and RNAase A, and the only antigenic similarity detected by this technique was between human plasma and pancreas RNAase.

Detection of antigenic cross-reactions between various human RNAases by precipitation of RNAase-antibody complexes with $(\text{NH}_4)_2\text{SO}_4$

Minden & Farr (1971) have shown immunoprecipitation and other secondary binding tests to be insensitive, at times, to the presence of antigen-antibody binding. To rule out this possibility of insensitivity, each enzyme was also made to react with each antiserum, and any immune complexes formed were precipitated subsequently with a cold $(\text{NH}_4)_2\text{SO}_4$ solution (Neuwelt *et al.*, 1976). In contrast with the immunodiffusion studies, this test revealed the presence of a cross-reaction between human pancreatic RNAase and RNAase A, by using anti-RNAase A serum (Table 4). It detected, moreover, antibody in the rabbits immunized with human liver RNAase, and demonstrated the existence of a cross-reaction between human liver and spleen RNAase. When the antibody was conjugated to Sepharose 4B so as to eliminate the need for $(\text{NH}_4)_2\text{SO}_4$ as a precipitant of immune complexes, the results were identical. On the basis of these studies there appeared to be at least two immunologically distinct groups of human RNAases, one the spleen and liver enzymes, and the second the plasma and pancreatic RNAases. The latter group also appeared to be related to RNAase A, when anti-RNAase A serum was used. It should be noted, however, that no cross-reaction was seen between anti-human

Table 4. *Detection of cross-reactivity of various RNAases by $(\text{NH}_4)_2\text{SO}_4$ precipitation of immune complexes*

Individual portions (0.1 ml) of antisera were added to 2.5 units of each of the enzymes listed in the first column of the Table: 0.85% NaCl (0.3 ml) in 0.01 M-Tris/HCl buffer, pH 7.5, was then added to each mixture. After incubation for 30 min at 4°C, 0.2 ml of a cold saturated solution of $(\text{NH}_4)_2\text{SO}_4$ was added and the immunoglobulins were allowed to sediment in the cold for 10 min (Neuwelt *et al.*, 1976). The cloudy mixture was clarified by centrifugation (30000 *g* for 10 min) and enzyme activity in the supernatant solution was determined by the increase in A_{260} of acid-soluble nucleotides (Kalnitsky *et al.*, 1959). Control reaction mixtures were prepared as above, except that they contained preimmunization sera in place of antisera. The values shown are averages of six determinations and reflect the percentages of enzyme bound to antisera compared with preimmunization sera. The variation between replicate determinations was less than 5% in all cases.

Enzyme	Enzyme bound to antibody (%)		
	Antibody to RNAase A	Antibody to human pancreas RNAase	Antibody to human liver RNAase
RNAase A	100	2	0
Human pancreas RNAase	99	100	0
Human plasma RNAase	100	100	2
Human spleen RNAase	0	5	99
Human liver RNAase	0	4	100

pancreatic RNAase) serum and RNAase A (Table 4). This disparity between results observed with the anti-(RNAase A) and the anti-(human pancreatic RNAase) sera suggests that there is a marked antigenic difference between RNAase A and human pancreatic RNAase.

Use of a primary binding test to detect reactions of identity, reactions of partial identity and reactions of non-identity between various human RNAases

Since the $(\text{NH}_4)_2\text{SO}_4$ test cannot differentiate easily between a reaction of identity and a reaction of partial identity between two proteins, an additional procedure was used. The blocking primary enzyme immunoassay utilized previously (Neuwelt *et al.*, 1973) to detect subtle antigenic differences between normal and mutant arylsulphatase A enzymes was adapted to study the human RNAases (Fig. 2). The assay, although analogous to the blocking solid-phase radioimmunoassays, differs in that the specificity of an enzyme for its substrate is used instead of radioactivity. That is, variable amounts of a test enzyme are used to saturate a solid-phase immunoadsorbent, and any test enzyme that does not bind is washed away. The standard enzyme, the one to which the antiserum on the immunoadsorbent was made, is then added to fill any remaining antigen-binding sites. After this, the immunoadsorbent is allowed to settle to the bottom of the tube, and any unbound standard enzyme in the supernatant solution is determined. If the test enzyme saturates all the antigen-binding sites on the immunoadsorbent, none of the subsequently added standard enzyme binds, so that all of the standard

enzyme activity remains in the supernatant solution. This result is a reaction of identity. If, on the other hand, the test enzyme does not fill all of the antigen-binding sites, some of the standard enzyme will bind to the immunoadsorbent, which results in less standard enzyme activity in the supernatant solution. This is a reaction of partial identity. If the test enzyme is not able to fill any of the antigen-binding sites, the standard enzyme binds maximally, giving a minimum enzyme activity in the supernatant solution, and is therefore a reaction of non-identity.

The theoretical curves that result from the blocking immunoassay are illustrated in Fig. 2(a), which shows that the maximum enzyme activity in the supernatant solution is obtained by adding the standard enzyme to buffer instead of to the immunoadsorbent. The minimum activity in the supernatant solution is obtained by adding the standard enzyme directly to the immunoadsorbent without the prior addition of any test enzyme. These maximum and minimum values are referred to as the upper and lower baselines. The relationship of the upper plateau values (arrows, Fig. 2a) to the upper and lower baselines directly reflects the ability of the test enzyme to fill the antigen-binding sites on the immunoadsorbent. In a reaction of identity the upper plateau is equal to the upper baseline; in a reaction of partial identity, the upper plateau is anywhere between the two baselines; and, in a reaction of non-identity, there is no upper plateau, simply a straight line equal to the lower baseline.

With this in mind, the blocking immunoassay indicates that the human liver and spleen enzymes are antigenically identical, since the upper plateau for both enzymes is equal to the upper baseline (Fig. 2b). The human plasma and pancreatic RNAases,

Fig. 2. Use of the blocking primary enzyme immunoassay (Neuwelt et al., 1973) to detect antigenic cross-reactions between various RNAases

The sources of the RNAases and antisera are described in the Experimental section, as are the RNAase assays. (a) Theoretical curve. As discussed in the text, the arrows indicate the upper plateau values of enzyme activity measured in the supernatant solution. As indicated, the upper baseline represents the RNAase activity of the supernatant solution of control mixtures containing only buffer plus standard enzyme. The lower baseline represents the RNAase activity of the supernatant solution of control mixtures containing rabbit antibody conjugated to Sepharose plus standard enzyme (see the text). (b) Reaction of identity between human liver and human spleen RNAases. Anti-(human liver RNAase) serum conjugated to Sepharose 4B was used as the immunoadsorbent. Human liver enzyme (specific activity 88000 units/mg of protein, $3\mu\text{g}$ of protein/ml) ($\cdots$), and human spleen enzyme (70000 enzyme units/mg of protein, $3\mu\text{g}$ of protein/ml) (—), were used as the test enzymes. The test enzymes were serially diluted, and 0.3 ml of each dilution was added to a mixture consisting of 0.15 ml of immunoadsorbent and 0.55 ml of

although very similar antigenically, can be differentiated, since the upper plateau for the plasma RNAase, in contrast with that for pancreatic RNAase, is slightly below the upper baseline (Fig. 2c). The curve for the human pancreatic enzyme is shifted to the right of the curve for the plasma RNAase because of a higher concentration of pancreatic-enzyme protein. The blocking immunoassay (Fig. 2c) confirms the results of the $(\text{NH}_4)_2\text{SO}_4$ test in that it suggests a reaction of non-identity between human liver and pancreas enzymes. That is, when the human liver enzyme is used as the test enzyme, a straight line of activity equal to the lower baseline is obtained. When anti-(RNAase A) serum is used as the immunoadsorbent, the upper plateau for human pancreatic RNAase is consistently just above the lower baseline (Fig. 2d). This suggests that RNAase A and human pancreas enzyme, although antigenically related, share few antigenic sites. These results, then, are quite consistent with both the immunodiffusion studies (Fig. 1) and the $(\text{NH}_4)_2\text{SO}_4$ test (Table 4), both of which also indicated a marked antigenic difference between the human and bovine RNAases.

Discussion

Although immunodiffusion techniques are the most commonly used immunoassays in enzymology, these methods are relatively insensitive (Minden & Farr, 1971) and their results can even be misleading (Neuwelt *et al.*, 1973). The results of the present study further delineate this inadequacy of immunodiffusion as a sole means of detecting the presence of antibody and/or of distinguishing antigenic differences between two enzyme proteins. Thus the immunodiffusion studies reported here did not detect the presence of any antibody in three rabbits immunized with purified liver RNAase, in contrast with the positive results obtained with the blocking immunoassay and the $(\text{NH}_4)_2\text{SO}_4$ test. In addition, the immunodiffusion

slides did not reveal any cross-reaction between the human and bovine pancreatic enzymes, which was detected when the two primary binding tests were used. In short, primary binding tests were considerably more sensitive and specific than immunodiffusion methods in the immunological evaluation of human and bovine RNAases.

The results of the primary binding tests using antisera against human RNAases revealed several clear differences between the enzymes studied. The four human RNAases fell into two non-cross-reacting groups immunologically; the pancreas and plasma enzymes were in one group and the liver and spleen enzymes in the other. Thus the pancreas and plasma RNAases can be unambiguously differentiated from the liver and spleen RNAases on an immunological basis. Antigenic differences were not found between the liver and spleen enzymes, in agreement with reports describing the properties of the two proteins (Neuwelt *et al.*, 1976; Frank & Levy, 1976). In contrast, immunological studies of the human and bovine pancreatic RNAases revealed a very large antigenic difference between the two enzymes, a finding which, of course, is not surprising in view of their very different enzymic properties (Schmukler *et al.*, 1975; Fruchter & Crestfield, 1965). The human plasma and pancreatic RNAases appear to be quite closely related, in spite of a small, but consistent, antigenic difference. A further difference should also be noted, i.e. the increased sensitivity of the human pancreatic enzyme to spermidine. The significance of these immunological and enzymic differences is unclear at this time, since by other criteria the human plasma and pancreatic proteins appear to be quite similar (Schmukler *et al.*, 1975). Clinical studies also suggest that the two enzymes may be related, since pathological processes involving the pancreas result either in an increase (Reddi & Holland, 1976) or in a decrease (Bardon *et al.*, 1976) in plasma RNAase activity. Thus, although the antigenic and enzymic differences described here are

0.1% bovine serum albumin in 0.85% NaCl. After the mixture was incubated at 4°C for 30 min, the standard enzyme, human liver RNAase (5 units), was added to the reaction mixture. After another 30 min incubation, the conjugated Sepharose was allowed to settle and a portion of the supernatant solution was assayed for RNAase activity. The upper and lower horizontal lines represent the upper and lower baselines as discussed in Fig. 2(a) above. The test was performed five times and the results were internally consistent each time. (c) Reaction of partial identity between human pancreatic and plasma RNAases; reaction of non-identity between human pancreatic and liver RNAases. Reaction mixtures were prepared as in (b), except that the anti-(human pancreas RNAase) conjugated to Sepharose 4B was used as the immunoadsorbent. Human pancreas RNAase (3815 units/g of protein; 8 µg protein/ml; ····), human plasma RNAase (77000 units/mg of protein; 4 µg of protein/ml; —) and human liver RNAase (88000 units/mg of protein; 10 µg of protein/ml; ----) were used as test enzymes. Human pancreas RNAase (10 units) was added to each tube as the standard enzyme. The test was conducted five times and the results were always internally consistent. (d) Reaction of partial identity between human pancreas RNAase and RNAase A. Reaction mixtures were prepared as in (b) except that the anti-(RNAase A) serum conjugated to Sepharose 4B was used as an immunoadsorbent. RNAase A (1100 units/g of protein; 10 µg/ml; —) and human pancreas RNAase (3815 units/g; 8 µg/ml; ····) were used as test enzymes. RNAase A (7.5 units) was added to each tube as the standard enzyme. The test was conducted three times and the results were all internally consistent.

slight, they probably warrant a careful and detailed analysis of the specificity of attack of the two enzymes on internucleotide linkages.

Finally, the absence of any immunological cross-reaction between the plasma pancreas-type RNAase and liver-spleen-type RNAase suggests that immunological probes may be a convenient way of determining the distribution of these two types of enzyme systems in human tissues. Such studies would provide not only information as to the potential tissue sources of plasma RNAase, but also a means of determining which RNAase(s) are present in plasma under certain pathological conditions (Bardon *et al.*, 1976; Reddi & Holland, 1976).

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