Biochimica et Biophysica Acta, 438 (1976) 461—473

BBA 67856

HUMAN PANCREATIC-TYPE RIBONUCLEASES WITH ACTIVITY AGAINST DOUBLE-STRANDED RIBONUCLEIC ACIDS

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(Received January 8th, 1976)

Summary

Purified acid-thermostable ribonuclease (Ribonucleate 3′-pyrimidino-oligonucleotidohydrolase, EC 3.1.4.22) from human pancreas degrades double-stranded RNA at 2% the rate for single-stranded RNA. The activities against single-stranded RNA and double-stranded RNA were shown to be due to a single enzyme with properties similar to bovine pancreatic RNAase A. For purposes of comparison the activities against double-stranded RNA of crystalline ribonucleases of the whale, rat and cow were assayed and found to be 0.4%, 0.03% and 0.003%, respectively, of their activities against single-stranded RNA.

Both human serum and urine contain RNAase components of pancreatic origin which hydrolyze double-stranded RNA at 2% and 0.4%, respectively, of the rates against single-stranded RNA. By contrast, purified acid-thermostable RNAases from human spleen and liver hydrolyze double-stranded RNA at least 20-fold more slowly than human pancreatic RNAase, relative to the corresponding rates against single-stranded RNA. The human pancreatic and serum enzymes exhibit appreciable activity against the poly(C) component of the double-stranded poly(I) · poly(C); they also attack poly(C) itself at approximately 25 times the rate for poly(U) and at more than 50 times the rate for single-stranded RNA.

Introduction

During the course of a study of RNAases of human origin [1], it was noted that human pancreatic RNAase (Ribonucleate 3′-pyrimidino-oligonucleotidohydrolase, EC 3.1.4.22) exhibited surprisingly high relative activity towards

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Abbreviation: Up-naphthyl, uridine-3′-(*a*-naphthylphosphate).

double-stranded RNA. In view of the well-known low activity of bovine pancreatic RNAase against double-stranded RNA, it appeared desirable to examine this phenomenon in greater detail.

Double-stranded RNA is fairly widespread in mammalian systems, constituting well-defined regions in heterogenous nuclear RNA [2,3]. Moreover, interferon induction in virus-infected cells is known to be mediated via double-stranded RNA [4], and interferon may be induced in normal cells by either natural or synthetic double-stranded RNAs [4,5]. One of the most striking effects of double-stranded RNA in mammalian systems is its very high specific inhibition of protein synthesis [6] by complexing with the initiation factor [7].

It is consequently not at all unusual that a number of investigators have investigated the presence in mammalian cells and body fluids of activity against double-stranded RNA [6,8-12]. There is, in fact, currently an active search under way for the mammalian equivalent of *E. coli* RNAase III [13], the activity specific towards double-stranded regions in, and involved in the maturation of, RNA [14—16].

Considerable attention has also recently been devoted to the enhanced activity against double-stranded RNA exhibited by oligomeric forms of bovine pancreatic RNAase [17—19] and by bull seminal plasma RNAase [17,20].

Materials

Substrates

Yeast RNA, highly polymerized, from British Drug Houses (Poole, Dorset, England) was employed as a single-stranded RNA substrate. The double-stranded RNA substrates included phage f2 double-stranded RNA [21], a gift from Dr. J. Doskocil of the Institute of Organic Chemistry and Biochemistry, Prague, Czechoslovakia; and mycoviral double-stranded RNA, from a virus of *Penicillium chrysogenum* [22], kindly provided by Dr. J. Heyes of Beecham Pharmaceuticals, Betchworth, Surrey, England. Poly(U), poly(A), poly(C), and the double-stranded poly(I) · poly(C) were purchased from Miles Laboratories (Elkhart, Indiana, U.S.A.); highly polymerized calf thymus DNA from Sigma Chemical Co. (St. Louis, Mo., U.S.A.); and thymidine 3′- and 5′-(*p*-nitrophenylphosphate)s from Raylo Chemicals (Edmonton, Alta., Canada). Uridine-3′-(*α*-naphthylphosphate) was prepared according to Kole et al. [23]. Crystalline bovine serum albumin was obtained from Pentex (Kankakee, Ill., U.S.A.), and gelatin was Bacto grade from Difco Laboratories (Detroit, Mich., U.S.A.).

Enzymes

Bovine pancreatic RNAase (RNAase A) was a product of Sigma. Pancreatic DNAase and *α*-chymotrypsinogen were purchased from Worthington Biochemical Co. (Freehold, N.J., U.S.A.). Crystalline RNAase from rat pancreas [24] was a gift of Dr. M. Gruber, Biochemisch Laboratorium der Rijksuniversiteit te Groningen, Netherlands; and crystalline RNAase W_1 from whale pancreas [25] was provided by Dr. M. Irie, Hoshi College of Pharmacy, Tokyo, Japan. Crystalline bovine pancreatic RNAase, isolated by trichloroacetic acid extraction [26], was contributed by Dr. Maria Malicka, Institute of Biochemistry, Wrocław, Poland.

The RNAases from human pancreas, liver, spleen, serum and urine were purified as described by Bardoń et al. [1]. The preparations, which consisted of the effluents from SE-Sephadex in buffered 0.3—0.45 M KCl, were heated at 100°C at pH 3 for 5 min, neutralized and centrifuged. As previously described, human serum and urine contain two types of RNAase, which readily separate on an SE-Sephadex column into two peaks, A and B. Peak A exhibits the specificity of pancreatic-type RNAase and attacks Up-naphthyl, whereas peak B exhibits the properties of spleen and liver RNAase and is inactive against Up-naphthyl [1]. Only the properties of peaks A of serum and urine were examined in this study.

The final enzyme preparations were devoid of activities against native and denatured DNA, thymidine-5′-(*p*-nitrophenylphosphate) and thymidine-3′-(*p*-nitrophenylphosphate).

Fresh bovine pancreas was collected on solid CO_2 from the slaughterhouse. Human tissues were obtained from autopsies 24 h following decease at the Wolski Hospital, Warsaw.

Methods

RNAase activity towards single-stranded RNA and double-stranded RNA was determined by incubation in 0.3 ml medium including 300 μg single-stranded RNA or 100 ug double-stranded RNA, 0.03 M Tris · HCl buffer pH 7.6 (for the pancreatic, urine and serum enzymes) or pH 7.0 (for the spleen and liver enzymes), 0.1 M NaCl and 300 μg bovine serum albumin. Incubation with single-stranded RNA was for 15 min at 37°C, with double-stranded RNA for 5 min, following which 0.3 ml of precooled 1 M HCl in 76% ethanol was added. The mixture was kept on an ice-bath for 15 min and centrifuged at 5°C. The supernatant was diluted 3-fold and the increase in absorption at 260 nm, relative to a control without enzyme, was measured spectrophotometrically. Activity against double-stranded RNA was linear for only 5 min, but results for this substrate were also expressed in terms of 15-min incubation periods.

For poly(I) · poly(C) activity was determined by incubation for 15 min in 0.3 ml medium containing 100 μg substrate, 0.03 M Tris · HCl buffer, pH 7.6, 0.15 M NaCl and 300 μg bovine serum albumin. For poly(C) and poly(U) the medium contained 0.1 M NaCl and 300 μg gelatin in place of albumin. The reaction was terminated by addition of a 2-fold excess of cold 12% perchloric acid containing 20 mM lanthanum acetate [27] and the mixture centrifuged as above. The absorbances of the supernatants were read at 260 nm for poly(U) and at 280 nm for poly(I) · poly(C) and poly(C).

Activities are expressed as μmol acid-soluble product/15 min per mg protein, using extinction coefficients of $11 \cdot 10^3$ for RNA, $9.9 \cdot 10^3$ for poly(U) and $12.8 \cdot 10^3$ for poly(C).

Activity against poly(A) and, in some instances, against single-stranded RNA and double-stranded RNA was determined by following the increase in hyperchromicity of 10^{-4} M substrate in 0.03 M Tris · HCl buffer, pH 7.0 or 7.6 (depending on source of enzyme) containing 0.05% albumin and 0.1 M NaCl in semi-micro 10-mm pathlength cuvettes with a Cary 118 spectrophotometer, also at 37°C.

RNAase activity against Up-naphthyl was determined as previously described [23].

The incubation mixtures were buffered to the same pH values regardless of the substrate employed in order to secure comparable results for enzymes from different sources; hence activities against some substrates vary somewhat from those at the pH optima.

Enzymes were diluted in 5 mM Tris · HCl buffer pH 8.2 [28] containing 0.1% gelatin; RNAase A and rat pancreatic RNAase were diluted in buffer alone.

The pH optima were established by incubating the enzyme as above, using the following buffer systems: 0.03 M Tris · HCl pH 6.8—9.2 or 0.03 M Davis buffer pH 5.8—8.6 for single-stranded RNA and double-stranded RNA; 0.03 M Tris · HCl and 0.02 M Tris/citrate [17] pH 5—8.5 for poly(C); and 0.02 M Tris/citrate for poly(U).

Heating of enzyme samples was performed by immersion of the enzyme solution, preadjusted to pH 5 for 5 min in a water bath at the desired temperature. Heating was terminated by immersion in an ice bath, and samples assayed for residual activity.

Results

The relatively low activity of bovine pancreatic RNAase against double-stranded RNA has been known for some time and, in fact, has been frequently employed in the past for distinguishing the replicative forms of viral RNA from their single-stranded counterparts [29]. As can be seen from Table I, the rate of hydrolysis of RNA by RNAase A, which is 500 000 μmol for single-stranded RNA, falls dramatically for double-stranded RNA to a value of only 16 μmol

TABLE I

ACTIVITIES OF VARIOUS PURIFIED HUMAN RNAases AND OF CRYSTALLINE PANCREATIC RNAases FROM THE WHALE, RAT AND COW TOWARDS SINGLE-STRANDED RNA AND DOUBLE-STRANDED RNA

Source of ribonuclease	Activity (in μmol acid-solubilized substrate/15 min/mg protein)		Activity against double-stranded RNA / Activity against single-stranded RNA
	Single-stranded RNA	Double-stranded RNA from phage f2	
Human pancreas	3 400	65	$2 \cdot 10^{-2}$
Human serum (peak A)	640	15	$2 \cdot 10^{-2}$
Human urine (peak A)	43 500	168	$4 \cdot 10^{-3}$
Human spleen	2 400	—	—
Human liver	1 100	—	—
Whale pancreas	90 000	340	$4 \cdot 10^{-3}$
Rat pancreas	130 000	41	$3 \cdot 10^{-4}$
Bovine pancreas (RNAase A)	500 000	16	$3 \cdot 10^{-5}$

acid-soluble products/15 min/mg protein, under analogous conditions.

A striking difference in behaviour is exhibited by the acid-thermostable RNAase from human pancreas. From Table I it will be seen that a purified preparation of human pancreatic enzyme [1], homogeneous in polyacrylamide gel electrophoresis and sedimentation velocity analysis, hydrolyzes single-stranded RNA at a rate of 3400 μmol/15 min/mg protein, whereas the corresponding value with double-stranded RNA as substrate is 65.

Measurements of rate of increase in hyperchromicity gave similar results; only a 40-fold higher concentration of the human pancreatic enzyme was required to produce an increase in hyperchromicity of double-stranded RNA equal to that with single-stranded RNA. This result was identical using two samples of double-stranded RNA from widely different sources, namely from phage f2 and from a virus of *Penicillium chrysogenum*. By contrast approximately 30000 times more RNAase A was required for hydrolysis of double-stranded RNA to an extent comparable with that for single-stranded RNA.

Since the foregoing results appeared to be at variance with the widely accepted notion of the low activity of pancreatic RNAase towards double-stranded polyribonucleotides, it appeared desirable to establish whether the relatively rapid cleavage of double-stranded RNA by our preparation is an intrinsic property of the human enzyme, and whether RNAases from other human organs, and from the pancreas of other mammalian species, exhibit similar properties.

The following data indicate that the activities of human pancreatic RNAase

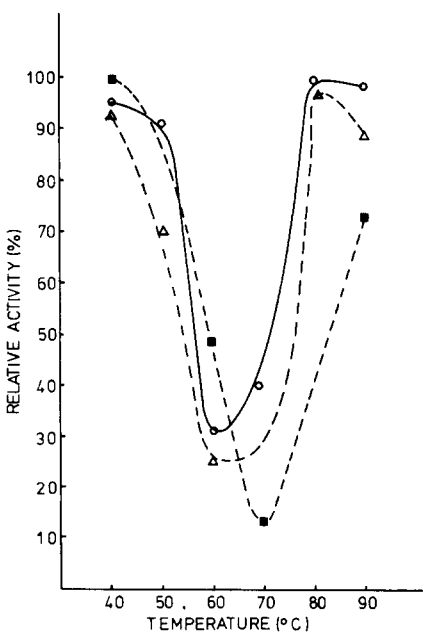

Fig. 1. Effect of heating for 5 min at pH 5 to various temperatures on activities of human pancreatic ribonuclease on: —△—△—△—, single-stranded RNA; —○—○—○—, double-stranded RNA; —■—■—■—, Up-naphthyl.

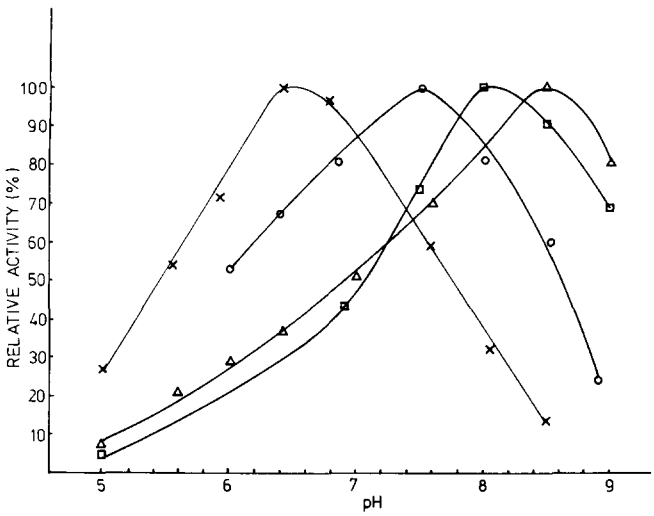

Fig. 2. pH-dependence of human pancreatic ribonuclease activity on: —△—△—△—, single-stranded RNA; —○—○—○—, double-stranded RNA; —X—X—X—, poly(U); —□—□—□—, poly(C).

against single-stranded RNA and double-stranded RNA are due to a single enzyme with the properties of a bovine pancreatic RNAase:

(a) The relative activities against single-stranded RNA, double-stranded RNA and Up-naphthyl remained unchanged throughout the purification procedure. There were small variations in the relative rates of hydrolysis of single-stranded RNA and double-stranded RNA dependent on the ionic strength at which the enzyme was previously stored (see below).

(b) Heating of the enzyme at 100° C at pH 8.5 for 5 min led to total loss of activities against single-stranded RNA, double-stranded RNA and Up-naphthyl.

(c) Heating of the enzyme at pH 5 at temperatures up to 60—70° C led to parallel decreases in activities against the aforementioned three substrates (Fig. 1). Somewhat unexpectedly, activities were regained after heating at temperatures above 70° C. The source of this enhanced stability following heating at more elevated temperatures is not clear.

(d) The enzyme hydrolyzes pyrimidine homopolyribonucleotides like poly(C) and poly(U) (see below for details). With double-stranded poly(I) · poly(C), the acid-soluble products exhibited the typical ultraviolet spectrum of CMP, pointing to the absence of significant activity against poly(I). As for bovine pancreatic RNAase A [30], activity against poly(A) was detectable only at high enzyme concentrations, and was less than 0.03% that against single-stranded RNA.

Addition of human pancreatic RNAase to RNAase A did not alter the low activity of the latter against double-stranded RNA, thus excluding the possibility that the human preparation contains some activator(s) for cleavage of double-stranded RNA. On the other hand, activities of the human enzyme exhibited different pH optima against single-stranded RNA and double-stranded RNA, the values being about 8.5 and 7.5, respectively (Fig. 2).

Since the human pancreas preparation was obtained by a purification procedure involving sulfonosalicylic acid treatment [1], as compared to traditional

TABLE II

EFFECT OF NaCl CONCENTRATION ON HUMAN PANCREATIC RNAase ACTIVITY, WITH THE VALUE IN 0.1 M NaCl TAKEN AS 100

NaCl (M)	Single-stranded RNA	Double-stranded RNA
0.1	100	100
0.15	125	55
0.3	260	15

sulfuric acid extraction, it may be argued that this is the source of the enhanced activity against double-stranded RNA. Consequently commercial RNAase A, crystalline RNAase from bovine pancreas purified by trichloroacetic acid extraction [26], and a sulfonosalicylic acid extract of bovine pancreas, were compared for ability to cleave double-stranded RNA. For all three preparations the relative rates of hydrolysis of single-stranded RNA to double-stranded RNA were approximately the same.

Human RNAases from other sources

The peak A components of human serum and urine ribonucleases, which are predominantly of pancreatic origin [1], hydrolyzed double-stranded RNA at 2% and 0.4%, respectively, of the rates for single-stranded RNA (Table I).

For purified preparations of RNAase from human liver and spleen, hydrolysis of double-stranded RNA was too slow to permit of assay by means of acid-solubility, and could be followed only by hyperchromicity measurements. For both activities, approximately 800 times more enzyme was required to produce an increase in hyperchromicity of double-stranded RNA equal to that with single-stranded RNA, whereas for human pancreatic RNAase only a 40-fold higher enzyme concentration sufficed.

Influence of ionic strength

Table II illustrates the effect of salt concentration on human pancreatic RNAase activity. Increasing the NaCl concentration in the incubation medium to 0.3 M differentially affected activities against single-stranded RNA and double-stranded RNA. Taking activities in 0.1 M NaCl as 100, the former increased to 260, while the latter dropped to 15. Hence, if the activity against double-stranded RNA is 2% of that against single-stranded RNA in 0.1 M NaCl, it decreased to about 0.1% in 0.3 M NaCl. The enhancement by 0.3 M NaCl of activity against single-stranded RNA was even further accentuated when the enzyme was subjected to dialysis against low ionic strength buffer (see below).

Activities against synthetic polynucleotides

Table III presents the rate of hydrolysis of double-stranded poly(I) · poly(C), relative to that for single-stranded RNA, by RNAases of human origin and bovine RNAase A. For pancreatic, serum and urine activities, these rates are 7%, 5% and 1%, respectively, as compared to 0.04% for bovine RNAase A. For human spleen RNAase the value is 0.2%. Somewhat unexpectedly it is 1% for the liver enzyme, i.e. equivalent to that for urine RNAase.

TABLE III

ACTIVITIES OF VARIOUS PURIFIED HUMAN RNAases AND OF BOVINE RNAase A, TOWARDS POLY(U), POLY(C) AND POLY(I) · POLY(C)

Source of ribonuclease	Activity (in μmol acid-solubilized substrate/15 min/mg protein)			Activity against poly(I) · poly(C) / Activity against poly(C)	Activity against poly(I) · poly(C) / Activity against single-stranded RNA	Activity against poly(C) / Activity against single-stranded RNA
	Poly(C)	Poly(U)	Poly(I) · poly(C)			
Human pancreas	221 000	9 000	220	$1 \cdot 10^{-3}$	$7 \cdot 10^{-2}$	65
Human serum (peak A)	38 000	1 400	30	$8 \cdot 10^{-4}$	$5 \cdot 10^{-2}$	59
Human urine (peak A)	5 274 000	65 400	585	$1 \cdot 10^{-4}$	$1 \cdot 10^{-2}$	121
Human spleen	4 100	940	5	$1 \cdot 10^{-3}$	$2 \cdot 10^{-3}$	2
Human liver	5 600	800	14	$3 \cdot 10^{-3}$	$1 \cdot 10^{-2}$	5
Bovine RNAase A	2 200 000	227 000	183	$8 \cdot 10^{-5}$	$4 \cdot 10^{-4}$	4

Poly(I) · poly(C) is perhaps not the most ideal double-stranded substrate, since only one of the strands is susceptible to the enzyme. Even when activities against poly(I) · poly(C) are compared to those against poly(C) alone (Table III), this does not clearly reflect the ability of the enzyme to cleave the double-stranded structure, because of the highly variable rates of hydrolysis of poly(C) under our assay conditions by the enzymes from different sources (see ratio of activity against poly(C) to that against single-stranded RNA, last column, Table III). Human RNAases of pancreatic origin hydrolyze poly(C) from 60 to 120 times more rapidly than single-stranded RNA, whereas for the enzymes from spleen and liver, and for bovine RNAase A, the ratios are at least ten times lower. The differences in rate of hydrolysis of poly(C) consequently mask the differences in rates of hydrolysis of poly(I) · poly(C) when poly(C) is taken as the basis of reference.

RNAase dimerization and activity against single-stranded RNA and double-stranded RNA

Human RNAase assayed in 0.1 M NaCl has been observed to vary in activity against single-stranded RNA and, to some extent, also against double-stranded RNA, depending on the treatment it has previously undergone. In view of reports on aggregation of human serum RNAase under certain ionic conditions [31], and on different molecular weight species of human urine RNAase [32], it appeared desirable to examine the possibility of a correlation between these changes in human RNAase activities against single-stranded RNA and double-stranded RNA and enzyme aggregation.

Fig. 3 displays the changes in elution pattern from Sephadex G-75 of urine RNAase previously subjected to various treatments. RNAase isolated from urine by sulfonosalicylic acid extraction and tannin-caffeine treatment, eluted at a position corresponding to a molecular weight of 30000. Its rate of hydrolysis of double-stranded RNA was 2% that for single-stranded RNA. The same preparation, following SE-Sephadex chromatography and elution with 0.3 M KCl, eluted from Sephadex G-75 at a position corresponding to a molecular weight of 14000 and hydrolyzed double-stranded RNA at 0.4% the rate for single-stranded RNA. If, however, after SE-Sephadex chromatography, it was dialyzed against 1 mM acetate buffer pH 5.6, it again eluted from Sephadex G-75 at a position corresponding to a molecular weight of 30000 and once again hydrolyzed double-stranded RNA at 2% of the rate for single-stranded RNA. Hence a shift in the position of elution of the enzyme from that for a dimer to that for a monomer was accompanied by a 5-fold decrease in the relative rates of hydrolysis of double-stranded RNA to single-stranded RNA.

Analogous changes in the Sephadex G-75 elution pattern are observed for human pancreatic RNAase, but with less striking modifications in the ratio of activities against double-stranded RNA and single-stranded RNA. An aqueous homogenate of pancreas hydrolyzes double-stranded RNA at 4% the rate for single-stranded RNA. Following sulfonosalicylic acid extraction and tannin-caffeine treatment, the RNAase elutes from Sephadex G-75 at a position corresponding to a molecular weight of 30000. After chromatography on SE-Sephadex and elution with 0.45 M KCl, it appears on Sephadex G-75 at a position corresponding to a molecular weight of 14000, and attacks double-

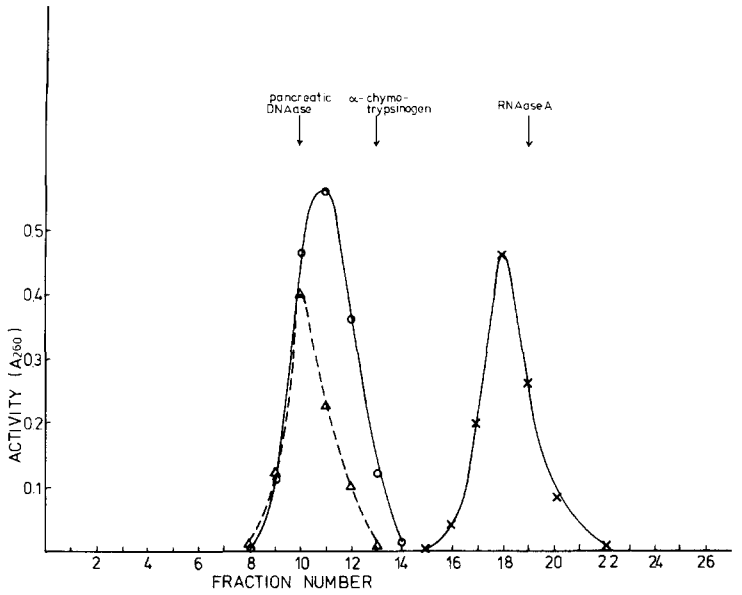

Fig. 3. Gel filtration chromatography of human urine ribonuclease, previously subjected to various treatments, on a 94 X 1.5 cm column of Sephadex G-75, at a flow rate of 15 ml/h with collection of 5-ml fractions. —o—o—o—, following sulfonosalicylic acid and tannin-caffeine treatment, activity expressed as A_{260} resulting from incubation of 2 µl effluent with single-stranded RNA for 15 min, as described in Methods; —X—X—X—, following purification on SE-Sephadex, activity expressed as A_{260} after incubation of 2 µl effluent with single-stranded RNA as above; —Δ—Δ—Δ—, following purification on SE-Sephadex and exhaustive dialysis against 1 mM acetate buffer pH 5.6, activity expressed as A_{260} after incubation of 1 µl effluent with single-stranded RNA as above. Arrows indicate the positions of elution of marker enzymes.

stranded RNA at 2% the rate for single-stranded RNA. After exhaustive dialysis against a low buffer concentration, its rate of cleavage of double-stranded RNA again attains a value of 4% that for single-stranded RNA.

An effect of enzyme monomerization resembling that resulting from SE-Sephadex chromatography was also observed when the enzyme was stored for over a week in 0.05 M phosphate buffer pH 6.5. Under such circumstances human pancreatic RNAase, which previously eluted from Sephadex G-75 at a position corresponding to a molecular weight of 30000, now rechromatographed to give two peaks eluting at positions corresponding to molecular weights of 14000 and 30000.

RNAases from the pancreas of rat and whale

The crystalline enzymes from the pancreas of the rat [24] and the whale [25] exhibited varying rates of hydrolysis of double-stranded RNA (Table I). The rat enzyme attacked double-stranded RNA at a rate of about 41 µmol acid-solubilized product/15 min per mg protein, compared to 130 000 µmol for single-stranded RNA, i.e. at 0.03% the rate for single-stranded RNA. The whale RNAase W_1 hydrolyzed double-stranded RNA at a rate of 340 µmol acid-soluble product/15 min per mg protein as compared to 90 000 µmol for single-stranded RNA, i.e. at 0.4% the rate for single-stranded RNA.

Discussion

Ribonucleases, whose mode of action involves intramolecular transphosphorylation, have been generally assumed to exhibit only very low, if any, activity towards double-stranded RNA at 0.15 M NaCl concentration [20,33]. The only exception hitherto reported is bovine semen RNAase BS-1 (see below) which cleaves double-stranded RNA, relative to single-stranded RNA, about 10 times more rapidly than bovine RNAase A [34]. The present findings call for a revision of the aforegoing assumption since ribonucleases from human tissues, as well as those from the pancreas of the rat and the whale, exhibit appreciable, albeit varying, activities towards double-stranded RNA. Amongst the RNAases embraced in this study, that from the human pancreas is the most active, its rate of cleavage of double-stranded RNA being 2% the rate for single-stranded RNA. Since bovine RNAase A hydrolyzes double-stranded RNA at only 0.003% the rate for single-stranded RNA (see Table I), it follows that the human enzyme is almost 700 times more active than the bovine against double-stranded RNA, relative to single-stranded RNA.

The activity against double-stranded RNA in the human pancreas may be considered an intrinsic property of the enzyme on the basis of its specificity, its behaviour during purification, and its stability under various conditions. The differences in pH optima displayed against single-stranded RNA and double-stranded RNA are not unexpected; bovine seminal plasma RNAase also displays a slightly lower pH optimum for double-stranded RNA than for single-stranded RNA [17].

The differences in specificity between human pancreatic RNAase and the corresponding liver and spleen enzymes are reflected not only in their varying rates of hydrolysis of double-stranded RNA, and of Up-naphthyl [1], but also in the degree of preference for poly(C) relative to poly(U) (Table III). The pancreas enzyme attacks poly(C) at more than 24 times the rate for poly(U), whereas the liver and spleen enzymes do so 7 and 4 times faster, respectively. For bovine pancreatic RNAase it is 10 times faster. These differences are less striking when activities are determined under other incubation conditions; e.g. the preference of RNAase A for poly(C) relative to poly(U) is appreciably accentuated when assays are carried out at low ionic strength [28]. With the human pancreatic RNAase, assay at pH 7.6 favours activity against poly(C), cleaved optimally at pH 8, relative to that against poly(U), for which the pH optimum is 6.4 (see Fig. 2).

The preference of human pancreatic-like RNAases for poly(C) relative to poly(U) is in agreement with such recently reported preferences for RNAase activities from human serum [31,35] and pancreas [35]. These similarities are further underlined by additional common properties such as a requirement for high ionic strength and inhibition by poly(G). However, unlike Schmukler et al. [31], we have not observed any preference for hydrolysis of internucleotide linkages involving cytidine residues in single-stranded RNA. Our results demonstrate that digests of single-stranded RNA with human pancreatic or serum RNAases contained fragments with 3′-termini of both uridine (60%) and cytidine (40%). If we assume the same enzyme was studied by Schmukler et al. [31], we are unable to account for this discrepancy; it should however, be noted

that the methods employed for determination of the 3′-termini in this study [36] differ from those used by Schmukler et al. [31].

Apart from the work on bovine semen RNAase [17], none of the various reports on mammalian enzymes active against double-stranded RNA is based on isolation and purification of an enzyme to an extent sufficient to warrant a comparison of its properties with those of the activities isolated in this study. The preliminary data reported for these enzymes does suggest that their activities cannot, in most instances [10—12], be attributed to acid-thermostable RNAases.

It is pertinent to examine the possible relevance to our findings of the increased rate of hydrolysis of double-stranded RNA by oligomeric forms of bovine RNAase A [17,18]. This enzyme hydrolyzes double-stranded RNA at only $3 \cdot 10^{-5}$ times the rate for single-stranded RNA (Table I). For dissociable dimers formed by simple aggregation, this rate increases 4 times [17], whereas for synthetic cross-linked dimers the increase may be more than 400-fold relative to the monomer [19]. Bovine semen plasma RNAase, a natural dimer, was found to attack double-stranded RNA more rapidly than monomeric RNAase A [17]. From these data, and on the basis of experiments with dimers formed between RNAase A and its inactivated derivative carboxymethylated at histidine residue 119, which exhibited no enhancement of the rate of hydrolysis of double-stranded RNA relative to monomeric RNAase A, it was initially postulated that hydrolysis of double-stranded RNA requires two active sites, one on each subunit of the dimer [37]. This interpretation was subsequently placed in doubt by the observation that a monomer of bovine semen plasma RNAase degrades double-stranded RNA as efficiently as the natural dimer [20]. Our own findings provide independent evidence that a dimeric structure is not a prerequisite for ability to hydrolyze double-stranded RNA by all RNAases which resemble, in specificity and other properties, the bovine pancreatic enzyme. Human urine RNAase, which cleaves double-stranded RNA at 0.4% the rate for single-stranded RNA, has a molecular weight of only 14000 [38], and is presumably a monomer. Following exhaustive dialysis the human urine enzyme appears to double in molecular weight, while its rate of cleavage of double-stranded RNA relative to single-stranded RNA apparently increases, largely as a result of a decrease in activity against single-stranded RNA. It would consequently appear that although activity of human RNAases against double-stranded RNA does not require a dimeric structure, such a structure does exhibit a higher rate of cleavage of double-stranded RNA relative to single-stranded RNA.

The possible physiological significance of the relatively high activity of human RNAases against double-stranded RNA is not immediately obvious. It would clearly be desirable to examine the mechanism of hydrolysis of double-stranded RNA by these enzymes, including a study of the products of hydrolysis, preferably with the use of radioactively labelled RNA. Such studies are being initiated in this laboratory. It is, however, pertinent to note that the high activity of human RNAases against double-stranded RNA may be of significance in relation to interferon induction by such RNA.

Acknowledgments

We are very much indebted to Dr. J. Doskocil for a gift of phage f2 double-stranded RNA, to Dr. J. Heyes of Beecham Pharmaceuticals for mycoviral double-stranded RNA, to Dr. Maria Malicka for bovine pancreatic RNAase, to Dr. M. Irie for the whale pancreas RNAase, and to Dr. M. Gruber for the rat pancreas RNAase. We are also grateful to Krzysztof Lang for help with some phases of this investigation, and to Halina Szemplińska and Jerzy Sosnowski for skilful technical assistance. This investigation was carried out as Project 09.3.1 of the Polish Academy of Sciences.

References

1 Bardoń, A., Sierakowska, H. and Shugar, D. (1976) Clin. Chimica Acta 67, 231—243
2 Harel, L. and Montagnier, L. (1971) Nat. New Biol. 229, 106—108
3 Jelinek, W. and Darnell, J.E. (1972) Proc. Natl. Acad. Sci. U.S. 69, 2537—2541
4 Tytell, A.A., Lampson, G.P., Field, A.K. and Hilleman, M.R. (1967) Proc. Natl. Acad. Sci. U.S. 58, 1719—1722
5 Field, A.K., Tytell, A.A., Lampson, G.P. and Hilleman, M.R. (1967) Proc. Natl. Acad. Sci. U.S. 58, 1004—1010
6 Robertson, H.D. and Mathews, M.B. (1973) Proc. Natl. Acad. Sci. U.S. 70, 225—229
7 Kaempfer, R. and Kaufman, J. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1222—1226
8 Norlund, J.J., Wolff, S.M. and Levy, H.B. (1960) Proc. Soc. Exp. Biol. Med. 133, 439—444
9 Stern, R. (1970) Biochem. Biophys. Res. Commun. 41, 608—614
10 Stern, R. and Wilczek, J. (1973) Fed. Proc. 32, 620
11 Torrence, P.F., Waters, J.A., Buckler, C.E. and Witkop, B. (1973) Biochem. Biophys. Res. Commun. 52, 890—898
12 Birge, C.H. and Schlessinger, D. (1974) Fed. Proc. 33, 1275
13 Robertson, H.D., Webster, R.E. and Zinder, N.D. (1968) J. Biol. Chem. 243, 82—91
14 Dunn, J.J. and Studier, F.W. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1559—1563
15 Dunn, J.J. and Studier, F.W. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3296—3300
16 Rosenberg, M., Kramer, R.A. and Steitz, J.A. (1974) J. Mol. Biol. 89, 777—782
17 Libonati, M. and Floridi, A. (1969) Europ. J. Biochem. 8, 81—87
18 Libonati, M. (1971) Biochim. Biophys. Acta 228, 440—445
19 Bartholeyns, J. and Moore, S. (1974) Science 186, 444—445
20 Libonati, M., Malorni, M.C., Parente, A. and d'Alessio, G. (1975) Biochim. Biophys. Acta 402, 83—87
21 Doskocil, J., Fuchsberger, N., Vetrak, J., Lackovic, V. and Borecky, L. (1971) Acta Virol. 15, 523
22 Buck, K.W., Chain, E.B. and Himmelweit, F. (1971) J. Gen. Virol. 12, 131—139
23 Kole, R. and Sierakowska, H. (1971) Acta Biochim. Polon. 18, 187—197
24 Beintema, J.J., Campagne, R.N. and Gruber, M. (1973) Biochim. Biophys. Acta 310, 148—160
25 Irie, M., Yamada, T. and Ukita, T. (1966) J. Biochem. 59, 344—352
26 Malicka, M. (1972) Arch. Immunol. et Ther. Exp. 20, 555—559
27 Schmukler, M., Friedling, S.P. and Levy, C.C. (1972) Biochim. Biophys. Acta 268, 403—410
28 Zimmerman, S.B. and Sandeen, G. (1965) Anal. Biochem. 10, 444—449
29 Weissmann, C., Billeter, M.A., Vinuela, E. and Libonati, M. (1966) in Viruses of Plants (Beemster, A.B.R. and Dijkstra, J., eds.), pp. 249—274, North Holland, Amsterdam
30 Beers, Jr., R.F. (1960) J. Biol. Chem. 235, 2393—2398
31 Schmukler, M., Jewett, P.B. and Levy, C.C. (1975) J. Biol. Chem. 250, 2206—2212
32 Naskalski, J. (1972) Przegląd Lekarski 29, 394—403
33 Uchida, T. and Egami, F. (1971) in The Enzymes (Boyer, P.D., ed.), Vol. 4, pp. 205—250, Academic Press, New York
34 Palese, P. and Koch, G. (1972) Proc. Natl. Acad. Sci. U.S. 69, 698—701
35 Reddi, K.K. (1975) Biochem. Biophys. Res. Commun. 67, 110—118
36 Uchida, T., Arima, T. and Egami, F. (1970) J. Biochem. 67, 91—102
37 D'Alessio, G., Doskocil, J. and Libonati, M. (1974) Biochem. J. 141, 317—320
38 Delaney, R. (1963) Biochemistry 2, 438—444