Basic Charges on Ribonuclease Molecules and Activity
towards Double-Stranded Polyrribonucleotides

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One of the properties by which RNA in a double-stranded form or hybridized to
DNA can be recognized, is its high resistance to digestion by bovine pancreatic
RNAase‡ A (EC 3.1.4.22) and by RNAase T₁ (EC 3.1.4.8) under ionic-strength
conditions that stabilize the secondary structure of the nucleic acid (Billeter & Weiss-
mann, 1966; Edy et al., 1976).

Under the same experimental conditions single-stranded RNA is rapidly
hydrolysed by these enzymes.

Some RNAase species acting on single-stranded RNA have been shown to be also
capable of efficiently degrading double-stranded polyrribonucleotides under conditions
‡ Abbreviation: RNAase, ribonuclease.
Table 1. Some characteristics of several RNAase species

Experimental conditions were not identical; the activity values (relative to those of bovine RNAase A) in the Table permit only a rough semi-quantitative comparison. Aggregated dimers and higher aggregates of bovine pancreatic RNAase A were prepared as described by Crestfield et al. (1962). Cross-linked dimers of bovine RNAase A were prepared, and the experiments with them were performed, by Wang et al. (1976). Viral double-helical RNA or the complex poly(A).poly(U) were used as double-stranded substrates.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Amino and guanidino groups</th>
<th>Carboxyl groups</th>
<th>Activity on yeast RNA</th>
<th>Activity on double-stranded RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine RNAase A</td>
<td>14</td>
<td>4</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Bovine RNAase A aggregated or</td>
<td>14</td>
<td>4</td>
<td>0.41-0.68</td>
<td>4-8</td>
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<tr>
<td>cross-linked dimers</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bovine RNAase A higher aggregates</td>
<td>14</td>
<td>4</td>
<td>0.48</td>
<td>10-16</td>
</tr>
<tr>
<td>Bovine RNAase BS-1</td>
<td>18*</td>
<td>4*</td>
<td>0.45</td>
<td>10-17</td>
</tr>
<tr>
<td>Bovine RNAase BS-1 (monomerized)</td>
<td>18</td>
<td>4</td>
<td>0.70</td>
<td>6-8</td>
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<tr>
<td>Whale pancreatic RNAase</td>
<td>17</td>
<td>5</td>
<td>0.25-0.33</td>
<td>25-38</td>
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<td>Rat pancreatic RNAase</td>
<td>17</td>
<td>5</td>
<td>0.6</td>
<td>2-4</td>
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<td>Guinea-pig pancreatic RNAase A</td>
<td>15</td>
<td>4</td>
<td>0.5</td>
<td>1.0</td>
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<td>Red-deer pancreatic RNAase</td>
<td>13</td>
<td>5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Reindeer pancreatic RNAase</td>
<td>12</td>
<td>5</td>
<td>1.0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Per subunit.

where bovine RNAase A is virtually inactive. These are seminal RNAase BS-1, a dimeric protein (D'Alessio et al., 1972a,b; Libonati & Floridi, 1969), and its monomeric catalytically active derivative (D'Alessio et al., 1975; Libonati et al., 1975a), cross-linked (Wang et al., 1976) or aggregated (Crestfield et al., 1962; Libonati & Floridi, 1969) dimers and higher aggregates (Libonati, 1969, 1971; Wang et al., 1976) of bovine RNAase A, and the RNAase isolated from the pancreas of the lesser rorqual or pike-whale (Balaenoptera acutorostrata) (Emmens et al., 1976; Libonati et al., 1976).

Other properties of these enzymes are: (1) a specific activity toward cyclic nucleotides similar to that of monomeric RNAase A; (2) a specific activity in the depolymerization of single-stranded RNA from yeast, which is about 30-50% that of bovine RNAase A; (3) the number of basic charges on the molecule (or the density of these charges in the case of RNAase A aggregates), which is significantly higher than that of bovine monomeric RNAase A (Table 1).

From these observations, the idea has been advanced (Libonati & Floridi, 1969; Libonati et al., 1975a,b, 1976) that a correlation may exist between the basicity of a RNAase molecule and its ability to degrade double-stranded RNA. Support for this hypothesis has come from several other observations. For instance, spermine RNAase A, prepared by cross-linkage with dimethyl suberimidate, is 115 times as active as the native enzyme at degrading the poly(A)·poly(U) complex and 380 times as active on the hybrid poly(dA)·poly(rU) (Wang, 1976). Moreover, the activity on double-stranded RNA of some other mammalian RNAases, like the pancreatic RNAase of guinea pig (A component) and of red deer (Van den Berg & Beintema, 1975; Leijenaar-van den Berg et al., 1974), having charge characteristics (Table 1) similar to those of bovine RNAase A, is indistinguishable from that of the latter enzyme (Libonati et al., 1976). On the contrary, pancreatic RNAase of reindeer (Leijenaar-van den Berg & Beintema, 1975), a protein significantly less basic than bovine RNAase A (Table 1), is also less active towards double-helical RNA than the latter enzyme (Libonati et al.,
Pancreatic RNAase of rat (Beintema et al., 1973), having a higher density of charges (both three basic and three acidic residues more) than bovine RNAase A (Table 1), shows a slightly but significantly higher activity towards double-helical RNA than does the latter enzyme (Libonati et al., 1976).

These facts are in line with recent observations on differences in activity toward poly(A), as a function of the structure of this polymer, shown by cross-linked dimers of RNAase A, by RNAase BS-1 and by whale RNAase. When poly(A) is subjected to the action of equal amounts (on a weight basis) of native monomeric RNAase A and of its cross-linked dimer in standard saline citrate (0.15 m-NaCl/0.015 m-sodium citrate) at pH 7.0, or adjusted to pH 5.0, i.e. under conditions favouring the formation of an ordered base-stacked single-stranded helix or of the 'acidic' double-helical form of poly(A), the homopolymer is degraded at a significantly higher rate by dimeric than by monomeric RNAase A. On the other hand, at a very low ionic strength (0.0025 m-Tris/HCl, pH 7.2, or 0.0001 m-citrate/phosphate buffer, pH 7.15), i.e. under conditions that favour random coils of the polymer, monomeric RNAase A is definitely more efficient than the dimeric enzyme at degrading poly(A) (M. Palmieri & M. Libonati, unpublished work). The same phenomenon has been observed in experiments performed under similar conditions with RNAase BS-1 and bovine monomeric RNAase A (Palmieri & Libonati, 1976b).

Whale pancreatic RNAase, on the other hand, shows a much higher activity than bovine RNAase A (and RNAase BS-1) towards any ordered form of poly(A) (Libonati et al., 1976), but this difference in activity toward ordered poly(A) appears greatly decreased when incubations are carried out at low ionic strength. Under these conditions the rates of degradation of randomly coiled poly(A) by whale and bovine pancreatic RNAases become much closer to each other (M. Libonati, M. Palmieri, S. Sorrentino & J. J. Beintema, unpublished work). Apparently all RNAase species characterized by a higher density of basic charges than bovine RNAase A show a relatively high activity in degrading the ordered structures of poly(A), both the double-helix and the base-stacked single-stranded helix.

A possible general mechanism of action at the molecular level may be advanced. It is known that, whereas basic proteins usually stabilize double-helical nucleic acids (Allfrey et al., 1963), histone F1 is capable of uncoiling supercoiled viral DNA, and a basic protein isolated from the prostate of rat unwinds double-helical DNA (Vogel & Singer, 1975; Mainwaring et al., 1976). Another notable exception is bovine pancreatic RNAase A, which destabilizes double-stranded DNA (Felsenfeld et al., 1963). A stronger destabilization of the secondary structure of calf thymus DNA is effected by seminal RNAase BS-1 and by cross-linked dimers of bovine RNAase A (Palmieri & Libonati, 1975, and unpublished work; see Fig. 1).

The destabilization has been demonstrated not only by following spectrophotometrically the thermal-transition profile of double-stranded DNA in the presence of the protein. Preliminary experiments show that 14C-labelled Escherichia coli DNA, when incubated with seminal RNAase BS-1 under conditions similar to those where a destabilization of the secondary structure of DNA is spectrophotometrically detectable, becomes partly susceptible to the action of S1 nuclease from Aspergillus oryzae, which is specific for single-stranded DNA. There is a significantly higher loss of acid-insoluble radioactivity by the action of S1 nuclease (purified as described by Vogt, 1973) during the interaction of RNAase BS-1 with the DNA than in control experiments with equal amounts of bovine monomeric RNAase A (M. Palmieri, G. Carsana & M. Libonati, unpublished work). The same phenomenon may be supposed to occur with double-helical polynucleotides.

As a consequence of the interaction with these basic proteins, double-stranded RNA and the ordered forms of poly(A) may become locally destabilized, i.e. single-stranded, and, at those sites, susceptible to enzymic attack. The latter should be, and is, endonucleic, as has been shown in gel-filtration experiments with double-stranded RNA subjected to controlled digestion by RNAase BS-1, bovine RNAase A dimers and higher aggregates, and monomeric RNAase A (Palmieri & Libonati, 1976a).
Fig. 1. Destabilization of calf thymus DNA by bovine pancreatic RNAase A and by seminal RNAase BS-1

Mixtures: in 1 ml of 0.0015M-sodium phosphate buffer, pH 7.0, containing 0.002M-NaCl, 9μg of DNA and 10μg of bovine RNAase A (a) or RNAase BS-1 (b) were contained. Absorbance was measured at 260nm with a Zeiss PMQ II spectrophotometer, equipped with a thermostatically controlled water bath. ○, DNA control (starting absorbance, 0.168); ●, DNA–RNAase mixture. Absorbance increase was plotted as percentage of maximum increase.

In conclusion, degradation of double-stranded structures of RNA by RNAases non-specific for double-helical RNA appears to be an essentially unspecific process, occurring at a rate determined by the basicity of the RNAase molecule.

Finally, from a practical point of view, the data presented could in part be used to emphasize the importance of the assay conditions to measure the change of RNAase activity in tissues of interest.

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ASPECTS OF GENE EXPRESSION IN EUKARYOTES

Organization of a Eukaryotic Genome

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Messenger Ribonucleic Acid Metabolism during Myogenesis

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Muscle cells in culture undergo a process of terminal differentiation resembling that seen during muscle development in vivo. After one or more cell divisions, mononucleated myoblasts, from foetal calf muscle, form multinucleate muscle fibres, with the plasma membrane characteristic of muscle and containing the contractile apparatus and enzymic equipment for muscle metabolism [for review see Merlie et al. (1976)]. Quantitatively, the overall pattern of protein synthesis undergoes little change, although increases in the synthesis of certain proteins such as the large subunit of myosin occur. Qualitatively, there is increasing evidence for isoenzymic changes, such as that documented for creatine kinase (Turner et al., 1974), where muscle-specific proteins replace the more universal form of the structural protein or enzyme. The use of two-dimensional gel techniques has indicated that there may be multiple forms of actin, one of which is