Biochem. J. (1992) 281, 343–348 (Printed in Great Britain)

Sensitivity of monomeric and dimeric forms of bovine seminal ribonuclease to human placental ribonuclease inhibitor

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We have studied the inhibition of bovine pancreatic RNAase (RNAase A) and bovine seminal RNAase in its native dimeric form (RNAase BS-1) and in monomeric carboxymethylated form (MCM RNAase BS-1) by human placental RNAase inhibitor (RNAase inhibitor) in order to understand the effect of enzyme structure on its response to the inhibitor. Study of the inhibition as a function of inhibitor concentration revealed that RNAase A and MCM RNAase BS-1 were inhibited fully and the inhibitor-sensitivities of the two were comparable. But under identical inhibitor concentrations RNAase BS-1 was found to be virtually insensitive to the inhibitor; at higher (3–10-fold) inhibitor concentrations marginal inhibition of the native enzyme could be observed. When RNAase BS-1 was pretreated with 5 mM-dithiothreitol (DTT) and assayed, it exhibited greater inhibitor-sensitivity, presumably as a result of its partial monomerization on exposure to DTT. This DTT-mediated change in the response of RNAase BS-1 to the inhibitor did not, however, seem to occur either in the assay conditions (which included DTT) or even when the enzyme was pretreated with DTT in the presence of the substrate, suggesting an effect of the substrate on the enzyme behaviour towards the inhibitor. Independently, gel-filtration runs revealed that, although DTT treatment caused monomerization of RNase BS-1, this change did not take place when DTT treatment was carried out in the presence of the substrate. From our observations, we infer that differential inhibitor-sensitivity of the dimeric and monomeric forms of RNAase BS-1, the relative contents of the two forms and the influence of the substrate on them may be important determinants of the net enzyme activity in the presence of the inhibitor.

INTRODUCTION

Pancreatic-type RNAases are widely distributed in animal tisues and extracellular fluids. Many mammalian tissues also contain an acidic protein that is inhibitory to such RNAases (Blackburn & Moore, 1982). Human placental RNAase inhibitor (RNAase inhibitor) belongs to this class of protein inhibitor of RNAases. *In vitro* the RNAase inhibitor is known to inhibit both secretory and intracellular pancreatic-type RNAases. It has also been shown to interact with angiogenin, a homologue of pancreatic RNAase (Shapiro & Vallee, 1987). Although the exact biological role of the inhibitor is not yet clearly understood, an unusually tight-binding nature of its interaction with RNAase(s) or angiogenin (Shapiro & Vallee, 1987) is highly suggestive of a role for this protein *in vivo*. With respect to RNAases, the inhibitor is considered to play a role in the regulation of cellular RNAase activity (Blackburn & Moore, 1982). This calls for cellular mechanisms to modulate RNAase–inhibitor interaction that are largely unknown.

Much of the work on the RNAase–inhibitor interaction has been done *in vitro*, with bovine pancreatic RNAase (RNAase A) and human placental RNAase inhibitor as the model system (Blackburn & Moore, 1982). Shapiro & Vallee (1991) have reported studies on interaction of placental RNAase inhibitor with a major RNAase from the same tissue. Bovine seminal RNAase (RNAase BS-1) is another interesting and well-characterized RNAase of pancreatic type. It is a dimeric protein of M_r 29000 in which two identical subunits are held together by two disulphide bridges and non-covalent forces (D'Alessio *et al.*, 1972, 1975). The subunit of RNAase BS-1 has significant sequence similarity to RNAase A (Di Donato & D'Alessio, 1979). The availability of methods to dissociate RNAase BS-1 and to purify the enzyme in catalytically active apparent monomeric (MCM RNAase BS-1) and dimeric forms (D'Alessio *et al.*, 1975), and an earlier observation by Blackburn & Gavilanes (1980) that the two forms of the enzyme could differ in their response to the inhibitor, prompted us to study further the sensitivity of RNAase BS-1, in different forms and in different conditions, to placental RNAase inhibitor. We observed that the two forms of RNAase BS-1 differed drastically in their sensitivity to the inhibitor, the dimer being virtually insensitive to the inhibitor. Further, the substrate seems to stabilize the enzyme in its dimeric state and thus influence its monomerization. So, under conditions that otherwise promote monomerization, the net sensitivity of the enzyme to the inhibitor and thus its activity (in the presence of the inhibitor) may be linked to substrate concentrations.

MATERIALS AND METHODS

Materials

RNAase BS-1 was a gift from Mr. N. Sitaram, of our Institute. Bovine pancreatic RNAase A was obtained from Boehringer Mannheim (Mannheim, Germany) and human placental RNAase inhibitor from Bolton Biologicals (St. Paul, MN, U.S.A.) or Promega (Madison, WI, U.S.A.). One unit of inhibitor (according to manufacturer's specification) is equivalent to the amount required to inhibit by 50% the activity of 5 ng of RNAase A.

RNA isolation

Escherichia coli strain D10 (RNAase I^-) was grown to exponential phase in Luria broth or in M9 medium (Maniatis *et al.*, 1982) with or without ^{3}H-labelled uridine (1 mCi/l), cells were harvested by centrifugation and RNA was extracted. For RNA extraction, the cells were lysed with 1% (w/v) SDS in 0.3 M-sodium acetate buffer, pH 4.6, the lysate was extracted

Abbreviations used: RNAase A, bovine pancreatic RNAase; RNAase BS-1, bovine seminal RNAase in native dimeric form; MCM RNAase BS-1, monomeric carboxymethylated form of RNAase BS-1; RNAase inhibitor, human placental RNAase inhibitor; DTT, dithiothreitol.

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with phenol and RNA was twice precipitated with cold ethanol. An A_{260} value of 1 unit was assumed to represent 40 μg of RNA.

Purification of different forms of RNAase BS-1

RNAase BS-1 used in this study (see above under 'Materials') was purified from bovine seminal plasma by the procedure of D'Alessio *et al.* (1972). We found the preparation to be homogeneous as assessed by gel filtration and SDS/PAGE. MCM RNAase BS-1 was prepared by treatment of the native enzyme with DTT, followed by treatment with iodoacetic acid and gel filtration through Sephadex G-75 in 0.1 M-ammonium acetate buffer, pH 5.0, as described by D'Alessio *et al.* (1975).

RNAase and RNAase inhibitor assays

RNAase reactions were carried out in 10 μl of buffer A (20 mM-Tris/HCl buffer, pH 7.4, containing 0.1 M-NH_4Cl and 5 mM-magnesium acetate) containing 5 mM- or 1 mM-DTT with ^{3}H-labelled or unlabelled total *E. coli* RNA (1 μg) and amounts of the nuclease as indicated. Incubations were carried out for 15 min at 37 °C in the absence or in the presence of various concentrations of the inhibitor as specified in the Figures. At the end of the incubations samples were processed by one of the following procedures.

(*a*) In 'chromatographic assays' reaction samples were spotted on Whatman no. 3 paper strips and chromatographed in the descending direction with 1 M-ammonium acetate/ethanol (1:1, v/v) as solvent (Reddy *et al.*, 1979). Degradation products equivalent to trichloroacetic acid-soluble material move away from the origin during chromatography. So degradation was assessed by determining the decrease in radioactivity at the origin. The assays were linear with respect to enzyme concentration, and the amount of RNAase preparation required for 50% degradation of 1 μg of ^{3}H-labelled *E. coli* RNA in 15 min was taken as 1 unit of the enzyme. The specific activities of different enzyme preparations were determined in pilot experiments, and appropriate amounts of enzymes equivalent to 1.5 activity units were generally used for assays in the presence or in the absence of the inhibitor and the percentage inhibition was calculated with reference to the observed degradation of the substrate in the absence of the inhibitor. Generally the same batches of the enzyme and the substrate were used in the assays under comparison.

(*b*) In some experiments samples were directly electrophoresed in 1% agarose gels, and RNA bands were detected by ethidium bromide staining. Assays were carried out with 0.25 activity unit [determined as above in (*a*)] of different enzyme preparations, and degradation of rRNA (from 1 μg of total *E. coli* RNA) to products of the size of 4–5 S was assessed visually. This procedure was used only for the purpose of qualitative comparisons and not for obtaining any quantitative data. The minimum amount of inhibitor required to inhibit RNAase activity such that 23 S and 16 S rRNA bands were clearly visible on the gel was considered for comparison of the inhibition. Assays using this procedure are referred to as 'gel assays' in the text.

RESULTS

As described in the Materials and methods section, in chromatographic assays degradation of RNA to products equivalent to trichloroacetic acid-soluble material was assessed, whereas in the gel assays electrophoresis of the reaction samples in agarose gels was carried out and fragmentation of macromolecular RNA was studied.

In Fig. 1 the effects of RNAase inhibitor, as a function of its concentration, on the activities of RNAase A, native RNAase BS-1 and MCM RNAse BS-1 are shown. The sensitivities of the three enzymes to the inhibitor were found to be different. RNAase A was found to be totally inhibited by about 8 units of the inhibitor, as also was MCM RNAase BS-1, which required 8–10 units of inhibitor for comparable inhibition. However, the behaviour of the native RNAase BS-1 was different. Up to about 20 units of the inhibitor the enzyme was insensitive to the inhibitor, and only marginal inhibition (a maximum of about 10–15%) could be seen with larger amounts of inhibitor (up to 90 units). Experiments to compare inhibition of the three enzymes as a function of time (up to 15 min) also indicated similar differences (results not shown).

When assays were carried out with lower levels of enzyme activities (gel assays; Fig. 2), this facilitated the use of conditions of greater inhibitor/enzyme ratios than used in Fig. 1 and a lower DTT concentration (1 mM). A more pronounced difference in the behaviour of the three enzymes could be seen in these assays: RNAase A and MCM RNAase BS-1 could be inhibited by 1 and 6 units respectively, whereas RNAase BS-1 was insensitive even at 60 units of the inhibitor.

To check the effect of reduction of interchain disulphide bridges on the sensitivity of RNAase BS-1 to the inhibitor, we studied the effect of RNAase inhibitor on the enzyme pretreated with 5 mM-DTT (Fig. 3). RNAase BS-1 pretreated with 5 mM-DTT even for a short period (5 min at 0 °C) was found to be inhibited at amounts of inhibitor comparable with those required for the inhibition of MCM RNAase BS-1 (Fig. 1). However, the inhibition reached a value of 50% and plateaued off thereafter. Since selective reduction of interchain disulphide bridges with DTT was earlier reported to result in conversion of about 30% of RNAase BS-1 into its apparent monomeric form (D'Alessio *et al.*, 1975), we also checked if there was any monomerization of the enzyme following DTT treatment under our experimental conditions. H.p.l.c. runs of RNAase BS-1 untreated or treated with DTT indicated that DTT treatment at 0 °C or 10 °C or 37 °C for as short a period as 2–5 min itself converted a significant portion (35–45%) of the enzyme into an apparent monomeric form. In addition, the extent of monomerization did not increase further when the time of DTT treatment at 37 °C was extended beyond 2 min, probably indicating an equilibrium situation (B. S. Murthy & R. Sirdeshmukh, unpublished work). The ob-

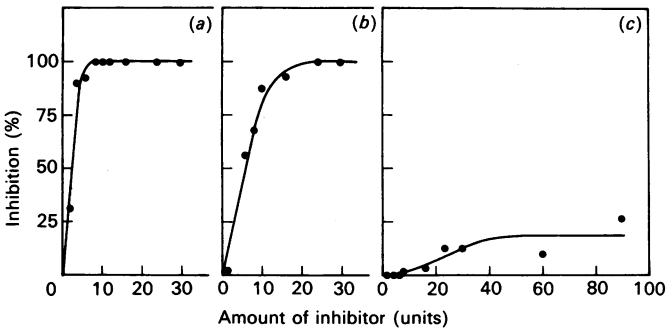


Fig. 1. Inhibition of RNAase A, MCM-RNAase BS-1 and RNAase BS-1 as a function of inhibitor concentration, studied by chromatographic assay

RNAase assays in the presence of buffer A with 5 mM-DTT in the absence and in the presence of various amounts of the inhibitor were carried out as described in the Materials and methods section, with 1 μg of ^{3}H-labelled total *E. coli* RNA and appropriate amounts of RNAase A (*a*), MCM RNAase BS-1 (*b*) or RNAase BS-1 (*c*) equivalent to 1.5 activity units. RNA degradation was determined by chromatography of the reeaction samples as described in the Materials and methods section and percentage inhibition was calculated with reference to degradation in the absence of the inhibitor.

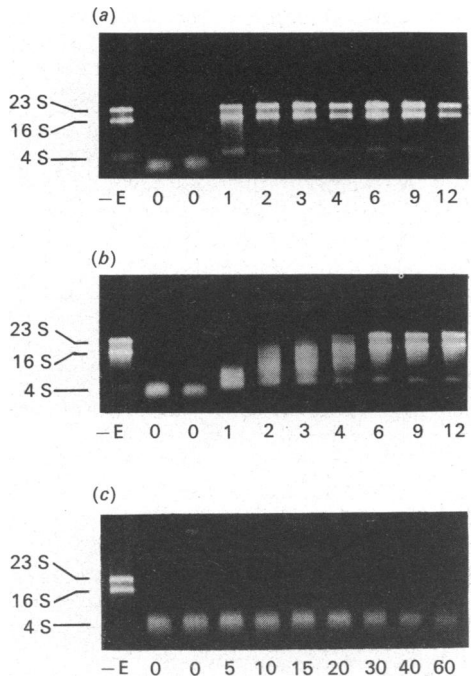


Fig. 2. Inhibition of RNAase A, MCM RNAase BS-1 and RNAase BS-1 as a function of inhibitor concentration, studied by gel assay

RNAase assays in the absence and in the presence of various amounts of inhibitor were carried out as described in the Materials and methods section in buffer A with 1 mM-DTT, with 1 μg of total *E. coli* RNA and appropriate amounts of RNAase A (*a*), MCM RNAase BS-1 (*b*) or RNAase BS-1 (*c*) equivalent to 0.25 activity unit. Reaction samples were electrophoresed in 1% agarose gels, and RNA bands were detected by ethidium bromide staining. Numbers below the lanes indicate the amounts of inhibitor (units) used in the respective reactions. Control RNA samples (−E) were incubated in the absence of the enzyme but in the presence of the largest amount of inhibitor used in that assay.

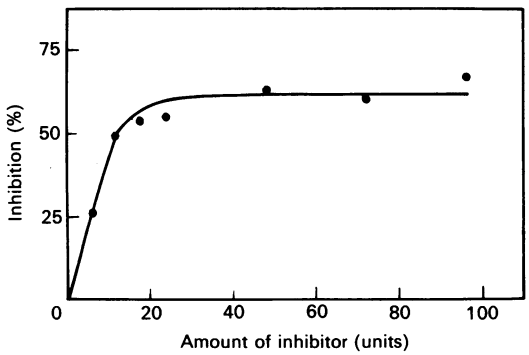


Fig. 3. Inhibition of RNAase BS-1 pretreated with DTT as a function of inhibitor concentration

RNAase assay were carried out in the absence and in the presence of various amounts of the inhibitor, with 1 μg of ^{3}H-labelled total *E. coli* RNA and 1.5 activity units of native RNAase BS-1 pretreated with 5 mM-DTT at 0 °C for about 5 min just before the assay. Degradation was determined and inhibition calculated as in Fig. 1.

served inhibition in Fig. 3 could therefore be grossly correlated with the monomerization of RNAase BS-1 observed as a result of treatment with DTT.

Since exposure of RNAase BS-1 to 5 mM-DTT renders it inhibitor-sensitive, presumably owing to monomerization, the difference in the inhibition of the native enzyme (assayed in the

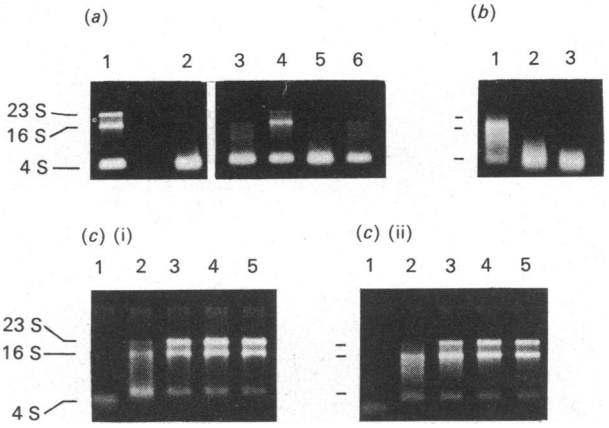


Fig. 4. Effect of DTT pretreatment in the absence and in the presence of substrate on the inhibitor-sensitivity of RNAase BS-1 and RNAase A, studied by gel assay

(*a*) RNAase BS-1 was pretreated with 5 mM-DTT in buffer A with or without the substrate, as described in the Results section, and samples equivalent to 0.25 activity unit were assayed in the absence and in the presence of 90 units of the inhibitor. Reaction samples were electrophoresed in 1% agarose gels, and RNA was detected by ethidium bromide staining as described in the Materials and methods section. Lane 1, control RNA; lane 2, reaction in the absence of inhibitor; lanes 3–6, reactions in the presence of inhibitor. Enzyme was preincubated without DTT (lanes 2 and 3), with DTT alone (lane 4), with DTT and 0.2 μg of total *E. coli* RNA/μl (lane 5) and with DTT and 0.2 μg of 2′,3′-(cyclic)CMP/μl (lane 6). (*b*) RNAase BS-1 was pretreated with 5 mM-DTT in buffer A containing various concentrations of total *E. coli* RNA: 0.01 μg/μl (lane 1), 0.05 μg/μl (lane 2) and 0.2 μg/μl (lane 3). The enzyme assays were carried out as in (*a*) with 0.25 activity unit of the enzyme and 90 units of inhibitor. (*c*) RNAase A was pretreated with 5 mM-DTT alone (i) or with 5 mM-DTT and 0.2 μg of total *E. coli* RNA/μl (ii) in buffer A and samples (0.25 activity unit) were assayed in the absence (lane 1) and in the presence of 1, 2, 3 and 4 units of the inhibitor (lanes 2–5 respectively).

presence of DTT; Fig. 1*c*) and that of RNAase BS-1 pretreated with DTT (Fig. 3) could be explained in terms of an effect of the substrate on the structure of the enzyme (see the Discussion section). We further examined this possibility by studying the inhibitor-sensitivity of the enzyme after DTT pretreatment in the absence and in the presence of the substrate, and the results are shown in Fig. 4. With the use of buffer A containing 5 mM-DTT and a specified amount of RNA, RNAase BS-1 was first passed through a series of quick dilutions at 37 °C, which takes about 2 min. The last 10-fold dilution was done with buffer A containing no DTT but containing RNA. In this way the enzyme was first exposed to DTT in the presence of RNA and used for the assay. The control sample consisted of the enzyme subjected to the above procedure with the use of buffer A containing 5 mM-DTT alone. The last dilution in this case also was done as in the other sample. Both the enzymes were then immediately assayed under identical conditions with additional amounts of fresh substrate (the proportion of the substrate from the enzyme addition was 10% of the total substrate used for the reaction). Use of lower enzyme activity, as in Fig. 2, helps in keeping RNA degradation, if any, to a minimum during DTT pretreatment, but this also necessitated use of the gel assay. As the results show (Fig. 4*a*), the enzyme pretreated with DTT alone was found to acquire greater inhibitor-sensitivity than the one pretreated with DTT in the presence of RNA (lane 4 compared with lane 5), the inhibition, if any, in the latter case (lane 5) being comparable with that of the enzyme untreated with DTT (lane 3). We made a similar observation when 2′,3′-(cyclic)CMP was used in the pretreatment

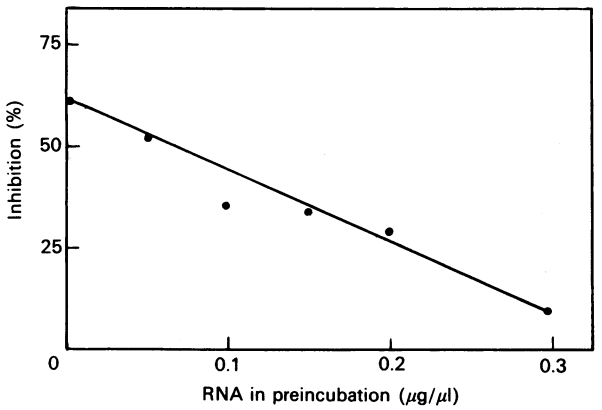


Fig. 5. Effect of DTT pretreatment in the absence and in the presence of the substrate on the inhibitor-sensitivity of RNAase BS-1, studied by chromatographic assay

RNAase BS-1 was pretreated with 5 mM-DTT in buffer A in the absence and in the presence of indicated amounts of the substrate as described for Fig. 4, and the enzyme equivalent to 1.5 activity units was assayed for inhibitor-sensitivity in the presence of 90 units of the inhibitor. Reaction samples were chromatographed, and degradation and inhibition were determined as indicated in the Materials and methods section.

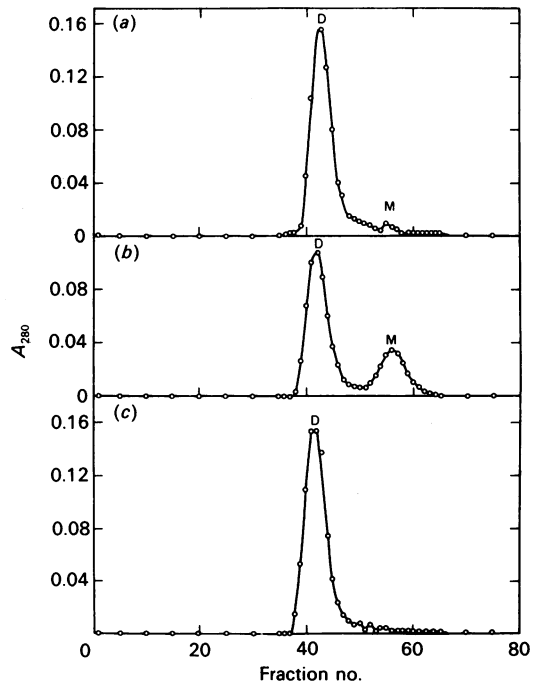


Fig. 6. Gel filtration on Sephadex G-75 of RNAase BS-1 untreated or treated with DTT in the absence and in the presence of 2′,3′-(cyclic)CMP

Portions (8 mg) of RNAase BS-1 were incubated as such and in the presence of 5 mM-DTT at 37 °C for 5 min in the absence and in the presence of 20 mg of 2′,3′-(cyclic)CMP in a total volume of 1 ml. The enzyme was immediately loaded on to a 1.6 cm × 100 cm Sephadex G-75 column and eluted with 100 mM-ammonium acetate buffer, pH 5.0. Fractions (2 ml) were collected and the absorbance of fractions at 280 nm was determined. (*a*) RNAase BS-1 incubated as such; (*b*) RNAase BS-1 incubated with DTT; (*c*) RNAase BS-1 incubated with DTT and 2′,3′-(cyclic)CMP. In (*c*) 2′,3′-(cyclic)CMP was found to be eluted after fraction no. 75 and is not shown in the Figure.

step (lane 6). This effect of the substrate was further found to vary in a concentration-dependent manner (Fig. 4*b*). Pretreatment of RNAase A with DTT in the absence (Fig. 4*ci*) and in the presence (Fig. 4*cii*) of total *E. coli* RNA did not show any significant effect on its inhibition by the inhibitor. In a similar experiment, shown in Fig. 5, but using the chromatographic assay procedure, we observed that the presence of different concentrations of the substrate (0.05–0.3 μg/μl) during DTT pretreatment decreased the inhibitor-sensitivity of RNAase BS-1, the extent of inhibition decreasing linearly from 50% (at 0.05 μg of substrate/μl) to about 10% (at 0.3 μg of substrate/μl).

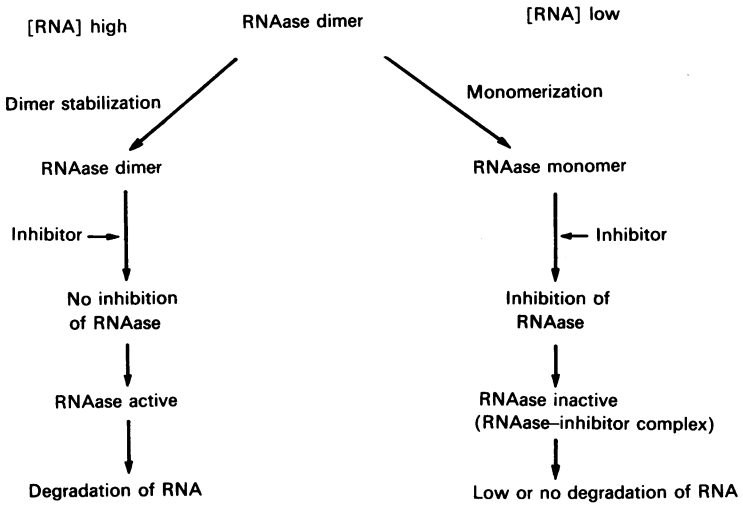
By using gel filration on Sephadex G-75, we assessed the status of the enzyme following DTT treatment in the absence and in the presence of the substrate. The protein untreated with DTT was eluted as a homogeneous peak (Fig. 6*a*), but following treatment with DTT it was eluted in two fractions as reported by D'Alessio *et al.* (1975), these corresponding to the dimeric and monomeric forms (designated as D and M respectively; Fig. 6*b*). However, the elution profile of the enzyme treated with DTT in the presence of 2′,3′-(cyclic)CMP (Fig. 6*c*) resembled that of the enzyme untreated with DTT, indicating that in the presence of the substrate the formation of the monomeric fraction as in Fig. 6(*b*) was not favoured. (We could not do this experiment with *E. coli* RNA as substrate owing to its obvious interference with the gel-filtration profiles of the enzyme.)

DISCUSSION

In this investigation we studied the sensitivity of different forms of RNAase BS-1 to the human placental RNAase inhibitor under different experimental conditions. These forms included the native enzyme, which is a dimeric protein, MCM RNAase BS-1, which is the purified stable monomeric form, and RNAase BS-1 pretreated with 5 mM-DTT, which is a mixture of dimeric and monomeric forms under the experimental conditions used.

The main conclusions of our experiments are as follows: (1) MCM RNAase BS-1 and RNAase A exhibit comparable sensitivities to the RNAase inhibitor; (2) the native RNAase BS-1 was inhibited very little, if at all, by the RNAase inhibitor, and therefore may be considered to be insensitive to the inhibitor; (3) pretreatment of RNAase BS-1 with DTT renders it inhibitor-sensitive, but the presence of substrate during DTT pretreatment of the enzyme resists its conversion into the inhibitor-sensitive form. Another useful piece of information related to the molecular interaction of the inhibitor with the enzyme also emerges out of this study. The binding of the inhibitor with RNAase A is believed to involve contacts at several amino acid residues, one of the contact points being lysine residues at position 31 and 37 (Blackburn & Gavilanes, 1982). In RNAase BS-1 these positions are occupied by cysteine and glycine respectively (Di Donato & D'Alessio, 1979). Since both RNAase A and MCM-RNAase BS-1 were inhibited comparably by the RNAase inhibitor, the presence of lysine residues at these positions may not be obligatory for bringing about effective binding of the inhibitor and inhibition of enzyme activity.

DTT treatment of the enzyme is expected to result in total reduction of the interchain disulphide bridges of RNAase BS-1 (D'Alessio *et al.*, 1975). Therefore the observation that the inhibition of DTT-treated enzyme was not complete and reached a maximum value of about 50% under the experimental conditions of Fig. 3 suggests that reduction of the interchain disulphide bridges alone may not be adequate for acquiring sensitivity to the inhibitor. More likely, the loss also of other non-covalent interactions present in the native dimeric structure resulting in monomerization of RNAase BS-1 may be necessary. The acquisition of inhibitor-sensitivity, presumably due to mono-



Scheme 1. Schematic diagram for inhibition of RNAase BS-1 by RNAase inhibitor under conditions of low and high RNA concentration and control of net enzyme activity

merization (as discussed in the Results section), following DTT treatment of RNAase BS-1 supports this conclusion. In the structure proposed for RNAase BS-1 (Capasso *et al.*, 1983; Suzuki *et al.*, 1987) and for RNAase A dimers (Fruchter & Crestfield, 1965*a*,*b*), hydrophobic interactions hold the *N*-terminal tail of one unit with the body of the other, which includes the putative binding sites for the inhibitor. In view of this structural similarity, loss of these interactions in RNAase BS-1 would be expected to result in the formation of RNAase A-like monomeric structure suitable for interaction with the inhibitor. Blackburn & Gavilanes (1980) have reported differences in the inhibitor-sensitivity of RNAase BS-1 and MCM-RNAase BS-1; the latter was more inhibitor-sensitive than the former. The differences observed by us are more pronounced and allow us to infer that the dimeric form of RNAase BS-1 is apparently insensitive to the inhibitor.

Although DTT pretreatment of the enzyme can significantly increase its sensitivity to the inhibitor, the presence of RNA during pretreatment was found to resist such a change, implying substrate stabilization of RNAase BS-1 in the dimeric state with lower inhibitor-sensitivity, or insensitivity to it (Figs. 4 and 5). This conclusion is again consistent in view of the proposed structure of RNAase BS-1 (Capasso *et al.*, 1983; Suzuki *et al.*, 1987) in which histidine-12 and histidine-119 (which play a fundamental role in the enzymic activity) of each chain are exchanged in the formation of composite active sites in the dimeric structure. Binding of the substrate may therefore stabilize this structure of the enzyme. The results shown in Fig. 6 also support this possibility.

Studies on the inhibition of RNAase A by RNAase inhibitors from different sources have suggested both competitive and non-competitive mechanisms from a classical point of view (Blackburn *et al.*, 1977; Blackburn & Jailkhani, 1979; Burton & Fucci, 1982; Turner *et al.*, 1983). However, some authors have argued against its classification as competitive or non-competitive on account of its tight-binding nature (see discussion by Fominaya *et al.*, 1988). Whatever may be the mechanism, the possible effect of the substrate to stabilize the dimeric inhibitor-insensitive structure of RNAase BS-1, influencing its net inhibitor-sensitivity, suggests a link between substrate concentrations, inhibitor-sensitivity and the enzyme activity, as illustrated in Scheme 1. The scheme implies that in the presence of DTT and with low RNA concentrations monomerization is favoured, resulting in a decrease in RNA degradation because the monomer is inhibitor-sensitive. It should be noted that low RNA condition may only facilitate monomerization (which may primarily be a result of reduction of interchain disulphide bridges by DTT) and may not itself be the cause of it. On the contrary, high RNA condition may have a more direct effect in stabilizing the enzyme in the dimeric inhibitor-insensitive form, resulting in RNA degradation under these conditions.

RNAase BS-1 is an extracellular enzyme. Although it is reported to exhibit some interesting biological effects (Matousek, 1973; Vescia *et al.*, 1980; Vescia & Tramontano, 1981), its biological role is still a puzzle. The enzyme is reported to be present in bovine seminal-vesicular tissue (Quarto *et al.*, 1987; Tamburrini *et al.*, 1986). Similarly, RNAase inhibitor is reported from bovine brain and liver (Burton & Fucci, 1982; Burton *et al.*, 1980). But the coexistence of RNAase BS-1 and the inhibitor in any bovine tissue has not been shown. For these reasons, at present we cannot assess any direct implication of our results in the cellular context. However, if RNAase BS-1 has any cellular function, its biochemistry and enzymic behaviour *in vitro* as shown in Scheme 1 at least allow us to think of a putative mechanism for the regulation of its activity. Whether the same could be extended to other inhibitor-responsive RNAases will be the next question. Multiple species of inhibitor-responsive RNAases reported in the literature (Brockdorff & Knowler, 1987; Kimball & Meyer, 1987) may also include dimeric and monomeric structures. Even RNAase A is known to form a dimeric structure in solution (Crestfield *et al.*, 1962); interchain disulphide bridges as found in RNAase BS-1 do not seem to be an obligatory requirement for dimerization. It will be interesting to study if any of these forms would exhibit a behaviour similar to RNAase BS-1 towards the inhibitor and give a wider perspective to the scheme shown in Scheme 1.

We thank several of our colleagues at the Centre for Cellular and Molecular Biology for useful discussions and comments on the manuscript, Mr. N. Sitaram for generously donating the RNAase BS-1 and Mr. M. V. Jagannadham for his enthusiastic help in h.p.l.c. runs.

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Received 8 May 1991; accepted 25 June 1991