Deletions at the C-terminus of interferon γ reduce RNA binding and activation of double-stranded-RNA cleavage by bovine seminal ribonuclease

Catherine H. SCHEIN*† and Monika HAUGG†

*Swiss Institute for Alternatives to Animal Testing (SIAT), Technopark, Pfingstweidstrasse 30, CH-8005 Zürich, Switzerland and †Laboratorium für Organische Chemie der E.T.H., CH-8092 Zürich, Switzerland

Recombinant interferon γ (IFN- γ) from three species activates the cleavage of double stranded (ds-) RNA by the dimeric RNAase isolated from bovine semen (BS-RNAase). Human and bovine IFN- γ bind RNA tightly enough to inhibit cleavage by RNAase A [Schein, Haugg and Benner (1990) FEBS Lett. 270, 229–232]. Murine IFN- γ and a proteolytic fragment of human IFN- γ , both of which lack part of the positively charged C-terminus, bind RNA weakly and do not inhibit RNAase A.

Their ability to activate BS-RNAase is proportional to their activity in the anti-viral assay. Two monoclonal antibodies that neutralize the anti-viral activity of human IFN- γ inhibit the activation of BS-RNAase by both full-length and proteolysed human IFN- γ . Our results demonstrate that the C-terminus of IFN- γ contributes to RNA binding and activation of BS-RNAase, as well as to anti-viral activity.

INTRODUCTION

Induction of an anti-viral state in cells after treatment with interferons is accompanied by changes in the stability [1,2] and processing [3,4] of cellular and viral RNAs. Destabilization of double-stranded (ds-)RNA by interferon (IFN)- α has been attributed to a three-step mechanism in which IFN- α binds to a receptor and by doing so activates DNA-binding factors [5]. These induce the transcription and synthesis of 2',5'-A_n synthetase, which then produces 2',5'-P-linked polyadenylic acid, a polynucleotide that activates latent RNAase-L to degrade ds-RNA [6].

However, IFN- γ is not a strong inducer of the synthesis of 2',5'-A_n synthetase [7,8]. In previous work, we have shown that it is capable, *in vitro*, of activating bovine seminal ribonuclease (BS-RNAase) directly. We also demonstrated that IFN- γ itself binds RNA tightly enough to inhibit the activity of RNAase A, one of the most potent RNAases known [9]. These results are particularly interesting as another suggested mechanism for IFN action is via the induction of proteins that bind RNA [10] and that a ds-RNAase activity is co-induced with IFN when cells are treated with various IFN-inducing molecules [11].

Different areas of IFN- γ are responsible for its effects on the activity of RNAases. By proteolytically removing C-terminal amino acids from human IFN- γ , we prepared a fragment which

bound weakly to RNA, did not inhibit RNAase A, but partially retained the ability to activate BS-RNAase. Murine IFN- γ , which lacks the last nine amino acids of the bovine and human proteins (Table 1), behaved similarly to this fragment in all assays. These results demonstrate that the positively charged C-termini of the human and bovine proteins enhance RNA binding, and deletions in this area decrease activation of BS-RNAase in proportion to their effect on the anti-viral activity.

MATERIALS AND METHODS

Assays

The RNAase assay and preparation of the ³²P-labelled ss- and ds-RNA substrates were as previously described [9]. For the assay, 4 μ l of assay mixture (containing ³²P-labelled ss- or ds-RNA in Tris/HCl, pH 8) was combined with 2–5 μ l of sample and incubated for the indicated time at 37 °C. Assays were stopped by the addition of 20 μ l of cold 10% trichloroacetic acid, and 4 μ l of a solution of 20 μ g/ml BSA/40 μ g/ml yeast tRNA was added as carrier. Samples were vortexed, incubated for 30–60 min on ice, centrifuged for 10 min at 4 °C in an Eppendorf centrifuge, the supernatant and pellet fraction counted separately, and the data expressed as the percentage of total RNA solubilized. RNAases and IFNs were diluted with TTG buffer (10 mM Tris/HCl, pH 7.9, 0.02% Tween 20, 20% glycerol) except where noted. For kinetic assays (Figure 3), larger volumes of sample were taken for the initial data points to reduce the counting error.

The Bradford protein assay was used to estimate IFN specific activity [12] and the amount of IFN added to assays. Anti-viral assays were performed by the external laboratories indicated.

Proteins

Recombinant human IFN- γ , which, like that isolated from human cells, lacks the initial N-terminal Cys-Tyr-Cys residues encoded in the gene sequence, was obtained from C. Weissman (Molecular Biology I, University of Zürich, Zürich, Switzerland). This material, which was used directly for the experiments

Table 1 C-Terminal amino acids of IFN- γ from three species and the deletion mutants of human IFN- γ used in this study

Species	C-Terminal sequence
Human IFN- γ	–PAAKTGKRKRSQMLFRGRRASQ
Human IFN- γ Δ12	–PAAKTGKRKR
Human IFN- γ Δ14	–PAAKTGKR
Bovine IFN- γ	–PKSNLRKRKRSQMLFRGRRASQ
Murine IFN- γ	–PESSLRKRKRSRC

Abbreviations used: BS-RNAase, bovine seminal ribonuclease; IFN- γ , interferon γ ; ss- and ds-RNA, single- and double-stranded RNA; IFU, interferon units.

† To whom correspondence should be addressed.

Figure 1 'Phast Gel' (Pharmacia; 20% acrylamide/SDS) of the purified human IFN- γ preparations (130 ng of full-length human IFN- γ , 180 ng of IFN- γ Δ 12, 190 ng of IFN- γ Δ 14) and the RNAases {330 ng of BS-RNAase isolated from semen, 135 ng of recombinant RNAase A (prepared as described previously [15])} used in this study

The gel was stained with Coomassie Blue.

described in Figures 2c and 2d and Table 4, was titred in Zürich (using WISH cells with Newcastle disease virus challenge) to have approx. 1×10^7 – 2×10^7 interferon units (IFU)/mg of protein. Some of this protein was used to prepare C-terminal deletions essentially as described by Döbeli et al. [13]. After overnight cleavage at room temperature with either the protease factor Xa (Biolabs or Boehringer-Mannheim) or endopeptidase Arg-C (Sigma), samples were diluted 5-fold with 10 mM sodium phosphate buffer, pH 5.7, applied to small columns of CM-Sephadex C-25 (Pharmacia) and eluted with a step gradient of NaCl in 10 mM sodium phosphate buffer, pH 7.3. IFN- γ was eluted between 300 and 500 mM NaCl, the factor Xa-treated (Δ 12) protein between 200 and 300 mM NaCl, and the Arg-C-treated (Δ 14) protein between 80 and 100 mM NaCl. The appropriate eluates were concentrated in Centricon 3 micro-concentrators (Amicon) where necessary. As the samples were diluted at least 25-fold for assay, the total amount of salt added was approximately the same for each assay.

The number of amino acids deleted was determined by electrospray-ionization m.s. on a Finnigan MAT (San Jose, CA, U.S.A.) TSQ710 instrument. To desalt before m.s., each protein preparation after CM-Sephadex chromatography was applied to a BU 300 (Brownlee) column (3 cm $\times$ 2 mm), and eluted with a 13 min gradient (0.2 ml/min) from 0.1% trifluoroacetic acid in water to 80% acetonitrile in 0.08% trifluoroacetic acid. The single protein peak from each preparation was concentrated in a Rotovac, and 1–2 μ l was used for the analysis. The mass-average M_r values were: 16908 for the starting protein (exactly that calculated for 143 amino acids plus one N-terminal methionine), 15492 for the factor Xa-treated material (exactly that calculated for a deletion of 12 amino acids from the C-terminus) and 15205 for the Arg-C-treated protein (exactly that calculated for a deletion of 14 amino acids from the C-terminus). The factor Xa-cleaved material also contained some shorter fragments, as did the starting material. N-terminal amino acid sequencing of the Arg-C-treated protein indicated that there was no deletion from the 5' end and that the N-terminal methionine was still present. The relative mobility of the three IFN preparations (before the desalting h.p.l.c.) on a 20% Phast Gel (Pharmacia) is shown in Figure 1. The anti-viral activities of the preparations, determined using WISH cells with endomyocarditis virus challenge at Hoffman-LaRoche, Basel, Switzerland as described previously [14], were wild-type IFN- γ , 7×10^5 IFU/mg; IFN γ Δ 12,

1.2×10^6 IFU/mg; IFN- γ Δ 14, 1.35×10^5 IFU/mg. These samples were used for the experiments in Figure 3.

Samples prepared in the same fashion were used for the experiments described in Figure 2 and Tables 2 and 3. These were titred by Dr. Michel Horisberger at Ciba Geigy, Basel, Switzerland using WISH cells with a vesicular stomatitis virus challenge, and the specific activities were determined to be: wild-type IFN- γ , 1.6×10^7 IFU/mg; IFN γ Δ 12, 4×10^7 IFU/mg; IFN- γ Δ 14, $(0.5\text{--}1) \times 10^7$ IFU/mg. Dr. Horisberger has found that IFN- γ requires much longer incubation with the cell layer than IFN- α for maximum assay sensitivity. The net incubation time is 48 h as the medium is not changed on virus addition; the incubation time at Hoffman-LaRoche is 24 h and the medium is changed before virus is added. The specific activity of the recombinant murine IFN- γ (from G. Garotta, Hoffman-LaRoche) was 3×10^6 IFU/mg (activity determined on L929 cells with vesicular stomatitis virus challenge). Purified recombinant bovine IFN- γ was a gift from Ciba-Geigy [9]. BS-RNAase, purified from bovine seminal fluid, was from Steven A. Benner (Organic Chemistry, ETH, Zürich, Switzerland).

PAGE assay of RNA binding

The protein-containing sample (1 μ l) was combined with 2–4 μ l of 32 P-labelled RNA in Tris/HCl, pH 8, at room temperature. Then 1 μ l of a 30% glycerol solution containing dyes was added and the samples were applied to 4.5–6% polyacrylamide minigels (Hoefer) in 50 mM Tris/borate/1 mM EDTA (TBE) buffer. Gels were run with cooling at 400 V, soaked for 30 min in acetic acid/methanol to fix, vacuum-dried at 60 °C on to Whatman 3M paper, covered with tape, and autoradiographed with or without intensifying screens at –70 °C.

Antibodies

Monoclonal antibodies to human IFN- γ were obtained from Hoffman-LaRoche. To remove background RNAase activity, antibodies were passed over CM-Sephadex (as for the IFN- γ preparation) and concentrated in Centricon 10 micro-concentrators (Amicon). Antibody concentration was estimated by dot-spotting on nitrocellulose, and specific binding to human IFN- γ measured by e.l.i.s.a. Both of the neutralizing antibodies, MC-69 and MC-123, bound IFN- γ in an e.l.i.s.a. to a dilution of 4×10^{-5} and detected all three forms of IFN- γ on immunoblots. The non-neutralizing antibody MC-127 bound very weakly to IFN- γ -coated e.l.i.s.a. wells and to immunoblots of the three derivatives, whereby it detected the Δ 12 and Δ 14 IFN- γ deletion proteins more efficiently than the full-length human IFN- γ .

RESULTS

Effect of deletions of human IFN- γ on RNAase

Human and bovine recombinant IFN- γ inhibit the degradation of ss- and ds-RNA by bovine pancreatic RNAase at the same concentrations at which they activate the cleavage of the same substrates by BS-RNAase ([9] and Table 2). Deletion with Factor Xa of the last 12 amino acids of human IFN- γ does not diminish its anti-viral activity, its ability to bind ss- or ds-RNA (Figures 2a and 2b), its inhibition of RNAase A or its activation of BS-RNAase (Table 2). The specific activity of this form of IFN- γ is higher than the full-length molecule in the anti-viral assay, consistent with previous results [13].

However, when 14 amino acids were deleted with the protease Arg-C, human IFN- γ retained about 20% of the specific anti-

Table 2 Effect of human IFN- γ and C-terminal deleted fragments thereof on the cleavage of ss- and ds-RNA substrates by BS-RNAase and RNAase A

Approx. 6 ng of RNA was used per 10 μ l of assay mixture. ss-RNA assay mixtures were incubated for 25 min at 37 °C; 0.1 μ g of RNAase A (from Boehringer-Mannheim mixtures) or 0.02 ng of BS-RNAase (purified from bovine seminal fluid) were added per assay. For the ds-RNA assay, 0.1 ng of RNAase A or 0.2 ng of BS-RNAase was added per assay and incubation was for 60 min at 37 °C. Approx. 10 ng of human IFN- γ or the indicated deletion thereof was added (according to assays carried out by Dr. Horisberger, 160 IFU/assay was added for the full-length IFN- γ , 400 IFU/assay for the IFN- γ Δ 12 protein, and 100 IFU/assay for the IFN- γ Δ 14 protein). A large excess of IFN- γ was used in this experiment to show the differential effect on the two nucleases (much larger amounts of IFN are required to inhibit RNAase A than to activate BS-RNAase). Duplicate samples are indicated.

Addition	ss-RNA solubilized (%)	ds-RNA solubilized (%)	
No RNAase, buffer alone	2	1	
+ IFN- γ	2	3	
+ IFN- γ Δ 12	3	2.5	
+ IFN- γ Δ 14	4	3.6	
RNAase A + buffer	44, 46	64, 56	
+ IFN- γ	15, 14	40, 41	
+ IFN- γ Δ 12	12, 12	40, 42	
+ IFN- γ Δ 14	49, 52	59, 57	
BS-RNAase + buffer	6, 7	Assay 1	Assay 2
+ IFN- γ	70, 76	48, 51	3.5, 3.1
+ IFN- γ Δ 12	66, 64	50, 49	40, 40
+ IFN- γ Δ 14	23, 23	49, 45	46, 41
			50, 46

viral activity of the full-length protein, but its ability to bind RNA was greatly reduced (Figures 2a and 2b). The Arg-C protease-treated IFN- γ did not inhibit RNAase A at the highest concentration possible to add to the assay, and its ability to stimulate cleavage of ss-RNA substrates by BS-RNAase was greatly diminished (Table 2). However, IFN- γ Δ 14 could still activate the cleavage of ds-RNA substrates by BS-RNAase (Table 2). In kinetic studies (see Figure 3) both C-terminally deleted IFN preparations were weaker than the full-length molecule in activating BS-RNAase.

Antibodies to IFN- γ block the activation of BS-RNAase

Our previous experiments [9]; C. H. Schein and M. Haugg, unpublished work) have shown that IFN- γ activates BS-RNAase in buffers of differing salt concentration and in the presence of other proteins. To demonstrate further that the activation was indeed due to the IFN in the preparation, we tested the effect of three monoclonal antibodies on the activation of BS-RNAase using a large excess of human IFN- γ and the Δ 14 deletion. Two of the antibodies, MC-69 and MC-123, both of which bind to human IFN- γ between amino acids 76 and 113, neutralized the anti-viral activity of human IFN- γ on human WISH cells. MC-127, which is specific for the C-terminal amino acids 120–146, was non-neutralizing [9]. As Table 3 illustrates, both of the neutralizing monoclonal antibodies greatly reduced the activation of BS-RNAase by full-length human IFN- γ or the Δ 14 deletion protein, whereas MC-127 had no effect.

Effect of murine IFN- γ on RNAase activity

Further evidence for the role of the C-terminus in inhibition of RNAase A is that recombinant murine IFN- γ , which lacks the last nine amino acids of human and bovine IFN- γ (Table 1),

Figure 2 Polyacrylamide-gel band-shift determination of the ability of various forms of IFN- γ to bind to 12 ng of ss-RNA (a, d) or ds-RNA (b, c)

(a,b) Effect of deleting C-terminal amino acids from human IFN- γ on binding to ss- and ds-RNA. Proteolysis and purification of the deletion proteins as well as the band-shift assay are described in the Materials and methods section; the gel contained 4.5% polyacrylamide. (c) Murine IFN- γ at the highest concentration it is possible to use in the assay binds only weakly to RNA, whereas (d) bovine IFN- γ binds ss-RNA nearly as efficiently as the human protein. The gels for (c) and (d) contained 6% polyacrylamide. Owing to variations in their specific activities, 100 ng of protein is approx. 1000 IFU for human, 400 IFU for bovine and 300 IFU for murine IFN- γ . See the legend of Table 2 for the specific activities of the IFN deletion proteins.

showed only slight binding of RNA at the highest concentration possible to add to the assay (Figure 2c). Bovine IFN- γ , in contrast, bound RNA nearly as efficiently as human IFN- γ ,

Table 3 Monoclonal antibodies (MC-69, MC-123) that neutralize the anti-viral activity of human IFN- γ neutralize its ability to activate the cleavage of ds-RNA by BS-RNAase

The results are shown as the percentage of added ds-RNA solubilized in 30 min at 37 °C. For the assay, 4 μ l of the indicated antibody solution or buffer (10 mM sodium phosphate, pH 7.3, 80 mM NaCl plus 1 mg/ml macaloid-treated BSA) was combined with 4 μ l of a dilution of IFN- γ in TTG buffer; the mixture was incubated for 2 h on ice and centrifuged for 5 min at 4 °C. Then 2 μ l of a 0.5 ng/ml dilution in TTG buffer of BS-RNAase (0.1 ng/assay) was combined with 1 μ l of the antibody/IFN mixture (160 IFU of the full-length or 50 IFU of the Δ 14 deletion protein) or buffer, RNA assay mixture (4 μ l) was added, samples were incubated, and the assays stopped with trichloroacetic acid as described in the Materials and methods section. Means $\pm$ S.D. of the indicated number of assays (in parentheses) are shown; where more than duplicate assays are indicated, the assays were repeated the next day.

Addition	No BS-RNAase, + IFN- γ	+ BS-RNAase no IFN- γ	+ BS-RNAase, + IFN- γ	+ BS-RNAase, + IFN- $\gamma\Delta$ 14
Buffer	3	2 $\pm$ 0 (3)	37 $\pm$ 1.83 (4)	31 $\pm$ 7.1 (2)
MC-127 (non-neutralizing)	1	6.3 $\pm$ 3.3 (4)	35 $\pm$ 2.8 (5)	32.5 $\pm$ 2.6 (4)
MC-69 (neutralizing)	1	6.5 $\pm$ 1.0 (2)	17 $\pm$ 3 (3)	11 $\pm$ 1.4 (2)
MC-123 (neutralizing)	2	5.5 $\pm$ 0.7 (2)	15 $\pm$ 2.9 (3)	9.5 $\pm$ 0.71 (2)

Table 4 Comparative effect of murine and human IFN- γ on the cleavage of ds-RNA substrates by three RNAases

Both murine and human IFN- γ activate BS-RNAase but only human IFN- γ inhibits recombinant murine or bovine pancreatic RNAases. The latter two proteins were secreted from *Escherichia coli* and purified as described [15]. Each assay mixture contained 0.1 ng of the indicated RNAase, about 10 ng of RNA, and the indicated amounts of murine or human IFN- γ , and was incubated for 30 min at 37 °C. Again, a large excess of IFN was added in this experiment to show the differential effects on the selected nucleases. The background in the assay, determined from samples of buffer or buffer plus IFN (murine or human), was only 1–2% solubilized ds-RNA. Means $\pm$ S.D. of duplicate samples are shown.

RNAase	Addition	ds-RNA solubilized (%)
BS-RNAase	Buffer	21 $\pm$ 9.8
	100 ng of murine IFN- γ (300 IFU)	53.5 $\pm$ 2.1
	10 ng of murine IFN- γ (30 IFU)	42.5 $\pm$ 2.1
	30 ng of human IFN- γ (300 IFU)	47 $\pm$ 1.4
Recombinant murine pancreatic RNAase	Buffer	67 $\pm$ 1.4
	100 ng of murine IFN- γ (300 IFU)	67.5 $\pm$ 3.5
	10 ng of murine IFN- γ (30 IFU)	83.5 $\pm$ 3.5
	30 ng of human IFN- γ (300 IFU)	37 $\pm$ 5.7
Recombinant bovine pancreatic RNAase (RNAase A)	Buffer	80.5 $\pm$ 2.12
	100 ng of murine IFN- γ (300 IFU)	74 $\pm$ 1.4
	10 ng of murine IFN- γ (30 IFU)	87 $\pm$ 1.4
	30 ng of human IFN- γ (300 IFU)	43.7 $\pm$ 5.0

emphasizing the role of the C-terminus of the protein in this activity (Figure 2d). As Table 4 illustrates, the murine protein did not inhibit RNAase A or recombinant murine pancreatic RNAase, but it did activate BS-RNAase. Table 4 also shows that recombinant RNAase A, secreted from *E. coli*, was inhibited by human IFN- γ in a similar manner to RNAase A and B isolated from bovine pancreas.

Time course of degradation of ds-RNA by BS-RNAase in the presence and absence of IFN- γ

The activation of BS-RNAase by IFN- γ is obvious from the earliest time point measured in the ds-RNA assay (Figure 3). Although human IFN- $\gamma\Delta$ 12 was nearly as active as the full-length protein in the assay, the Δ 14 deletion protein was significantly less able to activate BS-RNAase.

Figure 3 Time course of ds-RNA [(a) 10 ng/10 μ l time point, (b) 40 ng/10 μ l time point] degradation in the presence of BS-RNAase (2 ng/10 μ l time-point assay), alone (○) or +10 ng (7 IFU) of human IFN- γ (■), 10 ng (12 IFU) of human IFN- $\gamma\Delta$ 12 (△) or 10 ng (about 2 IFU) of human IFN- $\gamma\Delta$ 14 (▲)

Either TTG buffer alone or an IFN dilution in TTG buffer was added to the RNA assay mixture, and an aliquot was removed for the zero time point, which was held at 37 °C for the duration of the assay (20–30 min). The rest of the mixture was prewarmed for 5 min at 37 °C and the assay started by adding (prewarmed) BS-RNAase. At intervals, aliquots were removed into cold 10% trichloroacetic acid, protein/RNA carrier was added, and the assay mixtures were centrifuged and counted as usual.

DISCUSSION

As IFNs inhibit angiogenesis *in vitro* [16] and *in vivo* [17], and as factors capable of inducing angiogenesis include an RNAase

homologue, angiogenin [18], and ribonucleoproteins [19], we tested whether IFNs might directly interact with RNAases. Our initial studies demonstrate that concentrations of IFN- γ that inhibit bovine pancreatic RNAase (RNAase A) activate the closely related RNAase isolated from bovine semen [9]. BS-RNAase, which differs from RNAase A in only 23 of 124 residues, forms a covalent cysteine-linked dimer [20] and is about 100 times more active in cleaving ds-RNA substrates [9]. We conclude further that the major binding site for RNA in IFN- γ is located at the C-terminus of the molecule, at approximately the same site as previously shown for the binding of heparin sulphate [21]. However, a 15–20-fold weight excess of heparin sulphate is required to reverse binding of IFN- γ to RNA (results not shown). Substrate binding is the basis of the inhibition of RNAase A and recombinant murine pancreatic RNAase by human IFN- γ . The sequence specificity of IFN binding to RNA has not been determined.

The area of IFN- γ responsible for activating the cleavage of ds-RNA by BS-RNAase is within the binding region of the neutralizing antibodies MC-123 and MC-69. The three-dimensional crystal structure of human IFN- γ [22] locates the area between amino acids 76 and 113 at the dimer surface.

The C-terminus probably stabilizes receptor binding, as cleavage diminishes but does not eliminate activity in the anti-viral assay. Although there is some indication that the positively charged C-terminal 14–19 amino acids of human IFN- γ are essential for binding to its receptor and induction of an anti-viral state in cells [13,23,24], our result showing that the $\Delta 14$ C-terminal deletion proteins still possess 10–30% of the anti-viral activity of the full-length protein is consistent with those of others [25]. The differences may be due to instability of the C-terminally deleted proteins, as our samples were titred in the same laboratory at Hoffmann–LaRoche as those of Döbeli et al. [13] (who used, however, FT cells and Sindbis virus challenge). Both Wetzel et al. [24] and Luk et al. [25] used the same assay cells (human lung carcinoma A549) and challenge virus (endomyocarditis). Freezing and thawing of samples and even the method of dilution before assay can significantly alter assay results (C. H. Schein, unpublished work).

In addition, small differences in the assay conditions may also affect the overall result. Rubenstein et al. [26] showed that the sensitivity of the IFN assay is dependent on the type of cell, the type and titre of virus used, the length of time the cells are incubated with IFN before virus challenge, and whether a preformed cell layer is used or the IFN is added simultaneously with a cell suspension. If the C-terminal deletion proteins bind the receptor less tightly, longer preincubation of the cells with IFN- γ could account for the higher estimate of the specific activity of the IFN deletion proteins and relatively higher values for IFN- $\gamma\Delta 14$ measured by Dr. Horisberger. However, both assays indicated similar relative activities for the deletion proteins (roughly 1:2:0.3 for the full-length: $\Delta 12$: $\Delta 14$).

There are several possible mechanisms by which IFN could stimulate BS-RNAase. One is that IFN- γ may have an as yet unidentified enzyme activity. We were, however, unable to detect any RNA-cleavage product that appeared only in the presence of IFN- γ [9]. Our data are consistent with IFN improving the catalytic activity of BS-RNAase while not altering its cleavage mechanism. It is clear from Figure 3 that the degradation of ds-RNA has several phases, and that IFN- γ particularly accelerates the first (rapid) steps.

The survival of cells depends on their ability to control the

activity of RNAases. Negative control of RNAase activity has been demonstrated by the isolation and cloning of proteins that inhibit RNAases [27,28]. Activation of latent RNAases presents a rapid and efficient way for a cell to respond to changing environments. We have demonstrated that recombinant IFN- γ from three different species, prepared in four different laboratories, can directly stimulate the degradation of ds-RNA *in vitro* by a dimeric RNAase. As antibodies that neutralize anti-viral activity also interfere with the activation of ds-RNA cleavage, we suggest that direct interaction with RNAases may be one of the biologically important activities of IFN- γ . Measurement of the ability of purified preparations of IFN- γ to bind RNA and activate nucleases is also a rapid method of determining specific activity without the need for mammalian cell cultivation.

We thank G. Frank/M. Rümblei (Molecular Biology, E. T. H., Zürich, Switzerland) for protein sequencing, W. Staudenmann/P. James (Protein Analysis Laboratory, E. T. H., Zürich, Switzerland) for protein m.s., S. Benner (Organic Chemistry, E. T. H., Zürich, Switzerland) for BS-RNAase, G. Garotta/M. Fountoulakis (Hoffmann–LaRoche, Basel, Switzerland) for monoclonal antibodies to human IFN- γ and murine IFN- γ , M. Horisberger/Ms. Bendali (Ciba-Geigy, Basel, Switzerland) and G. Garotta and co-workers for performing the IFN anti-viral assay, and G. Folkers and J. Fetzer of the Pharmacy Institute of the E. T. H. Zürich. This work was supported by a grant from the Zürcher Krebsliga.

REFERENCES

- Friedman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. and Stark, G. R. (1984) *Cell* **38**, 745–755
- Dubois, M. F., Mezger, V., Morange, M., Ferrieux, C., Lebon, P. and Bensaude, O. J. (1988) *J. Cell. Physiol.* **137**, 102–109
- Harel-Bellan, A., Brini, A. T. and Farrar, W. L. (1988) *J. Immunol.* **141**, 1012–1017
- Samuel, C. E. (1988) *Progr. Nucleic Acid Res. Mol. Biol.* **35**, 27–72
- Williams, B. R. G. (1991) *Eur. J. Biochem.* **200**, 1–11
- Romeo, G., Fiorucci, G. and Rossi, G. B. (1989) *Trends Genet.* **5**, 19–24
- Rosenblum, M. G., Cheung, L. and Kessler, D. (1988) *J. Interferon Res.* **8**, 275–282
- Fish, E. N., Hannigan, G. E., Banerjee, K. and Williams, B. R. G. (1988) *Virology* **165**, 87–94
- Schein, C. H., Haugg, M. and Benner, S. A. (1990) *FEBS Lett.* **270**, 229–232
- Constantoulakis, P., Campbell, M., Felber, B. K., Nasioulas, G., Afonina, E. and Pavliakis, G. N. (1993) *Science* **259**, 1314–1318
- Meegan, J. M. and Marcus, P. I. (1989) *Science* **244**, 1089–1091
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Döbeli, H., Gentz, R., Jucker, W., Garotta, G., Hartmann, D. W. and Hochuli, E. (1988) *J. Biotechnol.* **7**, 199–216
- Garotta, G., Ozmen, L., Fountoulakis, M., Dembic, Z., van Loon, A. P. G. M. and Stuber, D. (1990) *J. Biol. Chem.* **265**, 6908–6915
- Schein, C. H., Boix, E., Haugg, M. et al. (1992) *Biochem. J.* **283**, 137–144
- Tsuruoka, N., Sugiyama, M., Tawaragi, Y. et al. (1988) *Biochem. Biophys. Res. Commun.* **155**, 429–435
- Sidky, Y. A. and Borden, E. C. (1987) *Cancer Res.* **47**, 5155–5161
- St. Clair, D. K., Rybak, S. M., Riordan, J. F. and Vallee, B. L. (1988) *Biochemistry* **27**, 7263–7268
- Benner, S. A. and Allemann, R. K. (1989) *Trends Biochem. Sci.* **14**, 396–397
- D'Alessio, G., DiDonato, A., Parente, A. and Piccoli, R. (1991) *Trends Biochem. Sci.* **16**, 104–106
- Lortat-Jacob, H. and Grimaud, J. A. (1991) *FEBS Lett.* **280**, 152–154
- Ealick, S. E., Cook, W. J., Vijay-Kumar, S., Carson, M., Nagabushan, T. L., Trotta, P. P. and Bugg, C. E. (1991) *Science* **252**, 698–702
- Shehan, K. C. F. and Schreiber, R. D. (1992) in *Tumor Necrosis Factors: the Molecules and their Emerging Role in Medicine* (Beutler, B., ed.), pp. 145–178, Raven Press, New York
- Wetzel, R., Perry, L. J., Veilleux, C. and Chang, G. (1990) *Protein Eng.* **3**, 611–623
- Luk, S. K., Jay, E. and Jay, F. T. (1990) *J. Biol. Chem.* **265**, 13314–13319
- Rubenstein, S., Familetti, P. C. and Pestka, S. (1981) *J. Virol.* **37**, 755–758
- Lee, F. S., Fox, E. A., Zhou, H.-M., Strydom, D. J. and Vallee, B. L. (1988) *Biochemistry* **27**, 8545–8553
- Vincentini, A. M., Kieffer, B., Matthies, R. et al. (1990) *Biochemistry* **29**, 8827–8834