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The cysteines in position 1 and 86 of rat interferon-α_1 are indispensable for antiviral activity

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The human, bovine, murine and rat interferon (IFN)-α families contain 4 conserved cysteines located at positions 1, 29, 99 and 139 that are involved in disulfide bridges. Rat and murine IFN-α subspecies carry a fifth Cys (Cys-86) which is not conserved in bovine and human IFN-α subspecies except for human IFN-α_1. Changing Cys-86 in rat IFN-α_1 into Ser or Tyr virtually abolished antiviral activity. As shown by others, the substitution of Cys-86 to Ser in human IFN-α_1 had no pronounced effect on activity. This suggests that in contrast to human and bovine IFN-α, Cys-86 in rodent IFN-α plays a crucial role in receptor binding. Changing Cys-1 to Gly in rat IFN-α_1 also destroyed activity, in agreement with results obtained in the human IFN-α_1 system.

Interferon-α_1; Antiviral activity; Disulfide bridge; Interferon receptor; (Rat)

1. INTRODUCTION

The interferon (IFN)-α genes constitute a true multigene family [1] that code for proteins that show on average 60% interspecies homology [2]. For instance, all human [1], bovine [2], murine [3–5] and rat IFN-α [6] proteins carry four cysteines at position 1, 29, 99 and 139. Cys-1 and 99 as well as Cys-29 and 139 are connected by disulfide bonds (Cys-1 is the first amino acid of the mature IFN-α peptide) [7].

Interestingly, all murine IFN-α genes and the only sequenced rat IFN-α gene (α_1) contain a fifth Cys at position 86. However, all bovine IFN-α proteins carry Arg or Gly and all human IFN-α subspecies (about 15) with one exception carry Ser or Tyr at this position. The exception is human IFN-α_1 which also contains a Cys at position 86 [8]. Here, we assess the importance of Cys-86 in rat IFN-α_1 for antiviral activity by substituting this amino acid by Tyr or Ser. In addition, we changed Cys-1 to Gly. All mutants are severely affected in biological activity. Comparison with mutations obtained by Beilharz et al. [9] in the human IFN-α_1 protein suggests that Cys-86 is essential for recognition of the receptor protein in the rat but not in humans.

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2. MATERIALS AND METHODS

2.1. *Bacterial strains and growth conditions*

Escherichia coli K-12 strain M5219 was used in all experiments [10]. This strain harbors a defective and nonexcisable λ prophage expressing the cIts857 gene and the gene for the antitermination factor *N*. Bacteria were grown in LC medium which contained (per l) 10 g tryptone, 5 g yeast extract (Difco), 8 g NaCl and 5 ml of 1 M Tris (pH 7.3). Cells were grown at 28°C to an A_{650} value of 0.2 and then induced at 42°C for 120 min.

2.2. *Construction of mutants of rat IFN-α_1*

The sequence encoding mature rat IFN-α_1 was cloned in *E. coli* expression vector pIF.D [11], which is derived from pPLc236 [10]. pIF.D contains a synthetic ribosomal binding site that adds a methionine to the N-terminus of mature IFN-α_1. Clones pIF.Ser-86 and pIF.Tyr-86 were obtained by site-directed mutagenesis of clone pIF.D essentially as described by Kunkel [12] and a mixture of two mutagenic heptadecamers. Clone pIF.Gly-1 was obtained as a gene fusion where the first

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four nucleotides of the bacteriophage MS2 coat gene [13] are connected to the second nucleotide of the mature IFN-α_1 sequence. This yields a product identical to our wild-type except for the Cys-1 ⟶ Gly substitution.

2.3. *Rat IFN-α_1 activity assay*

Bacterial lysates were prepared by suspending the bacteria in 6 M guanidine HCl, 1% β-mercaptoethanol. After centrifugation, the supernatant was isolated and tested for antiviral activity on RATEC cells as in [14]. The protein yields from the different plasmids were compared in Western blots [15] but did not differ significantly from one another (not shown).

3. RESULTS AND DISCUSSION

The mature rat IFN-α_1 sequence containing the 5 Cys residues is schematically represented in fig.1a. Cys-86 was mutated into either Ser (pIF.Ser-86) or Tyr (pIF.Tyr-86) (fig.1b). These clones were assayed for antiviral activity on rat cells and the results are listed in table 1. IFN.Ser-86 has only 10% of the activity of the wild-type protein and IFN-Tyr-86 even less, if any, antiviral activity. The Ser-86 mutation in the human IFN-α_1 gene still yields 40% activity [9]. Since Cys-86 is not known to be involved in a disulfide bond, this indicates that this residue might be crucial for direct recognition of the rat IFN-α_1 cell-surface receptor, whereas in humans the receptor-protein interaction appears less stringent at this particular position. These results agree with those obtained by Weber et al. [17] who used hybrids between human IFN-α_1 and human IFN-α_2 to locate regions that are important for antiviral activity on human, murine and bovine cells. Hybrids that contained Tyr at position 86 showed about 20-fold reduced specific activity on murine cells, whereas activity on human and bovine cells was unchanged. The fact that amino acid 86 is variable in human and bovine IFN-α also suggests this residue to be relatively unimportant.

We have also substituted Cys-1 for Gly-1 (fig.1b). In agreement with the findings for similar changes in human IFN-α_1 [9], this mutation almost completely inactivated rat IFN-α_1 (table 1).

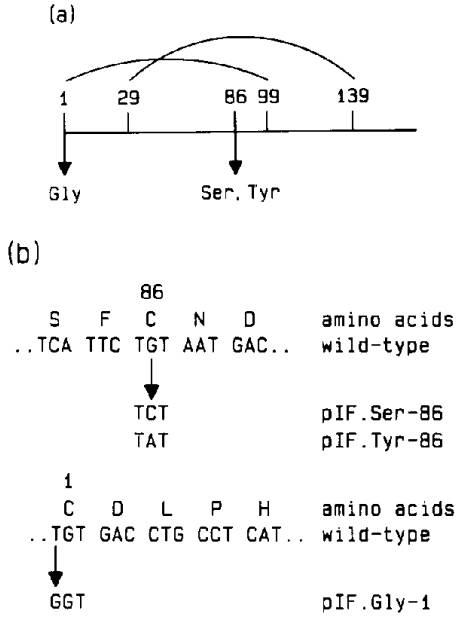

Fig.1. (a) Schematic representation of mature rat IFN-α_1. The position of the cysteine residues in the protein is shown by their amino acid number. The proposed disulfide bonds are indicated by connecting lines. Mutations discussed in section 3 are shown. (b) Relevant nucleotide and amino acid sequence of clones pIF.Ser-86, pIF.Tyr-86 and pIF.Gly-1.

Table 1

Specific activities of rat and human mutant IFN-α_1

	Human IFN-α_1[a] (%)	Rat IFN-α_1[b] (%)	Rat IFN-α_1 activity (U/l)
Wild-type	100	100	8.4×10^5
Ser-86	39	10	8.1×10^4
Tyr-86	n.c.	$\ll 1$	$\ll 9.8 \times 10^3$
Ser-1	5	n.c.	–
Gly-1	n.c.	2	1.6×10^4

[a] Tested on human cells (HEp-2) [9]. [b] Tested on rat cells (RATEC). See section 2 and [14]. n.c., not constructed

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