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Human interferon-γ lacking 23 COOH-terminal amino acids is biologically active

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We constructed five mutated cDNAs encoding human interferon-γ (IFN-γ) derivatives lacking 19–23 COOH-terminal residues and expressed them in *Escherichia coli*. All the derivatives were purified to homogeneity. They showed substantially the same order of antiviral activity in vitro as the intact molecule, and behaved as a dimer. The far- and near-UV circular dichroism spectra of the derivatives were quite similar to those of the intact one. These results indicate that the 23 COOH-terminal amino acids at least are not essential for achieving the full antiviral activity and constructing the higher structure of human IFN-γ.

IFN-γ; Antiviral activity; Higher structure

1. INTRODUCTION

Interferons (IFNs) possess a variety of biologic activities: antiviral, antiproliferative, and immunoregulatory. They are classified into three major groups, IFN-α, IFN-β, and IFN-γ, on the basis of the antigenicities, cellular origins, and biochemical properties [1]. Among these, IFN-γ is known to be sensitive to acid treatment [1] and to exist as a dimer [2–7].

Characterization of natural IFN-γ demonstrated that its COOH-terminal portion is processed to several species bearing different COOH-terminal amino acids [3,8]. We [6,9] and others [10,12] observed that the COOH-terminal portion of the recombinant human IFN-γ is also susceptible to proteolytic attack. Conflicting results have been obtained concerning the minimum structure of the COOH-terminal portion necessary for antiviral activity. We [6,9] and Rose et al. [11] observed that 15 COOH-terminal amino acids are not essential for antiviral activity, whereas Rinderknecht et al. [12] and Arakawa et al. [13] reported that the COOH-terminal portion is important.

To investigate further the relation between the COOH-terminal structure and the activity of IFN-γ, we prepared five derivatives lacking the COOH-terminal portion and have found that the IFN-γ derivative is still biologically active, even after 23 COOH-terminal amino acids are deleted.

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IFN-γ124, 123, 122, 121, 120 and 118 represent the human IFN-γ derivatives which lack 19, 20, 21, 22, 23 and 25 COOH-terminal amino acids, respectively

2. MATERIALS AND METHODS

pLC-2, the expression plasmid of human IFN-γ cDNA, was a gift from Hoffmann-La Roche Inc. (Nutley, NJ, USA). Bacterial strains, *Escherichia coli* N4830 (for IFN-γ 123 expression), DH-1/pRK248 (for expression of all the other IFN-γ derivatives) and JM-103 (for mutagenesis) were used.

Mutated cDNA coding for IFN-γ123 was constructed by site directed mutagenesis as described in [14] and was screened by a standard plaque hybridization method [15]. The other mutants were constructed with the aid of an 'oligonucleotide-directed in vitro mutagenesis system' (Amersham, Buckinghamshire, England). As shown in fig.1, the oligonucleotide for IFN-γ123 was designed to introduce the termination codon (TAG)

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              120   ***
ATG GCT GAA CTG - TAA TGG TTG TCC

              121   ***
GCT GAA CTG TCG - TAA TGG TTG TCC

              122   ***
GAA CTG TCG CCA - TAA TGG TTG TCC

              123 ...
  G TCG CCA GCA TAG AAA ACA GG

              124   ***
TCG CCA GCA GCT - TAA TGG TTG TCC
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Fig.1. Synthetic oligodeoxynucleotides used as a mutagenic primer. Numbers indicate those of amino acid residues of IFN-γ. Dots and asterisks represent mismatched bases and termination codon, respectively.

into position 124 and those for IFN-γ124, 122, 121, and 120 were designed to delete undesired DNA stretches. The nucleotide sequences of the altered regions were determined to confirm the maintenance of the designed sequence. The constructed cDNAs were inserted into the *Eco*RI site downstream of the λP_L-promoter of the pLC-2 vector.

These derivatives were expressed and purified as described in [6] with some modifications. The cell lysate obtained by 7 M guanidine hydrochloride (GuHCl) extraction was diluted 14-fold with phosphate buffered saline (PBS) containing 1 M urea. The mixture was applied to an immunoaffinity column using a monoclonal antibody (WN γ2-76.53) [16]. The derivative was eluted with 20 mM sodium phosphate buffer (pH 7.0) containing 2 M GuHCl. The eluate was then applied to a Sephacryl S-200 (Pharmacia, Uppsala, Sweden) column equilibrated with 25 mM ammonium acetate (pH 6.0) containing 2 M GuHCl. The desired fractions were subjected to dialysis against 25 mM ammonium acetate (pH 6.0) for renaturation to yield the active derivative.

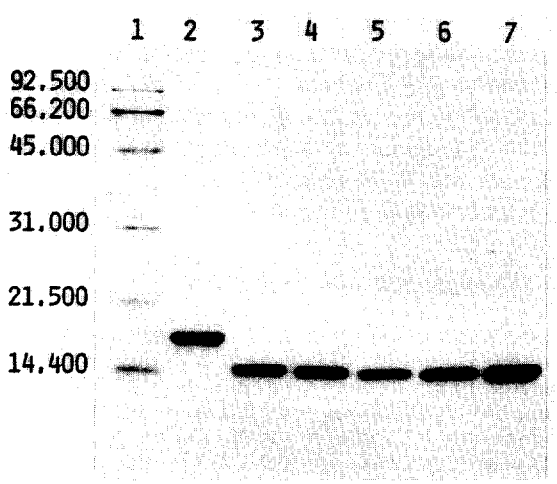

Fig.2. SDS-PAGE of the intact IFN-γ and its derivatives. Proteins were subjected to SDS-PAGE with a 15% gel under reducing conditions and the proteins were stained with Coomassie brilliant blue R250. Lanes: 1, molecular mass markers; 2, IFN-γ; 3, IFN-γ124; 4, IFN-γ123; 5, IFN-γ122; 6, IFN-γ121; 7, IFN-γ120.

Far- and near-UV circular dichroism (CD) spectra were measured under constant nitrogen flush at room temperature on a J-20A spectropolarimeter with a Data Processor DP-50IN (Jasco, Tokyo, Japan). The mean residue ellipticity, $[\theta]$, was calculated from the calculated mean residue weight of each protein.

The antiviral activity was determined as described in [6]. DNA manipulations [15] and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [17] were performed as described. Protein concentration was determined by the method of Lowry et al. [18] using bovine serum albumin as a standard. The amino acid composition and COOH-terminal amino acid were determined by the standard procedure [6]. To determine the molecular masses of the derivatives under nondenaturing conditions, Sephadex G-100 gel filtration was performed as described in [6].

3. RESULTS

To examine the role of the COOH-terminal portion in IFN-γ, we constructed five mutated cDNAs encoding IFN-γ derivatives lacking 19–23 COOH-

Table 1

Amino acid compositions of the IFN-γ derivatives

Amino acid	Number of residues per molecule					
	Intact	IFN-γ derivatives				
		124	123	122	121	120
Asx	20	20	20	20	20	20
Thr	4.9	3.8	3.9	3.7	3.8	3.9
Ser	9.7	7.6	8.2	6.8	7.2	6.6
Glx	19.4	16.9	17.2	17.3	17.2	17.3
Pro	2.3	2.0	2.0	2.1	1.0	1.0
Gly	5.2	3.5	3.1	3.2	3.2	3.2
Val	7.6	7.8	7.5	7.6	7.5	7.3
Ala	8.4	7.3	6.3	5.3	5.2	5.2
Met	5.1	3.7	4.0	3.9	3.8	3.8
Ile	6.7	6.0	6.6	6.6	6.5	6.5
Leu	10.4	8.8	9.3	9.5	9.5	9.4
Tyr	4.1	3.9	4.1	4.1	4.1	4.0
Phe	10.5	9.1	9.4	9.2	9.3	9.2
Lys	19.2	16.3	16.2	17.1	17.0	16.6
His	1.8	1.8	1.8	1.9	1.8	1.8
Arg	8.3	3.0	3.1	3.2	3.1	3.1
Trp	a	–	–	–	–	–
Cys	a	–	–	–	–	–

Samples were hydrolyzed in vacuo at 110°C for 24 h in constant boiling HCl containing 4% thioglycolic acid. a, not determined

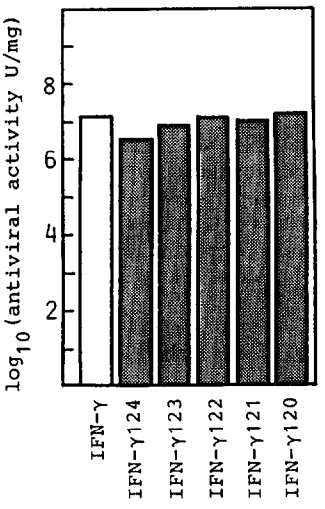

Fig.3. Antiviral activity of the IFN-γ derivatives.

terminal amino acid residues. The IFN-γ derivatives expressed in *E. coli* were purified by immunoaffinity chromatography, followed by gel filtration. The purity of each derivative thus purified was assessed by SDS-PAGE and all the derivatives gave a single band with a larger mobility than the intact one (fig.2). Amino acid analysis (table 1) and COOH-terminal amino acid analysis by hydrazinolysis (not shown) show that these derivatives possess the designed structure.

No dramatic change of the activity was observed among the intact molecule and the five derivatives (fig.3). This result indicates that at least 23 COOH-terminal amino acids are not essential for the antiviral activity.

Fig.4 shows the CD spectra of IFN-γ120, IFN-γ124, and the intact one. No significant change was observed in the either far- (fig.4A) or near-UV region (fig.4B) in any of them. Two other derivatives showed essentially the same CD spectrum (not shown). Gel filtration on a Sephadex G-100 column demonstrated that these derivatives behaved as a dimer under nondenaturing conditions (not shown). These results indicate that the deletion of at least 23 COOH-terminal amino acids affects neither the secondary nor tertiary structures.

4. DISCUSSION

In preceding papers [6,9], we reported that two lower-molecular-mass derivatives (17 kDa and 15 kDa) lacking 4 and 15 COOH-terminal amino acids generated by *E. coli* protease(s) are as active as the intact one. Rose et al. [11] also pointed out that IFN-γ lacking 15 COOH-terminal amino acids was active. On the other hand, Rinderknecht et al. [12] and Arakawa et al. [13] indicated that the COOH-terminal portion played an important role

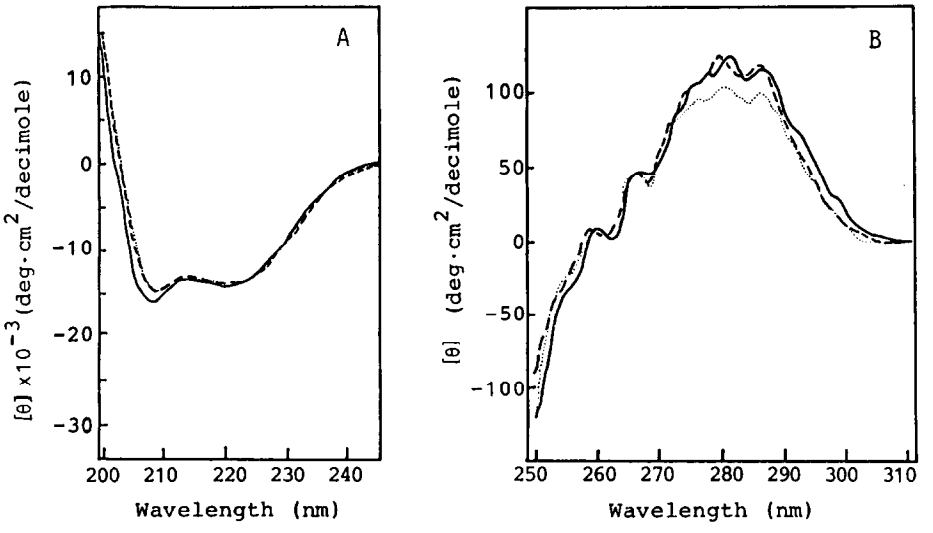

Fig.4. CD spectra of the intact IFN-γ, IFN-γ120 and IFN-γ124 in far-UV (A) and near-UV (B) regions. (——) IFN-γ; (– – –) IFN-γ120; (· · ·) IFN-γ124.

in eliciting antiviral activity. The results described in this paper clearly demonstrated that at least 23 COOH-terminal amino acids are not essential for antiviral activity. Though the apparent discrepancy remains unclear, it may have resulted from a difference in the cells used in the assay. The receptor for IFN-γ expressed on some cells might require the COOH-terminal portion for interaction.

The active site of IFN-γ, which interacts with receptor on cell surface, has not been identified. Antibodies against synthetic NH_2-terminal peptides [16,19] neutralize the antiviral activity, while antibodies against a synthetic COOH-terminal peptide [20] lack the ability to neutralize the activity. These observations indicate that the NH_2-terminal portion, rather than the COOH-terminal, might be essential to exert activity. The facts that: (i) the COOH-terminal portion is susceptible to protease attack [6,9,10,13], (ii) no significant change in CD spectra was observed among these derivatives and the intact one (fig.4), and (iii) that the derivatives behave as dimers, suggest that this portion might not have an essential role in retaining a higher structure. We also constructed IFN-γ118, but unfortunately it tends to form an insoluble aggregate at the renaturation step of the purification and could not be recovered (not shown). It is likely that the two residues (Glu^{119}–Leu^{120}) play a critical role for the folding of the protein.

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