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Crystallization of a recombinant form of the complete sequence of human γ-interferon: characterization by small-angle X-ray scattering, mass spectrometry and preliminary X-ray diffraction studies

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The crystallization conditions of a recombinant form of the *complete* sequence of human γ-interferon, designated r-hu IFN-γ (RU 42369), have been determined after studying the behaviour of this protein in solution by small-angle X-ray scattering (SAXS) as a function of pH and salt type. IFN-γ is difficult to crystallize without truncating at least the last five amino acids of the C-terminus; the SAXS results suggest viable crystallization conditions that led to crystals of r-hu IFN-γ suitable for X-ray diffraction analysis. The crystals were grown in the presence of ammonium sulfate using vapour-diffusion techniques. The crystals, which diffract to 5 Å resolution at best, belong to the primitive tetragonal space group $P42_12$ and have unit-cell parameters $a = b = 123.4$, $c = 93.4$ Å. The protein contained in these crystals was analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), which verified the presence of the complete amino-acid sequence of r-hu IFN-γ.

1. Introduction

Interferon-γ (IFN-γ) is a product of activated T lymphocytes and natural killer cells (Farrar & Schreiber, 1993; Pestka *et al.*, 1987). It exhibits pleiotropic biological activities (Dijkmans & Billiau, 1988) such as regulating the expression of class II major histocompatibility antigens (Wong *et al.*, 1983) and Fc receptors (Itoh *et al.*, 1980), activating human monocyte cytotoxicity (Nathan *et al.*, 1983), enhancing natural killer cell activity (Platsoucas, 1984) and regulating immunoglobulin production and class switching (Finkelman *et al.*, 1988). Expression of biological activity appears to be mediated through binding to specific cell receptors (Anderson *et al.*, 1982). The expression of human IFN-γ in *Escherichia coli* (Gray *et al.*, 1982; Tanaka *et al.*, 1983) has allowed the preparation of large quantities of highly purified recombinant human IFN-γ on which detailed structure–function relationship studies have been undertaken (Arakawa *et al.*, 1986; Leinikki *et al.*, 1987; Seelig *et al.*, 1988; Hogrefe *et al.*, 1989; Samudzi *et al.*, 1991; Ealick *et al.*, 1991; Samudzi & Rubin, 1993; Walter *et al.*, 1995; Randal & Kossiakoff, 1998). These studies suggest and show a high percentage of helical structure in these proteins. However, three-dimensional structural information concerning the C-terminal region of r-hu IFN-γ is not available and the implication of the C-terminus of r-hu IFN-γ in the receptor binding is not clear.

Residues of the C-terminal domain of human IFN-γ are critical in the interaction with the receptor (Jarpe & Johnson, 1993; Lundell *et al.*, 1994; Wetzel *et al.*, 1990) or in the triggering of biological response (Ealick *et al.*, 1991). For example, forms of recombinant human IFN-γ truncated at the C-terminal domain demonstrate substantially reduced antiviral activity (Leinikki *et al.*, 1987; Arakawa *et al.*, 1986; Seelig *et al.*, 1988; Trotta, 1986) and antibodies directed to the C-terminal domain neutralize *in vitro* bioactivity (Ealick *et al.*, 1991).

The proposed binding interface determined from crystallographic structures does not explain these results well. In the interferon-γ receptor complex (IFN-γRC), the C-terminal IFN-γ residues 122–138 extend into the solvent and do not appear to be important for receptor binding (Walter *et al.*, 1995). NMR studies also indicate that the C-terminal domain does not adopt a rigid conformation in solution (Grzesiek *et al.*, 1992). However, IFN-γ C-terminal deletion mutants such as 1–129 show a sharp drop in receptor binding, but the 1–132 mutant does not. This suggests that residues 130–132 (Lys-Arg-Ser) play a role in receptor binding (Walter *et al.*, 1995). Moreover, the biologically active IFN-γRC requires the species-specific interaction of the high-

affinity receptor IFN-γRα with at least one additional accessory factor, such as IFN-γRβ1 (Hibino *et al.*, 1992). Studies with a covalently linked IFN-γ mutant suggest that each domain of IFN-γ may function independently to transduce a biological signal (Lunn *et al.*, 1992). Structurally, this corresponds to each domain of IFN-γ binding one IFN-γRα molecule and one IFN-γRβ1 molecule. The potential interaction of different combinations of these accessory factors with IFN-γRC suggests an additional mechanism for IFN-γ to modulate its pleiotropic activities (Walter *et al.*, 1995). Based on antiviral assays with IFN-γ deletion mutants and receptor chimeras, the IFN-γRβ1 molecule interacts with the IFN-γRα molecule and with the C-terminal domain of IFN-γ (Hibino *et al.*, 1992). Comparison of IFN-γ and growth hormone (GH) bound to their receptors (Walter *et al.*, 1995) reveals a striking correspondence of the C-terminal residues of IFN-γ with the N-terminal residues of growth hormone that form a major proportion of the second growth-hormone receptor (GHR) binding site. While the receptor–receptor interaction sites used by GHR are not conserved in IFN-γRα, the second GHR molecule may be positioned without any poor contacts into IFN-γRC (Walter *et al.*, 1995). In this model, the interface of the second GHR interacts with the C-terminus of IFN-γ. The occurrence of different C-termini in natural IFN-γ may allow alteration of the second binding site, which could provide an additional control mechanism of IFN-γ signalling (Walter *et al.*, 1995). Recently, the crystal structure of a 3:1 cytokine receptor complex containing three glycosylated sIFN-γRα molecules and one non-glycosylated IFN-γ dimer, where the ten residues of the C-terminus are absent from each IFN-γ monomer, has been resolved (Thiel *et al.*, 2000). In this model, the C-terminal tail (residues 126–134) of each monomer of the IFN-γ dimer is disordered and has not been modelled.

In order to more clearly understand the role of the C-terminal residues, we have undertaken the task of crystallizing the complete peptide sequence of r-hu IFN-γ for X-ray structural analysis. In this paper, we describe the use of SAXS experiments to determine favourable crystallization conditions of a recombinant form of the complete sequence of human IFN-γ, which is difficult to crystallize without truncating at least five amino acids from the C-terminus. We also report the preliminary crystallographic studies of crystals of r-hu IFN-γ, which contain the complete sequence of the protein as verified by mass spectrometry, unlike the three-dimensional X-ray crystal structures previously reported to date that contain truncated forms of the protein.

2. Experimental procedures

R-hu IFN-γ (pI $\simeq$ 8) contains a sequence of 144 amino acids which is identical to that of native human interferon-γ except for the addition of an N-terminal methionine at position 0 (Perez *et al.*, 1990; Yamazaki *et al.*, 1986). As is usual for a recombinant protein produced by *E. coli* (Perez *et al.*, 1990), the molecule is neither glycosylated nor phosphorylated (Yamazaki *et al.*, 1986). It was purified by hydrophobic interaction chromatography, lyophilized and the biological activity was measured (Brafman, 1988). Peptide mapping by reverse-phase liquid chromatography with on-line identification by thermospray mass spectrometry and UV absorption spectrometry was used (Legrand *et al.*, 1993). The amino-acid sequence of r-hu IFN-γ contains several tyrosines and one tryptophan residue at position 36. It has a molecular weight of 16.9 kDa and in solution the functional protein is a dimer. The homogeneity of a recombinant protein preparation and the state of aggregation have been studied by time-resolved polarized fluorometry (Brochon *et al.*, 1993).

2.1. Purification

Prior to crystallization the protein was repurified. Analysis by HPLC shows the presence of a number of fragments arising from hydrolysis and deamidation of r-hu IFN-γ and therefore purification by HPLC was repeated. The protein sample dissolved in water was injected directly onto a C4 affinity chromatography HPLC column and r-hu IFN-γ was eluted at a flow rate of 16 ml min^{-1} with a gradient of solution *B* [0.1% TFA in 50% acetonitrile/50% water (*v*/*v*)] and solution *A* [0.1% TFA in 80% acetonitrile/20% water (*v*/*v*)] as follows: (i) initial equilibration of the column with the mixture 46% *A* + 54% *B*, 0–5 min; (ii) linear gradient from 46% *A* + 54% *B* to 40% *A* + 60% *B*, 5–70 min; (iii) linear gradient from 40% *A* + 60% *B* to 46% *A* + 54% *B*, 70–75 min.

A 15 min delay was imposed before the next reinjection to allow the column to re-equilibrate with 46% *A* + 54% *B*. The following steps of the purification were lyophilization, cation-exchange chromatography on a Fast Flow S-Sepharose column, dialysis with 20 m*M* HEPES buffer pH 7.0 and washing by centrifugation and concentration methods with water.

2.2. SAXS experiments

The X-ray scattering curves were recorded using the small-angle X-ray scattering station D24 on the DCI storage ring at the synchrotron laboratory LURE. The experimental setup used on D24 has been described previously (Vachette, 1979). The sample-to-detector distance used was 1573 mm and the X-ray wavelength λ = 1.448 Å. Three different pH conditions were tested, 50 m*M* sodium acetate pH 4.0, 50 m*M* sodium cacodylate pH 6.0 and 50 m*M* HEPES pH 8.0, with three different salts, 1.65 *M* $(NH_4)_2SO_4$, 1.7 *M* NaCl and 0.35 *M* KSCN. For each of these conditions, X-ray scattering patterns were recorded at room temperature (293 K) and at a protein concentration of 6 mg ml^{-1}.

2.3. Crystallization and X-ray studies

Crystals of r-hu IFN-γ suitable for X-ray structural analysis were grown by vapour-diffusion methods. 10 µl droplets containing a mixture of 8 µl protein solution (11 mg ml^{-1}) and 2 µl 2.8 *M* ammonium sulfate in 100 m*M* HEPES buffer pH 8.0 were allowed to equilibrate in hanging-drop experiments at 293 K against 1 ml 2.8 *M* ammonium sulfate in 100 m*M* HEPES buffer pH 8.0. After 2 d, the formation of a precipitate could be observed and after 14 d 1–3 small parallelepiped-shaped crystals grew from the protein precipitate (Fig. 1*a*). Finally, after 2–3 months the crystals had grown to dimensions of 0.3–0.5 mm (Fig. 1*b*).

Crystals were mounted in thin-walled glass capillaries. X-ray diffraction data from two crystals were collected at 293 K as rotation images on a MAR Research image plate with synchrotron radiation at the DW32 station, LURE-DCI, Orsay, France (Fourme *et al.*, 1992). Upon first exposure to X-rays the crystals diffracted to 5 Å resolution, but then rapidly degraded in the X-ray beam (see Table 1). The crystal form is primitive tetragonal, space group $P4_12_12$; crystal 1 had unit-cell parameters $a = b = 123.35$, $c = 93.37$ Å and mosaicity $\simeq 0.6^\circ$; crystal 2 had unit-cell parameters $a = b = 124.09$, $c = 95.03$ Å and mosaicity $\simeq 1.1^\circ$. The unit-cell volume and molecular weight of the protein give a Matthews volume of 2.61 and 2.69 Å^3 Da^{-1} for 32 protein molecules in the unit cell, presuming there to be four independent protein molecules in the asymmetric unit. These values fall within the range observed for crystallized proteins (Matthews, 1968). Assuming

Table 1
Crystallographic data for r-hu IFN-γ crystals.

Data scaled and reduced using *SCALA* and *TRUNCATE* (Collaborative Computational Project, Number 4, 1994).

Crystal	1	2
Unit-cell parameters (Å)	$a = b = 123.35$, $c = 93.37$	$a = b = 124.09$, $c = 95.03$
Mosaicity (°)	0.6	1.1
Exposure time (s)	300	300
Crystal-to-detector distance (mm)	300	400
Rotation angle (°)	1.0	1.5
Rotation range (°)	120	148.5
No. molecules per asymmetric unit	4	4
V_M (Å^3 Da^{-1})	2.61	2.69
Measured reflections	7047	9988
Unique reflections	1086	1289
Completeness (%)	87.5	97.3
Max. resolution (Å)	7.0	7.0
R_{sym} (7.6–7.0 Å shell)	0.098 (0.293)	0.131 (0.490)
R_{merge} (crystals 1 and 2)	0.136 (0.353)	

the partial specific volume of the protein in the crystal to be 0.800 and 0.799 cm^3 g^{-1}, the solvent contents of these crystals were calculated to be approximately 49.1 and 50.6% by volume. X-ray diffraction data to 7 Å resolution were indexed and integrated with *MOSFLM* (Leslie, 1994).

2.4. Mass spectrometry

Matrix-assisted laser desorption/ionization (MALDI) was first described in 1988 (Karas & Hillenkamp, 1988). During the past decade, there has been an increased use of this method for analyzing proteins and peptides. The main advantage of MALDI is its ability to selectively ionize biopolymers with a high tolerance for biological matrices (Carroll & Beavis, 1996). Application of this ionization mode associated with time-of-flight (TOF) detection in mass spectrometry have been described previously (Mann & Talbo, 1996; Andersen *et al.*, 1996).

2.4.1. Instrumentation. Mass spectra were recorded in positive mode with a MALDI-TOF mass spectrometer (Voyager Elite, Perspective Biosystems Inc.) equipped with a delayed extraction (DE) device at Laboratoire de Neurobiologie, Ecole Supérieure de Physique et Chimie Industrielles de la Ville de Paris. A nitrogen laser beam (λ = 337 nm, 3 ns wide pulse at 20 Hz) was focused for desorption on a gold-coated stainless-steel plate and the ions were detected with a multi-channel plate detector. Two ion analysis modes were used: (i) a linear mode for the analysis of proteins, with a total flight length of 2 m, an accelerating voltage of 25 kV and a DE time of 150 ns, and (ii) a reflection mode for the analysis of peptides, with a total flight length of 3 m, an accelerating voltage of 20 kV and a DE time of 100 ns.

2.4.2. Sample preparation. The matrix chosen was α-cyano-4-hydroxycinnamic acid (α-CHCA) mixed with a nitrocellulose (α-CHCA/NC). The thin-layer matrix surface was prepared by mixing a saturated solution of α-CHCA in acetone/isopropyl alcohol [1:1(*v*/*v*)] with a solution of nitrocellulose in acetone/isopropyl alcohol [1:1(*v*/*v*)] in a 1:1(*v*/*v*) ratio. 1 µl of this solution was deposited on the gold-coated target plate to obtain a thin regular translucent layer. 0.5 µl aliquots of a 100 pmole µl^{-1} solution of the protein recovered from one of the irradiated crystals dissolved in 0.1% aqueous TFA (trifluoroacetic acid) were spotted on the matrix layer and dried at room temperature under vacuum. Finally, 1 µl of the mixture of α-CHCA and H_2O/acetonitrile [1:1(*v*/*v*)] in 0.1% aqueous TFA was deposited on the matrix layer. The sample preparation was not washed.

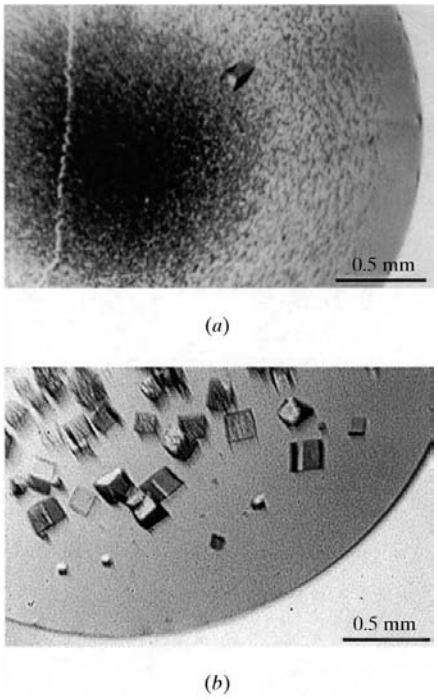

Figure 1
Crystal habit of r-hu IFN-γ grown in 2.8 *M* $(NH_4)_2SO_4$ pH 8 (*a*) after 14 d and (*b*) after three months.

3. Results and discussion

3.1. Diagnostics of crystallization conditions by SAXS experiments

Figs. 2 and 3 show the scattered intensity *I* as a function of the scattering vector *s* ($s = 2\sin\theta/\lambda$, with 2θ the scattering angle) at 293 K and at a protein concentration of 6 mg ml^{-1} in three different buffers (Fig. 2), 50 m*M* sodium acetate pH 4.0, 50 m*M* sodium cacodylate pH 6.0 and 50 m*M* HEPES pH 8.0, and in three different salts (Fig. 3), 1.65 *M* ammonium sulfate, 1.7 *M* sodium chloride and 0.35 *M* potassium thiocyanate.

The SAXS curve obtained at pH 4.0, far from the pI of the protein, shows an intense scattering profile in the regions of the lowest *s* values; this implies an aggregation of the protein (Fig. 2). On the other hand, the scattering curves at pH 6.0 and pH 8.0 do not show any interactions or aggregation or any differences (Fig. 2). These curves can be considered as a form factor for the protein and give a radius of gyration (R_g) of 23.0 ± 0.5 Å, which corresponds to a dimer for r-hu IFN-γ.

The effect of various salts on the interactions in solution of r-hu IFN-γ was studied at pH 6 (Fig. 3*a*) and at pH 8 (Fig. 3*b*). As expected, the same weak shielding effects by salts are observed in both cases, but no attractive interactions are observed. This is demonstrated by the curvature of the corresponding Guinier plots (data not shown). The small differences in screening efficiency observed with the various salts indicate that the protein follows the reverse order of the Hofmeister series, as would be predicted for a basic protein at a pH below its pI (Ries-Kautt & Ducruix, 1989; Guilloteau *et al.*, 1992; Lafont *et al.*, 1997). As for the effect of pH with different salts, the corresponding scattering curves at pH 6 and pH 8 show insignificant differences in all cases (Fig. 3). Since no aggregation was detected at these pH values, attractive interactions might be expected at higher concentrations of protein and salt. Consequently, we consider that these salts in the pH range 6–8 are suitable candidates for crystallization conditions.

3.2. Crystallization and X-ray studies

From the SAXS results above, we investigated the crystallization conditions of r-hu

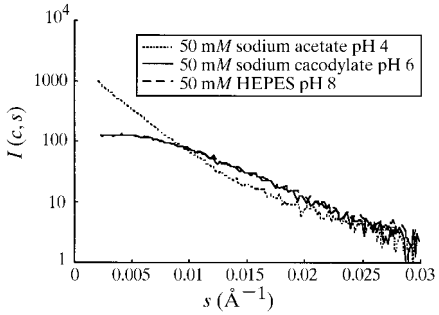

Figure 2
Small-angle X-ray scattering curves of r-hu IFN-γ (6 mg ml^{-1}) at 293 K as a function of pH.

Table 2
Comparison of the different crystalline forms of IFN-γ from different species, complete and truncated, available to date.

Crystalline form	Space group	Unit-cell parameters (Å)	Asymmetric unit		Diffraction limit (Å)
			Molecules	Dimers	
Complete r-human	$P42_12$	$a = b = 123.35$, $c = 93.37$	4	2	>5.0
Truncated r-human†	$R32$	$a = b = 114.0$, $c = 315.0$	4	2	3.5
r-bovine‡	$P2_12_12_1$	$a = 42.8$, $b = 79.9$, $c = 85.4$	2	1	3.0
r-bovine§	$P2_12_12_1$	$a = 36.15$, $b = 81.10$, $c = 83.86$	2	1	2.0
r-rabbit¶	$P6_122$	$a = b = 57.7$, $c = 169.2$	2	1	2.7

† Ealick *et al.* (1991). ‡ Samudzi & Rubin (1993). § Randal & Kossiakoff (2000). ¶ Samudzi *et al.* (1991).

IFN-γ using vapour-diffusion methods for three different salts, 0.1–0.35 *M* KSCN, 1.0–4.0 *M* NaCl and 1.5–3.0 *M* $(NH_4)_2SO_4$, at three different pH values (pH 6.0, 7.0 and 8.0) and for different concentrations of protein in the range 11–44 mg ml^{-1}. The final concentration of the protein in the droplets was obtained by mixing different volumes of protein stock solution (11 mg ml^{-1}) and reservoir solution. All crystallization trials were conducted at 293 K. The successful crystallization conditions were the following: 2.5–2.8 *M* ammonium sulfate, 100 m*M* sodium cacodylate pH 6.0 or pH 7.0 or 100 m*M* HEPES pH 8.0, with a final protein concentration in the range 33–44 mg ml^{-1}. In the case of the experiments using NaCl and KSCN salts, only precipitates were observed.

It should be noted that the equilibrium process in droplets using ammonium sulfate as a precipitating agent is unusual in that a pH change occurs during the equilibrium process owing to a transfer of ammonia gas from the reservoir to the droplet or *vice versa* (Mikol *et al.*, 1989). The pH in the crystallization droplet is controlled by that of the reservoir.

In the case of r-hu IFN-γ, at low protein concentrations we do not observe any effect on the protein–protein interactions in solution owing to pH (see §2.2). However, we suggest that at higher protein concentrations and as the pH approaches the pI, the interactions in solution change and the association of the two effects (the increase of the protein concentration and the increase of pH) generates the best conditions for crystallization.

However, we do not know the nature of the precipitate occurring during the equilibrium process in the droplets or the phase diagram of the protein. Consequently, we propose three hypotheses concerning this precipitate: (i) the observed precipitate is crystalline and the equilibrium process in the droplets is accompanied by Oswald's ripening as was observed by Ng *et al.* (1996) for several proteins, (ii) the precipitate is a liquid–liquid phase separation or (iii) the precipitate is an amorphous precipitate corresponding to the metastable phase of gel, as was proposed for lysozyme (Muschol & Rosenberger, 1997).

Crystals of the complete sequence of r-hu IFN-γ with a prismatic crystal habit (Fig. 1) have been prepared and are suitable for preliminary X-ray diffraction analysis. Crystallographic data for both crystals are presented in Table 1. They are primitive tetragonal, space group $P42_12$, with unit-cell parameters $a = b = 123.4$, $c = 93.4$ Å, and contain four r-hu IFN-γ molecules in the asymmetric unit. It is noteworthy that the C-terminal truncated form of human IFN-γ also crystallizes with four independent molecules in the asymmetric unit (Ealick *et al.*, 1991), where two dimers are related by a non-crystallographic twofold axis. Attempts to find a molecular-replacement model at 7 Å resolution were unsuccessful using the atomic coordinates of IFN-γ *D*′ molecules from Ealick *et al.* (1991) and the complex to its receptor (Walter *et al.*, 1995). However, because the crystals diffract to low resolution, these results do not suggest any major structural differences from the other known crystallographic structures. The self-rotation function (*POLARRFN*; Collaborative Computational Project, Number 4, 1994) of one of the crystals reveals a twofold non-crystallographic axis as might be expected for crystals containing four independent molecules in the asymmetric unit (Fig. 4). C-terminal truncated IFN-γ molecules from human, bovine and rabbit have been crystallized in a variety of different unit cells and space groups (Table 2). Most diffract to moderate or low resolution with one or two functional dimers in the asymmetric unit. The overall topology of the IFN-γ molecules, whether truncated or from different species, is conserved between the different forms. Strictly speaking, however, this is not true polymorphism (except for the bovine IFN-γ crystals; Randal & Kossiakoff, 2000) because the molecules have small differences in amino-acid sequence.

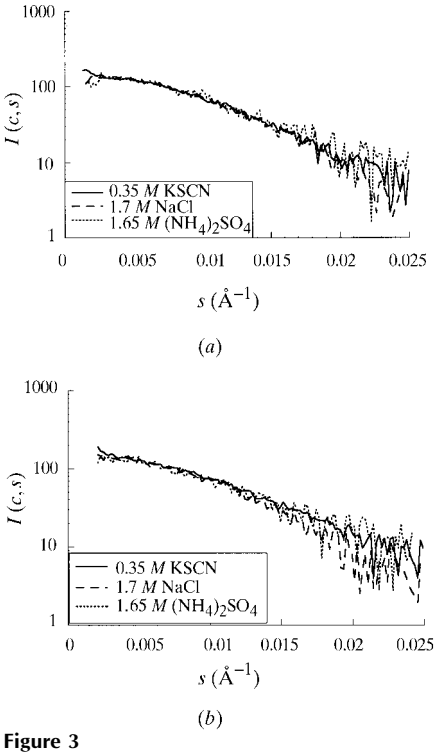

Figure 3
Small-angle X-ray scattering curves of r-hu IFN-γ (6 mg ml^{-1}) at 293 K as a function of different salts (*a*) at pH 6 and (*b*) at pH 8.

3.3. Mass-spectroscopy studies

MALDI-TOF mass spectra of an r-hu IFN-γ crystal are shown in Fig. 5. The complete mass spectrum with the two principal bulk peaks shown in Fig. 5(*a*) corresponds to simply charged molecules $(M + H)^+$ in the range m/z = 14 000–18 000 and doubly charged molecules $(M + 2H)^{2+}$ in the range m/z = 7000–9000.

Detailed analysis of the singly charged molecule range permitted us to detect the presence of three protein populations in the

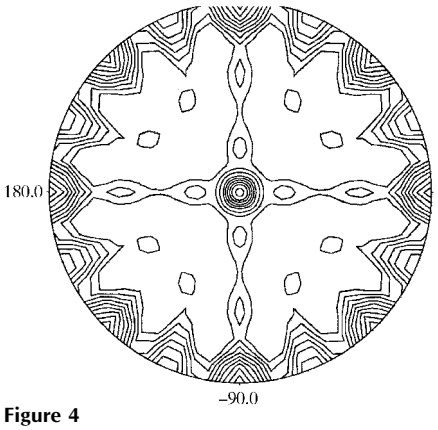

Figure 4
The self-rotation function at $\kappa = 180°$ of crystal 1 illustrating twofold non-crystallographic axes (low peaks) along with the crystallographic axes (high peaks).

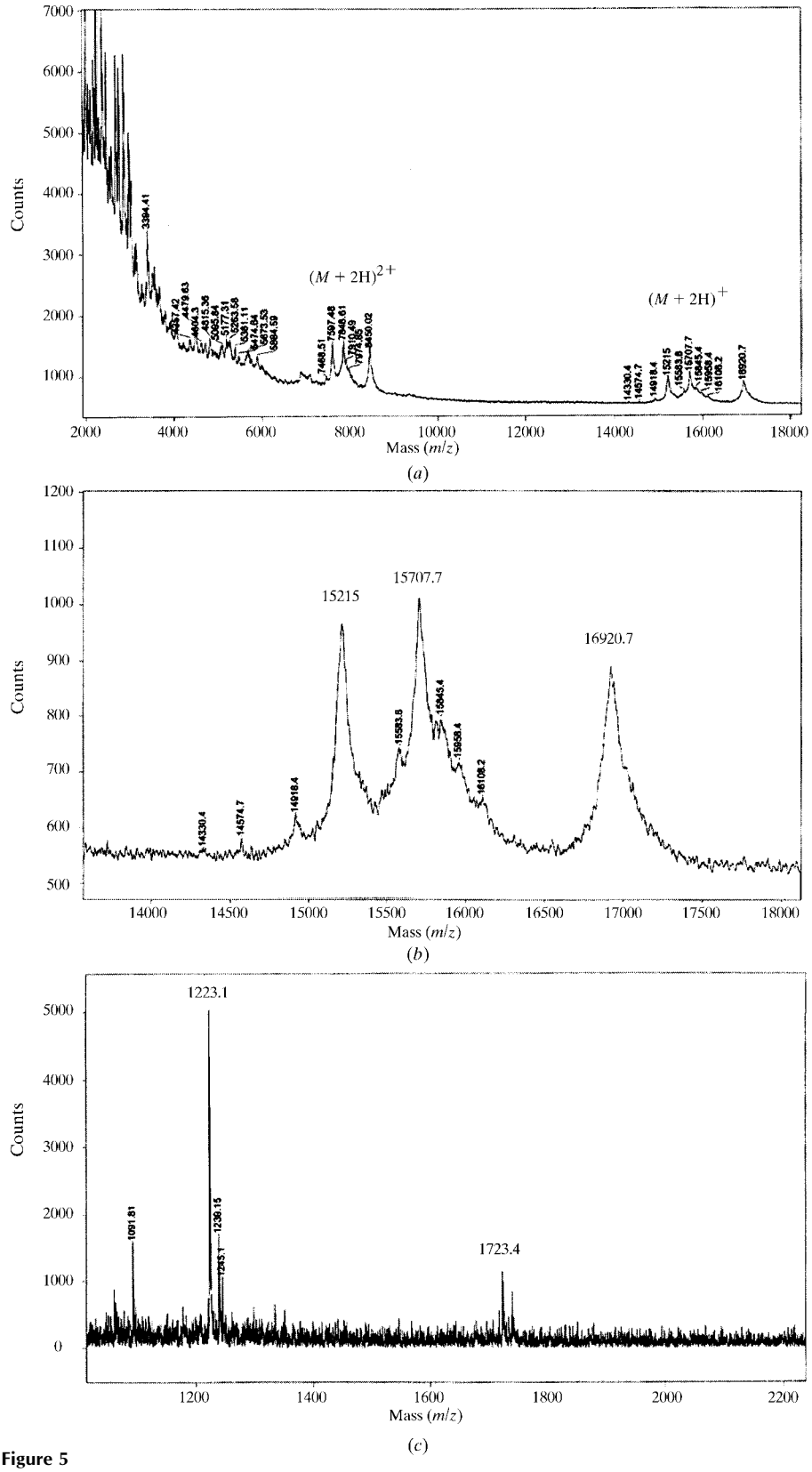

Figure 5
MALDI-TOF MS spectra of r-hu IFN-γ using α-CHCA/NC matrix. (*a*) The complete mass spectrum with two principal peak bulks corresponding to singly and doubly charged molecules $(M + H)^+$ and $(M + 2H)^{2+}$. (*b*) Detail of the singly charged molecule $(M + H)^+$ region of the mass spectrum showing the presence of complete r-hu IFN-γ detected at 16 920.7 Da and of the two other populations corresponding to the fragments 0–134 and 0–130 of r-hu IFN-γ, detected at 15 707.7 and 15 215.0 Da, respectively. (*c*) Detail of the peptide analysis showing the presence of the two complementary fragments of ten and 14 amino acids detected at 1223.1 and 1723.4 Da, respectively.

r-hu IFN-γ crystals in approximately equal quantities with the following molecular weights: 15 707.7, 15 215.0 and 16 920.7 Da (Fig. 5*b*). The latter value (16 920.7 Da) corresponds to the theoretical molecular weight, 16 908.4 Da, calculated from the amino-acid sequence of r-hu IFN-γ. Moreover, the two former populations determined by mass spectrometry at 15 707.7 and 15 215.0 Da correspond to the fragments 0–134 and 0–130 r-hu IFN-γ, with calculated theoretical molecular weights 15 705.0 and 15 205.4 Da, respectively.

Peptide analysis (Fig. 5*c*) permitted us to find the two complementary fragments of ten and 14 amino acids in length with molecular weights 1223.1 and 1723.4 Da, respectively. These values are in good agreement with the theoretical molecular weights of 1222.5 and 1722.0 Da calculated for sections 135–144 and 131–144 of the C-terminal domain of r-hu IFN-γ. This indicates that proteolysis takes place at positions Met134 and Lys130.

The mass-spectroscopy (MS) results clearly show the presence of the complete amino-acid sequence in the crystals of r-hu IFN-γ. We presume that the presence of two other protein fragments (0–130 and 0–134) along with their complementary peptides (135–144 and 131–144) is a result of protein degradation after crystallization and that this occurs either in the crystallization droplets, since the crystals were analyzed by X-rays six months after their formation, or as a result of exposure to intense synchrotron radiation X-rays two months prior to the mass-spectroscopy analysis. It is noteworthy, however, that the complementary peptides did not diffuse out of the crystals, strongly suggesting that degradation occurs after crystallization and that the peptides are locked in the crystal lattice. A more complete MS study of r-hu IFN-γ at different stages of crystallization would be necessary to determine precisely the moment of degradation.

4. Conclusions

This paper presents preliminary results on the characterization and crystallization of a complete recombinant form of human γ-interferon, designated r-hu IFN-γ (RU 42369). The behaviour of r-hu IFN-γ molecules in solution was studied by small-angle X-ray scattering (SAXS) as a function of pH (pH 4.0, 6.0 and 8.0) and as a function of salt type [NaCl, KSCN and $(NH_4)_2SO_4$]. This allowed us to propose suitable crystallization conditions and to grow crystals of a recombinant form of the complete sequence

of human γ-interferon, which has never previously been obtained. These crystals grow from ammonium sulfate solutions, diffract to 5 Å resolution and belong to the primitive tetragonal space group $P42_12$, with unit-cell parameters $a = b = 123.4$, $c = 93.4$ Å. The same crystals as used in the X-ray diffraction studies have been analyzed by MALDI mass spectrometry. The mass-spectroscopy results reveal the presence of the complete amino-acid sequence of r-hu IFN-γ in the crystals, as well as two other protein fragments which are a consequence of either aging or X-ray damage to r-hu IFN-γ.

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