after SDS-polyacrylamide gel electrophoresis, the eluate should be centrifuged at 20,000 rev/min (Sorvall SS-34 rotor) for 20 minutes to remove particulate matter before dialysis. Coomassie blue staining of the gels to locate protein bands does not interfere with subsequent sequenator analysis.

New technologies such as the improved amino acid sequencing method described above lead to new research opportunities. With the greater sensitivity provided by this technique, we now can obtain amino acid sequence information on both proteins and peptides with submicrogram (picomole) quantities. This sensitivity should permit analysis of biomedically relevant molecules—such as the interferons—that can only be obtained in microgram quantities, and this ability opens possibilities for further study of these molecules. For example, knowledge of the amino acid sequence permits the synthesis of corresponding DNA probes and opens the possibility of new strategies for isolating genes, such as those for interferons, that express low levels of messenger RNA's (*8*).

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Human Fibroblast Interferon: Amino Acid Analysis and Amino Terminal Amino Acid Sequence

Abstract. *The purification of human fibroblast interferon has been simplified to a two-step procedure consisting of affinity chromatography on Blue Sepharose and sodium dodecyl sulfate polyacrylamide gel electrophoresis. A preliminary amino acid composition and the sequence of the 13 amino-terminal residues of homogeneous interferon prepared by this method is reported.*

Since the discovery of interferon, its purification and chemical characterization have been primary goals of interferon research. Although their attainment has been slow because of the small quantities of interferon proteins available, purification to homogeneity has now been achieved with some interferons. However, only microgram quantities have been available for characterization—human fibroblast interferon (*1*, *2*), human lymphoblastoid interferon (*3*), human leukocyte interferon (*4*), mouse interferon (*5*)—and only limited structural information has been acquired (*4*, *6*).

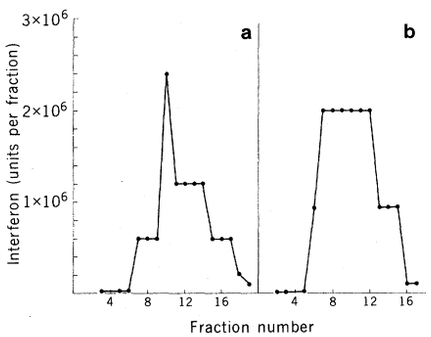

Fig. 1. (a) Fractionation of crude interferon on a large column of Blue Sepharose. Elution of interferon with 50 percent ethylene glycol in column buffer begins at fraction 1. (b) Small Blue Sepharose column. Fractions 7 to 17 in (a) were pooled, passed through the small column, and eluted with 50 percent ethylene glycol in column buffer (fractions 1 to 20).

A thorough understanding at the molecular level of the numerous phenomena that are caused by interferon in cells in culture and in animals will not be possible until the elucidation of primary and secondary structures of the interferon proteins is achieved. This structural information will permit (i) comparison of amino acid sequences of interferons from various cell types and animal species, (ii) identification of the polypeptide segments involved in binding to interferon-specific cell-surface receptors, and (iii) chemical synthesis of interferons.

We now report an improved procedure for the purification of human fibroblast interferon that can be used to provide enough protein for structural studies. Using the automated protein microsequencing technique described in (*7*), we have determined the sequence of the 13 amino acid residues at the amino terminus of the interferon prepared by this method. We also report a preliminary amino acid composition of the protein.

Human diploid fibroblast cells (FS-4) were cultured and interferon was produced (*1*). Interferon was assayed by a microtechnique (*8*) with vesicular stomatitis virus as the challenge virus. Interferon units are given in National Institutes of Health human fibroblast interferon units.

The crude interferon, 10 to 15 liters produced in the absence of serum, was made 1*M* in NaCl and passed at room temperature through a column (4 by 10 cm) of Blue Sepharose (Pharmacia, Inc.) equilibrated with 0.02*M* sodium phosphate buffer, *p*H 7.2, containing 1*M* NaCl. The interferon was retained whereas more than 95 percent of the total protein passed through the column. The interferon was eluted with a mixture of the column buffer and ethylene glycol (1:1), and each fraction was diluted immediately with 0.5 volume of the buffer (Fig. 1a). Fractions containing interferon activity were pooled, diluted with two volumes of the column buffer, and passed through a small (1 by 6 cm) Blue Sepharose column for concentration. The interferon was eluted as described above (Fig. 1b).

Fractions containing interferon were pooled, dialyzed against 1 m*M* tris-HCl,

Table 1. Amino acid composition of human fibroblast interferon.

Amino acid	Composition	
	Mole percent	Residues per 20,000 daltons
Asp	11.1	18.9
Thr	4.0	6.8
Ser	6.2	10.5
Glu	15.9	27.0
Pro	1.6	2.7
Gly*	4.6	7.8
Ala	5.9	10.0
Cys†	1.0	1.7
Val	3.5	6.0
Met	1.7	2.9
Ile	5.3	9.0
Leu	12.0	20.4
Tyr	4.4	7.5
Phe	5.5	9.4
His	2.9	4.9
Lys	6.8	11.6
Arg	6.4	10.9
Trp‡	0.6	1.0

*Includes correction for free glycine present in unhydrolyzed protein. †Determined after performic acid oxidation. ‡Determined after hydrolysis with mercaptoethanesulfonic acid.

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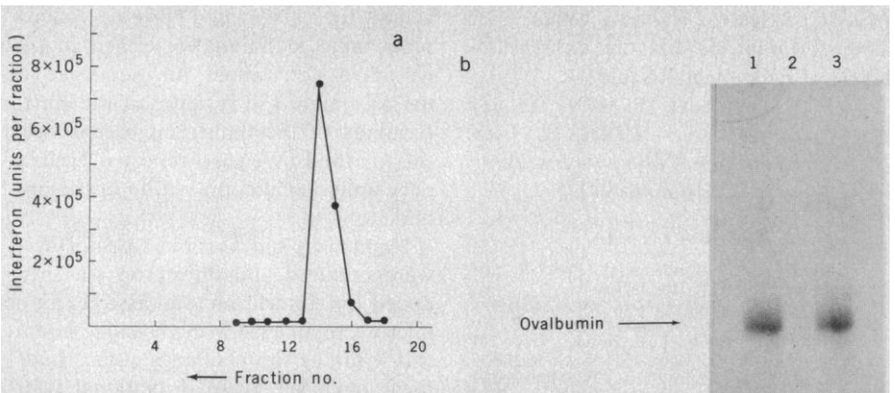

Fig. 2. (a) Preparative electrophoresis of interferon, activity profile. Fractions 6 to 15 in Fig. 1b were pooled, concentrated, and subjected to electrophoresis in a polyacrylamide slab gel, 0.75 mm thick. Fractions 14 and 15 were pooled and processed for amino acid sequencing. (b) Polyacrylamide slab gel, staining of proteins eluted from preparative gel in (a). Approximately 2 percent of the protein in fractions 14 and 15 (a) was subjected to electrophoresis and stained. Lanes 1 and 3, standard proteins; lane 2, interferon.

*p*H 6.8, containing 0.02 percent sodium dodecyl sulfate (SDS, Bio-Rad electrophoresis grade), and concentrated to dryness in a vacuum centrifuge. The interferon was then subjected to electrophoresis on a SDS-polyacrylamide slab gel and eluted (Fig. 2a). Fractions eluted from the gel were assayed for interferon activity (Fig. 2a). Approximately 0.2 μg of interferon from the peak activity fraction was subjected to electrophoresis in this system again, and the gel was stained with Coomassie blue (Fig. 2b).

The preparative electrophoresis fractions containing interferon were pooled and centrifuged for 30 minutes at 30,000 rev/min at 4°C to remove polyacrylamide gel particles. The interferon solution was dialyzed first against 0.15*M* NaCl containing 0.1 percent SDS and then against 0.02 percent SDS. The dialyzed interferon was concentrated to dryness in a vacuum centrifuge.

This purification procedure is simpler and shorter than that described previously (*1*). Recoveries from the large Blue Sepharose column have ranged from 50 to 100 percent, and those from the small column approach 100 percent. The interferon (5×10^7 U/mg) eluted from these columns is stable for at least 4 weeks at 4°C in 1*M* NaCl, 35 percent ethylene glycol, *p*H 7.2. Recoveries of activity from the SDS gels have ranged from 5 to 20 percent, and specific activities of this protein have ranged from 2×10^8 to 8×10^8 U/mg. Accurate specific activities are difficult to determine, and

1 4
H-Met-Ser-Tyr-Asn-Leu-Leu-
7 10 13
Gly-Phe-Leu-Gln-Arg-Ser-Ser-

Fig. 3. The amino-terminal amino acid sequence of human fibroblast interferon.

two- to fourfold differences above 1×10^8 U/mg are probably not meaningful. Overall yields of purified interferon from 10- to 15-liter batches of crude material (5×10^7 to 7×10^7 total units, 8×10^4 U/mg) have averaged around 10 percent. This gives 5 to 10 μg of homogeneous interferon.

Amino acid analysis on 1- to 2-μg portions was performed on a Durrum D-500 amino acid analyzer (Table 1). Automated Edman degradation on 0.4- to 2-μg portions of the purified interferon was performed on a spinning cup sequenator (*7*). Phenylthiohydantoin (Pth) amino acids were identified by high-performance liquid chromatography (HPLC) on a Du Pont Zorbax CN column (*9*).

The sequence of the 13 amino terminal amino acid residues of human fibroblast interferon was determined by this microsequencing technique (Fig. 3). Yields of Pth methionine at cycle 1 for three sequenator runs ranged from 60 to 100 percent (based on protein determination by amino acid analysis), and the sequenator repetitive cycle yields were 92 to 95 percent. Any unblocked minor peptide sequence present at > 5 percent of the reported sequence could have been detected by the methods used, but none has been observed. This result coupled with the high initial Pth yields confirmed the homogeneity of the interferon polypeptide preparation.

Determining the amino acid sequence of a protein is essential in order to identify its active site and to understand the molecular mechanism of action. Comparison of structural features of interferons from different species and from different cell types within an animal will prove or disprove whether they are different proteins. If there is an active site common to all interferons, it should be identifiable by comparison of the amino acid sequences. Comparison of the amino terminal sequence reported here for human fibroblast interferon does not as yet reveal any apparent homology with the amino-terminal sequence reported for human lymphoblastoid interferon (*10*). However, there is limited homology (3 of 13 residues identical) with the 37,000-dalton mouse Ehrlich ascites cell interferon (*11*).

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