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Amino acid sequence of a human leukocyte interferon

(microsequence determination/interferon diversity/automatic sequence determination/multigene family/protein primary structure)

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ABSTRACT **The primary structures of three major species of human leukocyte interferon differ from the structure predicted from the DNA sequence of recombinants containing leukocyte interferon-coding regions. Compared to the recombinant interferon produced in bacteria, three of the purified natural proteins isolated from leukocytes lack the 10 COOH-terminal amino acids suggested by the DNA sequence.**

Although interferon was discovered 23 years ago (1), the structure of the genes and proteins are only now being elucidated with the aid of recombinant DNA technology, DNA sequence analysis, and advances in protein purification and sequence determination. These results indicate that human leukocyte interferon consists of a family of proteins with similar primary structures.

Sensitive methods for protein sequence analysis at the nanomole level (2–5) have revealed NH_2-terminal amino acid sequences for lymphoblastoid (6) and leukocyte (7) interferon that differ in 2 out of 20 positions. Powerful protein purification techniques involving high-performance liquid chromatography (HPLC) have been used to resolve at least 10 different species of human leukocyte interferon, and tryptic maps of this family of proteins exhibit remarkable homology (8). Amino acid sequence analysis of tryptic and chymotryptic peptides from human lymphoblastoid interferon suggests the existence of at least five species (9).

The successful cloning of human leukocyte interferon has provided additional evidence in support of this diversity. Recombinant bacterial plasmids containing interferon cDNAs have been analyzed and reveal different restriction maps and DNA sequences (10–14). Extensive nucleic acid sequence determination of interferon cDNAs from a virus-induced myeloblast cell line indicates that at least eight distinct species of leukocyte interferon are transcribed during the induction process (14), and this result is corroborated by restriction endonuclease mapping of interferon sequences in a human gene bank (15–17). All of these reports suggest that every active species of human leukocyte interferon is 165 or 166 amino acids in length, even though many individual amino acid assignments differ within the family of proteins. We report here the partial amino acid sequence of major species of human leukocyte interferon. These proteins, which represent a significant fraction of the active interferon produced by these cells, lack the 10 COOH-terminal amino acids suggested previously (11–14) from the DNA sequences. Each of the three species is active although lacking the 10 COOH-terminal amino acids.

EXPERIMENTAL PROCEDURES

Human leukocyte interferon species α_1, α_2, and β_1 were isolated and purified from chronic myelogenous leukemia (CML) cells as has been described (8, 18–20). Samples of interferon (7–12 nmol) were digested with tosylphenylalanine chloromethyl ketone-treated trypsin (TPCK-trypsin, 0.2 nmol, Worthington) for 19 hr at 37°C in 50 μl of 0.2 M $NaHCO_3$. Then, 2-mercaptoethanol (2 μl) was added and the sample was incubated for 1 hr at 37°C. The sample was adjusted to 0.14 M pyridine/0.5 M formic acid, pH 3, and applied to an Ultrasphere-octyl column (4.6 × 250 mm, 5-μm-diameter resin spheres, Altex Scientific, Berkeley, CA). The column was eluted with a linear 0–40% (vol/vol) gradient of *n*-propyl alcohol in 0.5 M formic acid/0.14 M pyridine for 3 hr. The column effluent was monitored with an automatic fluorescamine system (21). Amino acid analyses were performed with a fluorescamine analyzer (22).

The sequences of the tryptic peptides were determined by Edman degradation (23). The sequences of small peptides were determined manually (2, 3) and the phenylthiohydantoin derivatives of the amino acids were identified either by HPLC (24–26) or quantitatively by amino acid analysis after back-hydrolysis (27). Automatic Edman degradations were performed in a modified Beckman 890C sequenator. The modifications, which are similar to those described by Wittmann-Liebold (4) and Hunkapiller and Hood (28), include an improved vacuum system, improved reagent and solvent delivery system, extensive solvent and reagent purification, and a device (29) that automatically converts anilinothiazolinone to phenylthiohydantoin derivatives of amino acids. Proteins are retained in the spinning cup with 6 mg of Polybrene, which together with 100 nmol of glycylglycine has been subjected to seven cycles of Edman degradation. Phenylthiohydantoin derivatives of amino acids were analyzed by HPLC on Du Pont Zorbax octadecylsilica or cyanopropylsilica columns on a Waters Associates chromatograph by monitoring absorbance at 254 nm and 313 nm. Peak assignments, except for serine, were made by chromatography on a Zorbax octadecylsilica column. The phenylthiohydantoin derivative of serine was identified as the "dehydro" derivative on a cyanopropylsilica column. Peaks were integrated and gradient elution was controlled by a Spectra Physics SP4000 integration system. All phenylthiohydantoin derivatives were detected by their absorbance at 254 nm, except for those of serine and threonine, which were detected at 313 nm.

The sequences of peptides with low yields of the NH_2 terminus were determined again after mild hydrolysis (30) with 25% aqueous trifluoroacetic acid for 2 hr at 55°C in the spinning cup. This procedure has been successfully used to *N*-deformylate peptides that may have become partially *N*-formylated by exposure to formic acid (and contaminating formaldehyde therein). This treatment causes very little (less than 10%) hy-

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Abbreviations: CML, chronic myelogenous leukemia; HPLC, high-performance liquid chromatography; IFLrA, recombinant A (rA) of human leukocyte interferon.

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drolysis of amide bonds. However, it remains to be proven whether *N*-formylation of the peptides is occurring in the buffers used for HPLC and is responsible for the low yields from some peptides analyzed without prior treatment with trifluoroacetic acid.

RESULTS AND DISCUSSION

Leukocytes isolated from patients with CML can be induced with Newcastle disease virus to produce large quantities of interferon (20). We have purified and characterized 10 interferon proteins produced by these cells: α_1, α_2, β_1, β_2, β_3, and γ_1, γ_2, γ_3, γ_4, and γ_5 (8). The molecular weights of these species range between 16,000 and 21,000. Structural analysis reveals many similarities among these proteins. Within experimental error, all 10 species exhibit similar amino acid compositions, and tryptic maps reveal many peptides that may be common to all species.

Three species that are virtually identical in molecular weight, amino acid composition, and tryptic profiles—α_1, α_2, and β_1—were chosen for sequence analysis. Each of these proteins is a major component of the active interferon produced by CML cells; together they represent 30–50% of the total interferon produced (unpublished data). The amino acid compositions of these species are presented in Table 1. Although the compositions of these three proteins are consistent with the composition of leukocyte interferon produced by normal cells (18), and their specific activities range between 2.6 and 4.4 $\times$ 10^8 units/mg, values that are among the highest that have been reported, the molecular weights are somewhat lower than expected from DNA (11–14) and protein (9) sequence analysis.

Species β_1 of human leukocyte interferon was digested with trypsin and the peptides were separated by HPLC (Fig. 1). The resolving power of this technique allowed us to purify 12 peptides produced by digestion with trypsin. Digestion of β_1 with aminopeptidase M had demonstrated that the NH_2 terminus of the protein is blocked (8), and therefore if the peptide contains an arginine at the COOH terminus, it would not react with fluorescamine and would not be visualized by this system. Hydrolysis of the nonpeak fractions followed by amino acid analysis localized the NH_2-terminal peptide. The sequence of this peptide has already been reported (7). Subsequent studies have suggested that the NH_2 terminus is probably blocked by *N*-formylation due to exposure to formic acid (unpublished data).

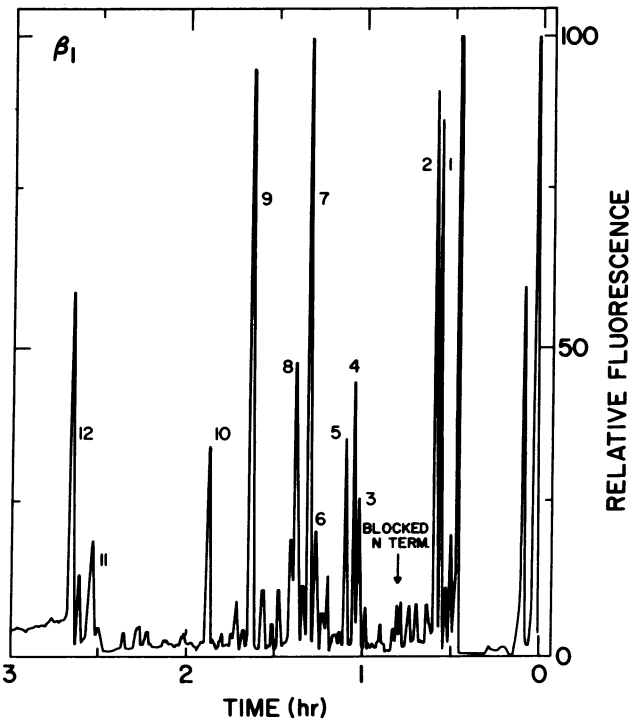

FIG. 1. Human leukocyte interferon species β_1 (10 nmol) was digested with trypsin and the peptides were resolved by HPLC. Peaks representing interferon peptides are numbered 1–12. The position of the NH_2-terminal peptide was determined by hydrolysis of the nonpeak fractions followed by amino acid analysis, because the free amino group of the peptide was found to be blocked and did not react with fluorescamine (7). Peaks eluting within the first 30 min include background contaminants such as amino acids and 2-mercaptoethanol, and hydrophilic amino acids and dipeptides produced by the digestion. Amino acid analysis of the remaining peaks that are not numbered revealed low yields of many amino acids (partial digestion products) or background levels, so these peaks were not analyzed further.

Table 1. Amino acid analyses of human leukocyte interferon species α_1, α_2, and β_1

Amino acid	Residues per molecule			
	α_1	α_2	β_1	Expected
Asx	14.9	12.9	12.5	11
Thr	8.3	9.2	9.7	10
Ser	9.9	11.0	11.2	12
Glx	21.9	22.6	22.6	23
Pro	6.6	5.5	5.7	5
Gly	5.5	4.9	5.4	5
Ala	9.1	9.2	8.0	8
Val	8.0	6.9	6.5	7
Met	3.9	4.2	5.3	5
Ile	8.0	7.8	7.0	8
Leu	19.4	19.9	19.9	19
Tyr	4.3	4.8	4.8	5
Phe	7.4	8.9	9.5	10
His	3.3	3.0	3.1	3
Lys	11.4	10.0	9.7	10
Arg	6.7	7.9	8.8	8
Cys	4.2	4.4	3.4	4
Trp	—	—	—	2
Total	152.8	153.1	153.1	155

Calculation of amino acid residues was normalized to 155 (including 2 for tryptophan) for all species on the basis of sequence data presented in this report. Analyses are the average of at least two determinations. Trp determinations were not done.

Digestion of species α_1 and α_2 with trypsin resulted in tryptic maps that were virtually identical to each other and to the tryptic map of β_1 (Fig. 2). The correspondence of these three tryptic profiles indicates that the primary structures of peptides from these three species must be largely similar.

The composition of each peptide was determined by amino acid analysis with a fluorescamine analyzer (Table 2). Peptides 1–4, 7, and 9–11 are identical in composition for all three species, representing 56% of the protein. Only α_1 contains peptides that differ from those of α_2 and β_1. Peptide 8 of α_1 lacks one phenylalanine from the described composition and has one additional serine, and peptide 12 appears to have a different composition.

The sequences of the peptides indicated in Table 2 were determined by the Edman procedure (23) either manually or automatically with a modified Beckman sequenator. We previously proposed an amino acid sequence for a species of human leukocyte interferon on the basis of DNA sequence analysis of the cDNA present in our bacterial clone that produces interferon (12, 13), and this suggested sequence was used to order the tryptic fragments (Fig. 3). This primary structure provides the best match of all the proposed sequences (9, 11, 14) for our amino acid sequence data.

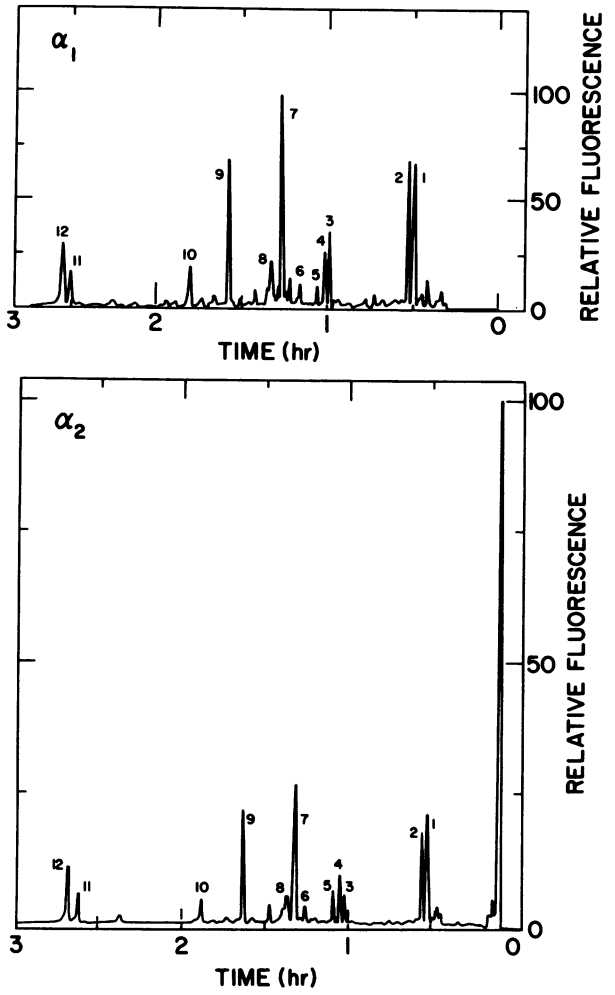

FIG. 2. Human leukocyte interferon species α_1 (7 nmol) and α_2 (4.4 nmol) were digested with trypsin and the peptides were resolved by HPLC.

Digestion of proteins with trypsin often provides information as to which peptide is located at the COOH terminus, because one peptide without lysine or arginine should be generated. Our data showed that both peptides 3 and 5 contained no arginine or lysine. Because the proteins used in this study have been shown to be homogeneous and both peptides contained aromatic amino acids, we postulated that one of these was produced by a chymotryptic-like activity of the TPCK-trypsin used. This assumption was substantiated when sequence analysis revealed that peptide 5 (positions 126–129) was a chymotryptic fragment of peptide 7 (positions 126–131). Therefore, peptide 3 is the COOH-terminal peptide.

The presence of two basic amino acids in peptides 2, 6, 8, and 11 indicated that tryptic cleavage did not occur at all possible positions. At positions where two basic amino acids were in tandem (12–13, 22–23, 120–121, and 133–134) one peptide bond was cleaved preferentially. At two positions located internally within tryptic fragments (position 33 in peptide 8 and position 83 in peptide 11) tryptic cleavage did not occur. Because trypsin can cleave peptide bonds adjoining these amino acids in other proteins, we assume that the tertiary structure of the protein renders these sites less susceptible to attack. The amino acid sequence best matches the proposed amino acid sequence of our cDNA clone IFLrA. Our species may be closely related to one of the interferon "A" species that have been reported (9), but confirmation must await resolution of these species into homogeneous proteins.

Table 2. Amino acid compositions of tryptic peptides

Peptide	Composition	Species* α_1	α_2	β_1
1	Glx, 1.2, Ala, 1.1, Met, 1.3, Ile, 1.0, Arg 1.0	Sc	Sc	Sc
2	Glx, 1.0, Tyr, 1.0, Phe, 1.0, Lys, 1.0, Arg 1.0	Sc	S	Sc
3	Thr, 1.0, Ser_3, 3.3, Leu, 1.2, Phe 1.1	Sc	S	Sp
4	Asx, 0.9, Ser, 1.0, Glx, 1.0, Ala, 1.1, Val, 1.1, Ile, 1.0, Leu, 1.1, Arg 1.0	S	Sc	Sc
5	Thr, 0.9, Ile, 0.9, Leu, 1.0, Tyr 1.0	Sc	NA	Sc
6	Asx, 1.3, Ser_2, 2.0, Glx_2, 1.7, Gly_2, 1.7, Ala, 1.1, Val, 0.8, Tyr, 0.6, Lys, 1.0, Arg, 0.7, Pro 1.1	Sc	S	NA
7	Thr, 1.1, Ile, 1.0, Leu_2, 2.1, Tyr, 1.1, Lys 1.0	S	S	Sc
8	Asx_3, 2.7, Glx_5, 4.8, Gly_2, 2.2, Phe_4, 3.8, His, 1.0, Lys, 1.0, Arg, 1.0, Pro 0.9	D†	Sp	Sp
9	Thr, 1.0, Glx, 1.2, Ala, 1.0, Met_2, 2.4, Leu_3, 3.1, Arg 1.0	Sc	Sp	Sp
10	Ser_2, 1.9, Ile, 0.9, Leu_2, 2.0, Phe, 1.0, Lys, 1.0, Cys 0.9	S	Sc	Sp
11	Asx_5, 4.8, Thr_4, 3.7, Ser_2, 2.3, Glx_7 7.0, Gly_2, 2.0, Pro, 1.2, Ala_3, 2.8, Val_3, 2.7, Met, 0.8, Ile, 0.9, Leu_6, 5.7, Tyr_2, 1.8, Phe, 0.8, Lys_2, 2.0, Cys 0.8	Sp	Sp	Sp
12	Asx, 0.9, Thr_2, 1.9, Ser, 1.0, Glx_4, 4.0, Pro, 0.8, Ala, 1.0, Val, 1.0, Met, 1.4, Ile_3, 2.8, Leu_2, 2.0, Phe_2, 2.0, His, 0.9, Lys 1.0	D‡	NA	Sc

The number under each amino acid represents the relative amount of that amino acid compared to the others as actually determined for the tryptic peptides of leukocyte interferon β_1, except for peptide 6, for which the value for the peptide derived from leukocyte interferon α_2 is given because the analysis for the corresponding β_1 peptide was not performed.

* S, same composition as indicated; D, different composition; c, sequence completely determined; p, sequence partially determined; NA, not analyzed.

† Composition has one less Phe and one additional Ser; the sequence of this peptide is Asp-Phe-Ser-Pro-Glu-Gly-Glx.

‡ Composition has multiple differences.

The surprising difference between interferon species α_1, α_2, and β_1 and the proposed amino acid sequences of our cDNA clones (12–14, 16, 17) is that species α_1, α_2, and β_1 all terminate at position 155, 10 amino acids earlier than has been observed or proposed in previous reports. Four arguments can be proposed to support the conclusion that peptide 3 is the COOH-terminal peptide: (*i*) the amino acid composition does not include arginine or lysine; (*ii*) it is not a chymotryptic fragment, because it ends with threonine; (*iii*) no other protease cleavages occurred in interferon species or other control proteins subjected to identical conditions; and (*iv*) all other tryptic peptides have been accounted for in the sequence. Amino acid compositions for peptide 3 in all three interferon proteins were identical and contained only the six amino acids that were determined by direct sequence analysis. The specific activity of each

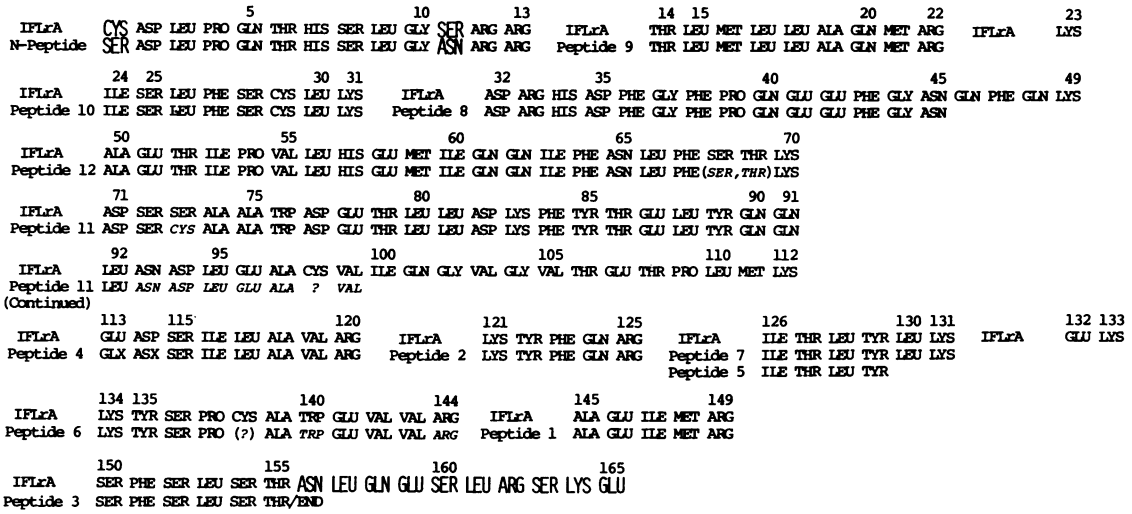

FIG. 3. Amino acid sequence of human leukocyte interferon species α_2 and β_1 peptides. Amino acid sequences of tryptic peptides are aligned with the suggested amino acid sequence of our cloned species of leukocyte interferon determined by DNA sequence analysis of IFLrA, the recombinant A of human leukocyte interferon (12, 13). Sequence A represents the sequence deduced from the cDNA sequence and the sequences directly below represent those determined by Edman degradation. A representative yield and assignment of sequence for peptides 3 and 11 are given. Sequence analysis of 2.2 nmol of peptide 3 from leukocyte interferon α_1 yielded 1.1 nmol (50%) of Phe>PhNCS (the phenylthiohydantoin) at cycle 2. The assignment of Thr>PhNCS at position 6 is based on amino acid composition. Sequence analysis of 0.8 nmol of peptide 11 from α_1 was performed after incubating the peptide in 25% (vol/vol) trifluoroacetic acid in water for 2 hr at 55°C. A yield of 0.2 nmol (25%) of Asp>PhNCS was obtained at cycle 1. Positive identifications were made for 21 of the 34 residues; tentative assignments were made for 7 residues noted by italics (*Cys*-73, *Asn*-93, etc.). Where no assignment could be made, a ? is present within the sequence and the positions are left blank at the end of the sequence. Residues in larger type (Cys-1 and Ser-1, Ser-11 and Asn-11, and residues 156–165 corresponding to the IFLrA DNA sequence) represent differences between the protein sequence and that predicted from the DNA sequence. Numbering of amino acids follows the cDNA sequence. We did not determine the amino acids representing positions 23, 46–49, 98, 100–112, and 138. The dipeptide Glu-Lys (positions 132–133) was not resolved by HPLC. Sequences in peptides 1, 2, and 12 were used in the construction of probes for the detection of bacterial clones containing leukocyte interferon cDNAs (13). Amino acids in italics represent best estimates that were consistent with the data, but were not greatly above background levels. Only two residues (nos. 1 and 11), noted in larger type, were not in accord with the IFLrA sequence. Both of these residues were determined from leukocyte interferon α_1. Because position 1 is Cys in all our recombinant cDNA and genomic clones (14, 16, 17) and those of Nagata *et al.* (15), we assume the Ser found by us (7) and Zoon *et al.* (6) may represent an artifact of the microsequencing procedures or modifications of the protein introduced during production and isolation. Although no NH_2-terminal cysteine has been found in any of the natural leukocyte interferons (6, 7, 9), we have found Cys at the NH_2 terminus of IFLrA produced in *Escherichia coli* (31, 32). As noted above, the Asn-11 was determined as the residue for leukocyte interferon species α_1 (7). Although IFLrA contains a Ser at position 11, all other recombinants we have isolated contain Asn in this position. Therefore, leukocyte interferon α_1 is clearly distinct from IFLrA. Were the residue at position 11 known for leukocyte interferons α_2 and β_1, we expect it would be Ser. Because almost all other peptides were isolated from leukocyte interferons α_2 and β_1 and because there is complete agreement between the sequences determined and those of IFLrA, it is likely that the primary sequences of leukocyte interferons α_2 and β_1 are identical to IFLrA and to each other.

of these proteins indicates that the 10 COOH-terminal amino acids apparently are not essential for activity.

The correspondence between our primary structure for human leukocyte interferon species α_2 and β_1 and the amino acid composition of the native protein is shown in Table 1 and Fig. 3. There is virtual agreement between the sequences determined, and all the tryptic fragments that would be generated from the sequence predicted for recombinant interferon IFLrA. Four and 13 COOH-terminal residues of peptides 8 and 11, respectively, were not determined (Fig. 3). However, because the compositions of peptides 8 and 11 are identical to the composition of the same fragments that would be produced by tryptic cleavage of an interferon with the sequence suggested by our cDNA data, we propose that the sequences of these peptides remaining to be identified are represented in the cDNA sequence. This assumption enables us to calculate the composition of human leukocyte interferon species α_2 and β_1. Values for each amino acid are within the accuracy of the analysis, and the molecular weights are virtually identical. The proposed amino acid sequence for these human leukocyte interferon species is presented in Fig. 4. The amino acid sequences of these two human leukocyte interferons (8) are probably identical to that of recombinant IFLrA (12–14). However, the 10 COOH-terminal amino acids predicted by the DNA sequence are missing, with no apparent decrease in specific activity. The calculated molecular weight and amino acid composition match well with the experimentally determined molecular weight and amino acid composition of the native protein. The NH_2 terminus of the protein appears to be blocked by *N*-formylation. All these three species of leukocyte interferon, α_1, α_2, and β_1, show this same structural feature. Species α_1 differs from α_2 and β_1 in at least two internal tryptic fragments, whereas α_2 and β_1 may be identical in sequence. Differences between species α_2 and β_1 may reside in modifications, produced in culture or during isolation, that change the oxidation state or net charge of the molecules. These proteins represent 30–50% of the total active interferon produced by cells from patients with CML.

The lack of the 10 COOH-terminal amino acids may represent a normal processing event after translation, and this can be substantiated by COOH-terminal sequence analysis of interferon produced by normal leukocytes. This implies that amino acid sequences of mature proteins predicted by DNA sequences may have significant limitations. Only direct sequence analysis of the proteins themselves can ascertain their primary structures. Because native human leukocyte interferon produced in cultures of Namalva cells (9) and a myeloblastic cell line (34) have higher molecular weights (34) and appear to contain the COOH-terminal amino acids (9), some of the human leukocyte interferon species isolated from cultures of buffy coats from normal or leukemic donors may lack the 10 COOH-ter-

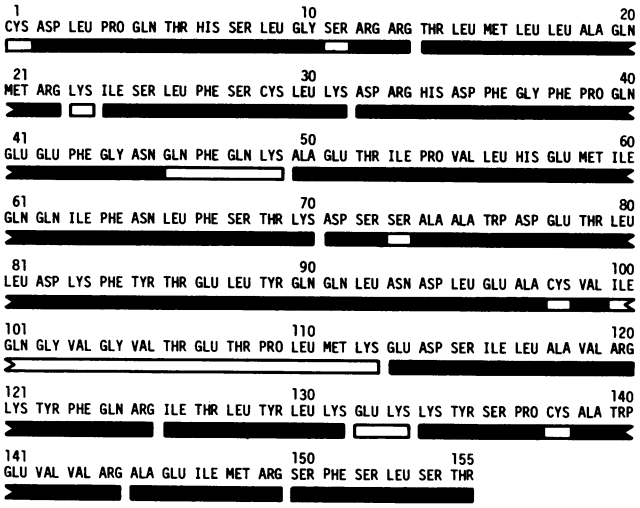

FIG. 4. Proposed amino acid sequence of human leukocyte interferon species α_2 and β_1. The beginning and end of each tryptic peptide isolated and sequenced are shown by the bars underneath the sequence. Solid bars represent amino acid sequences that were determined by microsequencing of the peptides. The unfilled areas represent sequences that were not directly identified by microsequencing but were consistent with the amino acid compositions of the peptides and microsequencing procedures. The sequences of these areas were deduced from the sequence of recombinant IFLrA. Position 23 could be Lys (13) or possibly Arg (33); it is possible that α_2 may represent one, and β_1, the other.

minal amino acids due to proteolytic cleavage. It is thus likely that much of the crude human leukocyte interferon used in clinical trials to date (35) lacks the 10 COOH-terminal amino acids. Because several of the natural species we have purified are larger (8), it is possible that the product secreted from buffy coat cells contains the full predicted sequence. Whether native interferon produced and active *in vivo* represents the full-length or shortened species remains to be determined.

It is of note that less than 200 μg of each species was used for determination of their respective amino acid sequence. This was made possible by the use of sensitive amino acid and peptide analysis (21, 22) as well as microsequencing technology (2–5, 7, 28, 29) in combination with DNA sequence analysis of recombinants containing the coding region for these human leukocyte interferons (12, 13). The integration of all these methods permits efficient use of microgram amounts of protein for sequence analysis. The fact that these human leukocyte interferons (α_1, α_2, and β_1) are shorter than the sequence predicted from the DNA emphasizes the necessity for determining the sequences of the proteins themselves.

Note Added in Proof. After this report was submitted, Zoon (36) reported the partial amino acid sequence of a leukocyte interferon species from Namalva cells that differs from the sequence we report here.

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1. Isaacs, A. & Lindenmann, J. (1957) *Proc. R. Soc. London Ser. B* **147**, 258–267.
2. Tarr, G. E. (1977) *Methods Enzymol.* **47**, 335–357.
3. Levy, W. P. (1981) *Methods Enzymol.* **79**, 27–31.
4. Wittmann-Liebold, B. (1973) *Hoppe-Seylers Z. Physiol. Chem.* **354**, 1415–1431.
5. Hunkapiller, M. W. & Hood, L. E. (1980) *Science* **207**, 523–525.
6. Zoon, K. C., Smith, M. E., Bridgen, P. J., Anfinsen, C. B., Hunkapiller, M. W. & Hood, L. E. (1980) *Science* **207**, 527–528.
7. Levy, W. P., Shively, J., Rubinstein, M., Del Valle, U. & Pestka, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5102–5104.
8. Rubinstein, M., Levy, W. P., Moschera, J. A., Lai, C.-Y., Hershberg, R. D., Bartlett, R. T. & Pestka, S. (1981) *Arch. Biochem. Biophys.* **210**, 307–318.
9. Allen, G. & Fantes, K. H. (1980) *Nature (London)* **287**, 408–411.
10. Nagata, S., Taira, H., Hall, A., Johnsrud, L., Streuli, M., Ecsödi, J., Boll, W., Cantell, K. & Weissmann, C. (1980) *Nature (London)* **284**, 316–320.
11. Mantei, N., Schwarzstein, M., Streuli, M., Panem, S., Nagata, S. & Weissmann, C. (1980) *Gene* **10**, 1–10.
12. Maeda, S., McCandliss, R., Gross, M., Sloma, A., Familletti, P. C., Tabor, J. M., Evinger, M., Levy, W. P. & Pestka, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7010–7013.
13. Goeddel, D. V., Yelverton, E., Ullrich, A., Heyneker, H. L., Miozzari, G., Holmes, W., Seeburg, P. H., Dull, T., May, L., Stebbing, N., Crea, R., Maeda, S., McCandliss, R., Sloma, A., Tabor, J. M., Gross, M., Familletti, P. C. & Pestka, S. (1980) *Nature (London)* **287**, 411–416.
14. Goeddel, D. V., Leung, D. W., Dull, T. J., Gross, M., Lawn, R. M., McCandliss, R., Seeburg, P. H., Ullrich, A., Yelverton, E. & Gray, W. P. (1981) *Nature (London)* **290**, 20–26.
15. Nagata, S., Mantei, N. & Weissmann, C. (1980) *Nature (London)* **287**, 401–408.
16. Maeda, S., McCandliss, R., Chiang, T.-R., Costello, L., Levy, W. P., Chang, N. T. & Pestka, S. (1981) in *Developmental Biology Using Purified Genes*, eds. Brown, D. & Fox, C. F. (Academic, New York), Vol. 23, in press.
17. Pestka, S., Maeda, S., Hobbs, D. S., Levy, W. P., McCandliss, R., Stein, S., Moschera, J. A. & Staehelin, T. (1981) in *Cellular Responses to Molecular Modulators*, eds. Scott, W. A., Werner, R. & Schultz, J. (Academic, New York), Vol. 18, in press.
18. Rubinstein, M., Rubinstein, S., Familletti, P. C., Miller, R. S., Waldman, A. A. & Pestka, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 640–644.
19. Rubinstein, M., Rubinstein, S., Familletti, P. C., Gross, M. S., Miller, R. S., Waldman, A. A. & Pestka, S. (1978) *Science* **202**, 1289–1290.
20. Rubinstein, M., Rubinstein, S., Familletti, P. C., Brink, L. D., Hershberg, R. D., Gutterman, J., Hester, J. & Pestka, S. (1980) in *Proteins: Structure and Biological Function*, eds. Gross, E. & Meienhofer, J. (Pierce, Rockford, IL), pp. 99–103.
21. Böhlen, P., Stein, S., Stone, J. & Udenfriend, S. (1975) *Anal. Biochem.* **67**, 438–445.
22. Stein, S., Böhlen, P., Stone, J., Dairman, W. & Udenfriend, S. (1973) *Arch. Biochem. Biophys.* **155**, 203–212.
23. Edman, P. (1950) *Acta Chem. Scand.* **4**, 283–293.
24. Zimmerman, C. L., Apella, E. & Pisano, J. J. (1977) *Anal. Biochem.* **77**, 569–573.
25. Abrahamsson, M., Gröningsson, K. & Castensson, S. (1978) *J. Chromatogr.* **154**, 313–317.
26. Bhown, A. J., Mole, J. E., Weissinger, A. & Bennett, J. C. (1978) *J. Chromatogr.* **148**, 532–535.
27. Lai, C.-Y. (1977) *Methods Enzymol.* **47**, 369–373.
28. Hunkapiller, M. W. & Hood, L. E. (1978) *Biochemistry* **17**, 2124–2133.
29. Wittmann-Liebold, B., Graffunder, H. & Kohls, H. (1976) *Anal. Biochem.* **75**, 621–633.
30. Elson, N. A., Brewer, H. B. & Anderson, W. F. (1974) *J. Biol. Chem.* **249**, 5227–5235.
31. Staehelin, T., Hobbs, D. S., Kung, H.-F. & Pestka, S. (1981) *Methods Enzymol.* **78**, 505–512.
32. Staehelin, T., Hobbs, D. S., Kung, H.-F., Lai, C.-Y. & Pestka, S. (1981) *J. Biol. Chem.* **256**, in press.
33. Streuli, M., Nagata, S. & Weissmann, C. (1980) *Science* **209**, 1343–1347.
34. Hobbs, D. S., Moschera, J., Levy, W. P. & Pestka, S. (1981) *Methods Enzymol.* **78**, 472–481.
35. Mogensen, K. E. & Cantell, K. (1977) *Pharmacol. Ther. Part A* **1**, 369–381.
36. Zoon, K. (1981) in *The Biology of the Interferon System*, eds., De Maeyer, E., Galasso, G. & Schellekens, H. (Elsevier/North Holland, Amsterdam), pp. 47–55.