Amino Terminal Sequence of the Major Component of Human Lymphoblastoid Interferon

Abstract. *Homogeneous human lymphoblastoid interferon with an apparent molecular size of 18,500 daltons was characterized by its amino acid composition. Analysis of the amino terminal sequence by Edman degradation indicates that the sequence is unique.*

Major advances in the large-scale production and purification of interferons have provided sufficient material for their chemical characterization (*1-6*). Recent improvements in the amino acid analysis (*6, 7*) and sequence determination of picomole quantities of proteins with the use of high-performance liquid chromatography (HPLC) for identification (*8, 9*) have permitted composition and sequence data to be obtained for several mouse (*5, 10*) and human interferons (*2-4, 6*).

Purified human lymphoblastoid interferon has been produced in nanomole quantities from Namalwa cells induced with Newcastle disease virus, strain B1. The major interferon species was isolated in homogeneous form by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and has an apparent molecular size of 18,500 daltons (Fig. 1). This component has a final specific activity of 2.2×10^8 to 2.5×10^8 inteferon units per milligram of protein (*4*). The amino acid composition and the first 20 amino terminal residues are presented in this report.

The amino acid analyses were performed (*11*) with the use of HPLC and fluorescence detection of *o*-phthalaldehyde derivatives of amino acids (*7*). Proline was measured by gas chromatography (*12*). The amino acid composition of the 18,500-dalton component (Table 1) is strikingly similar to those reported for mouse (*5*), human fibroblast (*2, 3*), and human leukocyte interferons (*6*), especially in the abundance of hydrophobic amino acids.

Table 1. The amino acid composition of the 18,500-dalton component of human lymphoblastoid interferon. Analyses were performed with 1 to 3 μg of protein. Unhydrolyzed, duplicate samples were analyzed to monitor contamination. The quantity of each amino acid detected in the unhydrolyzed samples was less than 5 percent of the amount present after hydrolysis, except for serine and glycine which were less than 10 percent. These blank values were subtracted from the values determined in the hydrolyzed samples. Cysteic acid and methionine sulfone were measured after performic acid oxidation (*13*). Tryptophan was determined after hydrolysis in a mixture of 6*N* HCl and 4 percent (by volume) thioglycolic acid (*14*).

Amino acid*	Percent†	Residues per 18,500 daltons‡
Asx	9.0	14.9
Thr	4.8	8.0
Ser	6.5	10.7
Glx	16.5	27.3
Pro	6.6	10.9
Gly	6.5	10.7
Ala	6.6	11.0
Cys	1.1	1.8
Val	4.6	7.7
Met	0.7	1.2
Ile	4.2	6.9
Leu	10.8	17.8
Tyr	2.3	3.8
Phe	4.3	7.1
His	2.6	4.4
Lys	6.3	10.4
Arg	5.8	9.6
Trp	0.4	0.6

*The amino acid residues are Asx, either aspartic acid or asparagine; Ala, alanine; Arg, arginine; Cys, half-cystine; Glx, glutamine or glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Trp, tryptophan; and Val, valine. †The percentage represents (nanomoles of amino acid residue per nanomoles of total amino acid residues) × 100. ‡These numbers are based on the apparent molecular size of 18,500 daltons obtained by SDS polyacrylamide gel electrophoresis.

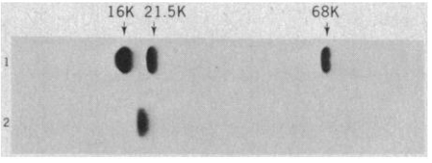

Fig. 1. Analytical sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of the purified 18,500-dalton component of human lymphoblastoid interferon. (Lane 1) Molecular size standards: 2.5 μg of bovine serum albumin (68,000 daltons) and 5.0 μg each of soybean trypsin inhibitor (21,500 daltons) and myoglobin (16,000 daltons); (lane 2) 2.2 μg of the 18,500-dalton component prepared from the preparative SDS polyacrylamide gel.

1 5
H-Ser-Asp-Leu-Pro-Gln-Thr-His-
10
Ser-Leu-Gly-Asn-Arg-Arg-Ala-
15 20
Leu-Ile-Leu-Leu-Ala-Gln-

Fig. 2. The amino terminal sequence of the major 18,500-dalton component of human lymphoblastoid interferon. After a 100,000-fold purification, the 18,500-dalton component was dialyzed twice against 6-liter volumes of phosphate-buffered saline (*p*H 7.4) with 0.1 percent SDS and then twice against 1 liter of 0.01 percent SDS (Bio-Rad Laboratories) in HPLC-grade water (Baker) at 25°C for 24 hours. The sequence was based on the results of six determinations.

The amino terminal sequence was determined with a spinning cup sequenator and slight modification of the Edman Quadrol program (*9*). The amino terminal sequence of the first 20 amino acids is shown in Fig. 2. Multiple analyses (six determinations) on three independent preparations of interferon (20 to 500 pmole of protein) yielded the sequence through residue 20. A second sequence (approximately 10 percent) was found in one earlier preparation. A fraction migrating slightly faster than the major 18,500-dalton component had equal amounts of both sequences and half the specific activity. Improved fractionation of the 18,500-dalton component from the preparative SDS polyacrylamide gels essentially eliminated the second sequence. Two preparations have yielded the sequence shown in Fig. 2 with undetectable quantities (< 3 percent) of the contaminant.

The amino acid sequence of the amino terminus of the 18,500-dalton component of human lymphoblastoid interferon does not appear to be homologous with either the amino terminal sequence of the band A, the 33,000-dalton component of mouse Ehrlich ascites tumor cell interferon (*10*), or of human fibroblast interferon (*3*). Amino terminal sequence homology does exist, however, between the 18,500-dalton component of human lymphoblastoid interferon and mouse Ehrlich ascites tumor cell interferon band C (the 18,000- to 22,000-dalton component). Of the first 20 amino acids, 13 appear to be identical (*10*).

KATHRYN C. ZOON
MARK E. SMITH
PAMELA J. BRIDGEN
CHRISTIAN B. ANFINSEN
Laboratory of Chemical Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20205
MICHAEL W. HUNKAPILLER
LEROY E. HOOD
Division of Biology, California Institute of Technology, Pasadena 91125

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Mouse Interferons: Amino Terminal Amino Acid Sequences of Various Species

Abstract. *Mouse interferons of three size classes (A, 35,000 to 40,000 daltons; B, 26,000 to 33,000 daltons; and C, 20,000 daltons) were purified from Ehrlich ascites tumor cells infected with Newcastle disease virus. The sequences of the first 24 amino acids (No. 17 has not been identified) of interferons A and B are identical. The sequence of the first 20 amino acids of interferon C differs from that of A and B in 18 positions. There is partial homology in amino terminal sequence between mouse interferons A (or B) and a human fibroblast interferon and between mouse interferon C and a human lymphoblastoid interferon.*

Interferons are glycoproteins that are produced by various vertebrate cells in response to viral infection or some other stimuli. They are released from the producing cells, interact with other cells, and alter the biochemical and immunological characteristics of these in various ways. Interferons are species specific: they are more active usually in homologous than in heterologous cells (*1*).

There are several reports on the purification of mouse interferons induced by infection with Newcastle disease virus in cultured mouse cells. The purified interferons cover a wide molecular weight range. Those isolated from L929 and C243 cells were found to consist of two species, a larger one of 35,000 to 40,000 daltons and a smaller one of 22,000 to 28,000 daltons (*2*). We reported earlier the isolation of mouse interferons from cultures of Ehrlich ascites tumor cells infected with Newcastle disease virus (*3*, *4*). These consist of three size classes: A, about 35,000 daltons; B, about 26,000 daltons, and C, about 20,000 daltons. The specific activity of each is about 2×10^9 NIH units per milligram of protein. A and B migrate as broad bands on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS), and C gives a sharper band. The amino acid compositions of interferons A, B, and C are similar. The tryptic peptide maps of A and B are very similar, that of C is different (*3*, *4*).

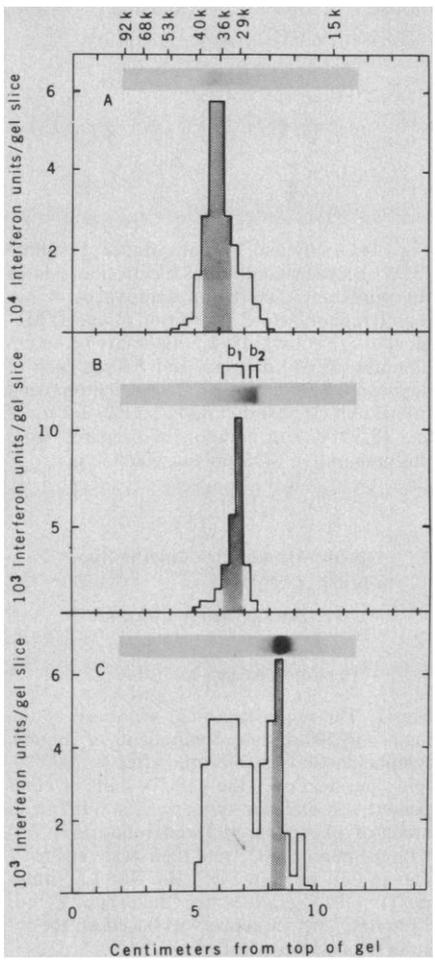

Fig. 1. Analyses by polyacrylamide gel electrophoresis in the presence of SDS of three protein fractions enriched in interferons, showing the size distributions of interferon active materials and of proteins. Panels A, B, and C show the analyses of preparations enriched in interferon classes A, B, and C, respectively. The horizontal inserts at the tops of the panels are photographs of the appropriate gel tracks after they were stained for proteins with Coomassie brilliant blue. For detection of interferon activity, the gel tracks were sliced, the slides were eluted, and the eluted material was tested in an assay based on the protection of cells treated with interferon from the cytopathic effect of vesicular stomatitis virus. The amino terminal amino acid sequences of the proteins eluted from the shaded regions of the gel tracks shown in panels A, B and C, and in the case of the gel track shown in panel B also, from the region b_2 were determined.

Here we report the NH_2-terminal amino acid sequences of mouse interferons from size classes A, B, and C. The detailed description of the improved and highly sensitive amino acid sequencing technique used to establish these has been provided by Hunkapiller and Hood (*5*).

The interferons were produced in Ehrlich ascites tumor cells and purified according to a modified version of our described procedure (*4*). This involves chromatography on controlled pore glass, carboxymethyl-Sephadex, and phosphocellulose (*6*). These steps result in three fractions, each of which is enriched in a different size class of interferons. Each of these is further fractionated by chromatography on octyl-Sepharose and isoelectric focusing (*3*, *4*). At this stage the specific activities of interferons A, B, and C are about 1.6×10^9 to 1.8×10^9 reference units per milligram of protein. The final step of purification is preparative polyacrylamide gel electrophoresis in the presence of SDS (*7*). This serves to improve the separation of the interferons A, B, and C from each other and to remove minor contaminating proteins, together with the ampholines remaining from isoelectric focusing.

The distributions of proteins and of interferon activity in the gel electropherograms of the preparations enriched in interferon class A, B, and C, respectively, are shown in Fig. 1. The interferon A preparation (Fig. 1A) gives rise to one major diffuse protein band (35,000 to 40,000 daltons) which comigrates with interferon activity. The specific activity of this preparation does not decrease during the gel electrophoresis.

The interferon B preparation (Fig. 1B) gives rise to a broad and diffuse band (25,000 to 40,000 daltons). The most heavily stained portion coincides with the 26,000- to 30,000-dalton region, whereas most of the interferon activity migrates in the 29,000- to 35,000-dalton region. This apparent discrepancy might

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