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PRESENCE OF A COMMON STRUCTURE IN THE TWO MOLECULAR SPECIES OF MOUSE L CELL INTERFERON

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SUMMARY Tryptic digests of the two molecular species of purified mouse L cell interferon, labeled with [^{125}I] and [^{3}H] methionine, were analyzed chromatographically. The 40,000 dalton-species yielded 4 methionine-containing and 6 [^{125}I]-labeled fragments, whereas the 24,000 dalton-species gave rise to 4 methionine- and 7 [^{125}I]-labeled fragments. Of these, 3 methionine-containing and 3 [^{125}I]-labeled fragments were found chromatographically identical between the two species. These results suggest that the two distinct species of interferon contain a common polypeptide structure.

Heterogeneity of interferon molecules in a given animal species is well known, as regards the molecular weight, isoelectric point, and some biological activities such as cross-species antiviral effects (1). Mouse L cell interferon induced by NDV[1] is known to have two molecular species of molecular weight 35,000-40,000 (S interferon) and 22,000-24,000 (F interferon) (2-5). On the other hand, Knight found that L cell interferon induced by MM virus was composed of 10 to 11 polypeptides of molecular weight ranging from 20,000 to 32,000 (6). Kawakita et al. also observed a similarly broad distribution in apparent molecular size for NDV-induced interferon from Ehrlich ascites tumor cells (7).

The molecular basis of the heterogeneity of interferon has remained largely unresolved. One of the central issues concerning the nature of the molecular heterogeneity is whether or not the different species have any common polypeptide structure. Recently, we succeeded to obtain essentially pure L cell interferon preparations induced by NDV, doubly labeled by [^{125}I]

[1]Abbreviations used are: TPCK, L-(1-tosylamide-2-phenyl)ethyl chloromethyl ketone; NDV, Newcastle disease virus

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and [^{3}H] methionine (5). In this report, we present the results of tryptic peptide analysis of the two molecular species, which showed that some peptides were common to them.

EXPERIMENTAL PROCEDURES

Chemicals-Dowex 50 x8 type PA-35 was obtained from Beckman Instrument Inc., Sephadex G-10 from Pharmacia, and TPCK-treated trypsin and silica gel plates (precoated, 10 x 20 cm, 0.25 mm thick) were from E. Merck AG., Darmstadt.

Preparation of interferon-L cell interferon was prepared as described previously (5), which was labeled by feeding [^{3}H] methionine to the NDV-induced culture. It was also labeled with [^{125}I] using chloramine T at a late stage of purification, and purified to an essentially pure state. The S and F interferons separated from each other by gel filtration were shown to have less than 5% cross-contaminations as analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Peptide analysis (8)-The interferon preparation having 5,000 to 10,000 cpm of [^{125}I]-radioactivity (2.22 x 10^{7} cpm/mg protein)was precipitated by 10% ice cold trichloroacetic acid with 1 mg bovine plasma albumin as carrier, and washed with cold acetone followed by ether. It was oxidized with performic acid (5% H_2O_2 (30%) in formic acid) at 0°C for 1 h, lyophilized, suspended in 5 ml of redistilled water, and digested with 100 µg of TPCK-trypsin for 2 h at 37°C, then for 4 h with freshly added 50 µg of TPCK-trypsin. During digestion, the pH was maintained at about 8 by adding NaOH, and after the 6 h incubation, the pH became almost constant. Formic acid was added to pH 2.5, and after low speed centrifugation, the supernatant was applied onto a Dowex 50 column (1.0 x 27 cm) equilibrated with 0.2 M pyridine-acetate buffer (pH 2.74). The column was washed with 0.2 M pyridine-acetate buffer (pH 3.01) and eluted with a convex gradient of pyridine-acetate from 0.2 M, pH 3.01 (138 ml in a mixing chamber)to 2 M, pH 5.15 (262 ml in a reservoir), and further eluted with 4 M pyridine-acetate buffer (pH 5.60). Elution was performed at 55°C at a flow rate of about 25 ml/h with an applied pressure of 5 Kg/cm^{2}, and fractions of 120 drops (ca. 2 ml) were collected.

Sephadex G-10 column chromatography-Flow-through fractions of the Dowex 50 column were lyophilized, redissolved in 1 ml of an elution buffer (10 mM phosphate buffer at pH 7.1, 0.5 M NaCl) containing Blue dextran 2000 and tyrosine as markers, and chromatographed on a Sephadex G-10 column (0.9 x 76 cm) at a flow-rate of 8 ml/h. Fractions of 50 drops (1.28 ml) were collected.

Silica gel thin layer chromatography-Peak fractions of the Sephadex G-10 column eluate were collected, concentrated by evaporation, and desalted by a Sephadex G-10 column. They were developed, together with methionine added as a marker, on silica gel thin layer plates with methanol:acetic acid:water (350:6:144) at room temperature. The position of methionine was detected by ninhydrin reaction, and the radioactivity was measured on silica gel scraped from the plate in 5 mm width.

RESULTS

As shown in Fig. 1, the tryptic digest of S interferon yielded, upon chromatography on a Dowex 50 column, five [^{125}I]-labeled peptides in the pH gradient, besides the one in flow-through fractions at pH 2.74, whereas seven

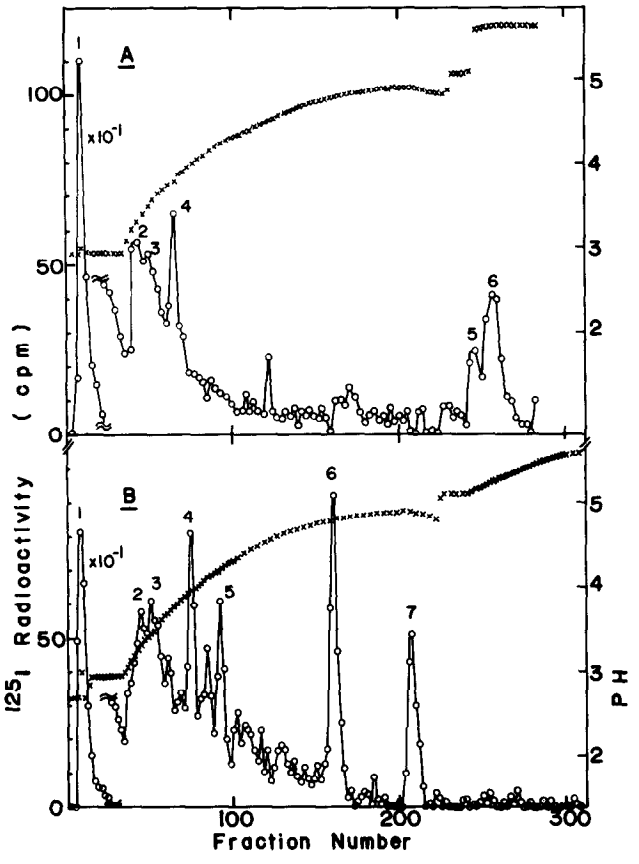

Fig. 1 Chromatograms of tryptic digests of (A) S and (B) F interferons on a Dowex 50 column: [^{125}I]-labeled peptides. Interferons were treated with trichloroacetic acid and performic acid followed by TPCK-trypsin, and the digests chromatographed as described in Experimental Procedures.
———O———, [^{125}I]-radioactivity. -----X-----, pH.

peptide peaks were observed in the case of F interferon. The number of the radioactive peaks varied to some extent from experiment to experiment due to minor peaks, but those peaks mentioned above were observed reproducibly. Comparing the two chromatograms, some of the peptides were clearly different from each other in the elution positions, but peaks 1, 2, and 3 of the two interferons were at the same positions, as judged by the pH values (precise to 0.01 unit). Moreover, the flow-through fractions, peak 1, both from S and F eluted at the void volume on Sephadex G-10 column chromatography (data not shown).

When the same digests of the S and F interferons were examined for [^{3}H] methionine-containing peptides on the Dowex 50 column, the chromatograms shown in Fig. 2 were obtained. Both yielded two peptides, numbered 3 and 4, in the pH gradient fractions. Peak 4 of the two interferons were at an identical position.

The flow-through fractions were chromatographed on a Sephadex G-10 column. As shown in Fig. 3, each showed two [^{3}H] methionine-containing fragments, 1 and 2, of apparently the same molecular sizes. To further substantiate their identity, the four peptides were separately recovered and chromatographed on silica gel thin layer plates with methionine added as a marker. As shown in Fig. 4, the peak 1 peptides from both S and F interferons migrated to the same position, and the same was true for the peak 2 peptides.

DISCUSSION

Tryptic digestion thus gave rise to 4 methionine-containing and 6 [^{125}I]-labeled (presumably tyrosine-containing)peptides from S interferon (40,000 daltons), and 4 methionine-containing and 7 [^{125}I]-labeled ones from F (24,000 daltons). Although the amino acid composition of the interferon is not known, the number of the fragments observed would appear reasonable considering their molecular weights and the average contents of methionine and tyrosine among proteins (9).

The exact numbers of the tryptic peptides are yet to be determined, and the heterogeneity of each interferon must be further investigated, but what we want to stress in this report is that the S and F interferons showed several common tryptic fragments, 3 methionine-containing and 3 [^{125}I]-labeled ones. That is, they share some common polypeptide structure. It is unlikely, however, that they are in a monomer-dimer relationship (10), because the larger molecule could not be dissociated into the smaller one by treatment with hot sodium dodecyl sulfate even under reducing conditions (3, 4), and also because there are some clear differences in their tryptic peptide patterns.

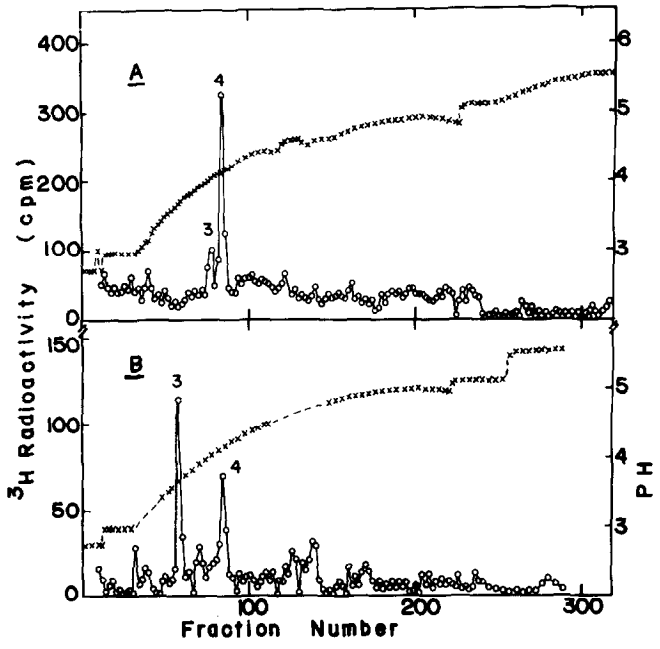

Fig. 2 Chromatograms of tryptic digests of (A) S and (B) F interferons on a Dowex 50 column: [^{3}H] methionine-containing peptides. Chromatography was carried out as described in the legend to Fig. 1.

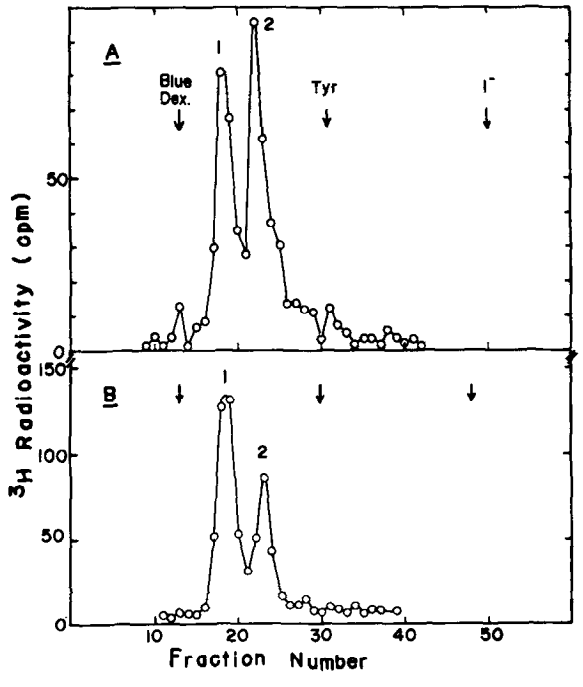

Fig. 3 Sephadex G-10 column chromatography of [^{3}H] methionine-containing peptides in the flow-through fractions from the Dowex 50 column (pH 2.74, Fig. 2). A, S interferon. B, F interferon.

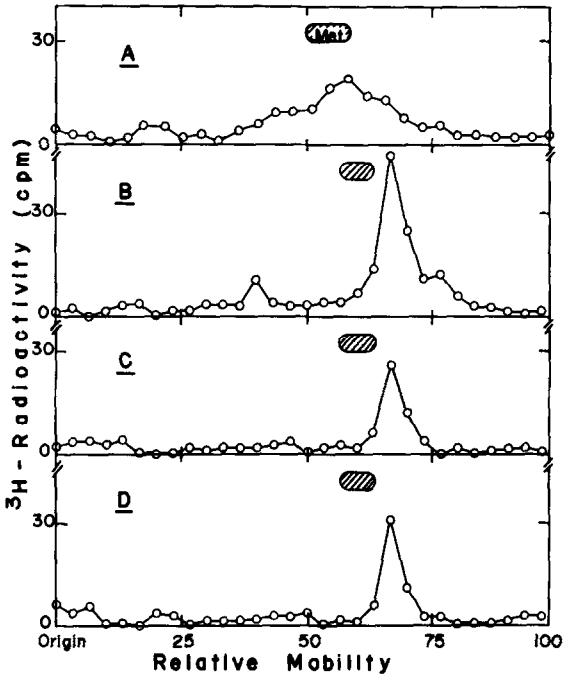

Fig. 4 Silica gel thin layer chromatography of the [^{3}H] methionine-containing peptides. Each of the peak fractions of the Sephadex G-10 column chromatography in Fig. 3 was collected separately, and after desalting, developed on silica gel thin layer plates. Methionine was added as the internal marker and detected by ninhydrin reaction. A, Peak 1 peptide of the S interferon digest. B, Peak 1 peptide of the F interferon digest. C, Peak 2 peptide of the S. D, Peak 2 peptide of the F.

Several ways of explanation may be conceived for the size heterogeneity of interferon molecules which contain some common polypeptide structure. First, these molecules may be coded by different genes containing partially identical structure, which could possibly be derived by gene duplication from an ancestral gene, or produced by a mechanism similar to the generation of antibody genes. No experimental evidence is available to support or deny this. Second, the heterogeneity may reside in the carbohydrate moiety. There is evidence indicating the microheterogeneity of the carbohydrate in at least some interferon preparations (11, 12), and the apparent molecular weights of human interferons were reduced when their carbohydrate contents were diminished artificially (13, 14). Third, the heterogeneous molecules may be produced

by proteolytic cleavage of a large molecule, as suggested by Chadha et al.(15) for human leukocyte interferons.

Theoretically, these possibilities are not mutually exclusive, but the first one seems unlikely to us, because we recently observed that the S interferon could be converted to smaller molecules similar in size to the F interferon in vitro (Yonehara et al., manuscript in preparation). Investigations are in progress to distinguish the last two possibilities.

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