Antibodies to Synthetic Peptides of Human Interferon-β
USE IN BIOSYNTHETIC STUDIES*

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Two peptides from the amino terminus of human interferon-β were synthesized corresponding to amino acids 1–21 and 18–45. The peptides were conjugated to bovine serum albumin, and rabbits were immunized with either the (1–21)- or the (18–45)-peptide conjugate. Antibodies to the synthetic peptides were detected in the sera using a radioimmunoassay with 125I-labeled peptide. Two of the antisera, one against peptide 1–21 and one against peptide 18–45, immunoprecipitated [35S]interferon-β. The former was used to study the biosynthesis of interferon-β in human diploid fibroblasts. In cells induced with double-stranded RNA when induced cells were treated with the glycosylation inhibitor tunicamycin. Neither the antibody to peptide 1–21, the antibody to peptide 18–45, nor a combination of both antibodies neutralized the interferon-β antiviral activity. We conclude that the amino terminus of interferon-β may not be involved in the binding of interferon-β to its receptor.

Antibodies to synthetic peptides that recognize the full-length parent protein have become useful tools in the study of macromolecular processes (see Ref. 1 for review). Synthetic peptides can be chosen from amino acid sequences determined from proteins, or they can be chosen from an amino acid sequence which has been predicted from DNA sequence. In the latter case, the actual protein may not have been observed or isolated. By this technique, previously unknown proteins have been partially characterized using antibodies to synthetic peptides (2). The advantages of synthetic peptides as antigens are 1) that it is not necessary to have the full-length protein as an antigen, and 2) that essentially unlimited amounts of the antigen in the form of the synthetic peptide are available.

Antibodies have been prepared to a synthetic peptide of human interferon-β, and these antibodies were subsequently used to purify natural IFN-β (3). A monoclonal antibody has been made to a synthetic peptide of a human IFN-α and was used to study the binding of IFN-α to cells (4). In this report, we show that antibodies produced in rabbits to two synthetic peptides of IFN-β will immunoprecipitate but not neutralize native IFN-β. These antibodies were used to study the biosynthesis and processing of IFN-β in human diploid fibroblast cells.

EXPERIMENTAL PROCEDURES

Materials—[35S]Methionine (>400 Ci/mmol) and 125I (17 Ci/mg) were purchased from New England Nuclear. Formaline-fixed Staphylococcus aureus cells (Immuno-Precipitin) was purchased from Bethesda Research Laboratories. Eagle's minimal essential medium and fetal calf serum were purchased from GIBCO. Peptides were purchased from Peninsula Laboratories, Inc., San Carlos, CA. Crude IFN-β labeled with [35S]methionine was prepared as previously described (5).

Peptide Synthesis, Labeling, and Immunizations—The complete amino acid sequence of IFN-β containing 166 amino acids has been previously reported (6). Synthetic peptides with amino acid sequences 1–21 and 18–45 at the N terminus were chosen for preparation of antibodies. The N-terminal peptide, 1–21, contained the sequence MSYNLLFGLQRSSNFQHQKLL and the (18–45)-peptide contained the sequence QKLLWQLNGRLYCLDRMNFDPDIEEK. (1–21)-Peptide was purchased from Peninsula Laboratories, Inc. and contained a single major peak upon reverse-phase HPLC (95% of UV peaks). The peptide of sequence 18–45 contained an acetamido-methyl group blocking its cysteine sulfhydryl. It was purchased as a crude mixture of peptides which was further purified by size-exclusion chromatography (Bio-Gel P-6, 10% aqueous acetic acid) to give a product which contained one major peak (40% of UV-absorbing material) upon reverse-phase HPLC. Immunizations were performed with this material after conjugation to bovine serum albumin as described below. For purposes of testing antisera, the peptide of sequence 18–45 was further purified by partition chromatography using Sephadex G-25F and the two-phase system: butanol/acetate acid/water (4:1:4, v/v/v). Typically, 50 mg of crude peptide were charged onto a column (2.5 × 70 cm) and the peptide appeared as a single symmetrical peak with Rf = 0.3 and a yield of 8.25 mg. The product gave an amino acid analysis which was consistent with its structure and showed a single major peak upon reverse-phase HPLC. Peptides were coupled to bovine serum albumin with glutaraldehyde as follows. Ten mg of peptide were mixed with 5 mg of bovine serum albumin in 2 ml of 0.02 M potassium phosphate, pH 7.0; 1 ml of a 10% solution of glutaraldehyde was added dropwise over a 10-min period, and the mixture was stirred for 2 h at 25 °C. After conjugation, the peptide-BSA conjugates were dialyzed versus phosphate-buffered saline at 4 °C to remove unconjugated peptide. Peptides were radioiodinated with 125I by the chloramine-T method (7). [125I]-Peptides were used to monitor the conjugation and to screen antisera in a radioimmunoassay. Conjugation of initial peptide to BSA varied from 25 to 50%. Nine New Zealand White rabbits were immunized with the (1–21)-conjugate and seven rabbits with the (18–45)-conjugate. Each rabbit was immunized with 100 µg of peptide-BSA conjugate in complete Freund's adjuvant every 2 weeks. Bleedings were done prior to immunization but only once every 4 weeks.

Radioimmunoassay for Antibodies to Peptides—Antibody-peptide complexes were precipitated with the aid of Immuno-Precipitin. Anti-
sera or dilutions thereof were mixed with 20,000 cpm of \(^{125}\)I-peptide in 0.3 ml of phosphate-buffered saline containing 10 mg/ml BSA and incubated for 4 h at 37 °C. Immuno-Precipitin, 0.1 ml of a 10% solution, was added and incubated for 30 min at 37 °C. The Immuno-Precipitin, with the antigen-antibody complex bound, was collected by centrifugation and washed four times with phosphate-buffered saline. After the final wash, the pellets were counted in a \(\gamma\) counter and compared to the \(^{125}\)I-peptide precipitated by the preimmune serum.

Neutralization of IFN-β—Peptide antisera, diluted or undiluted (5-20 \(\mu\)l) were added to 100 \(\mu\)l of cell growth medium containing 4 units/ml IFN-β. The mixtures were incubated for 1 h at 37 °C, and then IFN-β was assayed as previously described (8). Polyclonal antibodies to native IFN-β were a gift of A. Billau, Riga Institute, Leuven, Belgium. This antibody had a titer of 1:125,000 as determined by the procedure described above.

Labeling of Cellular Proteins with \(^{35}\)S/Methionine and Immunoprecipitation—Human diploid fibroblast cells were grown in Eagle’s minimal essential medium supplemented with 7% fetal calf serum at 37 °C in a 55% air, 5% CO\(_2\) humidified atmosphere. Cells, 14 days old, were superinduced with ribo-poly(I):poly(C) to synthesize IFN-β as previously described (5). After the 6-h superinduction process, the induced cells (1-2 \(\times\) 10\(^6\) cells/culture dish) were washed three times with Earle’s balanced salt solution. One ml of methionine-free medium containing \(^{35}\)S/methionine was added to each reaction and left for 1 h at 4 °C. After the final wash, the pellets were resuspended in 100 \(\mu\)l of cell growth medium containing 4 units/ml IFN-β. The mixtures were incubated at 37 °C. At designated times, the medium was removed and retained for immunoprecipitation and assay of the secreted \(^{35}\)S]IFN-β. Interferon was assayed on human diploid fibroblast cells as previously described (8). Cells were lysed on ice in 0.5 ml/dish of a buffer of 0.02 M Tris-HCl, pH 7.2, 0.15 M NaCl, 0.5% deoxycholate, 1% Nonidet P-40, 0.1% SDS, 1% β-mercaptoethanol. Cell lysates were heated at 100 °C for 2 min and then centrifuged for 10 min at 15,000 \(\times\) g. Antiserum (1-10 \(\mu\)l) was added to each supernatant and to each corresponding exocellular medium for incubation at 4 °C for 16 h. Precipitation of the antigen-antibody complex was accomplished by the addition of Immuno-Precipitin. One-hundred \(\mu\)l of a 10% solution of Immuno-Precipitin was added to each reaction and left for 1 h at 4 °C. The Immuno-Precipitin was collected by centrifugation, and the pellets were washed four times with a buffer of phosphate-buffered saline, pH 8.6, 0.5% Nonidet P-40, 0.1% SDS, 0.02% sodium azide, 1 mg/ml BSA. The pellets after the final wash were resuspended in 30 \(\mu\)l of gel loading buffer containing 1% β-mercaptoethanol and heated at 100 °C for 2 min. The supernatants after centrifugation were loaded onto gels for SDS-PAGE analysis of the immunoprecipitated proteins.

**RESULTS**

Antibodies to the synthetic peptides of IFN-β were detected in the serum by a radioimmunoassay using \(^{125}\)I-labeled pep-

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**FIG. 1. Antibodies to synthetic peptides of IFN-β.**

A, antibodies to (1-21)-peptide at the fourth bleeding as determined by binding to \(^{125}\)I-(1-21)-peptide. B, antibodies to (18-45)-peptide at the fourth bleeding as determined by the binding to the antibody to \(^{125}\)I-(18-45)-peptide. Open bars, preimmune serum; hatched bars, immune serum. C, immunoprecipitation of \(^{35}\)S]IFN-β by antibodies to (1-21)-peptide. Five \(\mu\)l of each antiserum were used as described under “Experimental Procedures.” Lanes 2, 4, 6, 8, 10, 12, 14, 16, and 18 are preimmune serum for rabbits A, B, C, D, E, G, H, I, and J, respectively. Lanes 1, 3, 5, 7, 9, 11, 13, 15, 17 are the sixth bleed immune serum from the respective rabbits. D, immunoprecipitation of \(^{35}\)S]IFN-β by antibodies to (18-45)-peptide. Lanes 2, 4, 7, 9, 11 14, and 16 are preimmune serum for rabbits K, L, M, O, P, and Q, respectively. Lanes 1, 3, 5, 6, 8, 10, 13, and 15 are the sixth bleed immune serum from the respective rabbits. Lane 5, immune serum from rabbit B, sixth bleed. Lane 12, immune serum from rabbit B, eighth bleed.
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tides and Immuno-Precipitin to precipitate the antibody-[35S]-peptide complex. Fig. 1A shows the amount of [35S]-(1-21)-peptide bound to antibody in the serum of nine rabbits. All nine rabbits made some antibody detectable above the preimmune serum. Maximum antibody was obtained at the fourth, fifth, and sixth bleedings and declined thereafter. Fig. 1B shows the amount of [35S]-(18-45)-peptide bound to antibody in the serum of seven rabbits. All seven rabbits made detectable antibody above the preimmune serum. Antibodies from all the rabbits were tested for immunoprecipitation of [35S]IFN-β (Fig. 1, C and D). Antisera from rabbits immunized with (1-21)- and (18-45)-peptides, respectively. Antibodies from rabbits B and O had the highest titer for the immunoprecipitation of [35S]IFN-β and were used in all further experiments. A high antibody titer against the peptide did not necessarily indicate a high titer for immunoprecipitation, since some antisera showed substantial binding of peptide but would not immunoprecipitate [35S]IFN-β. Precipitated proteins were analyzed on SDS-PAGE, and Fig. 2A shows the immunoprecipitation of IFN-β by antisera from the rabbits immunized with (1-21)- and (18-45)-peptides, respectively. Antibodies from rabbits B and O had the highest titer for the immunoprecipitation of [35S]IFN-β and were used in all further experiments. Therefore, we decided to use it to study the intracellular biosynthesis and exocellular secretion of the IFN-β. Antibodies have been used in studying the intracellular biosynthesis of a number of polypeptide hormones, and pro and prepro intracellular forms of these hormones have been detected (see Ref. 10 for review). Therefore, our initial experiments were directed toward the immunoprecipitation of intracellular [35S]IFN-β from human diploid fibroblast cells that had been induced by poly(I:C) to synthesize IFN-β. Immunoprecipitations were performed with cell lysates and with the exocellular medium after various periods of labeling with [35S]methionine. Fig. 3A shows the precipitation of the secreted IFN-β after labeling for 40, 60, and 80 min. (lanes 10-12, respectively). IFN-β can be detected in the medium after 40 min of labeling (Fig. 3A, lane 10), and its concentration increases thereafter (Fig. 3A, lanes 11 and 12).

![Fig. 2. Immunoprecipitation of [35S]IFN-β by antibodies to synthetic (1-21)- and (18-45)-peptides.](image)

A. precipitation of [35S]IFN-β by antibody to (1-21)-peptide. SDS-PAGE of immunoprecipitates versus bleeding serum proteins. Ten μl of antisera 5 × 10^-5 nmol of [35S]IFN-β were used for each immunoprecipitation. Lane 1, preimmune serum; lanes 2-9 contain immunoprecipitated proteins by antisera at bleedings 1-8, respectively. B. precipitation of [35S]IFN-β by antibody to (18-45)-peptide. Ten μl of each antisera were used. Lane 1, preimmune serum; lanes 2-7 contain immunoprecipitated proteins by antisera at bleedings 1-6, respectively. C. inhibition of the precipitation of [35S]IFN-β by (1-21)-peptide and IFN-β. Unlabeled peptide or IFN-β was added to 10 μl of antisera B, bleeding 5, 1 h prior to the addition of [35S]IFN-β (5 × 10^-6 nmol). Lane 1, no unlabeled peptide or IFN-β; lanes 2 and 3, 0.5 and 5.0 nmol of (1-21)-peptide, respectively; lanes 4-6, 2.5 × 10^-7, 1.25 × 10^-7, and 2.5 × 10^-7 nmol of IFN-β, respectively.
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A

MW $\times 10^{-3}$

104

MW $\times 10^{-3}$

102

MW $\times 10^{-3}$

101

MW $\times 10^{-3}$

100

MW $\times 10^{-3}$

10

IFN-β

18,000

30,000

GEL FRACTION

FIG. 3. Immunoprecipitation of intracellular and exocellular [35S]IFN-β. Human diploid fibroblasts were induced with poly(I:C) and labeled with [35S]methionine as described under "Experimental Procedures." Immunoprecipitations were performed with 10 μl of antiserum added to either the lysate from 1-2 $\times 10^6$ cells or the 1 ml of medium surrounding the same cells. A, labeling of intra- and exocellular IFN-β at 40, 60, and 80 min. Lanes 1-3, cell lysates of uninduced cells; lanes 4-6, cell lysates of induced cells; lanes 7-9, medium of uninduced cells; lanes 10-12, medium of induced cells. B, antiviral activity of crude IFN-β analyzed by SDS-PAGE. Five ml of crude IFN-β were concentrated, subjected to electrophoresis, and eluted from gel slices as previously described (12). C, labeling of intra- and exocellular IFN-β at 10, 20, and 30 min. Lanes 1-3, cell lysates of induced cells; lanes 4-6, medium of induced cells.

specific for IFN-β since no [35S]IFN-β is precipitated from the medium of uninduced cells. Intracellular IFN-β can also be detected at 40, 60, and 80 min (Fig. 3A, lanes 4-6; respectively). In addition, a protein of estimated molecular weight of 18,000 is precipitated from both cell lysates and the exocellular medium of induced cells (Fig. 3A, lanes 4-6 and 10-12). This protein has a similar molecular weight as that reported previously for deglycosylated IFN-β (11) and is similar to a molecular weight of 19,600 calculated from the amino acid content of recombinant IFN-β (6). In order to detect the 18,000-dalton protein, significant amounts of the native $M_\text{r} = 23,000$ IFN-β must be produced and precipitated. When only small amounts of the $M_\text{r} = 23,000$ IFN-β are precipitated (Fig. 3A, lane 10, and Fig. 2A), the 18,000-dalton protein is not observed.

A protein of 10,000 daltons is also precipitated from the medium of induced cells (Fig. 3A, lanes 10-12) but not from the medium of uninduced cells (Fig. 3A, lanes 7-9). As with the 18,000-dalton protein, the 10,000-dalton component is not detected unless significant amounts of the $M_\text{r} = 23,000$ IFN-β are precipitated. Although there is a similar size protein precipitated from induced cell lysates, it is also precipitated from lysates of uninduced cells (Fig. 3A, lanes 1-3). This 10,000-dalton protein in the medium of induced cells may be a degradation product of IFN-β.

It was of interest that unglycosylated IFN-β may be secreted by induced cells since a protein of approximately 18,000 daltons is detected in the exocellular medium (Fig. 3A). We, therefore, performed an experiment to determine if crude IFN-β contained a protein with interferon activity which migrated on SDS-PAGE at a faster rate than the normal IFN-β of 23,000 daltons. Fig. 3B shows that a small peak of
interferon activity can be detected migrating faster than the normal IFN-β. This activity, migrating coincident with the precipitated 18,000-dalton protein, represents only 0.5% of the recovered interferon activity and may be unglycosylated IFN-β (11). We detected no interferon activity migrating faster than the 18,000-dalton component.

The next experiments were directed at determining the briefest labeling period in which IFN-β could be detected in cell lysates and in the exocellular medium. The data in Fig. 3C show that IFN-β can be detected in cell lysates in a labeling period as brief as 10 min. It can also be seen that the IFN-β continues to increase in cell lysates after 20 and 30 min of labeling (Fig. 3C, lanes 1–3). In the medium, however, virtually no IFN-β was detected until 40 min of labeling (Fig. 3C, lanes 4–6). This period of time is similar to that required for the synthesis and transport of other polypeptide hormones (10). Although intracellular IFN-β is detected prior to exocellular IFN-β, 10 min versus 40 min, this does not prove that the one is a product of the other. To further establish a precursor-product relationship kinetically, we labeled IFN-β in induced cells with [35S]methionine for 10, 20, and 30 min and then performed a 30-min chase with unlabeled methionine. The data in Fig. 4 show that [35S]IFN-β is immunoprecipitated from extracts of induced cells after labeling periods of 10, 20, and 30 min. A small amount of [35S]IFN-β can be detected outside the cell with a 30-min label, consistent with previous experiments. When cells from 10-, 20-, and 30-min labeling periods are chased for 30 min, intracellular interferon completely disappears, while the exocellular IFN-β increases (Fig. 4A). These results are shown graphically in Fig. 4B.

Since IFN-β is a glycoprotein, it was of interest to determine the effect of an inhibitor of glycosylation such as tunicamycin on the intracellular and exocellular components precipitated by the antibody. The data presented in Fig. 5 show that the antibody precipitates primarily a component of 18,000 daltons from lysates and medium of induced cells treated with tunicamycin. This protein is presumed to be the unglycosylated form of IFN-β. A small amount of the glycosylated IFN-β is precipitated from cell lysates and from the medium, indicating that the tunicamycin does not completely inhibit the glycosylation. As shown earlier with interferon activity (14), most of the unglycosylated IFN-β generated by tunicamycin does not exit the cell.

Since antisera to (1-21)- and (18-45)-peptides immunoprecipitated IFN-β, it was of interest to determine if these antisera individually or in combination neutralized IFN-β activity either individually or collectively. The antisera were tested for neutralization of small amounts of IFN-β, and the results of these tests are shown in Table I. Neither of the antisera individually nor in various combinations neutralized the IFN-β. It is unlikely that a low neutralizing activity was missed since relatively large amounts of undiluted antisera were used (Table I). We conclude that although these two antisera bind to the N

![FIG. 4. Pulse-chase experiments to measure intracellular and exocellular IFN-β.](image)

**Fig. 4.** Pulse-chase experiments to measure intracellular and exocellular IFN-β. Diploid fibroblast cells were induced with poly(I:C) to synthesize IFN-β as described under "Experimental Procedures." A, induced cells were labeled with [35S]methionine for 10, 20, and 30 min, and the intracellular and exocellular IFN-β was determined by immunoprecipitation. One 60-mm dish of cells was used for each time period. An identical set of induced cells were labeled for either 10, 20, or 30 min, washed three times with minimal essential medium containing methionine, and then all cells were incubated a further 30 min (chase) at 37 °C before immunoprecipitation of intracellular and exocellular IFN-β. B, amounts of intracellular IFN-β after a 10-, 20-, and 30-min label (O) and exocellular IFN-β after a 10-, 20-, and 30-min label followed by a 30-min chase (Q). IFN-β bands were scanned with a Joyce-Loebl densitometer, and relative absorbance was plotted versus time.

**Fig. 5.** Effect of tunicamycin on the synthesis of IFN-β. Lanes 1–4, cell lysates of induced cells after 10, 20, 30, and 40 min of labeling, respectively; lanes 5–8, medium of induced cells. The experiments were performed as described under "Experimental Procedure" and in the legend to Fig. 3. Tunicamycin was added to the medium at 1 µg/ml concurrent with the addition of actinomycin D in the superinduction procedure and maintained at 1 µg/ml during the labeling period.

**TABLE I**

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<th>Conc IFN-β added</th>
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*Undiluted antiserum.
Antiserum diluted 100-fold.
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DISCUSSION

Earlier studies on the biosynthesis of human IFN-β showed that antiviral activity could be detected inside the cell (13) and that tunicamycin inhibited the appearance of exocellular IFN-β with a concomitant increase of IFN-β within the cell (14). In these experiments, we have used an antibody to a synthetic peptide of IFN-β to study intra- and exocellular IFN-β induced in human diploid fibroblasts by poly(I:C). We have concluded the following from our experiments. 1) Antibodies raised to synthetic peptides corresponding to sequences at the N terminus of IFN-β immunoprecipitate native IFN-β but do not neutralize its antiviral activity. 2) The antibody to (1–21)-peptide precipitates a protein of \( M_r = 23,000 \) from cell lysates and the exocellular medium. We conclude that this protein is native IFN-β. 3) Intracellular IFN-β can be detected approximately 30 min before IFN-β appears in the medium. The intracellular IFN-β can be chased into the exocellular medium, suggesting that the former is a precursor of the latter. 4) The antibody precipitates a protein of 18,000 daltons from cell lysates and from the exocellular medium. This protein is presumed to be unglycosylated IFN-β since its electrophoretic mobility is similar to deglycosylated native IFN-β (11) and recombinant IFN-β (6); furthermore, its concentration is increased inside the cell by the inhibitor of glycosylation, tunicamycin.

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REFERENCES