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Biochemistry

Modification and secretion of human interleukin 2 produced in insect cells by a baculovirus expression vector

(lymphokine/signal peptide/glycosylation/eukaryotic expression/*Autographa californica* nuclear polyhedrosis virus)

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ABSTRACT **A cDNA coding for human interleukin 2 (IL-2) was inserted into the genome of *Autographa californica* nuclear polyhedrosis virus adjacent to the polyhedrin promoter. Cells infected with recombinant virus produced high levels of M_r 15,500 IL-2 polypeptide, the majority of which was secreted into the culture medium during infection. The recombinant IL-2 was able to stimulate the growth of an IL-2-dependent cell line. The N-terminal amino acid sequence of the insect-derived IL-2 was identical to that of natural IL-2. Thus, a mammalian signal peptide was recognized and properly removed in insect cells.**

A variety of prokaryotic and eukaryotic host/vector systems are available for the production of large quantities of proteins from cloned genes. For many applications, such as structural analysis, determination of biological specificity, vaccine production, diagnostics, and therapeutics, it may be desirable or even necessary that a recombinant gene product be identical to the natural protein made *in vivo*. Therefore, not only must a cloned gene be transcribed faithfully and the message translated, but the product must be properly modified, through such processes as glycosylation, cleavage of a signal peptide, disulfide-bridge formation, and proper folding. The development of host/vector systems that will process and modify recombinant proteins, in addition to producing them at high levels, is thus an important prerequisite for the production of such proteins.

Recently, the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) was shown to be suitable as a helper-independent, viral expression vector for the efficient production in cultured insect cells of proteins from cloned genes. Human fibroblast interferon (IFN-β; ref. 1) and a fusion protein between AcNPV polyhedrin and *Escherichia coli* β-galactosidase (2) are expressed at very high levels in cells infected with AcNPV recombinant viruses. More important, IFN-β is glycosylated by an N-linked glycan, the pre-IFN-β signal polypeptide is removed, and the protein is secreted efficiently (1). We are undertaking studies to determine how proteins from higher eukaryotes are processed and modified in insect cells infected with AcNPV expression vectors. Such studies will help to determine whether there are fundamental differences between invertebrate and vertebrate cellular mechanisms for protein modification and will provide the information needed to define the advantages and limitations for using baculoviruses as expression vectors.

Human interleukin 2 (IL-2), also referred to as T-cell growth factor, is a lymphokine required for the proliferation of activated T cells and the long-term *in vitro* growth of certain T-cell lines (3, 4). The primary structure of the human IL-2 gene product has been deduced from nucleotide sequence analyses of cDNA (5, 6) and genomic (7–9) clones. IL-2 is first synthesized as a precursor polypeptide of 153 amino acids (5, 6). Taniguchi *et al.* (5) proposed that cleavage of the 20 amino acid signal peptide results in the production of the mature IL-2 protein, which is secreted into the extracellular fluid. cDNA clones have been used to express recombinant human IL-2 in *E. coli* (6, 10) and in transfected monkey cells (5).

Natural IL-2 has been purified to homogeneity from the human T-cell leukemia line Jurkat (11, 12). Amino acid sequencing of the Jurkat-derived material confirmed the primary structure of the protein, deduced from the cDNA sequence, and established the N-terminal residues after signal peptide cleavage (12, 13). IL-2 appears to undergo some posttranslational modifications that account for the heterogeneity observed in size and charge (14). Approximately 40% of Jurkat-derived IL-2 is not glycosylated (15). In contrast to earlier reports, the majority of the IL-2 synthesized by normal human peripheral blood lymphocytes appears to be glycosylated (13). Minor species of IL-2 that are truncated at the N terminus also exist (15).

Here we describe the production of IL-2 in insect cells, using AcNPV as an expression vector. IL-2 was efficiently produced and the majority of the protein was secreted from the infected cells as a nonglycosylated protein. Sequence analysis of the N-terminal end of the secreted IL-2 indicated that the 20 amino acid signal peptide was accurately removed.

EXPERIMENTAL PROCEDURE

Construction of Recombinant Viruses. The expression vectors used in this study are diagrammed in Fig. 1. The plasmid transfer vector pAc380 has a unique *Bam*HI site at the putative transcription initiation, or cap, site for AcNPV polyhedrin mRNA (1). A similar vector, pAc373, was produced as described (1) with a single *Bam*HI site 50 bp downstream from the polyhedrin cap site; that is, 8 bp before the polyhedrin ATG translation initiation codon. Genetic sequences inserted at the *Bam*HI site in pAc380 and that utilize the polyhedrin control signals will be transcribed without the polyhedrin leader sequences, whereas genes inserted at the *Bam*HI site in pAc373 will be transcribed as fused mRNAs containing most of the polyhedrin leader region.

The sequence coding for human IL-2 was obtained from a cDNA library of poly(A)$^+$ RNA from induced Jurkat cells. This library was screened with synthetic oligonucleotide probes derived from the published Jurkat IL-2 cDNA se-

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Abbreviations: AcNPV, *Autographa californica* nuclear polyhedrosis virus; IL-2, interleukin 2; bp, base pair(s); hpi, hr postinfection.

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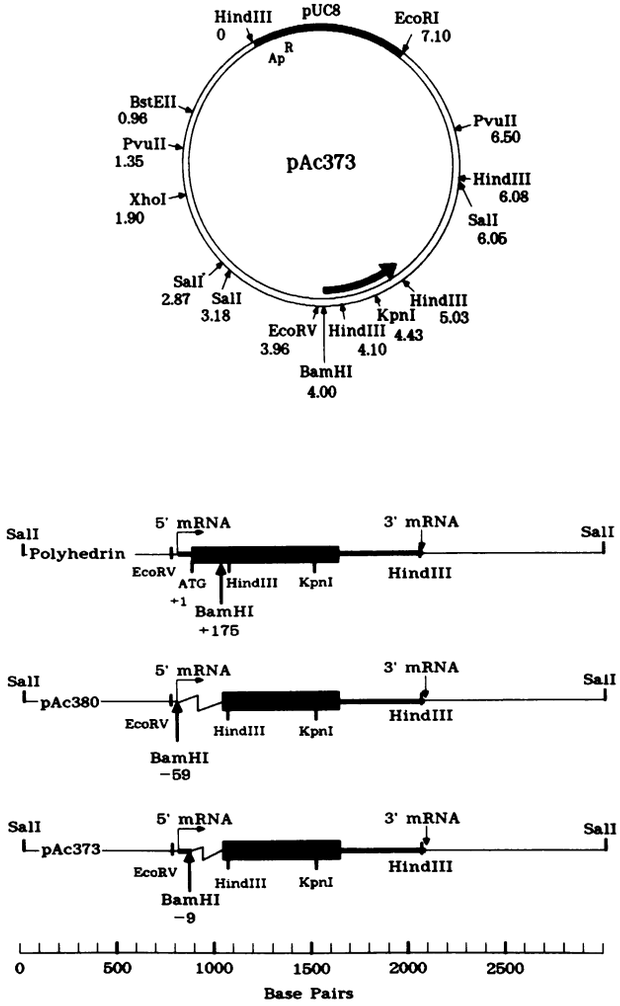

FIG. 1. AcNPV transfer vectors pAc373 and pAc380. The circular restriction map is of the AcNPV transfer vector pAc373. The thick arrow marks the location of the sequence coding for polyhedrin mRNA; numbers at restriction sites indicate distance in kilobase pairs (kbp) from the *Hin*dIII end of the pUC8 backbone; Ap^R, ampicillin resistance gene. The linear map is a detailed restriction map of the 3-kbp *Sal* I fragment containing the polyhedrin gene. The plasmid pAc380 has a deletion between −59 and +175 and a unique *Bam*HI site at −59, which is the cap site for polyhedrin mRNA (1). pAc373 has a deletion between −9 and +175 and a unique *Bam*HI site at −9, which is 50 bp downstream from the polyhedrin mRNA cap site (1).

quence (5). A plasmid cDNA clone that contained the entire coding region for human IL-2 (pIL2-2b) was isolated. This clone has been used to express active IL-2 in *E. coli* (unpublished work). To express IL-2 in insect cells, a 1-kbp *Bam*HI fragment from pIL-2B, which included 31 bp of 5′ nontranslated leader sequence, the entire IL-2 coding region, and 309 bp of 3′ nontranslated sequence, was excised and inserted into the *Bam*HI site of both pAc373 and pAc380. Recombinant plasmids containing inserts in the correct transcriptional orientation were designated pAc373-IL2 and pAc380-IL2.

Transfer of IL-2 cDNA from these plasmid vectors to the AcNPV genome was achieved by cotransfection, using the calcium phosphate precipitation procedure. *Spodoptera frugiperda* cells (10^6) were transfected with a mixture of 2 μg of pAc373-IL2 or pAc380-IL2 and 1 μg of purified AcNPV DNA as described (16). Three days after transfection, the medium had a virus titer of about 10^7 plaque forming units (pfu)/ml. Approximately 1% of the plaques from these viral progeny were formed from viruses that did not produce nuclear-viral occlusions (occlusion-negative viruses). Several of the occlusion-negative viruses were plaque-purified and, by means of restriction enzyme analysis, were found to have acquired the IL-2 gene fragment adjacent to the polyhedrin promoter. Recombinant viruses derived from pAc373-IL2 and pAc380-IL2 are referred to as Ac373-IL2 and Ac380-IL2, respectively.

Production of IL-2 in *S. frugiperda* Cells. AcNPV recombinant virus stocks were grown in *S. frugiperda* cells (IPLB-Sf21-AE) using TNMFH medium (17) plus 10% fetal bovine serum. To produce IL-2, *S. frugiperda* cells (10^7 per ml) were infected for 1 hr with Ac373-IL2 or Ac380-IL2 at 20 pfu per cell. The infected cells were washed once with TNMFH medium (no serum) and then resuspended in TNMFH medium plus serum at a cell density of 10^8 cells per ml. The infected cells were seeded in 100- to 500-ml spinner flasks (Bellco Glass) or in a 2-liter suspension cell fermentor (Biocul 20; Queue Systems) and incubated at 27°C.

Analysis of Proteins Produced by Recombinant Viruses. Infected *S. frugiperda* cells were labeled metabolically with either L-[^{35}S]methionine (New England Nuclear; 80 Ci/mmol; 1 Ci = 37 GBq) at 50 μCi/ml or D-[2-^{3}H]mannose (Amersham; 2 Ci/mmol), L-[6-^{3}H]fucose (ICN; 30 Ci/mmol), acetyl-D-[1,6-^{3}H]glucosamine (New England Nuclear; 33.1 Ci/mmol), or D-[6-^{3}H]galactose (ICN; 25 Ci/mmol) at 500 μCi/ml. Labeled proteins were analyzed by $NaDodSO_4$/12% PAGE. Infected cells were incubated with 5 μg of tunicamycin/ml and then labeled with 50 μCi of [^{35}S]methionine/ml in the presence of tunicamycin.

IL-2 mRNA was purified from the cytoplasm of infected cells by hybridization-selection with pIL2-2B plasmid DNA as described (18). The selected RNA was translated in a wheat germ extract (Bethesda Research Laboratories) or rabbit reticulocyte lysate prepared as described (19). In certain experiments, dog pancreas membranes (20) were included during translation in a reticulocyte lysate. Purified rotavirus mRNAs were included as a positive control for the protein-modification activity of membranes (20). Immunoblot analysis was performed as described (21), with minor modifications. The development of monoclonal anti-IL-2 antibodies will be described in detail elsewhere. Rabbit anti-IL-2 antisera was produced by multiple subdermal injections of recombinant human IL-2 (100 μg) mixed in complete Freund's adjuvant. The rabbits were bled 4 and 8 weeks later. Immunoprecipitation with the rabbit anti-IL-2 antisera was done as described (20).

Assay for IL-2 Activity. The IL-2 activity of the recombinant material was measured by using the IL-2-dependent murine cell line CTLL. The assay was performed in a manner similar to that described in ref. 22. One unit of activity was defined as the reciprocal of the dilution that yielded half-maximal incorporation of [*methyl*-^{3}H]thymidine.

Purification and Sequence Analysis of IL-2. IL-2 from insect cells was purified by a modification of the procedure described previously (11). The recombinant IL-2 in culture medium from Ac373-IL2-infected *S. frugiperda* cells was first concentrated by batch adsorption to silica gel under gentle agitation for 1 hr at 4°C. The gel was recovered, washed with 0.15 M NaCl/5 mM phosphate (pH 7.5), and packed into a chromatographic column. The IL-2 was specifically eluted with 0.05 M sodium borate, pH 8.0/1.5 M tetramethylammonium chloride and further purified by HPLC on a Synchropak RP-P column using an acetonitrile gradient buffer system. For final purification, the protein was loaded onto a Supercosil LC-308 column (20 × 4.6 mm, 5-μm particle size) in 10 mM trifluoroacetic acid and eluted with an acetonitrile gradient from 0 to 60% in 1 hr. Automated Edman degradation was carried out on a gas-vapor sequencer, and

the resulting phenylthiohydantoin amino acids were analyzed according to a method described by Hawke *et al.* (23).

RESULTS

Production of IL-2 Activity in Insect Cells. The major objectives of this study were to determine whether human IL-2 could be produced at high levels in insect cells using a baculovirus expression vector and whether the pre-IL-2 signal peptide would be correctly removed and the protein secreted from the infected cells. We also wished to examine the importance of the 5′ leader sequences of the AcNPV polyhedrin gene in the expression of foreign genes. The first step in cloning IL-2 in AcNPV expression vectors was to insert the human IL-2 coding sequence adjacent to the polyhedrin promoter in the specially constructed plasmid transfer vectors pAc373 and pAc380. A 1-kbp fragment containing a full-length IL-2 cDNA was inserted into the *Bam*HI site at −9 in pAc373 and at −59 in pAc380 (Fig. 1). The resulting chimeric transfer vectors pAc373-IL2 and pAc380-IL2 were mixed with AcNPV DNA and then co-precipitated with calcium phosphate. *S. frugiperda* cells were transfected and the resulting viral progeny were screened for the presence of recombinant viruses in which the polyhedrin gene had been inactivated by the insertion of the IL-2 gene fragment. These recombinants do not form occlusions in infected cells and thus have a distinctive plaque morphology (1, 16). Restriction analysis of DNA from several of these occlusion-negative viruses confirmed that IL-2 had been transferred to the AcNPV genome and was in the expected location relative to the polyhedrin promoter (data not shown).

Cells infected with Ac373-IL2 and Ac380-IL2 viruses secreted high levels of IL-2 activity into the culture medium (Fig. 2). Ac380-IL2, in which the IL-2 gene was fused to the polyhedrin promoter at a position that would exclude the polyhedrin 5′ leader sequences, produced about one-third the level of IL-2 activity produced in cells infected with Ac373-IL2. The IL-2 sequence in Ac373-IL2 was fused to the polyhedrin promoter at a position such that the hybrid gene would include the majority of the polyhedrin 5′ leader sequences. Cells infected with either of these vectors released IL-2 activity into the culture medium up to 72 hpi at which time the infected cells were still intact. In a separate experiment, the intracellular and extracellular levels of IL-2 were measured. At 56 hpi, approximately 67% and 72% of the activity was present in the media of cells infected with Ac373-IL2 and Ac380-IL2, respectively. Since the cells remained intact during infection and the majority of the IL-2 activity was in the medium, IL-2 was most likely being secreted, as opposed to being released as a result of cell lysis. Media samples from uninfected cells and cells infected with AcNPV had no detectable IL-2 activity.

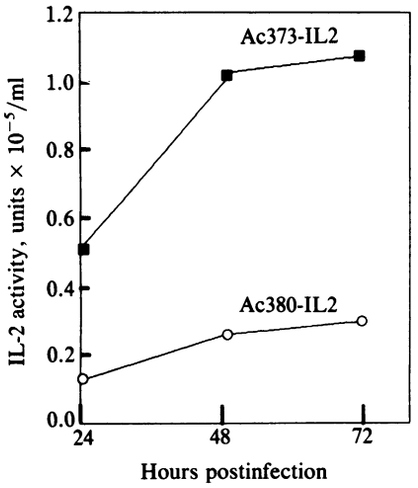

FIG. 2. Expression of IL-2 in Ac373-IL2- and Ac380-IL2-infected *S. frugiperda* cells. Cells were infected with Ac373-IL2 or Ac380-IL2 at a cell density of 10^7 cells per ml. The infected cells were diluted to 10^6 cells per ml in a final volume of 100 ml. At 24, 48, and 72 hr postinfection (hpi), samples were taken and the cells were removed by centrifugation. Cells and media were stored frozen until measured for IL-2 biological activity. The IL-2 activity in the media of pAc373-IL2- (■) and pAc380-IL2- (○) infected cells was determined as described (22).

Identification of IL-2 Produced in Infected Cells. In cells infected with AcNPV, polyhedrin was the major cellular protein at 48 and 72 hpi (Fig. 3, lanes 1 and 2). In cells infected with the recombinant virus Ac373-IL2, polyhedrin was not made and a new M_r 15,500 intracellular polypeptide accumulated (Fig. 3, lanes 3 and 4). The molecular weight of natural IL-2, as measured by NaDodSO$_4$/PAGE, is reported to be about 15,000 (12, 14). A M_r 15,500 protein was also secreted and by 72 hpi was one of the major polypeptides present in the medium (Fig. 3, lane 8). Accumulation of a protein of M_r 15,500 was observed in cells infected with Ac380-IL2, but at one-third the level in Ac373-IL2-infected cells (data not shown).

To identify the IL-2 polypeptides being made, Ac373-IL2-infected cells were incubated with [^{35}S]methionine at 36–39 hpi, and then the labeled proteins from the infected cells and the culture medium were immunoprecipitated with IL-2 antiserum. The M_r 15,500 polypeptide detected in stained gels was a major labeled protein in the infected cells (Fig. 4, lane 1) and in the culture medium (Fig. 4, lane 2), and this protein was immunoprecipitated with IL-2 antiserum (Fig. 4, lanes 3 and 4). In addition, an intracellular M_r 16,000 protein was

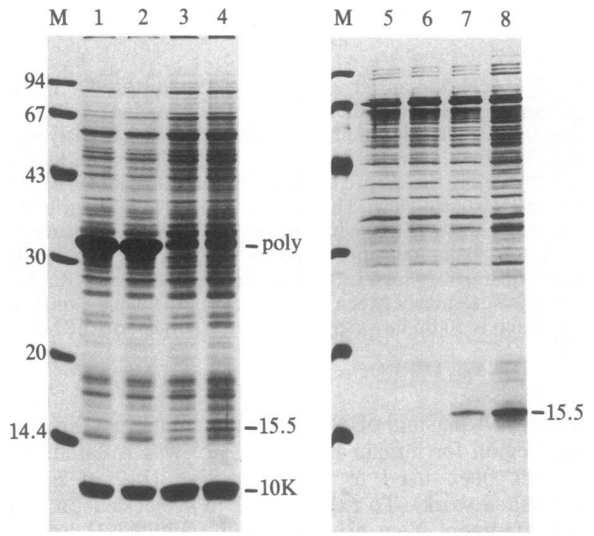

FIG. 3. IL-2 in the cells and medium of Ac373-IL2-infected *S. frugiperda* cells. Cells (3×10^6) were seeded in 25-cm^2 tissue culture flasks and infected for 1 hr with AcNPV or Ac373-IL2. The infected cell monolayers were washed twice with TNMFH medium and then incubated at 27°C in 3 ml of TNMFH medium. At 48 and 72 hpi, 5.0 $\times 10^4$ cells (lanes 1–4) and 50 μl of medium (lanes 5–8) were treated with sample buffer containing 2-mercaptoethanol and electrophoresed in a 12% NaDodSO$_4$/polyacrylamide gel; the gel was then stained with Coomassie blue. Proteins from AcNPV-infected cells at 48 hpi (lanes 1 and 5) and 72 hpi (lanes 2 and 6) and from Ac373-IL2-infected cells at 48 hpi (lanes 3 and 7) and 72 hpi (lanes 4 and 8) are shown. The locations of the highly expressed M_r 10,000 viral protein (10K) and of polyhedrin (poly) are indicated (lanes 1–4). Protein standards (lane M) and their molecular weights ($M_r \times 10^{-3}$, at left) are shown.

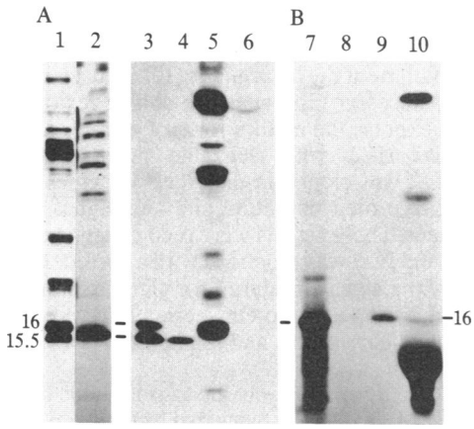

FIG. 4. Immunoprecipitation and cell-free translation of IL-2. *S. frugiperda* cells were infected with Ac373-IL2 as described in the legend to Fig. 3. (*A*) At 36 hpi, the infected cells either were washed once with methionine-free Grace's medium and then labeled for 3 hr with [^{35}S]methionine (50 μCi/ml) or were washed once with Grace's medium with 1/10th the normal amount of glucose and then labeled for 3 hr with D-[2-^{3}H]mannose (50 μCi/ml). Lanes 1 and 2: [^{35}S]methionine-labeled cellular and medium proteins, respectively. Lanes 3 and 4: [^{35}S]methionine-labeled cellular and medium proteins immunoprecipitated with anti-IL-2 antibody. Lane 5: [^{3}H]mannose-labeled cellular proteins. Lane 6: [^{3}H]mannose-labeled cellular proteins immunoprecipitated with anti-IL-2 antibody. (*B*) IL-2 mRNA was obtained by hybrid-selection and translated in a cell-free translation system with [^{35}S]methionine in the presence or absence of dog pancreas membranes. Lane 7: IL-2 mRNA translated in wheat germ extract. Lanes 8 and 10: IL-2 mRNA translated in a rabbit reticulocyte lysate in the absence (lane 8) or presence (lane 10) of membranes. Lane 9: Immunoprecipitate, obtained with anti-IL-2 antibody, of products of IL-2 mRNA translated in a reticulocyte lysate in the presence of membranes. All samples were electrophoresed in a NaDodSO$_4$/12% polyacrylamide gel. The locations of the M_r 15,500 IL-2 protein in lanes 1–4 and the M_r 16,000 IL-2 protein in lanes 1, 3, 7, 9, and 10 are indicated.

immunoprecipitated with IL-2 antiserum (Fig. 4, lane 3), but only very low levels were detected in the culture medium (Fig. 4, lane 4).

To investigate whether IL-2 produced in insect cells was glycosylated, Ac373-IL2-infected cells were labeled with tritiated D-mannose, acetyl-D-glucosamine, L-fucose, or D-galactose. Although each of these labeled sugars was incorporated into one or more infected cell proteins (data not shown), only D-[2-^{3}H]mannose labeled a polypeptide with a molecular weight similar to either of the two recombinant IL-2 proteins (Fig. 4, lane 5). However, this M_r 16,000 glycoprotein was found only in the infected cells and not in the medium and was not precipitated with IL-2 antiserum (Fig. 4, lane 6). In addition, when infected cells were labeled with [^{35}S]methionine in the presence or absence of the glycosylation inhibitor tunicamycin, the inhibitor had no effect on the size of either the M_r 15,500 or the M_r 16,000 IL-2 proteins (data not shown), indicating that they did not have N-linked carbohydrates. This finding is consistent with reports that the fraction of IL-2 produced in human Jurkat cells that is glycosylated contains no N-linked oligosaccharides (12, 13, 15). Overall, these results indicated that the insect-derived recombinant IL-2 was not glycosylated.

The M_r 16,000 IL-2 protein was somewhat larger than the major secreted form of recombinant IL-2 and was present primarily in the cells, suggesting that it might be pre-IL-2. To investigate this possibility, IL-2 mRNA was obtained from the cytoplasmic fraction of Ac373-IL2-infected cells translated *in vitro* in wheat germ and rabbit reticulocyte translation systems. A M_r 16,000 polypeptide was made in the wheat germ lysate (Fig. 4, lane 7), and a very low level of M_r 16,000 polypeptide was synthesized from IL-2 mRNA in the reticulocyte lysate (Fig. 4, lane 8). Because signal peptides are not cleaved in a wheat germ translation system (24), only pre-IL-2 would be made. Therefore, the apparent molecular weight of pre-IL-2 in our polyacrylamide gels was 16,000, the same molecular weight observed for the intracellular, nonsecreted IL-2 produced in Ac373-IL2-infected cells. The translational block of IL-2 mRNA in the reticulocyte lysate system was probably due to the binding and arrest of translation of newly synthesized IL-2 signal sequences by signal recognition particles, and the addition of dog pancreas membranes should supply the appropriate factors to overcome this block (see ref. 25 for review). With the addition of membranes to the reticulocyte lysate, a M_r 16,000 polypeptide was synthesized from hybrid-selected mRNA (Fig. 4, lane 10), and this protein was specifically immunoprecipitated with IL-2 antiserum (Fig. 4, lane 9). Together, these results indicate that the M_r 16,000 recombinant protein is pre-IL2. Because this protein did not accumulate in the cells during infection (Fig. 3), there was insufficient quantity available for purification and sequence analysis to confirm this hypothesis.

Purification and N-Terminal Sequence Analysis of Recombinant IL-2. Recombinant IL-2 was produced in serum-free medium in a 2-liter suspension-culture fermentor containing Ac373-IL2-infected *S. frugiperda* cells. IL-2 was partially purified from about 200 ml of culture medium by batch adsorption to silica gel and reversed-phase HPLC. The IL-2 was purified by an additional HPLC step on Suppelcosil LC-308. The IL-2-containing fraction was submitted to Edman degradation. The first 10 amino acids were determined and found to be identical to the N-terminal sequence of mature IL-2 (12, 13). The analysis of the phenylthiohydantoin threonine derivative at the third amino acid residue indicated that the recombinant protein was not glycosylated at this position, as is observed in about 40% of the IL-2 isolated from induced Jurkat cells (12, 13).

Comparison of Natural and Recombinant IL-2. Purified recombinant IL-2 produced in the insect cells was compared

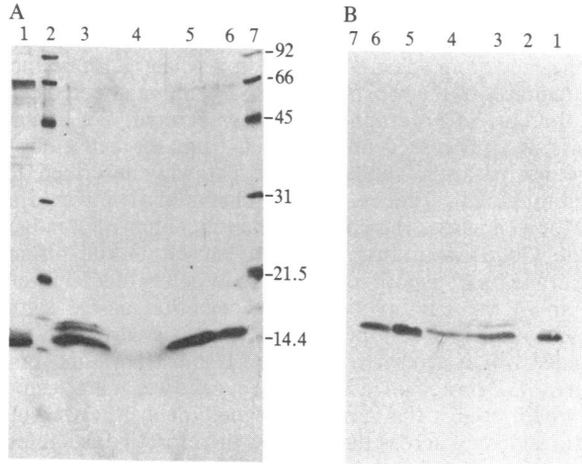

FIG. 5. Analysis of purified recombinant IL-2 by gel electrophoresis and immunoblotting. IL-2 samples and protein standards (lanes 2 and 7, $M_r \times 10^{-3}$ at right in *A*) were reduced and electrophoresed in NaDodSO$_4$/15% polyacrylamide gels and then either silver-stained (26) (*A*) or electrophoretically transferred to nitrocellulose for immunoblot analysis (21) (*B*). The nitrocellulose sheet was probed with monoclonal anti-IL-2 antibodies and then developed with an anti-antibody coupled to horseradish peroxidase. Lanes 1: Recombinant IL-2 produced in insect cells. Lanes 5 and 6: 500 ng and 100 ng of recombinant IL-2 produced in *E. coli*. Lanes 3 and 4: IL-2 produced in Jurkat cells. Lanes 3 and 4 represent different amounts of the same sample.

to both Jurkat-derived and *E. coli*-synthesized IL-2 (Fig. 5). The IL-2 species could be detected either by silver stain (Fig. 5*A*) or by immunoblot analysis (Fig. 5*B*). Analysis in $NaDodSO_4$/polyacrylamide gels revealed that the purified insect-cell material separated into two components, a major species at M_r 15,500 and a minor species at M_r 16,000 (Fig. 5*A*, lane 1). The M_r 15,500 species comigrated with purified recombinant *E. coli* IL-2 (Fig. 5*A*, lanes 5 and 6) and with the major species of IL-2 purified from Jurkat cells (Fig. 5*A*, lanes 3 and 4). Immunoblot analysis showed that these components were all immunoreactive with monoclonal anti-IL-2 antibodies (Fig. 5*B*). The minor components in the Jurkat-derived material, which migrate slightly slower than the major component, may represent variants in glycosylation (14, 15).

The purified recombinant IL-2 from insect and *E. coli* cells and purified natural IL-2 from human Jurkat cells were compared for their ability to stimulate the growth of an IL-2 dependent T-cell line. The IL-2 specific activity, measured as described in ref. 22, for these proteins were between 0.5 and 1.0×10^8 units/mg, or within the factor-of-2 error inherent in this assay.

DISCUSSION

In this study we demonstrated that (*i*) recombinant IL-2 was expressed at high levels under the transcriptional control of the AcNPV polyhedrin promoter; (*ii*) higher levels of expression were obtained when the IL-2 gene was inserted into an AcNPV expression vector that included the polyhedrin 5′ leader sequences; (*iii*) protein-processing mechanisms within the insect cell recognized the pre-IL-2 signal peptide and cleaved the amino acid sequence at the correct position; and (*iv*) the majority of the IL-2 was secreted. There was no evidence that the recombinant IL-2 produced in insect cells was glycosylated. However, about 40% of the natural IL-2 produced in human Jurkat cells is not glycosylated (12), and modifications such as glycosylation do not appear to be required for IL-2 bioactivity (10).

There were two intracellular forms of IL-2 produced in Ac373-IL2-infected cells: a M_r 15,500 protein that was efficiently secreted and a M_r 16,000 protein that was detected primarily in the cells and was the same size as pre-IL-2 synthesized from selected mRNA in a wheat germ extract. The majority of the recombinant IL-2 made in infected cells had the correct N-terminus and was secreted. We assume that it was processed and released from the cell along a secretory pathway similar to the pathway described for vertebrates (25). That is, nascent chains of IL-2 would be translocated across the endoplasmic reticulum (ER), moved to the Golgi apparatus, and then carried to the plasma membrane by transport vesicles. The targeting of a secretory protein to the ER involves the recognition of the signal peptide, on the nascent polypeptide, by a signal-recognition particle, which arrests translation. If the translation of a nascent IL-2 protein in infected insect cells was not arrested, one would predict that a pre-IL-2 would be synthesized but not translocated across the ER, and therefore, not secreted. One explanation for the presence of the M_r 16,000 IL-2 (presumably pre-IL-2) in Ac373-IL2-infected cells is that the available signal-recognition particles were titrated out by the excess number of pre-IL-2 signal peptides that were being synthesized. If this is true, then there may be an upper limit on the efficiency at which secretory or membrane-bound proteins with signal peptides can be processed in the insect cell/baculovirus vector system.

The amount of IL-2 produced and secreted from Ac373-IL2-infected cells was similar (per ml of medium) to that obtained in an *E. coli*/plasmid vector system (10). However, the amino acid sequence of IL-2 made in *E. coli* is not identical to natural IL-2 because of the addition of a bacterial formylmethionine at the N terminus (10). In bacterial vectors, it is usually necessary to delete the coding region for signal peptides to express the mature form of eukaryotic genes for proteins, like IL-2, with cleavable signal sequences. In contrast, IL-2 was cloned in the AcNPV expression vector with the IL-2 protein initiation site and signal peptide sequences intact. These signals were recognized in the infected insect cells and IL-2 was produced with a correct N terminus. Apparently, the signals required for signal peptide recognition and processing and protein secretion have been largely conserved between insects and mammals.

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