Targeting of interleukin-2 to the periplasm of *Escherichia coli*

GABRIELE HALFMANN,† HERVE BRAILLY,† ALAIN BERNADAC,† FELIX A. MONTERO-JULIAN,† CLAUDE LAZDUNSKI† and DANIEL BATY†*

†Laboratoire d’Ingénierie et Dynamique des Systèmes Membranaires du CNRS, 31 chemin J. Aiguier, BP 71, 13402 Marseille Cedex 20, France

†Immunotech SA, 130 Avenue De Lattre De Tassigny, B.P. 177F-13276 Marseille Cedex 9, France

(Received 10 February 1993; revised 27 April 1993; accepted 10 May 1993)

A synthetic gene coding for interleukin-2 (IL-2) was used to produce large amounts of recombinant IL-2 (met-IL-2) in *Escherichia coli*. Met-IL-2 was found to accumulate in the cytoplasm in an insoluble, aggregated form. Inclusion bodies located at the pole caps of cells were detected using immunogold labelling. Constructs were designed to fuse the IL-2 gene to DNA fragments encoding signal peptides for an outer-membrane protein (OmpA) or for a periplasmic protein (PhoA) of *E. coli*. No significant maturation was observed with these fusion proteins which were found in an insoluble form in the cytoplasm. The influence of charge disposition at the N-terminus of the mature portion of the protein was investigated by replacing positively charged amino acids with glutamic acid. None of the introduced substitutions had any effect. Various factors that might affect expression, secretion and folding were examined in an attempt to obtain secretion. By fusing IL-2 to the precursor maltose-binding protein (preMBP) a large fraction of the preMBP-IL-2 protein was correctly processed and transported to the periplasmic space. IL-2 derived from MBP-IL-2 after FXa cleavage possessed similar specific activity to recombinant IL-2 produced in Chinese Hamster ovary cells.

**Introduction**

The lymphokine IL-2 plays a central role in the development of the immune response. It is an inducible protein synthesized and secreted by activated T lymphocytes with the N-terminal extension of a signal peptide. As a growth factor, IL-2 acts via a specific receptor and stimulates the proliferation of IL-2-dependent T cells (Taniguchi et al., 1983; Smith, 1988). Interest in IL-2 has increased with the finding that this lymphokine can be used in the treatment of solid tumours (Rosenberg et al., 1986) and for partial restoration of T-cell function (Pahwa et al., 1989).

The cloning and synthesis of human IL-2 in *E. coli* was first described by Devos et al. (1983), but most of the protein produced remained insoluble in the cytoplasm. Biological activity therefore could not be restored completely and was not proportional to total synthesis. Expression of recombinant proteins in *E. coli* has become a valuable tool for basic research and industrial applications. However, high levels of expression of eukaryotic proteins lead in most cases to the formation of insoluble inclusion bodies in *E. coli* (Marston, 1986). The extraction, denaturation and renaturation of these proteins in their active form are time-consuming and expensive. The efficiency of renaturation and the activity of the renatured protein depend on the correct formation of its disulphide bridges (Marston, 1986). Expression via secretion offers several advantages over intracellular expression. If the signal sequence is processed correctly, the N-terminus of the recombinant protein will be identical to the authentic product. Export of proteins to the periplasm can prevent the degradation of the polypeptide. Disulphide bond formation in *E. coli* has been shown to occur concomitantly with export from the cytoplasm (Pollit & Zalkin, 1983; Derman & Beckwith, 1991). Folded eukaryotic proteins with correct disulphide bonds can therefore be produced (Oka et al., 1985; Parker & Wiley, 1989), which is a major advantage.
In some cases the export of heterologous proteins to the periplasm of E. coli requires only the correct fusion between a prokaryotic signal peptide and the mature portion of the foreign protein provided that the protein itself is normally secreted (Ghrayeb et al., 1984; Duffaud et al., 1987; Better et al., 1988; Parker & Wiley, 1989). Some eukaryotic proteins, such as interleukin-1β, can be recovered by simple osmotic shock (Joseph-Liauzun et al., 1990); others, however, are unable to enter the export pathway. For these proteins, additional steps must be taken to obtain their secretion.

IL-2 was used as a model protein to investigate the possible export of a protein known to form insoluble particles in the cytoplasm. This study of the export of IL-2 in E. coli represents a systematic analysis of different proteins to determine the prerequisites for the export of heterologous proteins. The influence of prokaryotic signal sequence has been investigated for several proteins (Denefle et al., 1989; D. Baty, unpublished results).

In this study, different factors influencing protein maturation and export, such as (i) the nature of the signal peptide per se (OmpA or PhoA), (ii) the role of charge distribution near the leader peptidase cleavage site, (iii) the influence of chaperones (GroES and GroEL), (iv) the incubation temperature and inducer concentration, (v) the use of lysis proteins and (vi) fusion to a known, secreted protein (preMBP) efficiently targeted by SecB to the export apparatus, were investigated.

**Methods**

**Strains and media.** E. coli TB1 araA(lac proAB) rpsL (Δ80 lacZ AM15) (Johnston et al., 1986) was used as host for the expression of the MBP-IL-2 fusion protein. In all other experiments, the plasmids were expressed in E. coli W3110 (Lloubès et al., 1986). M9 minimal medium and LB media have been described by Miller (1972). Antibiotic resistant plasmids were maintained in transformed strains by addition of 100 µg ampicillin ml⁻¹ and, when required, chloramphenicol (50 µg ml⁻¹) or kanamycin (50 µg ml⁻¹) to the growth medium.

**DNA manipulation.** The synthetic gene encoding IL-2 was provided by BBL. Oligomers were synthesized using a model 380A automatic DNA synthesizer (Applied Biosystem). The nucleotide sequence analysis by the T7 polymerase chain termination method was carried out using bacteriophage M13 or NaOH-denaturated plasmid templates (Messing, 1983; Hattori & Sakaki, 1986) and [α-³²P]dATP [1080 Ci (40 MBq) mmol⁻¹; Amersham]. Site-directed mutagenesis was performed as described by Baty et al. (1987b). To exchange lysines (K) either at position K8 and/or K9 of IL-2 for glutamic acid (E), the following oligonucleotides were used: K8E, 5'-GAGTTTTCTCAGTGCTCG-3'; K9E, 5'-GTTGAGTTCTCAGTGGC-3'; K8/9E, 5'-GTTGAGTTCTCAGTGGC-3'.

**Plasmid construction.** The plasmid pJF118EH (Furste et al., 1986) was used as a source to express IL-2 under the control of the tac promoter regulated by the lacP repressor. The 420 bp EcoRI–HindIII fragment of BBG3 replicative form (BBL) was inserted between the EcoRI and HindIII sites of pJF118EH to create pGF25 (Fig. 1). The ssOmpA-IL-2 fusion was created by linking the synthetic IL-2 gene to the signal peptide of OmpA by inserting the Xhol–BglII fragment of BBG3 between the BamHI and HindIII sites of plasmid pIN-III-ompA-Hind (Rentier-Delrue et al., 1988) connected by the oligonucleotides AGCGCTCTCTAGGC-3' and TCAGGGTAGTGAGGC-3' to create pFQ (Fig. 2). The ssPhoA-IL-2 fusion in pFP6 was constructed by inserting the Xhol–HindIII fragment of BBG3 between the HindIII sites of plasmid pBX4.1 (D. Baty, unpublished result) connected by the oligonucleotide cassette used for the previous construction (Fig. 2). The Xhol–HindIII fragment of BBG3 was inserted between the Stul and HindIII sites of pMal-p (New England Biolabs) using the oligonucleotides GCTCTCTAGGC-3' and TCTGCAGTGTAGGC-3' to give pLS28.

**Gene expression and protein characterization.** The plasmid-containing strains were grown in rich medium at the indicated temperatures. Gene expression was induced by the addition of 0.1–1 mM IPTG. Cell fractionation (Neu & Heppel, 1965) was performed as follows: cells in late exponential growth were harvested and washed in TE (10 mM-Tris/HCl pH 7.5, 1 mM-EDTA). After centrifugation, the cell pellet was gently resuspended in 30 mM-Tris/HCl, pH 7.5, 20% (w/v) sucrose, 0.5 mM-PMSE, 1 mM-EDTA to a density of 10⁶ bacteria ml⁻¹. After 10 min at room temperature, the suspension was centrifuged for 5 min at 6000 g. The sucrose solution was carefully drained from the tube and the pellet was resuspended in a same volume of cold water. The resuspended cells remained on ice for 10 min and were centrifuged at 15000 g for 10 min at 4°C. The supernatant is referred to as osmotic shock fluid (periplasmic fraction). The pellet was resuspended in 30 mM-Tris pH 7.5, 0.5 mM-PMSE, 1.25 mM-MgCl₂, 25 µg DNAse I ml⁻¹, freeze-thawed six times and centrifuged for 5 min at 15000 g. The pellet and the supernatant are referred to as the membrane and cytoplasmic fraction, respectively. All samples were resuspended in SDS loading buffer [60 mM-Tris/HCl pH 6.8, 1% (w/v) SDS, 10% Tris loading buffer [60 mM-Tris/HCl pH 6.8, 1% (w/v) SDS, 10%}

![Fig. 1. Construction of pGF25. Plasmid GF25 was constructed by inserting the EcoRI–HindIII fragment (black) containing the coding sequence for IL-2 into pJF118EH. The arrow indicates the direction of transcription from the tac promoter. The bla (white) and the lacP (stippled) genes are indicated.](image-url)
Targeting of IL-2 to periplasm of E. coli

Fig. 2. Construction of the ssOmpA-IL-2 and ssPhoA-IL-2 fusion proteins. The sequences coding for the signal sequence of OmpA (bold dashed) and PhoA (bold dotted) are indicated. pBX4.1 contains the growth release factor (GRF) gene (dashed) which is removed during the construction. The other genes are as described in Fig. 1 except that lacI is used in place of lacI`. The amino acid sequences of the signal sequences preceding the IL-2 sequence are indicated in bold at the bottom of the figure.

(v/v) glycerol, 2% (v/v) β-mercaptoethanol] heated to 95 °C for 5 min, analysed by 14% SDS-PAGE and immunoblotted on nitrocellulose as described by Towbin et al. (1979). The met-IL-2 and the IL-2 fusion proteins were visualized by immunodetection with monoclonal antibodies directed against IL-2.

Pulse-chase experiments. E. coli W3110 harbouring pFQ (preOmpA-IL-2) or pFP6 (prePhoA-IL-2) were co-transformed with plasmid groES/EL (Goloubinoff et al., 1989). Pulse-chase experiments were performed by diluting overnight cultures of the samples 100-fold in M9 medium. The presence of both plasmids was maintained by the addition of ampicillin (100 μg ml⁻¹) and chloramphenicol (50 μg ml⁻¹). At an OD₆₀₀ of 0.4 simultaneous induction of the IL-2 fusions and GroES/GroEL was obtained by the addition of 1 mM IPTG. After 10 min, 10 μCi (370 kBq) [³⁵S]methionine was added. After a 20 s pulse, the culture was chased for varying periods of time with 1% unlabelled methionine. Aliquots corresponding to 60000 c.p.m. were loaded on a 14% SDS-polyacrylamide gel.

IL-2 bioassay. The biological activity of human IL-2 was tested using the IL-2-dependent murine T lymphocyte cell line CTLL-2 (Gillis et al., 1978). The CTLL cell line was cultured in RPMI 1640 (Gibco) containing 1 mm-pyruvate, 2 mm-L-glutamine, 50 μg streptomycin and penicillin ml⁻¹ (all chemicals were from Flow), 10% (v/v) foetal calf serum (Gibco) and supplemented with 1 ng rhIL-2 ml⁻¹ (Sanofi). The IL-2 bioassay was then performed as described previously. Briefly, 100 μl per well of the cell suspension at 10⁶ cells ml⁻¹ giving 10⁴ cells per well was added to 96-well culture plates together with 100 μl per well of the IL-2 containing samples. The cells were cultured for 48 h at 37 °C with 5% (v/v) CO₂ in a humidified incubator and were then pulsed for 8 h with 0.5 μCi (18.5 kBq) [³H]thymidine (Amersham) per well. The cells were then harvested and the radioactivity incorporated in the cell pellets was determined in a beta counter (Kontron). All determinations were performed in triplicate. A standard range of serial dilutions of calibrated IL-2 (Sanofi) was included in every experiment. One Unit of activity was defined as the amount of IL-2 that leads to half maximum proliferation of CTLL cells. Concentrations of IL-2 in the samples were then expressed in Units. In parallel with bioactivity determinations, IL-2 concentrations were determined using a commercial immunoassay (Immunotech) calibrated against the international standard NIBSC 86/564. From these determinations specific activities, expressed as CTLL Units per ng IL-2, were derived for the different preparations.

Electron microscopy. The detailed procedure for fixation, embedding, cryosectioning and staining has been described previously (Bernadac & Lazdunski, 1981). The only difference was the use of the monoclonal antibody directed against IL-2 and of gold-coated protein A instead of ferritin-labelled anti-rabbit antibodies.

MBP-IL-2 purification. The protocol used for the purification of MBP-IL-2 and its cleavage by FXa was that described by New England Biolabs with some modifications. The amylose column was washed...
with 50 mM-Tris/HCl, pH 7.5, 150 mM-NaCl (TNA buffer). The periplasmic shock fluid was circulated through the column overnight at 60 ml h⁻¹ with TNA. The column was then washed with TNA until the A₅₅₀ reached the base line. The MBP-IL-2 was eluted with TNA containing 5 mM-maltose.

**Results**

High-level expression of mature IL-2 from pGF25 (Figs 1 and 3) resulted in its aggregation into inclusion bodies that were located either in the middle of the cells or at the polar caps (Fig. 5). This confirmed previous findings by Devos et al. (1983) that IL-2 aggregates in the cytoplasm of E. coli. To overcome this, we have investigated the targeting of IL-2 to the periplasm of E. coli by fusing it either to bacterial signal peptides or to the periplasmically-located maltose-binding protein. IL-2 was fused to two different E. coli signal sequences, one from the outer membrane protein (OmpA) and the other from the periplasmic alkaline phosphatase (PhoA) in pFQ and pFP6, respectively (Fig. 2). The synthesis of IL-2 and of the fusion proteins was compared in the same E. coli W3110 strain under identical experimental conditions. About 5% of total cellular protein was expressed as met-IL-2 or ssOmpA-IL-2 and 15% as ssPhoA-IL-2 (Fig. 3). The induced E. coli pGF25 (met-IL-2), pFQ (ssOmpA-IL-2) and pFP6 (ssPhoA-IL-2) were fractionated and separated into spheroplasts, periplasmic and membrane fractions (Fig. 4). The hybrid proteins, ssOmpA-IL-2 (16.9 kDa) or ssPhoA-IL-2 (16.7 kDa), were neither properly processed to the mature form of IL-2 (14.5 kDa) nor secreted to the periplasm of E. coli (Fig. 4). The majority of the fusion proteins remained unprocessed in the membrane fraction. Only a weak processing of the precursor to the mature form could be observed (Fig. 4, arrows in lanes T). To localize the fusion proteins found in the membrane fraction, immunogold labelling was performed. Immunogold labelling of E. coli W3110 revealed no difference in localization of the proteins of the cytoplasmic version or those derived from the ssPhoA-IL-2 (Fig. 5) or ssOmpA-IL-2 fusion proteins (not shown). These observations were consistent with the absence of processing of the fusion proteins and clearly demonstrated that they did not even enter the secretion pathway.

The N-terminus of mature IL-2 has two consecutive positively charged lysines at positions 8 and 9 that may perturb the export process. To test this possibility, in vitro mutagenesis was performed to substitute the lysine residues (K) at positions 8 and 9 by glutamic acid residues (E) which should not influence the biological activity of IL-2 (Ju et al., 1987). K8, K9 or K8/9 were replaced by E8, E9 or E8/9, respectively. The mutant IL-2 gene sequences were fused to the signal sequence of either OmpA or PhoA. Secretion competence and protein maturation were examined by analytical cell fractionation. The OmpA-IL-2 and PhoA-IL-2 constructs showed a level of synthesis similar to that of the wild-type IL-2. The positions of prestained molecular mass standards (Gibco-BRL) are shown on the left.
Targeting of IL-2 to periplasm of E. coli

2469

type IL-2, as detected by immunoblotting (data not shown). None of the substitutions of lysine by glutamic acid (E8, E9 and E8/9) improved precursor maturation or translocation of the fusion proteins as compared to the wild-type (data not shown).

IL-2 contains three cysteine residues, two forming a disulphide bridge. To assess whether the free cysteine residue 125 (C125), which is not essential for the biological activity of IL-2 (Ju et al., 1987) but may be responsible for the inter- and/or intramolecular formation of disulphide bridges, might be involved in the insolubility of the protein (and therefore increased protein aggregation), C125 was substituted by an alanine residue in the plasmids containing ssPhoA-IL-2 and ssOmpA-IL-2. C125 was also mutated, in addition to the replacement of the lysine residues by glutamic acid in the same plasmids. The expression was comparable to that of the wild type. No difference in protein maturation or translocation could be detected in the IL-2-A125 mutants and their fusion derivatives (data not shown). C125 therefore seemed not to be the dominant factor in the formation of intracellular aggregates.

We attempted to suppress premature folding and aggregation of the ssOmpA-IL-2 and ssPhoA-IL-2 fusion proteins that may have prevented translocation by increasing the intracellular level of the chaperones GroEL and GroES. Plasmids encoding groES and groEL (Goloubinoff et al., 1989) were introduced into E. coli W3110 harbouring pFQ (ssOmpA-IL-2) or pFP6 (ssPhoA-IL-2). Simultaneous induction of the IL-2 fusions (ssOmpA-IL-2 or ssPhoA-IL-2) with groES and groEL did not result in any significant increase in the maturation of these protein fusions (data not shown).

The pMal-p vector was used to construct a maltose-binding protein (MBP)-IL-2 fusion protein. This contains the sequence IEGR encoding the recognition site of the specific protease factor Xa, located inside the polylinker insertion site of the vector (Fig. 6a). After
Fig. 6. Expression, secretion and purification of the MBP-IL-2 hybrid protein. (a) The IL-2 gene was inserted in the pMal vector that encodes the maltose-binding protein (MBP), the signal sequence (SS) of preMBP and the factor Xa cleavage site (IEGR). The vertical arrow heads indicate the sites of cleavage by the signal peptidase (SP) and the factor Xa (FXa), respectively. The horizontal arrows indicate the different relevant genes and promotors of the plasmid pLS28. (b) The preMBP-IL-2 hybrid was induced with 1 mM-IPTG in LB at 37 °C at an OD₆₀₀ of 0.5. Two hours after induction, samples were taken and cell fractionation was performed as described in Methods. Protein samples corresponding to 0.5 OD₆₀₀ units were applied to a 14% SDS-polyacrylamide gel. After immunoblotting, the MBP-IL-2 fusion was visualized with anti-IL-2 and anti-MBP antibodies. Anti-β-lactamase (anti-Bla) was used to control the quality of the periplasmic fraction. MBP-IL-2* represents a degradation product of MBP-IL-2. (c) After purification the preMBP-IL-2 protein was treated for 0, 4 or 8 h with FXa. After immunoblotting, the proteins were visualized with anti-IL-2. The positions of prestained molecular mass standards (Gibco-BRL) are shown on the right.

Table 1. IL-2 activities in CTLL assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>IL-2 Specific bioactivity (CTLL; Units ml⁻¹)</th>
<th>IL-2 activity (CTLL; (ng ml⁻¹)</th>
<th>Specific activity (CTLL; Units ng⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhIL-2*</td>
<td>1</td>
<td>0.07</td>
<td>14.29</td>
</tr>
<tr>
<td>MBP-IL-2†</td>
<td>412</td>
<td>92</td>
<td>4.48</td>
</tr>
<tr>
<td>IL-2‡</td>
<td>660</td>
<td>62</td>
<td>10.65</td>
</tr>
<tr>
<td>Buffer§</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Recombinant Chinese Hamster ovary-derived IL-2.
† MBP-IL-2 fusion protein purified by maltose affinity chromatography.
‡ IL-2 derived from MBP-IL-2 by FXa treatment.
§ 10 mM-Tris/HCl, pH 7.5.

Discussion

In general, recombinant polypeptides can be accumulated to higher levels when retained intracellularly than when secreted. However, export of a polypeptide may be preferred for several reasons: (i) to allow formation of disulphide bridges required for function; (ii) to decrease proteolytic degradation, although severe proteolysis of heterologous proteins may occur in the periplasmic space; (iii) to facilitate the purification of recombinant proteins. For these reasons, we tried to obtain an E. coli construct able to produce and export IL-2. The fusion of a prokaryotic signal peptide to the mature IL-2 did not promote export which contrasted with results observed for other recombinant proteins produced in E. coli (Ghrayeb et al., 1984; Better et al., 1988; Parker & Wiley, 1989; Denéfle et al., 1989). Not only, were the ssOmpA-IL-2 and the ssPhoA-IL-2 not exported but they did not even enter the export pathway and it is therefore likely that they aggregated very early after proteolysis with factor Xa, the MBP-IL-2 fusion protein should be cleaved to give authentic IL-2 protein. Induction resulted in about 5% of the total cellular protein being preMBP-IL-2 and MBP-IL-2 fusion proteins (Fig. 6b, lanes T). Analytical cell fractionation revealed that most of the MBP-IL-2 hybrid protein was secreted to the periplasmic space of E. coli (Fig. 6b, lanes P). The fusion protein was degraded during purification (Fig. 6c, lane 0). After incubation with FXa, the hybrid proteins yielded MBP and IL-2 (Fig. 6c, lanes 4 and 8). The MBP-IL-2 fusion protein and the IL-2 derived from FXa cleavage were tested for their ability to sustain the proliferation of the IL-2-dependent cell line CTLL-2 (Table 1). Both recombinant proteins were found to be active in the assay. Interestingly, FXa cleavage yielded a protein with higher specific activity which was very similar to that of a preparation of Chinese Hamster ovary-derived recombinant IL-2 (rhIL-2).
synthesis. The signal peptides may have become buried very soon after synthesis and were unable to direct the protein to the export machinery. By using IL-2 as a model polypeptide, we have investigated the effects of different factors that may interfere with the export of recombinant proteins.

First we altered the charge distribution near the cleavage site since it had been demonstrated that by replacing basic residues in this region, defective export could be corrected (Summers & Knowles, 1989; Parker & Wiley, 1989). Although the mechanism of export in prokaryotes and eukaryotes appears to be similar, the presence of basic residues near the cleavage site is more frequent in eukaryotic secretory proteins (Watson, 1984; von Heijne, 1986). Whether this reflects some difference between early events in the secretory process of prokaryotes and eukaryotes remains an intriguing question. Expression and secretion of IL-2 in Streptomyces lividans resulted in active IL-2 being secreted to the culture supernatant with, however, inefficient processing and translocation of the precursor (Bender et al., 1990). The inability of the IL-2 fusions to enter the export pathway in E. coli may be explained mainly by thermodynamic considerations of the conformation of the mature region, independently of the signal sequence used. By contrast, experiments on the export of human interleukin-β showed a remarkable influence of the bacterial peptide on the secretion of this eukaryotic protein (Denèfle et al., 1989).

Observations concerning the nature of the N-terminal residues of a protein as a key factor in secretion (for review, see Boyd & Beckwith, 1990) led us to introduce mutations in the non-secretable hybrid proteins that comprise the signal peptides of PhoA and OmpA fused to the complete IL-2 gene. Some of the mutations were designed to change the net charge near the N-terminus of the mature form of IL-2, while other alterations were made to avoid the incorrect formation of disulphide bridges (leaving the N-terminal sequences unchanged). We found that the substitution of the basic lysine residues by glutamic acid did not improve the secretion competence of the fusion proteins, regardless of the replacement of one or the other (K8 or K9) or both (K8/9) residues. Results obtained in studies of the influence of the net charge at the N-terminus of the mature form of prokaryotic proteins on protein export (MacIntyre et al., 1990; Yamane & Mizushima, 1988) therefore do not apply to the secretion of heterologous proteins when these proteins tend to aggregate spontaneously.

For export, the precursor must be maintained in a translocation-competent conformation and the signal peptide must remain exposed to the secretory machinery. Early folding or aggregation of the precursor leads to loss of translocation. Bacterial chaperone factors, including trigger factor, GroEL, GroES, DnaK and SecB appear to be required to prevent early protein folding and to keep the precursor in a translocation-competent conformation (for review, see Saier et al., 1989; Lee & Olins, 1992). However, the rapid folding of IL-2 in the cytoplasm could not be inhibited by increasing the intracellular amount of GroEL and GroES.

Two general problems encountered in the synthesis of eukaryotic proteins in E. coli at a high level are protein aggregation and/or incomplete processing by bacterial peptidase activity. Altering growth conditions has been shown to influence favourably the yield of correctly processed material, e.g. by lowering the temperature (Schein & Noteborn, 1988) or by limited induction (Ghrayeb et al., 1984). Because induction with 1 mM IPTG leads to a high production level of the ssOmpA-IL-2 and ssPhoA-IL-2 fusions, we reduced the level of IPTG to 10 μM in LB and/or decreased the growth temperature to 28 °C. We could not observe any significant difference in the extent of maturation (precursor stability and processing competence) under different conditions (data not shown).

Since we could not promote export by the different techniques described above, we tried to apply our experience in the use of lysis proteins to promote non-specific release of proteins to the extracellular medium. Neither the colicin A lysis protein (Baty et al., 1987a), nor the bacteriophage φX174 lysis protein E (Witte et al., 1989) promoted the extracellular release of putatively soluble forms of IL-2.

IL-2 in its original environment may have some additional factors which influence its expression and secretion, either as directed by its original signal peptide or by other factors such as eukaryotic chaperones. In the case of the maltose-binding protein, attainment of a stable tertiary structure in the cytoplasm correlates with the loss of competence for secretion (Randall & Hardy, 1986). Thus, once a bacterial protein is properly folded in the cytoplasm, it seems that there is no mechanism of repackaging it for export. Binding proteins and chaperones appear to prevent the nascent polypeptide chains from crossing the energy barrier to a folded stable state, either stabilizing the partially folded structure or protecting these incompletely folded, flexible molecules from premature aggregation (Fischer & Schmid, 1990).

In a further attempt to promote secretion of IL-2, the mature protein was fused to the C-terminus of the maltose-binding protein. The export of the latter has been studied extensively and plasmid vectors have been designed based on these studies to promote high levels of expression and secretion of recombinant proteins (for review, Bankaitis et al., 1985; Randall et al., 1987). This system allows the protein to fold by itself without
interference of an adjacent signal sequence and has been used successfully with various proteins to obtain export in *E. coli* (Martineau et al., 1992; Brégègère & Bedouelle, 1992). This approach was successful and allowed export, processing of the MBP-FX-IL-2 fusion protein and production of active IL-2. Export of preMBP-IL-2 presumably occurs because export begins cotranslationally before completion of the synthesis of the IL-2 moiety. However, we cannot rule out the hypothesis that interaction of the hybrid protein with the SecB chaperone plays a part in preventing misfolding and aggregation.

We are grateful to M. Delage for helpful discussions. We thank J. M. Clement for the anti-maltose-binding protein. We also thank M. Green and H. Rickenberg for careful reading. This work was supported by the Ministère de la Recherche et de la Technologie and the Fondation pour la Recherche Medicale. G. H. was the recipient of an EC postdoctoral fellowship.

References


Targeting of IL-2 to periplasm of E. coli


