Purification and renaturation of recombinant human interleukin-2

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INTRODUCTION

Interleukin 2 (IL-2) or T-cell growth factor, is a 15500-Mr, single-subunit protein produced by T-lymphocytes that promotes the proliferation of activated T-cells (Robb, 1985). In addition it modulates growth and differentiation of other lymphocyte subsets, for example causing maturation of cytotoxic T-cells (Ezard et al., 1985), induction of γ-interferon (Farrar et al., 1981) and promotion of natural killer- and lymphokine-activated-killer-cell activity (Hinney et al., 1981; Mule et al., 1985).

IL-2 has been purified from natural and recombinant sources by a variety of methods, usually involving RP-h.p.l.c. (Henderson et al., 1983; Stern et al., 1984) as the principal purification step. The human IL-2 gene has been cloned and expressed in Escherichia coli as insoluble ‘inclusion bodies’ (Devos et al., 1983). Inclusion bodies are dense aggregates, largely of recombinant protein and 2–3 μm in diameter, that can be separated from soluble bacterial proteins by low-speed centrifugation after cell lysis (Scheron et al., 1985). In most cases inclusion bodies need to be dissolved in denaturing solvent and the protein then refolded and purified (Marston et al., 1984). Proteins vary considerably in their optimal conditions for renaturation; various factors such as pH, salt concentration and type, rate of removal of denaturant, concentration of the target protein and of contaminants may strongly affect the recovery of authentic protein (Ghelis & Yon, 1982). Since IL-2 has one disulphide bridge and one free cysteine residue, redox conditions must also be considered so as to provide oxidation without excessive formation of wrong disulphides. The purification of recombinant IL-2 has been described in outline and the protein thoroughly characterized (Kato et al., 1985; Liang et al., 1985; Yamada et al., 1986).

In the present paper we describe in detail the solubilization, refolding and purification of recombinant human IL-2. The strategy employed has been to purify IL-2 partially in denatured form and then, following preliminary refolding experiments aimed at identification of the correctly folded species, to improve the recovery of this species by systematic examination of the folding conditions and to develop a purification scheme based on these data.

MATERIALS AND METHODS

Molecular genetics

A synthetic gene coding for the mature 133-residue protein was constructed on the basis of the cDNA sequence determined by Taniguchi et al. (1983), cloned and expressed in E. coli RB791. The IL-2 expression vector pTIL2E/2TT is a derivative of pAT153 (Twigg & Sherratt, 1980) containing the hybrid tac promoter from pDR540 (Russell & Bennett, 1982) inserted between the restriction-endonuclease-HindIII and BamHI sites, a synthetic IL-2 gene inserted between the BamHI and Sall sites and a synthetic Trp A transcription terminator (Christie et al., 1981) between the Sall and AatI sites. The synthetic IL-2 gene was composed of single-stranded oligonucleotides which were synthesized by the solid-phase phosphotriester methodology using a Biosearch Synthesis Automation Machine (Applied Biosystems) and ligated as described by Sarama & Khorana (1972). Codon usage was chosen for optimal expression in E. coli. The gene sequence was checked and was found to be correct.

Growth and disruption of cells

A 50 ml sample of an E. coli RB791 culture containing the IL-2 plasmid was grown overnight to stationary phase and this culture was used to inoculate 5 litres of medium in shake flasks. After 1 h at 37° C cells were induced to produce IL-2 with isopropyl β-thiogalactoside, grown for 3 h and chloroform-killed. Cells (15–20 g wet wt.) were harvested in a Beckman J2-21 centrifuge at 2800 g for 5 min and disrupted by

Abbreviations used: RP-h.p.l.c., reversed-phase h.p.l.c.; DTT, dithiothreitol; PAGE, polyacrylamide-gel electrophoresis; 2-ME, 2-mercaptoethanol; f.p.l.c., fast protein liquid chromatography; GdmCl, guanidinium chloride; TFA, trifluoroacetic acid; g.p.c., gel-permeation chromatography; TX-100, Triton X-100; BSA, bovine serum albumin.

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sonication in 50 ml of 10 mm-phosphate buffer, pH 7.4, containing 0.15 m-NaCl/10 mm-EDTA/10 mm DTT, the IL-2 inclusion bodies pelleted by centrifugation at 10000 g for 10 min and the pellet resuspended in an equal volume of the above buffer for use in subsequent experiments.

**Solubilization of inclusion bodies**

Solubilization was monitored by SDS/PAGE. Samples (100 μl) of pellet suspension were centrifuged at 10000 g for 5 min and pellets were resuspended in 100 μl of solvent and left for 10 min at 20°C. After centrifugation supernatants were dialysed against 50 mm-Tris/HCl, pH 6.8; 2-ME (5% v/v) and SDS (1%) were added and samples boiled for 5 min. Pellets were washed with 190 mm-Tris/HCl, pH 6.8, boiled in 100 μl of sample buffer to extract the remaining proteins, and re-centrifuged to remove particulate matter. Samples (50 μl) of supernatants and pellet extracts were loaded on to gels and, after electrophoresis, proteins were revealed by staining with Coomassie Blue.

**G.p.c.**

Analytical g.p.c. was performed in 6 M-GdmCl/50 mm-Tris/HCl (pH 8.5)/10 mm-DTT (running buffer) with a 1 cm × 30 cm Pharmacia Superose 12 f.p.l.c. column and a Pharmacia f.p.l.c. apparatus; the flow rate was 0.5 ml/min. Samples (200 μl) of pellet suspension were centrifuged at 10000 g for 5 min and the pellet dissolved in 8 M-GdmCl/10 mm-DTT, pH 8.5, for 1 h at 37°C to ensure complete reduction and solubilization before chromatography. Preparative g.p.c. was performed on a 5 cm × 90 cm Sepharose CL-6B column equilibrated in running buffer and run at a flow rate of 70 ml/h; 40 ml of pellet suspension was centrifuged in a Beckman J2-21 centrifuge (JA-17 rotor) at 7000 g for 10 min and the pellet resuspended in 15 ml of 8 M-GdmCl/10 mm-DTT to give a final volume of 20 ml. IL-2-containing fractions were identified by silver staining after SDS/PAGE, pooled and stored at −70°C. Pools were respectively termed 'g.p.c.-f.p.l.c.' and 'g.p.c.-6B'. To study oxidation in the absence of DTT, g.p.c.-6B pools were desalted in 6 M-GdmCl/0.1% TFA, pH 2.2, using a Pharmacia PD-10 gel-filtration column; this material ('g.p.c.-DS') was used within a few hours, since autoxidation is still appreciable, even at this pH (Saxena & Wetlauffer, 1970).

**Refolding**

Renaturation was effected by dilution of 1–3 ml (per experiment) of the g.p.c. pool into 50 mm-Tris/HCl, pH 8.5. All renaturations were performed at 20°C. In order to vary the protein concentration at a constant GdmCl concentration, samples were appropriately diluted with 6 M-GdmCl or 6 M-GdmCl/10 mm-DTT before the final dilution in renaturation buffer. Renatured samples were often slightly cloudy. TFA was added to give a pH of 2.5–2.8, and samples were analysed by RP-h.p.l.c. Yields of IL-2 species, peak X and peak Y, were measured from RP-h.p.l.c. peak areas (see below).

**RP-h.p.l.c.**

RP-h.p.l.c. was performed on a Varian 5020 liquid chromatograph fitted with a UV-100 detector. Acetonitrile (HPLC Grade S; Rathburn Chemicals) and TFA (Fierce Sequenual grade) in Milli-Q-purified water (Millipore) were filtered on a 0.22 μm-pore-size filter before use. Synchronpak RP-P C8 columns (0.41 cm × 25 cm; Synchron, Linden, IN 47955, U.S.A.) were obtained from Anachem. Fractions were stored at −70°C. The amount of IL-2 protein was determined from peak areas using an A280 value of 9.58 × 10³ M⁻¹ cm⁻¹ (Kato et al., 1985).

**SDS/PAGE and gel scannning**

SDS/PAGE was performed with 15%-(w/v)-polyacrylamide gels as described by Laemmli (1970). H.p.l.c. column fractions in acetonitrile were 3-fold diluted in sample buffer [190 mm-Tris/HCl (pH 6.8)/3% (w/v) SDS/30% (v/v) glycerol/0.75% (v/v) Bromophenol Blue/5% (v/v) 2-ME]. Gels were silver-stained (Wray et al., 1981) or Coomassie Blue R-250-stained. Protein standards (Pharmacia) were phosphorylase b (molecular mass 94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and a-lactalbumin (14.4 kDa). For quantification of IL-2 in g.p.c. pools, silver-stained gels were scanned densitometrically with a Joyce-Loebl Chromoscan-3 densitometer. Loads (50–500 ng) of h.p.l.c.-purified IL-2, of known concentration (determined by RP-h.p.l.c. peak area), were used for calibration, and samples were loaded at such dilution that their IL-2 content fell within this range. Total protein was measured by the Bradford method (Pierce Protein Assay Reagent).

**Bioassay**

Amounts (in units) of IL-2 were estimated with an IL-2-dependent cell-proliferation assay, essentially as described by Gillis et al. (1978), the murine T-cytotoxic cell line CTL-L being used. One unit of IL-2/ml was defined as that concentration giving 50% maximal incorporation of [3H]thymidine, and units were standardized to JURKAT IL-2 obtained from Dr. G. Thurman, NCI–Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

**Chemicals**

Isopropyl β-thiogalactoside was obtained from Gibco. Acrylamide and bisacrylamide were from Bio-Rad; tetramethylethylenediamine, SDS and ammo-

### Table 1. Solubilization of IL-2 inclusion bodies

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Approximate proportion of IL-2 dissolved (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 M-GdmCl/50 mm-Tris, pH 8.5</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>1% SDS/50 mm-Tris, pH 8.5</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>70% Formic acid</td>
<td>&gt; 95</td>
</tr>
<tr>
<td>8 M-Urea/50 mm-Tris, pH 8.5</td>
<td>70–90</td>
</tr>
<tr>
<td>50% Acetic acid</td>
<td>30–50</td>
</tr>
<tr>
<td>5% Triethanolamine/HCl, pH 10.9</td>
<td>10–20</td>
</tr>
<tr>
<td>40% Propan-2-ol/0.1% TFA</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>1 M-NaCl/50 mm-Tris, pH 8.5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>0.5 M-2-ME/50 mm-Tris, pH 8.5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>1% TX-100/50 mm-Tris, pH 8.5</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

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Fig. 1. Solubilization of IL-2 inclusion bodies: SDS/PAGE analysis

Samples of pellet suspension were centrifuged and redissolved in the indicated solvent plus 10 mM-DTT. Supernatants (SN) were dialysed before boiling in SDS/2-ME; pellets (P) were re-extracted in an equal volume of sample buffer. Equal volumes of SN and P were then loaded. Tracks 1 and 2, 50% acetic acid extraction, SN and P; tracks 3 and 4, 8 M-GdmCl extraction, SN and P; tracks 5 and 6, 70% formic acid extraction, SN and P; track 7, Mr standards. The cause of the slight difference in mobility between acetic acid-solubilized (track 1) and insoluble (track 2) IL-2 is unclear; however, it does not result from hydrolysis or derivatization, as no change in apparent Mr or pl was observed on extensive incubation of pure IL-2 in 50% acetic acid.

Fig. 2. G.p.c. of pellet extracts

(a) G.p.c. of a 200 µl (5.4 mg/ml) pellet suspension dissolved in 6 M-GdmCl/10 mM-DTT, pH 8.5, using a 1 cm × 30 cm Superose 12 f.p.l.c. column equilibrated in the same buffer; the flow rate was 0.5 ml/min and the fraction size 1 ml. Mr markers were denatured γ-globulin (158000), ovalbumin (44000) and myoglobin (17000). (b) Preparative g.p.c. of a 20 ml pellet extract (9.25 mg/ml) on a 5 cm × 90 cm Sepharose CL-6B column in 6 M-GdmCl/DTT. The flow rate was 70 ml/h and 9.5 ml fractions were collected.

G.p.c.

G.p.c. in 6 M-GdmCl/10 mM-DTT achieved a 2.5–3-fold purification of IL-2 before the renaturation step (Fig. 2). IL-2 was detected by SDS/PAGE and by bioassay of fractions and was eluted at a position corresponding to an Mr of about 15000, indicating that it was unfolded and monomeric under these conditions. This purification is evident in densitometer scans (Fig. 3), which were used to estimate IL-2 concentrations in g.p.c.-purified samples, a method which was found to be more reliable (possibly owing to the denatured state of the protein) than radioimmunoassay. Data for g.p.c.-f.p.l.c., g.p.c.-6B and g.p.c.-DS pools are summarized in Table 2.

Renaturation of g.p.c.-purified IL-2

For initial refolding experiments, g.p.c.-6B material was diluted 20-fold in 50 mM-Tris/HCl, pH 8.5, to initiate renaturation/autoxidation and two peaks (X and Y) of IL-2 were observed on RP-h.p.l.c. analysis (Fig. 4). These species were over 95% pure by SDS/PAGE, as is
shown for peak X in Fig. 5; the apparent $M_r$ was 15030 (peak Y was approximately the same), which is consistent with the theoretical value of 15513 and with the value of 15000 reported for recombinant E. coli IL-2 by Liang et al. (1985). Specific bioactivity, relative to the JURKAT native IL-2 standard (10 $\times$ 10$^4$ units/mg), was 15.5 $\pm$ 6.5 $\times$ 10$^4$ units/mg for peak X (mean $\pm$ s.e.m., $n = 6$); the specific activity of peak Y was 2.4 $\times$ 10$^4$ units/mg. Peak X grew at the expense of peak Y as renaturation time increased and, furthermore, thiol titration with 5,5'-dithiobis-(2-nitrobenzoic acid) (Anderson & Wetlauffer, 1975) showed a 2.6-fold higher cysteine content in peak Y, strongly suggesting that peak Y was reduced IL-2 and was being oxidized to X. This assignment is confirmed by reported specific activities (Liang et al., 1986) and RP-h.p.l.c. retention times (Kunitani et al., 1986) for reduced and oxidized recombinant IL-2. In conclusion, peak X has the basic characteristics of native IL-2. Refolding and oxidation were thus optimized on the basis of peak-X yield as determined by RP-h.p.l.c. peak area; this method of quantification was found to be accurate when checked by amino acid analysis. The peak-X yield was divided by the total IL-2 in the g.p.c.-pool starting material (determined densitometrically; see Fig. 3) to give the percentage renaturation recovery. The principal parameters investigated with respect to renaturation recovery were protein concentration, IL-2 concentration and redox conditions.

**Protein and IL-2 concentration**

In order to monitor the effect of these variables on renaturation recovery, oxidation was allowed to go to completion. A progressive increase in peak-X recovery
was seen as both protein and IL-2 concentrations were decreased concomitantly (Table 3); recovery was increased 1.7-fold with a 10-fold decrease in concentration. Presumably aggregation or misdirected folding was responsible. Protein concentration alone had no significant effect on recovery, whereas the concentration of IL-2 did (Table 3), indicating that a specific process was responsible. However, recoveries after renaturation of unpurified GdmCl extracts were only about 5%, possibly owing to interference from high-M₆ membrane fragments or protein aggregates.

The fate of aggregated or misfolded IL-2 on RP-h.p.l.c. is unclear; a weak IL-2 band comprising less than 5% of the total IL-2 was seen, on SDS/PAGE, at around 45% (v/v) acetonitrile (19 min) in the gradient (Fig. 4), but no other species were detected. Aggregates are most likely to be found in the unretained fractions, although no bioactivity was detected in these fractions. Sub-denaturing concentrations of chaotrope have been seen to inhibit aggregation (Orsini & Goldberg, 1978), but 2 mM-GdmCl had no effect on peak-X recovery, nor did 20% (v/v) acetonitrile or 25% (v/v) ethylene glycol.

Table 3. Renaturation: variation of protein and IL-2 concentration

<table>
<thead>
<tr>
<th>Pool</th>
<th>[Protein] (µg/ml)</th>
<th>[IL-2] (µg/ml)</th>
<th>Recovery of peak X (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) G.p.c.-f.p.l.c.a</td>
<td>29</td>
<td>2.4</td>
<td>23</td>
</tr>
<tr>
<td>G.p.c.-f.p.l.c.b</td>
<td>8.7</td>
<td>0.7</td>
<td>30</td>
</tr>
<tr>
<td>(b) G.p.c.-f.p.l.c.a</td>
<td>16.5</td>
<td>0.7</td>
<td>31</td>
</tr>
<tr>
<td>G.p.c.-f.p.l.c.b</td>
<td>8.7</td>
<td>0.7</td>
<td>30</td>
</tr>
<tr>
<td>G.p.c.-f.p.l.c.b</td>
<td>8.7</td>
<td>0.4</td>
<td>38</td>
</tr>
</tbody>
</table>

Oxidation

In the presence of 1 mM-DTT at pH 8.5, it was necessary to leave IL-2 solutions for 6–24 h to oxidize fully, hence the g.p.c.-DS pool in 0.1% TFA was used to study oxidation by Cu²⁺ (with and without added Cu²⁺) and by thiol/disulphide mixtures (Table 4). Clearly, Cu²⁺ speeds the rate of autoxidation, as has been observed for ribonuclease (Ahmed et al., 1975) and lysozyme (Yutani et al., 1968).

Yields of IL-2 were similar to those obtained in the presence of DTT; however, if g.p.c.-DS samples were left at 4 °C in air for 24 h, substantial decreases in peak-X yield were observed that were reversed by reduction

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Fig. 4. RP-h.p.l.c. purification of IL-2

RP-h.p.l.c. of refolded IL-2 was performed on a 0.41 cm × 25 cm column of Synchropak RP-P C₁₈. Refolding conditions: 1.5 ml of g.p.c.-6B diluted 20-fold in Tris/HCl, pH 8.5; t = 60 min. H.p.l.c. conditions: solvent A, 0.1% TFA; B, 65% acetonitrile/0.1% TFA. Gradient: 0–82%B, 10 min; 82%B, 10 min; 82–100%B, 24 min; the flow rate was 0.5 ml/min. Two IL-2 peaks, X and Y, were identified by SDS/PAGE; they were eluted at 56.5% and 59.5% acetonitrile respectively. Peak X was oxidized IL-2 and Y was reduced IL-2.

Fig. 5. SDS/PAGE analysis of purified IL-2

IL-2 was purified by g.p.c. and RP-h.p.l.c. Proteins were revealed by silver staining. Track 1, Mₑ standards; track 2, 500 ng of IL-2 peak X (see Fig. 4) from C₁₈ h.p.l.c. of refolded g.p.c.-f.p.l.c. protein (samples for tracks 1 and 2 were reduced with 2-ME); track 3, sample as track 2, but unreduced, indicating little dimer in this preparation (although the latter is sometimes observed). The apparent Mₑ of IL-2 (calculated from the standards) was 15030.
and reoxidation, indicating the formation of wrong disulfide isomer(s) in the unfolded polypeptide.

GSSG/GSH mixtures also led to efficient oxidation (Table 4). The improved rate in the presence of GSH compared with GSSG alone indicates that thiol-disulfide reactions were rate-limiting in glutathione oxidation of IL-2, with mixed disulfide accumulating in the absence of free thiol (Creighton, 1978). Although rates were good with GSSG/GSH, there was the possibility that the third thiol of IL-2 had become derivatized, hence Cu²⁺/O₂ was considered the better oxidant.

Renaturation and purification protocol

On the basis of observations that IL-2 must be kept reduced during purification in GdmCl but that its oxidation/renaturation is inhibited by high levels of DTT, f.p.l.c. gel permeation was performed in 6 M-GdmCl/1 mm-DTT/20 mm-acetate buffer, pH 5; IL-2 remained reduced under these conditions for several days. Renaturation and autoxidation was effected at pH 8.5 in the presence of Cu²⁺; oxidation was attempted at lower pH values in the hope of increasing the yield, but was found to be extremely slow below pH 6. With IL-2 concentrations in the region of 1–2 µg/ml, highly purified IL-2 could be prepared conveniently at the 50 µg level of analytical RP-h.p.l.c. (Table 5). Since IL-2 will remain reduced on the time scale of preparative g.p.c. and, furthermore, renaturation is possible in bulk solutions, then this methodology is amenable to scaling up to at least the milligram level.

DISCUSSION

Many heterologous proteins are expressed in E. coli as aggregates (Marston et al., 1984); given the harsh conditions required to solubilize totally such IL-2 aggregates, it seems likely that much of this protein is improperly folded. The causes of inclusion-body formation are unclear; it may be that the nascent polypeptide aggregates in a manner analogous to that seen here in vitro; precipitation of folding intermediates has been demonstrated in vitro for P22 tailspike endorhamnosidase (King, 1986). IL-2 is a secreted protein and, in the eukaryotic cell, has a 19-residue signal sequence (Taniguchi et al., 1983) that is lacking in our construct and may prevent aggregation by binding to the signal-recognition particle/endoplasmic reticulum (Walter & Blobel, 1982) and immobilizing the chain during folding. The lack of glycosylation of recombinant IL-2 (Liang et al., 1985) is unlikely to play a part in aggregation, since 20% of native human IL-2 from cell culture is also unglycosylated (Robb et al., 1984).

The assignment of peak X as conformationally correct IL-2 was based on specific activity and on evidence of oxidation. The M₆ determination by SDS/PAGE showed that the polypeptide was largely intact; N- or C-terminal proteolysis remains a possibility on this evidence, although none has been reported for recombinant IL-2 from E. coli, except for variable aminopeptidase removal of the initiator methionine (Kato et al., 1985; Liang et al., 1985; Yamada et al., 1986).

Assignment of peak X as correctly oxidized protein, with the Cys-58–Cys-105 disulfide bridge formed, was confirmed by its retention time on RP-h.p.l.c.; the mispaired isomers Cys-58–Cys-125 and Cys-105–Cys-125 are eluted much earlier in the gradient (Browning et al., 1986) and may be responsible for the minor IL-2 peak at 45% acetonitrile. Recovery of Cys-58–Cys-105 IL-2 was much diminished if the protein was allowed to oxidize when denatured, since the Cys-105–Cys-125 bridge will predominate under these conditions (Browning et al., 1986) and, in the absence of added thiol, reshuffling to the correct isomer did not occur at a significant rate on refolding.

In summary, the recovery of IL-2 renatured from

### Table 4. Oxidation to peak X

<table>
<thead>
<tr>
<th>Added catalyst/oxidant</th>
<th>Oxidation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>34</td>
</tr>
<tr>
<td>1.5 µM-CuSO₄</td>
<td>100</td>
</tr>
<tr>
<td>1 mm-GSSG</td>
<td>61</td>
</tr>
<tr>
<td>5 mm-GSSG/0.5 mm-GSH</td>
<td>100</td>
</tr>
<tr>
<td>1 mm-GSSG/1 mm-GSH</td>
<td>100</td>
</tr>
<tr>
<td>0.5 mm-GSSG/5 mm-GSH</td>
<td>95</td>
</tr>
</tbody>
</table>

### Table 5. Purification and renaturation of IL-2 by g.p.c. and h.p.l.c.

Notes: a, equivalent to 80 ml of E. coli culture (A₆₀₀ = approx. 1) or 280 mg of cell paste; b, recovery of denatured IL-2 assumed to be 100% for these steps (owing to difficulties in accurately measuring IL-2 in crude samples); c, IL-2 renatured by dilution to give a final [IL-2] of 1.5 µg/ml. Other conditions were: protein concentration, 17 µg/ml; [GdmCl], 0.3 M; [DTT], 0.05 mM; buffer, 50 mm-Tris/HCl (pH 8.5)/1.5 µM-CuSO₄; t, 3 h; temperature, 20°C. Since recoveries from the C₁₈ column were near 100%, most losses of IL-2 occurred during renaturation. Abbreviation: ND, not determined.

<table>
<thead>
<tr>
<th>Total protein (mg)</th>
<th>Total vol. (ml)</th>
<th>Total IL-2 (µg)</th>
<th>Purity (%)</th>
<th>Purification (fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cellsa</td>
<td>28</td>
<td>–</td>
<td>150</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>GdmCl extractsb</td>
<td>4.4</td>
<td>0.8</td>
<td>150</td>
<td>3.4</td>
<td>6.4</td>
</tr>
<tr>
<td>of cell pellet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superose 12 g.p.c.b</td>
<td>1.7</td>
<td>5.0</td>
<td>150</td>
<td>8.8</td>
<td>16.5</td>
</tr>
<tr>
<td>Renaturation²</td>
<td>0.033</td>
<td>1.5</td>
<td>33</td>
<td>95</td>
<td>180</td>
</tr>
<tr>
<td>Synchropak RP-P C₁₈</td>
<td></td>
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<td></td>
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</table>
semi-purified extracts of *E. coli* has been improved by examination of the folding and oxidation conditions; such a strategy should also be useful for the production of other recombinant proteins that need to be denaturant-extracted and do not refold easily in high yield.

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