Purification of Glucagon3·Interleukin-2 Fusion Protein Derived from *E. coli*

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INTRODUCTION

Human interleukin-2(hIL-2)

- known as T Cell Growth Factor
- activation of T cell, B cell and NK cell
- Induction of interferon-gamma
- effective in treatment of cancer and HIV
- composed of 133 amino acid(MW : 15kDa)
- hydrophobic protein
- α-helical secondary structure
- one disulfide bond between Cys58-Cys105

E. coli derived rhIL-2

Expessed in the form of inclusion bodies(IBs)

INTRODUCTION

Previous Investigation

Expression : Using *E. coli* system
hIL-2 gene → use of fusion partner

Purification :
IB isolation
Solubilization of IB
Refolding
Chromatography(ion exchange, gel filtration)
RP-HPLC(reverse phase HPLC)

INTRODUCTION

Glucagon3·Interleukin-2(G3 ·IL-2)

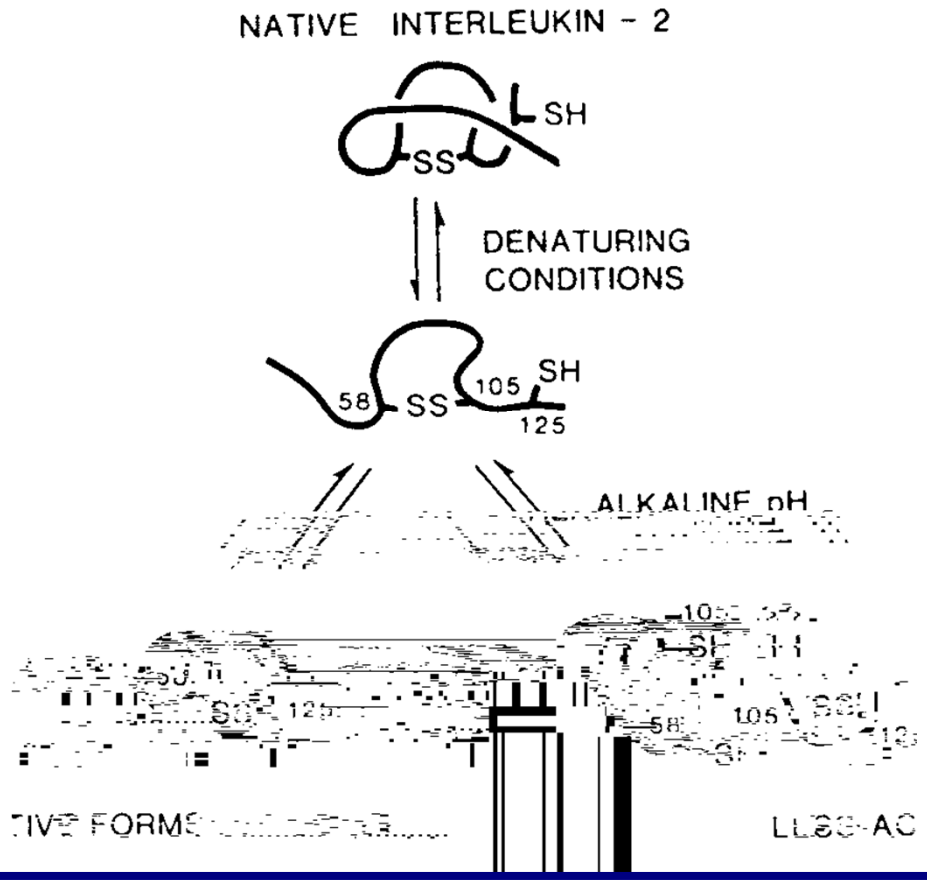
☐ The role of glucagon fusion partner

- Formation of homogeneous aggregates avoiding intermolecular disulfide bond
- Due to the hydrophilicity of glucagon, G3·IL-2 is more soluble in aqueous solution.

* The Cys125 residue was replaced to Ser125 to avoid the two possible isomers

INTRODUCTION

Structure of native hIL-2 and its isomers



INTRODUCTION

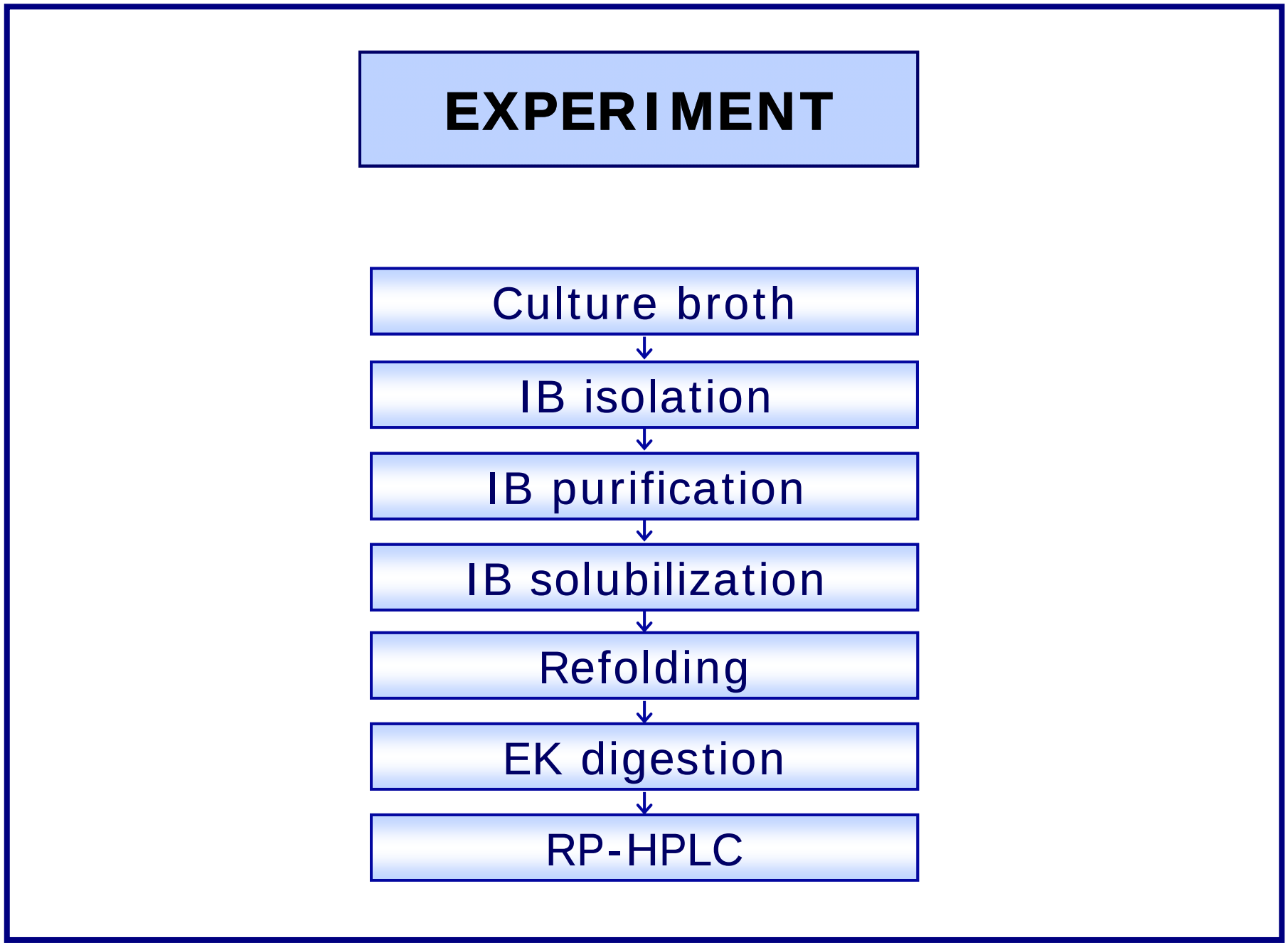
Objective

Economical and efficient process development in the purification of *E. coli* derived recombinant fusion protein using a model protein, G3·IL-2

INTRODUCTION

Strategies

- The ease of IB solubilization and refolding
- Only using RP-HPLC, obtain the highly purified recombinant protein


EXPERIMENT
Culture broth
IB isolation
IB purification
IB solubilization
Refolding
EK digestion
RP-HPLC

EXPERIMENT

Culture broth

Kindly provided by Bioprocess Research Unit at KRIBB

EXPERIMENT

IB purification

1. 10mM phosphate buffer(pH 7.4) containing 0.15M NaCl, 10mM EDTA and 1mM DTT
2. 2M urea for 30min at room temp.
3. 0.5(v/v)% Triton X-100 and 10mM EDTA

After each washing step, the solution was centrifuged at 6000g or 7000g for 30 min at 4C

EXPERIMENT

IB solubilization

Add the alkaline solution to the purified IB

* Alkailne solution : 0.05(w/v)% NaOH(pH12)

Incubate at 37C for above 1hr

Refolding

Simply pH shift down to pH 8

(with 0.5M Tris-HCl, pH 8)

EXPERIMENT

Enterokinase(EK) digestion

* Remove the glucagon fusion partner from the G3IL-2 fusion protein

* **Reaction condition**

  0.1U of EK per 1ug of fusion protein
  incubation at 37C for 16hr
  reaction buffer : 50mM Tris-HCl(pH 8)
  and add to urea to 2M

EXPERIMENT

RP-HPLC

- Column : Kromasil **C8** column
- Mobile phase : binary system of **acetonitrile** and **water**
- Detection at **254nm**

RESULT AND DISCUSSION

IB purification

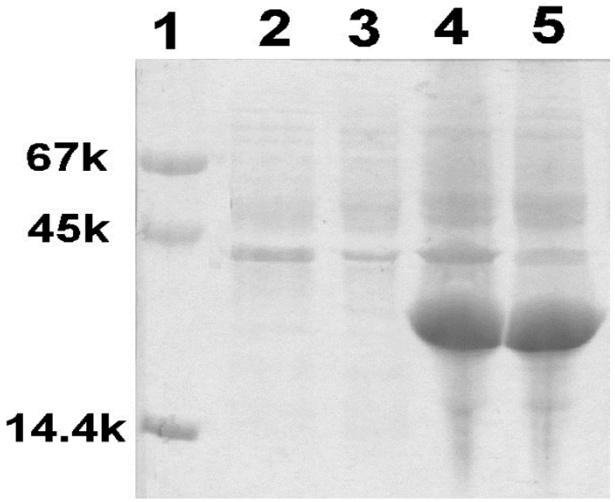


Fig. 1 SDS-PAGE during IB purification

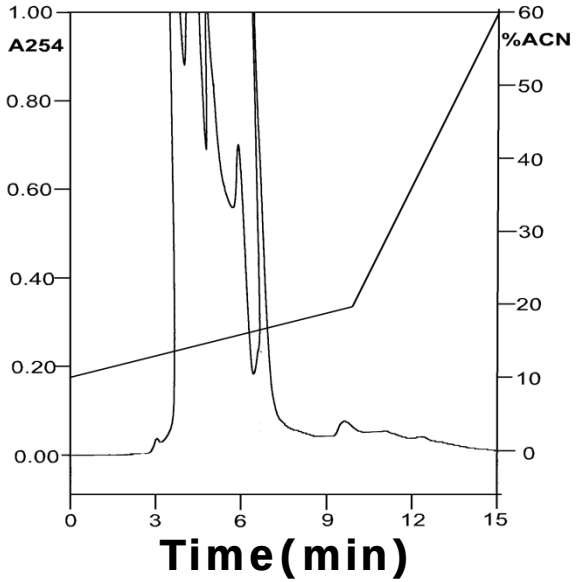


Fig. 2 RP-HPLC analysis of lane 2 in Fig.5

RESULT AND DISCUSSION

IB solubilization and refolding

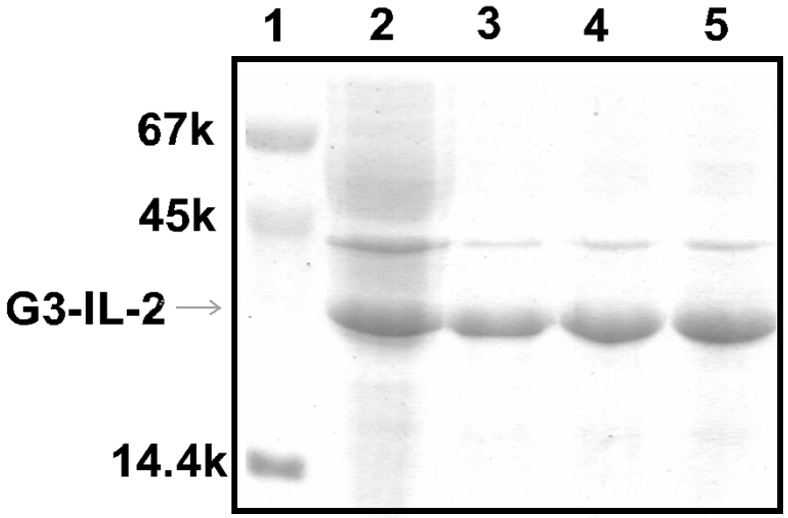


Fig. 3 SDS-PAGE analysis :lane 1 : MW marker, lane 2 : cell homogenate, lane 3 : solubilized IB with 8M urea, lane 4 : solubilized with alkaline solution, lane 5 : renaturation by pH shift

RESULT AND DISCUSSION

Enterokinase digestion

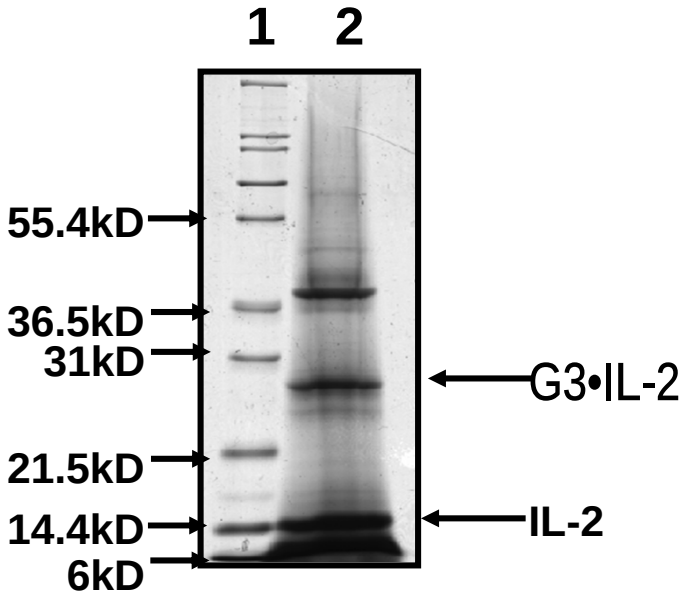


Fig. 4 SDS-PAGE analysis : EK digetion ; lane 1 : MW marker, lane 2 : digested G3-IL-2;

RESULT AND DISCUSSION

RP-HPLC of G3·IL-2

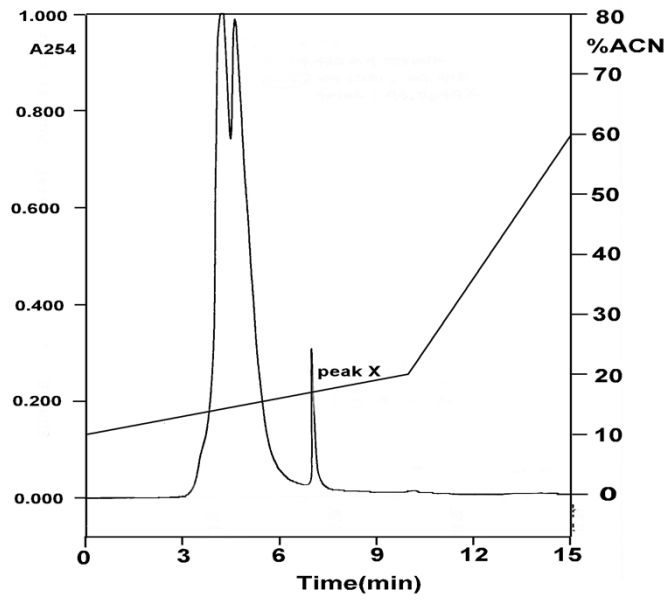


Fig.5 RP-HPLC chromatogram of G3·IL-2 ; peak X is G3·IL-2

RESULT AND DISCUSSION

RP-HPLC of G3·IL-2

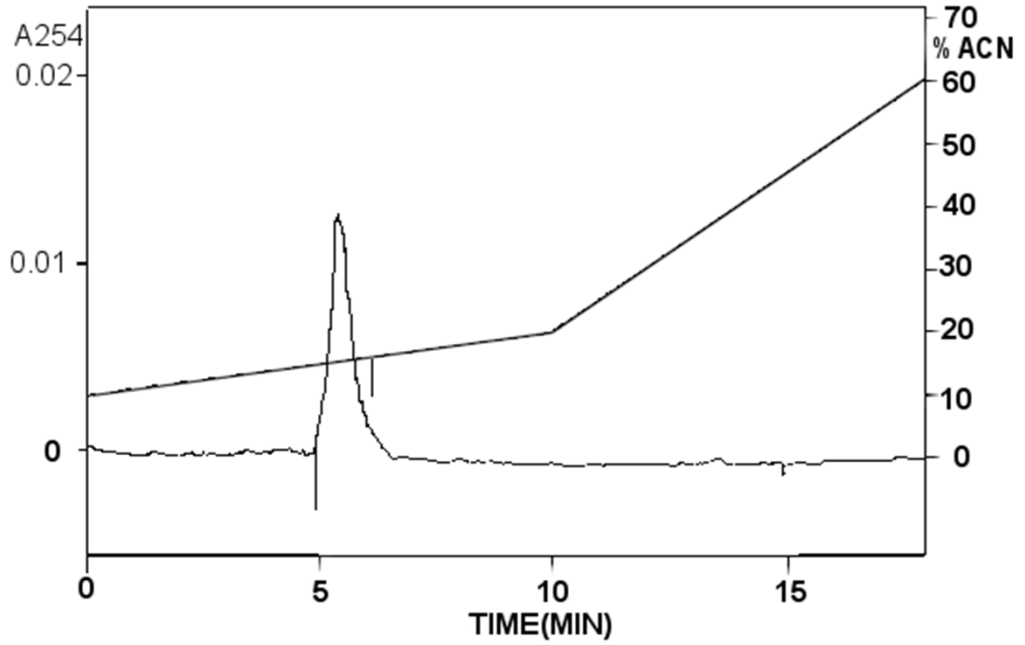


Fig. 6 RP-HPLC of purified G3·IL-2

RESULT AND DISCUSSION

Purified G3IL-2

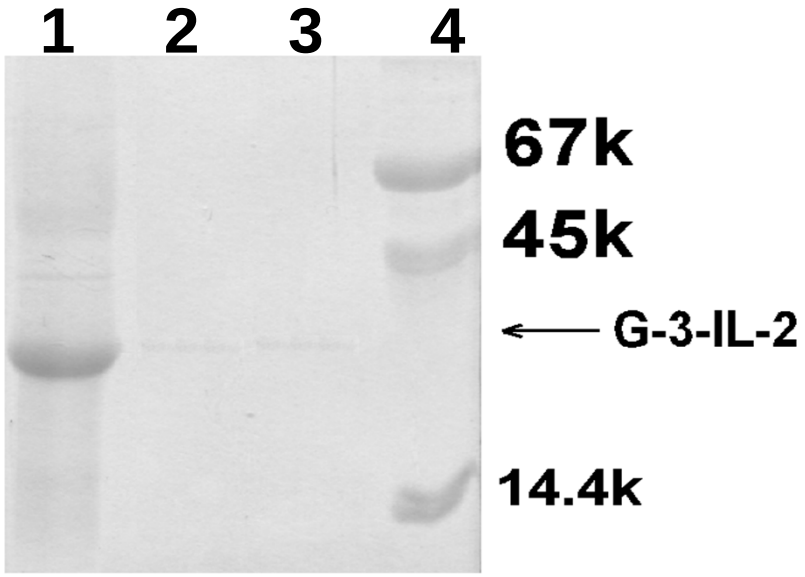


Fig. 7 SDS-PAGE of purified G3-IL-2

RESULT AND DISCUSSION

RP-HPLC of hIL-2

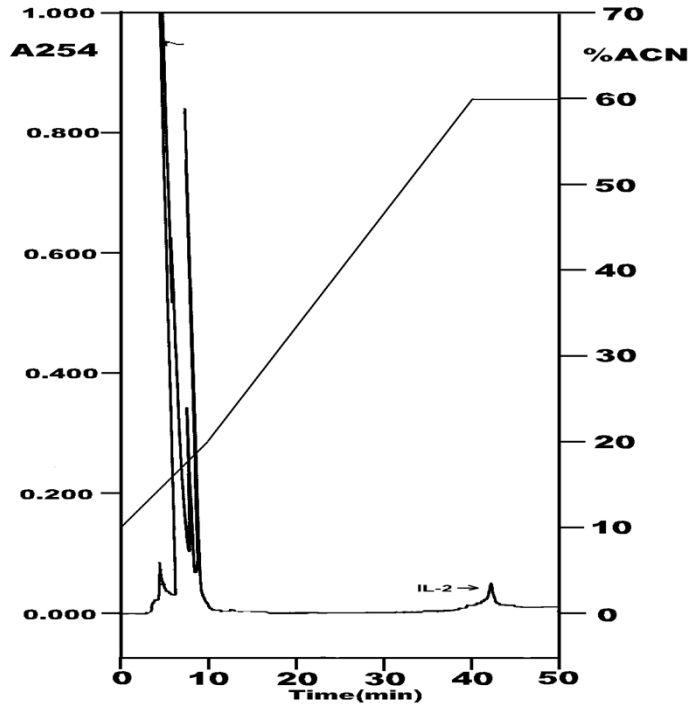


Fig. 8 RP-HPLC of EK digested sample

RESULT AND DISCUSSION

Purified hIL-2

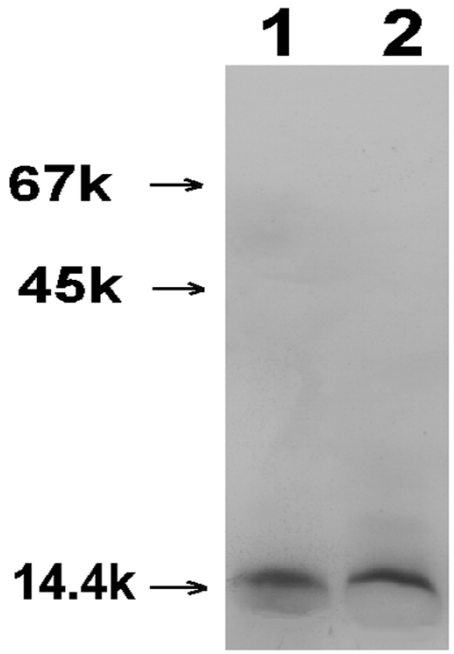


Fig. 9 SDS-PAGE of purified rhIL-2

RESULT AND DISCUSSION

Result of recombinant hIL-2 purification

Purification step	Component	Milligrams	Step yield (%)	Overall yield (%)
Insoluble aggregates	G3•IL-2	231	100	100
Solubilization of IB	G3•IL-2	238	100	100
Enterokinase cleavage	IL-2	71	30	30
Reverse phase HPLC	IL-2	67	94	28

CONCLUSION

- Cell membrane protein has strong absorbance at 254nm. So, the purification of IB was helpful to the RP-HPLC purification step.

- The insoluble aggregates of G3·IL-2 was simply solubilized by alkaline solution. The efficiency of alkaline solution is much higher than the chaotropic agent such as urea and guanidine chloride. It means that this method is economical and efficient.

CONCLUSION

- Due to the nature of glucagon fusion partner that makes homogeneous aggregates, refolding of G3·IL-2 was perfomed by pH shift. By SDS-PAGE analysis, this refolding process made no dimer or misfold form. At this step, we can save the refolding buffer and the time.

CONCLUSION

- The yield of enterokinase cleavage reaction was about 30%. It might be caused by the sterically hindered cleavage site of G3·IL-2 molecule.

- Because hIL-2 was much more hydrophobic than other proteins such as G3IL-2, G3 tag and enterokinase, hIL-2 was highly purified by one-step reverse phase HPLC.