Method for increasing the yield of properly folded recombinant human gamma interferon from inclusion bodies

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1. Introduction

@ Interferon - α, β and γ

- viral desease에 대한 방어기작이 있어 치료원으로 사용됨.
- 항원 및 구조적인 차이에 의해 α, β and γ로 구분

@ interferon γ 의 정제

- PEI침전, ammonium sulfate침전
- QAE, phenyl Sepharose, Sephadex G-100

@interferon γ의 성질

- 높은 등전점, cystein 잔기의 결핍, self associate경향

--> L-arginine을 이용한 최적 refolding 조건

2. Experiments

(1) Expression vector

E. Coli BL-21 including expression plasmid for the human IFNγ

(2) Cell growth and harvesting

cell harvest by centrifugation (5000rpm for 10min)

(3) Sonication

homogenation buffer(buffer A) :

0.1M Tris, pH 8.0, 10mM EDTA, 0.1M NaCl and 1mM PMSF

(4) Washing and harvesting of IB

- Add buffer A+8M urea to the sonicated cells(for 1h at 4'C) and centrifugation at 10000rpm for 30min
- Add buffer B(0.1M Tris pH 8.0, 1mM EDTA and 1M NaCl) to the pellet and centrifuge at 10000rpm for 30min
- Add water and centrifuge
- Discard the supernatant and Ib pellet was collected

(5) Solubilization of IB

- IB pellet 2.1g + 20ml of [6M GuHCl per 0.1M Tris-HCl, pH 8.0 per 2.0M EDTA, pH 8.0)
- Stirred for 2h at room temp.
- Cetrifuge at 17000rpm for 30min at 4'C

(6) Renaturation

- dilution of the IB in refolding buffer(0.1M Tris pH 8.0 + 0.2mM EDTA) without or with increasing amounts of L-arginine.
- light scattering at 500nm.

(7) Dialysis

- against 20mM Tris-HCl, pH 8.0 containing 100mM Urea until conductivity was between 3-3.7 mMhos.
- centrifuge : 10000rpm for 10min.

(8) S-Sepharose chromatography

- sample : 1200ml dialysed supernatant
- column : 20ml S-sephasose
- mobile phase : 20mM Tris-Hcl, pH 8.0
- wash : 200ml 50mM Tris pH 8.0
- elution : 160ml of linear gradient of 50mM Tris pH 8.0 + 1M NaCl

(9) S-100 chromatography

- column : S-100(2.5X80cm) column
- elution rate : 30ml/h
- mobile phase : phosphate buffered saline

(10) viral cytopathic assay

1U of antiviral activity : the amount of rh-IFN γ that is required to produce equivalent antiviral activity.

3. Results and discussion

(1) Refolding of rh-IFN γ

- urea and L-arginine : potent suppressor of aggregation.
- L-arginine has a dose dependent effect on the aggregation.
- 10-fold increase in the yield and the specific activity with 0.5M L-arginine

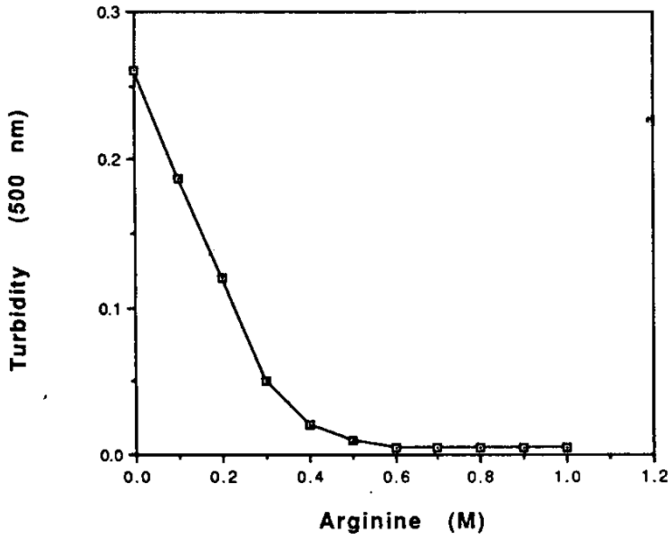

Fig. 1 Effect of arginine on aggregationof diluted IBs :
Guanidine solubilized Ibs were rapidly diluted(1:100) in the refolding buffer in the presence of increasing amounts of arginine.
After 1h the turbidity of the samples was measured at 500nm.

(2) Purification of the refolded rh-IFN γ on S-Sepharose.

- major protein peak eluted at 0.9M NaCl.

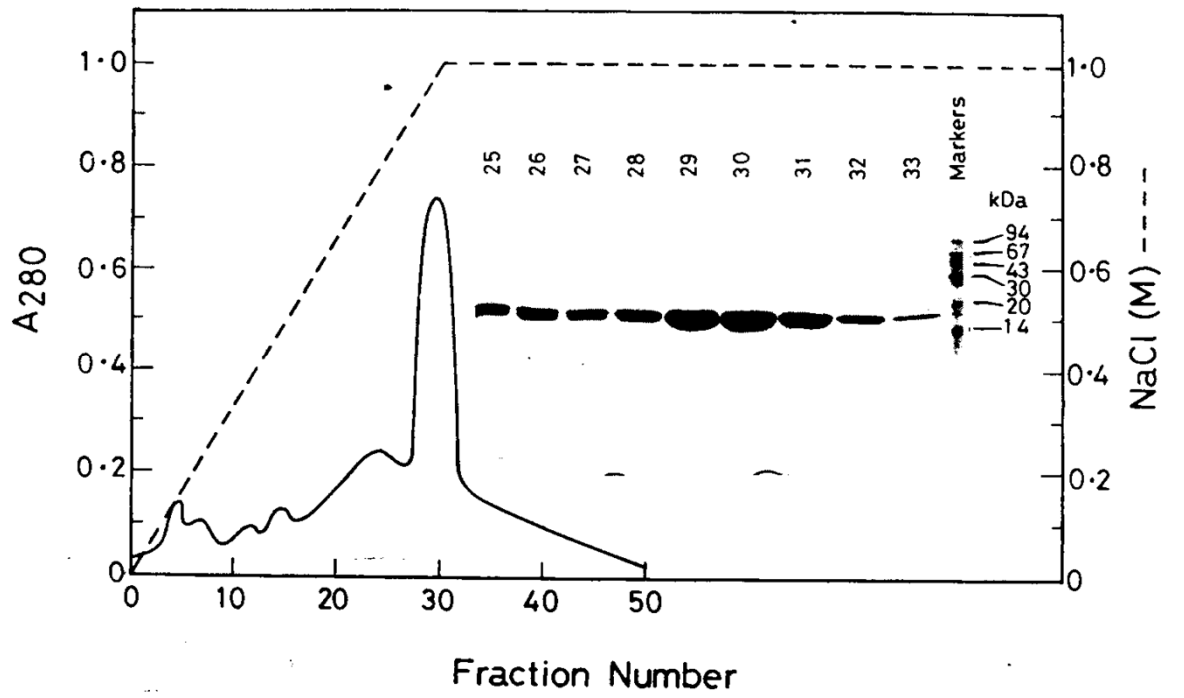

Fig. 2 Elution profile of rh-IFN on S-Sepharose :
Inset shows the silver stained SDS-PAGE of the fractions through the major protein peak.

(3) Gel filtration of rh-IFN γ

- Sephacryl-100 clumn chromatography.

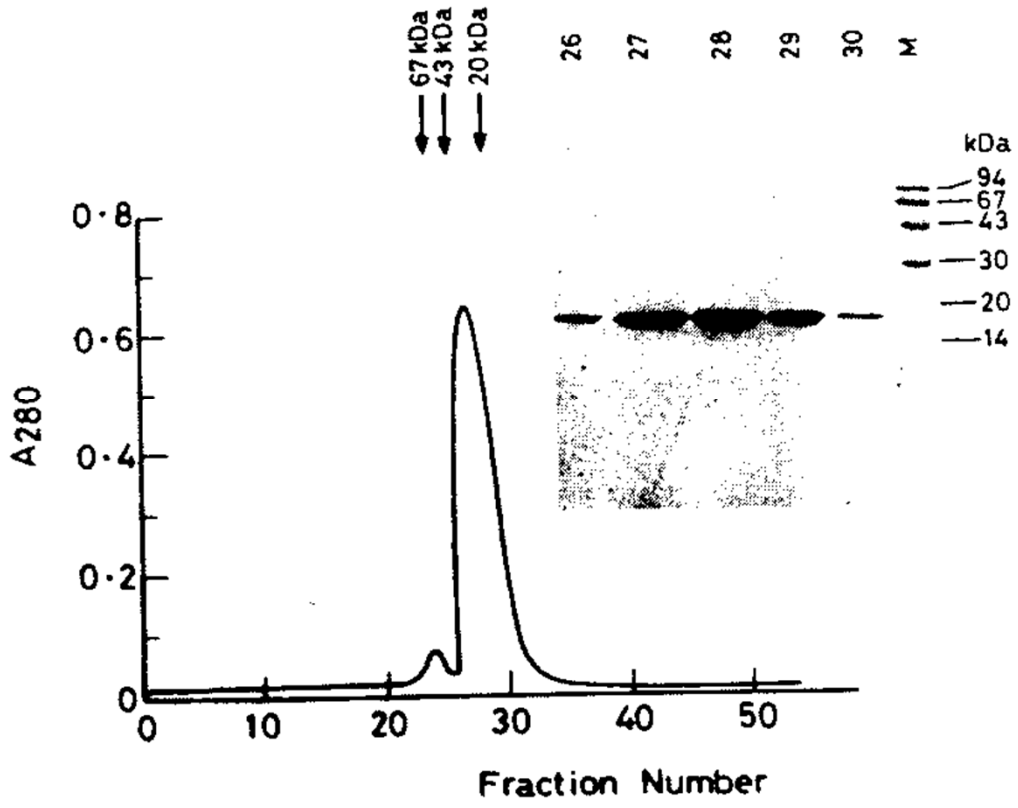

Fig.3 Elution profile of purified rh-IFN sn S-100 column :
Inset shows the silver stained SDS-PAGE of the rh-IFN peak.