

A NEW APPROACH FOR PURIFICATION OF RECOMBINANT HUMAN INTERFERON GAMMA EXPRESSED IN *ESCHERICHIA COLI*

S. Petrov, E. Ivanova, D. Chakarova, V. Posheva, M. Redzheb, G. Nacheva and I. Ivanov
Bulgarian Academy of Sciences, Institute of Molecular Biology "Roumen Tsanev", Sofia, Bulgaria
Correspondence to: Stefan Petrov
E-mail: stefart@abv.bg

ABSTRACT

A new approach for improving and scaling up the purification of recombinant human interferon gamma (hIFN γ) expressed in *Escherichia coli* and accumulated in the form of inclusion bodies was investigated. The new strategy involves denaturation of the inclusion bodies in 7.4M guanidine hydrochloride, loading on a hydrophobic (Octyl-Sepharose) column and elution with urea/ammonium chloride. The method is fast, simple and low in cost. For maximum recovery of the hIFN γ biological activity, an optimization of the refolding step is proposed.

Keywords: human interferon gamma, biological activity, purification, inclusion bodies

Introduction

Human interferon γ (hIFN γ) belonging to the type II interferons, is endogenously produced by lymphoid cells in response to antigenic stimuli (8) and is endowed with multiple biological activities (10). Recombinant hIFN γ (rhIFN γ) is obtained by expression of the hIFN γ gene in *E. coli* more than two decades ago (for review see ref. 10). It aggregates in the *E. coli* cells in the form of inclusion bodies where the protein is biologically inactive. The general strategy for purification and recovery of the activity of the rhIFN γ from inclusion bodies includes the following consecutive steps: a) isolation of inclusion bodies; b) solubilization of the inclusion bodies in chaotropic solutions; c) purification of the denatured rhIFN γ ; d) refolding and e) purification of the refolded (biologically active) rhIFN γ .

Many strategies have been developed for purification of rhIFN γ during the last two decades (see Table 1) and most of them suffer from shortcomings hampering their scaling up.

Selected methods for purification of rhIFN γ

TABLE 1

Reference	Procedure
Geng et al. (2)	USRPP-PHIC
Guan et al. (3)	SEC
Jin et al. (5)	EBA chromatography
Marekov et al. (7)	CM-Sepharose
Reddy et al. (9)	RPC

In this study we propose a new method for purification of rhIFN γ from *E. coli* based on a single step chromatography procedure, which may be applied also for a large scale production of the protein.

Materials and methods

Bacterial Strains and Chemicals

The *E. coli* strain LE 392 was used as a host for rhIFN γ expression.

All reagents for chromatography and electrophoresis were products of Merck (Darmstadt, Germany) and Sigma (USA).

Expression Vector

A gene coding for cysteineless hIFN γ was expressed constitutively (4) in *E. coli* LE392.

Production of rhIFN γ Inclusion Bodies

Transformed cells were cultivated overnight at 37°C in 1-liter flasks containing 250 ml of LB medium supplemented with 10 μ g/ml tetracycline and 0.1% glucose. Bacteria were harvested by centrifugation, suspended in buffer containing 1 M urea, 0.4 M GdnHCl, and 20 mM Tris-HCl (pH 8.8) and disrupted by sonication. After centrifugation (20 min, 14 000 rpm), the crude pellet containing rhIFN γ in the form of inclusion bodies was obtained.

Solubilization of Inclusion Bodies

The pellet of inclusion bodies was suspended in 7.4 M GdnHCl (pH 7.0), slowly diluted to 1 M GdnHCl with ice-cold water, and clarified by centrifugation.

Hydrophobic Chromatography

About 400 ml extract, containing denatured hIFN γ was loaded onto an Octyl Sepharose column equilibrated with 1 M GdnHCl (pH 7). The protein was eluted by a single step elution with 6M urea/1M ammonium chloride at a flow rate of 0.5 ml/min.

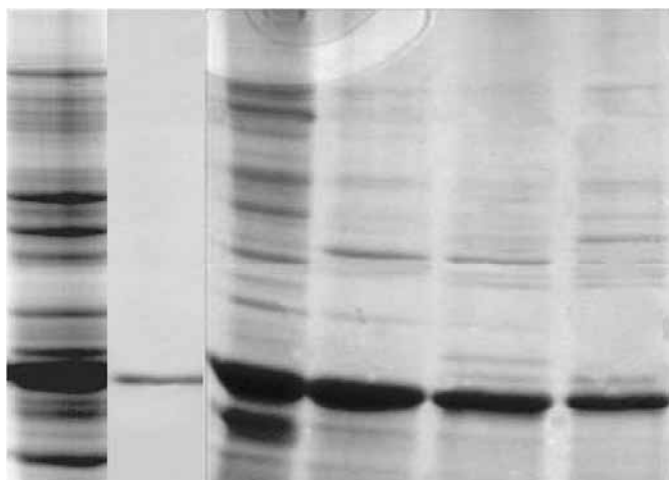
PAA-SDS Gel Electrophoresis

PAA-SDS gels (15%) were prepared according to Laemmly (6) and run at 20 mA for 1.5 h. After electrophoresis, the gels were stained with Coomassie blue R250.

6 Results and Discussion

5 According to a rhIFN γ purification protocol patented by Marekov et al. (7), inclusion bodies were isolated from bacterial cell lysates and solubilized in 7.4 M GdnHCl (pH 7.0). In order to absorb the protein on Octyl-Sepharose column, the extract was diluted up to 1M GdnHCl and the protein was eluted by a GdnHCl gradient. Refolding of the denatured rhIFN γ was carried out by dilution with deionized water to 0.7 M GdnHCl. This step is critical because the low GdnHCl concentration causes a rapid aggregation of the protein and concentrations higher than 0.7 M GdnHCl prevent its absorption on the resin. After refolding, the rhIFN γ is purified to homogeneity on a CM-Sepharose column eluted with a gradient of NaCl in 20mM Tris, pH 8.2.

7 In a previous study we have investigated the unfolding/folding transition of rhIFN γ in both urea and GdnHCl solutions (1). Our results showed firstly, that GdnHCl was more potent denaturant than urea and secondly, that the transition between the two (folded/unfolded) states of rhIFN γ occurred in a narrower GdnHCl concentration range in comparison with the urea. Taking into consideration the importance of the unfolding/folding step for the recovery of rhIFN γ biological activity, the purification procedure is now modified to replace the GdnHCl with urea-ammonium chloride. In the new procedure the dissolved (in GdnHCl) inclusion bodies were absorbed on an Octyl-Sepharose column and the latter was eluted with 6M urea/1M ammonium chloride. The SDS-PAA gel electrophoresis analysis (Fig. 1) shows that the purity of rhIFN γ obtained by this method is comparable to that of the preparations purified by the older protocol (7).



10 Fig. 1. SDS-PAAE analysis of selected fractions, eluted in a gradient of GdnHCl and by single step elution with urea/ammonium chloride. 1: Crude rhIFN γ -extract (inclusion bodies); 2: purified hIFN γ used as a marker; 3 - 4: selected fractions, eluted in a gradient of GdnHCl; 5 - 6: fractions, eluted with urea/ammonium chloride single step elution. The arrow indicates the position of the hIFN γ .

Conclusions

The main advantages of the optimized method for purification of rhIFN γ are as follows:

- It is faster (because of the single step elution of the Octyl-Sepharose column);
- It is cheaper (due to the substitution of urea for GdnHCl);
- The refolding of the rhIFN γ is more efficient and reproducible (because of the milder and better controlled experimental conditions).

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