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

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ABSTRACT

A new approach for improving and scaling up the purification of recombinant human interferon gamma (hIFNγ) 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 hIFNγ biological activity, an optimization of the refolding step is proposed.

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

Introduction

Human interferon γ (hIFNγ) 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 hIFNγ (rhIFNγ) is obtained by expression of the hIFNγ 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 rhIFNγ 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 rhIFNγ; d) refolding and e) purification of the refolded (biologically active) rhIFNγ.

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

TABLE 1

Selected methods for purification of rhIFNγ

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 rhIFNγ 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 rhIFNγ expression.

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

Expression Vector

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

Production of rhIFNγ Inclusion Bodies

Transformed cells were cultivated overnight at 37°C in 1-liter flasks containing 250 ml of LB medium supplemented with 10 μg/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 rhIFNγ 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 denaturated hIFNγ 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.

Results and Discussion

According to a rhIFNγ 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 denaturated rhIFNγ 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 rhIFNγ is purified to homogeneity on a CM-Sepharose column eluted with a gradient of NaCl in 20mM Tris, pH 8.2.

In a previous study we have investigated the unfolding/folding transition of rhIFNγ 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 rhIFNγ 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 rhIFNγ 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 (**Figure**) shows that the purity of rhIFNγ obtained by this method is comparable to that of the preparations purified by the older protocol (7).

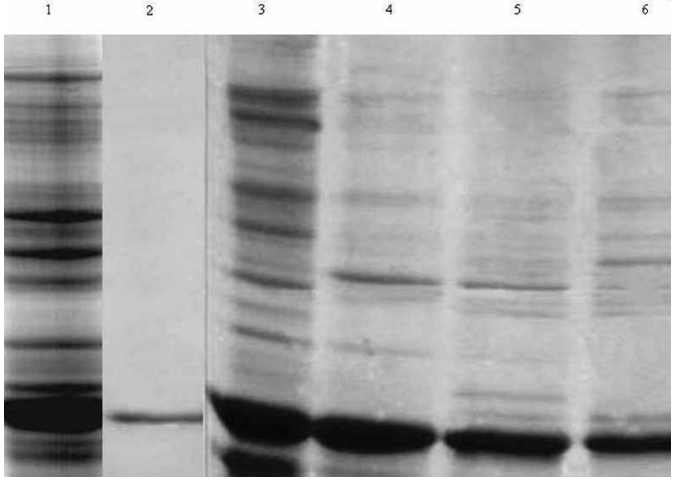

Fig. SDS-PAAGE analysis of selected fractions, eluted in a gradient of GdnHCl and by single step elution with urea/ammonium chloride. **1**: Crude rhIFNγ-extract (inclusion bodies); **2**: purified hIFNγ used as a marker; **3 - 4**: selected fractions, eluted in a gradient of GdnHCl; **5 - 6**: fractions,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

Conclusions

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

a) It is faster (because of the single step elution of the Octyl-Sepharose column);

b) It is cheaper (due to the substitution of urea for GdnHCl);

c) The refolding of the rhIFNγ is more efficient and reproducible (because of the milder and better controlled experimental conditions).

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