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Purification of RhIFN- in Bacteria Bodies with Acetic Acid-Water as Mobile Phase in RPLC

Ye Hua SHEN[1], Qi Dong ZHANG[1], Xin Du GENG[1]*,
Zhi Qing ZHANG[2], Yun De HOU[2]

[1]Institute of Modern Separation Science, Shaanxi Provincial Key Laboratory of Modern Separation Science, Northwest University, Xi'an 710069
[2]Institute of Virus, Chinese Academy of Preventive Medicine, Beijing 100052

Abstract: The extract of *E.coli* containing recombinant human interferon- (rhIFN-) with 7.0 mol/L guanidine hydrochloride (Gu·HCl) was directly injected into a column of reverse phase liquid chromatography (RPLC) to separate and purify rhIFN- with acetic acid-water as mobile phase. Gu·HCl and most impure proteins can be separated by this way. Compared with the usual dilution method, the bioactivity recovery of the purified rhIFN- was found to be over 500%. In addition, compared to common organic solvents employed in RPLC, acetic acid has higher freezing point, and therefore, it is easy to concentrate the aim-protein by freeze-drying when acetic acid-water is used as mobile phase in RPLC.

Keywords: Reversed-phase liquid chromatography, recombinant human interferon-, purification, acetic acid, mobile phase.

Introduction

Recombinant human interferon- (rhIFN-) is a cytokine exhibiting antiviral, antipro-liferative, and immunomodulatory activities, and has certain therapeutic value in clinical treatment. Geng *et al*[1] first used hydrophobic interaction chromatography（HIC）successfully to separate and purify rhIFN- simultaneously in inclusion body in one step. Several years later, size exclusion chromatography[2], ion exchange chromatography[3], and affinity chromatography[4,5] were employed in renaturation and/or simultaneous purification of proteins produced from DNA recombinant. However, no report in this area with RPLC has been found.

As the extract of the *E.coli.* engineering bacteria with 7 mol/L Gu·HCl is very complicated, in which it may contain many kinds of impure proteins, nucleic acids, lipoids and other substances, it is very difficult to use chromatographic method to separate and purify the aim-proteins directly. Before injecting the sample to a chromatographic column, a series of procedures, such as splitting cells, washing with 2.0 mol/L urea for many times to remove some impure proteins and centrifuging to collect

the purified inclusion body of rhIFN-, have to be done. After resolving the inclusion body with a high concentration of denaturant solution, the extrat solution obtained can be directly injected to a chromatographic column. The expense would be increased and the bioactivity of the aim-proteins be lost seriously.

Although RPLC is the best method in liquid chromatography to separate a complicated sample into many single components, the organic solvent used as mobile phase, such as methanol, acetonitrile, and tetrahydrofuran, make many proteins denature easily. However, some recombinant proteins, such as human insulin and interleukins could be purified by RPLC firstly and their bioactivity could be recovered after removing organic reagents. Many organic solvents used in RPLC are easy to burn, explode, and toxic. In addition, the freezing-points of these organic solvents are too low to concentrate aim-proteins by usually freeze-drying method. All of those weak points mentioned above confine their applications in industry. Acetic acid -water employed in this study as mobile phase would avoid the above problems.

Experimental

The pBV220 IFN- DH5α *E.coli* employed was given by professor Zhang from the Institute of Virus, Chinese Academy of Protective Medicine. The LB culture media, pH7.4 (add certain amount of Amp during fermentation). Solid media contained 2% agarose. The bacteria body from the fermentation liquid was firstly washed with a buffer and then extracted with 7.0 mol/L Gu·HCl [6]. The protein concentration in the extract was measured with Lowry method[7]. The bioactivity of rhIFN- was measured with cytopathic patient inhibition method[8].

The packing material employed (synthesized in our laboratory) was packed into a stainless steel column(100× 4.0 mmI.D) by slurry method. Acetic acid-water was used as mobile phase under linear gradient from 40% CH_3COOH to 100% CH_3COOH. Chromatographic separation was run at a flow rate of 1.0 mL/min, with detection wave length of 280 nm, at ambient temperature.

Results and Discussion

Separation and purification of rhIFN- in bacteria bodies with acetic acid- water

Two hundred microliters of sample solution in 7.0 mol/L Gu·HCl was directly injected into RPLC column and the chromatogram obtained is shown in **Figure 1**. Fractions were collected every 5 min and then were freeze-dried, respectively. The residue was dissolved in a buffer consisting of 0.02 mol/L phosphate solution of pH 7.0 to measure the bioactivity of rhIFN-. The bioactivity recovery denoted by rectangular shadow is also shown in **Figure 1**. From **Figure 1** the rhIFN- mainly elutes in the time interval from 25 to 30 min and Gu·HCl could be removed and better result of separation and purification

could be achieved by RPLC. Though the extract in 7 mol/L Gu·HCl contains many kinds of impure proteins, the mass recovery in the 25 to 30 min fraction was more than 50 %. Compared to the usual elution method, the bioactivity recovery of the rhIFN- in the same fraction was over 500%. In addition , there was a single zone of rhIFN- by SDS-PAGE in the same fraction. (result not shown)

The effect of sample size on the resolution with acetic acid - water as mobile phase

Sample size usually affects chromatographic resolution. **Figure 2** shows this effect with sample size changing from 200 L to 1000 L with an increment of 200L rhIFN- sample solution. The chromatograms were denoted with arabic numbers. The separation efficiency, as expected, became worse gradually and the peak height as well as mass recovery of rhIFN- became lower.

Figure 1 Chromatogram and bioactivity recovery of rhIFN- with acetic acid-water
------ time gradient
bioactivity area

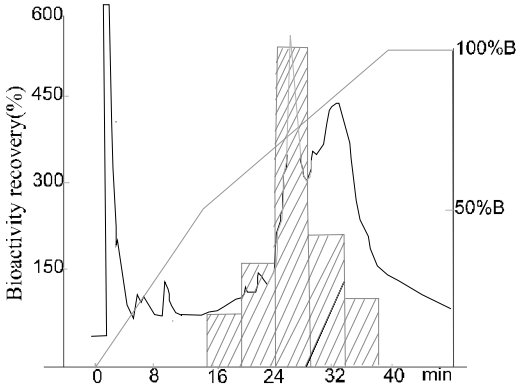
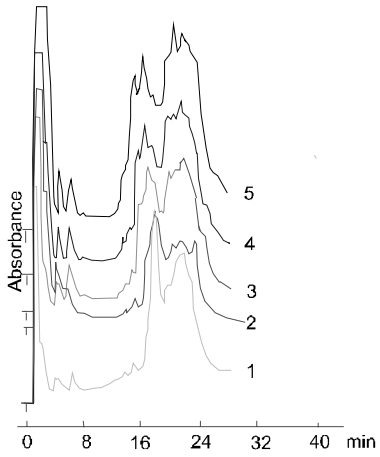


Figure 2 Chromatogram of various amounts of rhIFN-(RP-C_{18}, acetic acid-water)
1. 200l, 2. 400l, 3. 600l
4. 800l, 5. 1000l



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