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Magnetic Affinity Nanoparticles for Bevacizumab Adsorption

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Introduction

The vascular endothelial growth factor (VEGF) stimulates tumor angiogenesis by targeting its receptor (VEGFR) [1]. The IgG monoclonal antibody bevacizumab, produced in CHO cells, is used for cancer treatment due to its capability of targeting the endothelial growth factor A (VEGF-A) and inhibiting angiogenesis [2]. Bevacizumab purification step may account for as much as 70% of the total manufacturing cost because of the high purity necessary for its parenteral administration. Nowadays, its purification is achieved by protein A affinity chromatography (AC). However, protein A is a very expensive ligand and harsh elution conditions are required to recover bevacizumab from the AC column, which can damage the mAb as well as the protein A ligand [3].

Recently, we have reported a short peptide contained in VEGF that binds to bevacizumab with high affinity and selectivity and demonstrated that this peptide bound to agarose can be used to purify bevacizumab at a lower cost and no harsh elution conditions [4]. However, conventional chromatography requires a previous clarification step to avoid column clogging.

Unlike conventional chromatography, magnetic nanoparticle (MNP) purification allows the extraction of the target directly from the cell culture without needing clarification steps [5]. In this work, bevacizumab purification with MNP with a peptide ligand immobilized is assessed.

Results and Discussion

Ac-PHQGQHIGVSK-NH_2 was synthesized by solid phase peptide synthesis (SPPS) with Fmoc/tBu chemistry using Rink amide-MBHA and analyzed by matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectrometry (MS) and RP-HPLC (Figures 1-2). Lys was incorporated at the *C*-termini to facilitate its subsequent immobilization through its ξ-amino group on *N*-hydroxysuccinimidyl ester (NHS)-activated magnetic beads according to Pierce instructions (https://static.thermoscientific.com/images/D20904~.pdf).

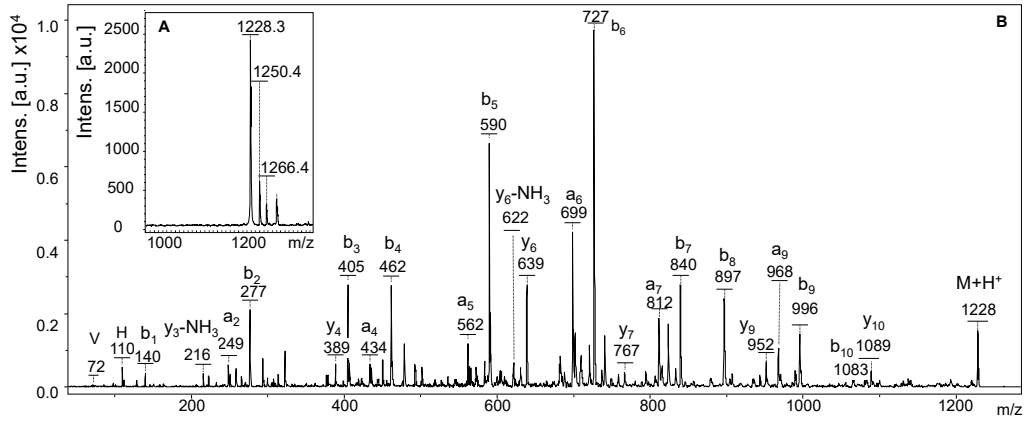

Fig. 1. PHQGQHIGVSK-NH_2 MS and MS/MS analysis (A) MALDI TOF MS: Signals at m/z 1228.3; 1250.4 and 1266.4 correspond to the $M+H^+$; $M+Na^+$ and $M+K^+$ respectively. (B) MS/MS: ions form the series, "b","a" and "y" are indicated.

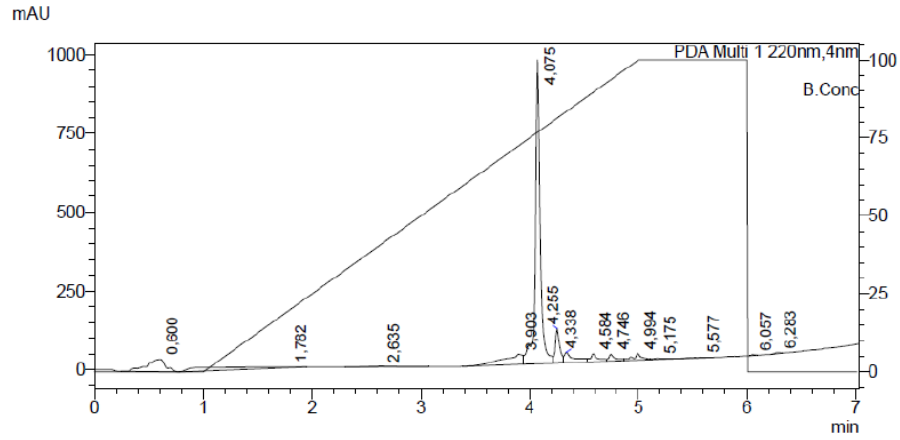

Fig. 2. Ac-PHQGQHIGVSK-NH_2 RP-HPLC analysis. RP column (C18 3.5µm, 4.6x50mm). Solvent A: 0.045% TFA in H_2O, Solvent B: 0.036% TFA in acetonitrile.

The amount of peptide immobilized, measured indirectly by quantifying the *N*-hydroxysuccinimide released as a result of peptide immobilization, was 1.1 nmol/mg of MNP. This value was higher than that reported for IgG immobilization in the data sheet (0.17 nmol of IgG/ mg of MNP; https://static.thermoscientific.com/images/D20904~.pdf).

MNP with the peptide immobilized were added to microtubes with a suspension of CHO cell culture containing bevacizumab in adsorption buffer (20 mM phosphate, 1M $(NH_4)_2SO_4$, pH 7.0). The MNP were separated after a short period of incubation by using a magnetic rack. After washing the beads with the adsorption buffer, elution buffer (20 mM phosphate buffer, pH 7.0) was added, and MNP were separated from the eluted bevacizumab for their reuse (Figure 3).

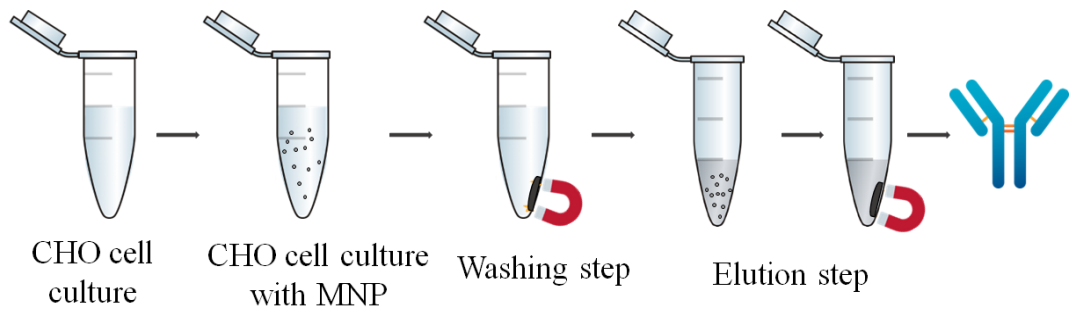

Fig. 3. Bevacizumab purification using Ac-PHQGQHIGVSK-MNP. Adsorption buffer: 20 mM phosphate buffer, 1 M $(NH4)_2SO_4$, pH 7.0. Elution buffer: 20 mM phosphate buffer, pH 7.0.

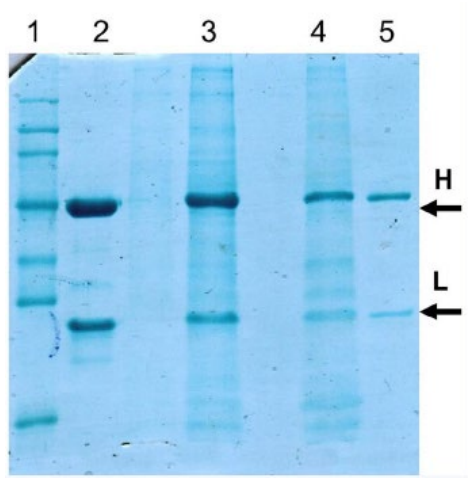

Fig. 4. SDS-PAGE under reduction conditions. 1) Molecular weight marker. 2) Pure bevacizumab. 3) Bevacizumab-producing CHO cell culture. 4) Washing fraction. 5) Elution fraction. H: Bevacizumab heavy chain. L: Bevacizumab light chain.

An excess of bevacizumab crude sample was added to determine the MNP maximum adsorption capacity by measuring the elution fraction protein content by Bradford reagent [6]. The capacity was 16 µg of bevacizumab/mg of MNP, corresponding to 32 mg of bevacizumab/ml of MNP (considering MNP density of 2.0 g/cm^3). Samples were evaluated by SDS-PAGE under reduction conditions. As it is observed in the elution fraction (line 5), MNP adsorbed bevacizumab selectively (Figure 4). As an excess of sample had been added, bevacizumab was also observed in the elution fraction together with the contaminants (lane 4).

Ac-PHQGQHIGVSK-MNP could purify the bevacizumab, although with some loss of it due to the small amount of MNP used for this test.

Peptide-MNP can be useful for bevacizumab purification from culture media without prior clarification and for easy sampling for quality control during the production process.

Acknowledgments

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