SEPARATION OF Fab FRAGMENTS OF HUMAN IgG USING PSEUDOAFINITY CHROMATOGRAPHY IN AGAROSE-OPT

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Abstract

The antibodies and fragments represent an important class of molecules employed in clinical diagnosis in vitro and in vivo, and in the area of biosensors. The need for research development in this area is great, aiming an efficient purification, using low cost techniques. This work studies the separation of Fab fragments of human immunoglobulin G (IgG) through pseudoaffinity chromatography using ortho-phosphotyrosine (OPT) as the ligand.

Key words:

Adsorption, monoclonal antibodies, chromatography.

Introduction

Immunoglobulins are defense proteins that react with unfamiliar molecules in the body. IgG fragments can be obtained by proteolytic treatment of IgG with the enzyme papain forming two Fab (antigen binding fragment) and Fc (crystallizable fragment).

IgG and its fragments are used in the areas of biosensors, clinical in vitro diagnostics and in vivo imaging and can be used for cancer treatments¹. Separation of IgG fragments is desirable since the Fc portion is unnecessary or deleterious in several therapeutic applications¹.

The pseudobiospecific ligands are a viable alternative: (traditional ligands are proteins A, G and L, which are costly among other disadvantages) they are smaller, simpler and more chemically stable in the chromatographic system. Amino acids containing ionic groups or aromatic side chain in its structure are pseudobiospecific ligands with the potential for IgG purification according to recent studies².

In this context, the ligand ortho-phospho-L-tyrosine (OPT) has been proposed and this study evaluates the potential of pseudoaffinity chromatography with OPT to adsorb the Fab of a human IgG solution. Different buffer systems were studied (Tris-HCl, MES, MOPS, sodium acetate, HEPES, and Bis-Tris).

Results and Discussion

The buffers Tris-HCl, MES, MOPS, sodium acetate, Bis-Tris, and HEPES were chosen as mobile phases for the chromatographic experiments. All these have pH between 5.5 and 8.2 (the range normally used for adsorption of biomolecules) and were selected because they provided good adsorption of pure human IgG, evidenced in studies developed by our research group. Elution was carried out by adding NaCl and consequent desorption of Fab fragments, Fc and cleaved fragments.

The condition Tris-HCl adsorbed the least total protein (1.7%) and HEPES adsorbed the most (38.4%). This behavior may be associated with the possible electrostatic interactions between the grouping NH_3^+ in the Tris buffer with OPT ligand, making protein adsorption difficult.

For the conditions that most adsorbed, electrophoresis was carried out and if fragments were separated, Western Blotting was performed.

The HEPES buffer provided the selective recovery of Fab fragments, confirmed after analysis of Western Blotting (Image 1).

Image 1. Western Blotting of the chromatographic fractions of agarose-OPT in experiments using HEPES buffer.

Conclusions

This study demonstrated successfully that the adsorbent agarose-OPT has the potential to separate fragments of IgG, cleaved with papain in order to obtain biomolecules used for various therapeutic and clinical applications. Among the evaluated conditions, the buffer HEPES, pH 7.0, separated Fab fragments and simultaneously was the condition that most adsorbed proteins (38.4%). We found that the nature of the buffer influenced the adsorption of protein on agarose-OPT. This work contributes to future application of this technique for the purification of non-recombinant or recombinant IgG fragments.

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¹ Holliger, P.; Hudson, P. J. Engineered antibody fragments and the rise of single domains. *Nature biotechnology*, **2005**, 23 1126–1136.

² Bresolin, I. T. L.; Fioritti, R. R.; Bueno, S. M. A. IgG purification by negative chromatography in amine-based ligands: A comparison of L-lysine and poly-L-lysine. *Process Biochemistry*, **2011**, 46, 2277–2285.