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CHITIN BINDING DOMAINS FOR IMMOBILIZING ANTIBODY FRAGMENTS IN IMMUNAFFINITY CHROMATOGRAPHY

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INTRODUCTION

Immunaffinity chromatography (IAC) [1] is a powerful tool using specific antibodies to purify desired ligands. In practice, however, the need for monoclonal antibodies, which must be produced in animal cells, makes this method rather unattractive because of high costs in man-power and material. Moreover, the chemical coupling of the antibody to the matrix results in random orientation and low yield of functional immobilized molecules.

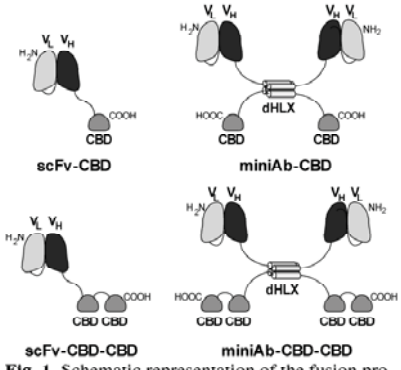

Fig. 1. Schematic representation of the fusion proteins containing one, two or four CDBs. The scFv-CBD constructs have one CBD, while the dimeric molecules miniAb-CBD bind via two CBDs. The scFv-CBD-CBD constructs represent a tandem CBD. The combination of the dimerization motif and the tandem CBDs results in functional units possessing four CBDs, "miniantibodies, miniAbs".

Here, we present an easy-to-perform, versatile and inexpensive method that overcomes the above-mentioned drawbacks of conventional IAC and exploits the power of recombinant antibody technology, allowing the antibodies to be expressed in bacteria: We produced genetic fusions of single-chain Fv-fragments to chitin-binding domains (CBDs) in *Escherichia coli* and immobilized this fusion protein on chitin beads directly out of the crude extract. To keep a stable complex between the CBD and the chitin column, our constructs contained 1-4 CBDs and/or several antibody domains (Fig. 1). After opti-mization of functional expression, binding and elution conditions, we are now able to purify antigens in either a 1- or 2-step procedure, depending on the specificity of the antibody [2]. Since the procedure does not require any chromatographic equipment nor any gradient elution, it can be carried out in a highly parallel fashion.

Supplementary files 1,

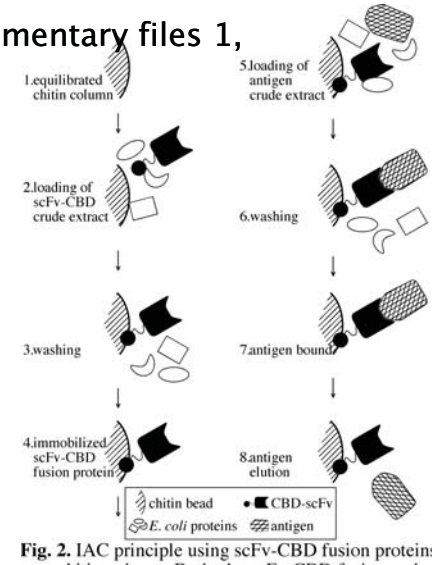

Fig. 2. IAC principle using scFv-CBD fusion proteins on a chitin column. Both, the scFv-CBD fusion and the antigenic protein are expressed in *E. coli*. Directly out of the soluble crude extract, the fusion protein is applied to the equilibrated chitin column material. After washing, the antigen-containing crude extract is loaded, washed again and then the antigen is eluted.

EXPERIMENTAL

The gene for the CBD of *Bacillus circulans* WL-12 chitinase A1 (Swiss Prot. Nr. P20533) [3] was cloned into a pHB-based vector [4]. The single cysteine of the CBD (PDB code 1ED7 [5]) was exchanged for alanine or serine to avoid undesired disulfide formation upon folding in the *E. coli* periplasm. The soluble fraction of the crude extract was directly applied to the chitin column (Fig. 2). Similarly, the crude extracts of various antigens were prepared. The steps illustrated in Fig. 2 were performed in various scales on automated chromato-graphy

systems or in a parallel fashion on a positive pressure manifold ("Cerex", Varian Analytical Instruments Inc., Walnut Creek, CA, USA) without photometric follow-up.

RESULTS AND DISCUSSION

In this paper we introduce a new concept of IAC using fusions of anti-body fragments and the *B. circulans* CBD recombinantly expressed in *E. coli*. While such fusions have been used to purify the fusion with the protein of interest on a carbohydrate column (e.g. cellulose or chitin) with subsequent cleavage of the partners[3,6] or simply to immobilize protein[7], in our approach the immobilized fusion is used to purify a ligand of the scFv. As an example, we present a scFv di-rected against the widely used His tag[8] and thus demon-strate a generic rapid protein purification method for his-tagged proteins using two different purification principles on the same tag if an IMAC[9] is done as a second step (Fig. 3). To avoid any dialysis steps and allow direct coupling of the columns we elute the his-tagged GFP at pH 10. This convenient elution behavior is due to the fact that this scFv fragment recognizes a protonated, C-terminally located his tag[8] which becomes deprotonated with increasing pH.

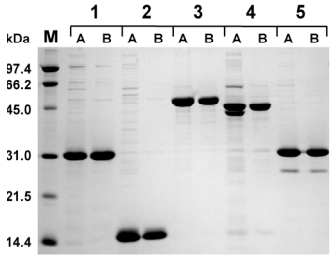

Fig. 3. Two-step purification of different his-tagged proteins. Lanes A show the eluted fractions of the anti-his tag affinity column, lanes B the fractions after the anti-his tag affinity column coupled with IMAC. (M) molecular weight marker, (1) scFv 4D5-his, (2) GroES-his, (3) PhoA-his, (4) CS-his and (5) GFP-his.

Originally, we demonstrated this method with scFv fragments of different specificities[2] which were selected out of the Human Com-binatorial Antibody Library (HuCAL), a naïve, fully synthetic scFv-library[10]. Purifi-cations have been carried out without any detector using gravity flow columns in com-bination with a positive pressure manifold, allowing the parallel purification of mg amounts of 24 different samples in a few hours.

Other approaches for immobilizing recombinant antibodies, be it by chemical coupling[11,12] or using Streptavidin matrices[13] have their disadvantages either in cost, time or labor investments. We believe that the technology described here may be of great value in the purification of proteins from small scales to larges scales and may finally lead to a generic protein purification.

CONCLUSION

Here we present a novel strategy for the directed immobilization of recombinant antibody scFv fragments. Fusions with cysteine-free tandem CBDs were designed for high expression level and tight binding to the chitin matrix. Our results demonstrate that this concept can be generally applied for IAC and yields highly pure protein.

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