BACTERIAL EXPRESSION AND CHARACTERIZATION OF A MODIFIED scFv FRAGMENT FROM THE ANTI-CARCINOEMBRYONIC ANTIGEN MONOCLONAL ANTIBODY CB.CEA.1

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INTRODUCTION

Single-chain antibody fragments (scFv) are defined as the smaller antigen-binding molecule derived from an antibody (1-2). We report the cloning bacterial expression and molecular characterization of an anti-carcinoembryonic antigen (CEA) scFv with potential use for the localization of tumors in CEA-positive carcinoma pâttients.

EXPERIMENTAL PROCEDURES

The scFv gene (VH-Linker-VL) was assembled using PCR amplification (3) and cloned into expression vectors developed by our group. The vectors were denominated pPACIB.1, pPACIB.3 (4), for export to periplasm proteins displaying 6 histidines either in the N- or C-termini, respectively. Protein expression was induced in LB media and using 10 representative *E. coli* strains. Also a vector called pTrp/OmpA, that produces proteins without histidines but with a myc-tag peptide (5) at the C-terminus, was used for express the scFv. Active scFv was detected after induction by specific ELISA and Western blot. IMAC purifications (6) was assayed for proteins tagged with histidines, while a monoclonal antibody-based affinity chromatography was used for scFv.myc-tag protein. Association and dissociation constants to antigen were calculated using a biosensor (BIAcore), and recognition of CEA-positive carcinoma cell lines were done by Fluorescence Analysis Cell Surface (FACS).

RESULTS AND DISCUSSION

Active scFv (concentration ca. 100 mg/L) was found in the periplasm of 5-10 *E. coli* strain (W3110, LE392, BMH71.18, TG1 and coliB), either employing pPACIB.1 or pPACIB.3 vectors. The scFv.myc-tag was produced in the only tested strain (coliB).

One step chromatographic purification of the 6 histidine-tagged scFv by IMAC was unsatisfactory, with purities of around 60%, independently of the position of the His-tag. However, the monoclonal antibody 9E10 affinity purification rendered very pure preparations of the scFv.myc-tag protein (purity ca. 98%).

Affinity constant for the scFV fragment (10^7M) resulted in a circa 2 order of magnitude lower than the value calculated for the biochemically produced Fab fragments. Values in this range have been reported either for scFv (7) or Fab fragments (8). Finally, in a FACS analysis with human CEA-positive carcinoma cells (LoVo), as a preliminary step to *in vivo* distribution assays, the scFv.myc-tag shows a recognition pattern similar to the parental antibody CB.CEA.1, as shows figure 1.

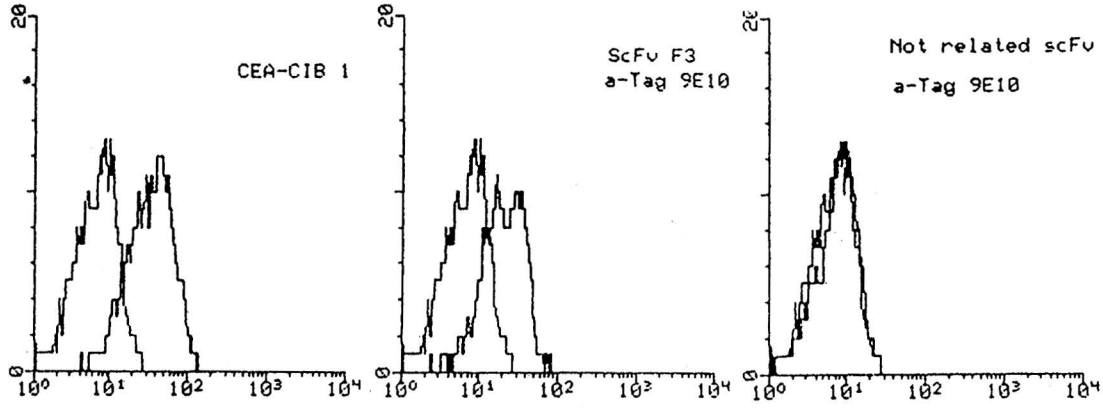

Fig. 1.- FACS analysis of CB.CEA.1 and derived scFv fragment using CEA-expressing carcinoma cells (LoVo)

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VARIABLE REGION SEQUENCES MODULATES PERIPLASMIC EXPORT OF A scFv ANTIBODY FRAMENT, SPECIFIC FOR HEPATITIS-B SURFACE ANTIGEN, IN *E. coli*

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INTRODUCTION

Recent developments in recombinant DNA technology make possible the production of Fab, Fv, and single-chain Fv (scFv) antibody fragments in genetically engineered microorganisms (1). Applications of recombinant Fab and scFv include the elucidation of antigen-antibody interaction, imaging, drug and toxin targeting, catalysis, immunomodulation, immunotherapy, neutralization, and detoxification. Recently authors have also show that antibody fragments could have potential for the immunopurification (2). We have produced a bacterial scFv specific for a recombinant Hepatitis B virus surface antigen (HBsAg). Using expression vectors designed for periplasmic export we found that the scFv nevertheless remained associated to bacterial insoluble material. We will show evidence suggesting that positively charged aminoacids of the heavy chain V-region (V_H) could be responsible for the association of the scFv to the bacterial inner membrane.

EXPERIMENTAL PROCEDURES

RNA was extracted from the anti-HBsAg mouse hybridoma CB-Hep.1, and cDNA synthesized. PCR was used for the assembly of the scFv (VL-L-VH or VH-L-VL) gene, including a 14 aminoacid spacer between VH and VL regions, and for site-directed mutagenesis of the VH domain. The sequenced gene was inserted for export into the secretion vectors pPACIB.1 and pPACIB.3, bearing OmpA secretion signal and for intracellular expression into pPACIB.4 and pPACIB.5 vectors. All vectors bear 6-histidine coding domain that are fused at the N- or C- terminal of the mature protein. Several *E. coli* strains were transformed and expression induced with beta indoleacrylic acid. Western Blots (WB) of SDS-PAGE bacterial material were developed with a specific anti Fab rabbit antibody. The activity of fragments was monitored by specific ELISA against HBsAg. The proteins were purified by immobilized metal affinity chromatography (IMAC).

RESULTS AND DISCUSSION

A high expression level was found for the scFv (VH-L-VL) in pPACIB.1 and pPACIB.3 when strain MM294 was used (ca. 15% of total bacterial protein). While both vectors have a bacterial secretion signal, the synthesized protein is not secreted into the periplasm. Extraction studies and electron microscopy indicate that the scFv is associated to the insoluble membrane fraction. This fraction was solubilized with 6M Urea, and the scFv with a 60% of purity renatured by extensive dyalisis against PBS. The renatured scFv binds to the antigen in a direct ELISA. Two different versions of the scFv fragment were cloned into the pPACIB.1 vector; one of them with mutated VH domain (Arg 16-Gly) and the other with change in domain order (VL-L-VH). These changes brought forth the export active scFv to periplasm, suggesting that framework 1 positively charged aminoacids of the VH region could be responsible for the association of the scFv to the bacterial membrane, as have been suggesting by other different studies (3).