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# High expression and purification of the recombinant camelid anti-MUC1 single domain antibodies in *Escherichia coli*

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**Abstract**

In contrast to the murine and human VHs, camels' single domain antibodies (sdAb) have sufficient solubility. These antigen-specific fragments are expressed well in *Escherichia coli*. Here, we report high expression and purification of sdAbs against MUC1 mucin. MUC1 is a high molecular weight glycoprotein with an aberrant expression profile in various malignancies. The sdAb genes were sub-cloned into a pET32a$^+$ vector to overexpress the protein coupled with fusion tags in *E. coli* BL21(DE3). The expressed single domain antibodies were purified by immobilized metal affinity chromatography and antigen affinity chromatography. Analysis by SDS–PAGE and Western blotting demonstrated the integrity of the sdAbs-tags, while ELISA results confirm that the activity of these molecules compare favorably with that of the parent recombinant antibodies. Enterokinase treated sdAb showed a band at the molecular weight around 12 kDa which demonstrated the naked protein in its natural structure with activities comparable to that of native protein. The high binding activity to MUC1 antigen purified from ascitic fluid (of patients with small-cell lung aggressive carcinoma and metastasis to peritoneum) and the very close similarity of these molecules to human VHs illustrated the potential application of these novel products as an immunodiagnostic and immunotherapeutic reagent.


*Keywords:* Single domain antibody; MUC1; Expression; Purification

The molecular nature of antibody molecules allows for an almost unlimited number of domain rearrangements. Antibody engineering allows the researcher to design and use a variety of binding and effector domains [1]. Number of industrial applications for functionalized antibody fragments can be envisaged including waste water treatment, industrial scale separation processes (e.g., of chiral molecules), abzymes, and as an ingredient in novel consumer goods with new or improved functionalities [1–4]. These applications require large amount of inexpensive molecules. Although significant progress has been made with respect to the production of single chain Fv (scFv)[1] fragments by the *Escherichia coli*, bulk production

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[1] *Abbreviations used:* BSA, bovine serum albumin; $CH_1$, constant heavy-chain domain; D-HMFG, deglycosylated human milk fat globule membrane; ELISA, enzyme linked immunosorbant assay; Fvs, non-covalently associated heterodimers of VH and VL domains; HMFG, human milk fat globule membrane; HRP, horseradish peroxidase; Ig, immunoglobulin(s); NSB, non-specific binding; OD, optical density; PBS, phosphate-buffered saline; scFv, single chain variable domain fragment; sdAb, single domain antibody; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; VH, variable domain from the heavy chain of conventional antibody; VHH, the VH fragment from a camel heavy-chain antibody; VL, variable domain from the light chain of conventional antibody.

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(>100 kg) at low costs is still not feasible [5]. The major reason for this is the structural and functional properties of these fragments. The conventional Fv containing the two variable domains (VH + VL) of immunoglobulins and the engineered fragment (e.g., scFv) have been considered as the smallest antibody fragments with retention of the full antigen-binding capacity. PCR and the development of powerful panning techniques led to the generation of large libraries of scFvs from which several specific binders could be selected successfully [6,7]. This strategy was a major breakthrough in molecular biology. However, the application of the technique is not straight forward. The cloning of the two correctly spliced gene fragments (VH + VL) is a difficult step, generation of a representative library is tedious, and the genetic constructs are often unstable in the bacterial host. Also, the expression yield, stability, and functionality of scFv often turn out to be problematic [8].

The work of some investigators indicated that isolated VH domains are expected to bind antigen in absence of VL domains [9]. This matter led to attempts to obtain an even smaller antigen-binding unit in a VH format. Unfortunately, the poor solubility, the reduced affinity for the antigen and the irreproducible outcome of manipulation of the murine and human VHs showed that additional protein engineering would be required to successfully generate single domain antibody fragments. By serendipity, it was discovered that this engineering is already performed continuously in nature. The discovery of camelidae heavy-chain antibodies naturally devoid of light chains opened up a new opportunity to develop novel sdAbs with improved solution properties [10]. These unique antibody isotypes interact with antigen by virtue of only one single variable domain, referred to as VHH. Despite the absence of the VH–VL combinatorial diversity, the VHH antibody fragments are expressed well in *E. coli*, extremely stable, highly soluble, and react specifically and with high affinity to the antigens. VHH antibody fragments and some VH fragments derived from camels' conventional antibodies are highly soluble and stable in solution [11,12].

Several sdAbs have been raised against different hapten and protein antigens [13,14]. Previously, we have generated and reported two sdAb libraries (from *Camelus dromedarius* and *Camelus bactrianus*) displayed on phage particles [15]. We used the MUC1-related peptide to evaluate the possibility of obtaining antigen-specific sdAb fragments against peptide antigens. The MUC1 membrane mucin has an extensive extracellular domain composed of variable numbers of a highly conserved 20 amino acid repeat sequence (PDTRPAPGSTAPPAHGVTSA), which is abundant in O-glycosylated regions rich in serine, threonine, and proline [16]. The dominant feature of epitopes within the MUC1 protein core is the presence, in full or part, of the hydrophilic sequence of PDTRPAP [16,17].

There are some reports on the generation of recombinant antibody fragments specific for tandem repeat region of MUC1 [15,18,19]. However, transgenic protein levels were relatively low, making future improvements necessary. Although many expression systems are available, the T7 promoter-driven system is among the most successful, due in large part to its ability to stringently control basal expression levels. In this study, the anti-MUC1 VHs genes were cloned into a pET32a$^+$ vector to overexpress the protein coupled with fusion tags in *E. coli* BL21(DE3).

Here, we report our efforts for the construction of two anti-MUC1 sdAbs, their overexpression, purification, and characterization.

## Materials and methods

### *Materials*

Synthetic mucin peptide (TSA-P1-24-TSAPDTRPAP GST APP AHGVTSA PDTR), corresponding to the mucin core protein, which was chemically conjugated to bovine serum albumin (BSA) by reaction with glutaraldehyde, was purchased from Q-BIO-GENE (Kayserberg, France).

All other reagents used in this study were at least of analytical grade and purchased from Sigma Chemical (St. Louis, MO).

### *Methods*

#### *Purification of MUC1 from various sources*

Fresh human milk samples from healthy mother were collected. Briefly, floated cream from the milk was obtained, disrupted with a homogenizer, and the membranes were pelleted at 80,000*g* for 90 min at 5 °C [20]. The pellets were resuspended in 0.3 mol/L sucrose, 70 mmol/L KCl, 2 mmol/L $MgCl_2$, and 10 mmol/L Tris–HCl buffer, pH 7.4. The cream fraction was extracted twice with two volumes of chloroform and twice with one volume of ether, and stored at −70 °C.

Preparation of chemically deglycosylated HMFG (D-HMFG), which closely resembles cancerous MUC1, was performed by incubation of the extensively dried sample (1 mg) in trifluoromethanesulfonic acid for 2 h at 0 °C, followed by neutralization with pyridine/water (3:2) at −20 °C, and dialysis against phosphate buffer (10 mM, pH 7.2) [21].

The native cancerous MUC1 was purified from ascitic fluid of a patient with aggressive small-cell lung carcinoma and metastasis to peritoneum, by an antibody–Sepharose affinity column as described before [15].

*Preparation of rabbit anti-camel labeled-HRP as a tracer*

Anti-camel IgG fractions were prepared in four rabbits and purified by protein A–Sepharose affinity chromatography (Amersham Pharmacia Biotech, Vienna, Austria) as described by the manufacturer. Immunoglobulin fractions purified from antisera were conjugated to HRP following a simplified $NaIO_4$ method. A suitable concentration of enzyme–conjugate was selected based on a titration assay [22].

*Media composition*

The Luria–Bertani (LB) medium contained 1% tryptone, 1% NaCl, and 0.5% yeast extract (plus 1.5% agar in plates) that was supplemented with 85 μg/ml ampicillin for the selection of transformants.

The LB medium supplemented with 0.01 M $MgCl_2$ and 0.02 M glucose was used as electroporation medium.

The Terrific Broth (TB) was prepared with 1.2% tryptone, 2.4% yeast extract, 0.4% glycerol, 0.23% $KH_2PO_4$, 1.25% $K_2HPO_4$, and 70 μg/ml ampicillin.

*Strains and vectors*

*Escherichia coli* Top10 strain (Invitrogen, San Diego, CA) was used as a host for plasmid manipulations and cultures in LB medium. The pET32a$^+$ vector, and *E. coli* BL21(DE3) strain (Novagen, Madison, WI) were used for sdAb overexpression.

*Construction of plasmid*

Construction and screening of anti-MUC1 sdAb libraries have been described in detail elsewhere [15]. Selected clones (RR-EB of *C. bactrianus* and RR-ED of *C. dromedarius*) were sequenced and characterized. These two sdAbs isolated in this manner were conventional antibody VH sequences that represented a minor subpopulation in the library. The VHs encoding sequences in these clones were modified by PCR to include flanking *Eco*RI and *Not*I restriction sites. Polymerase chain reaction (PCR) was carried out using the *Pfu* DNA polymerase (MBI, Fermantase, Opelstr., Germany), and cloned into the pET32a$^+$ expression vector. A pair of PCR primers, 5′-AGCGGCCGCCT AGTGAGGAG-3′ and 5′-GAATTCGTGCAGCT GCAGCAGCTGCAGCAGTC-3′, was designed to generate products with vector cohesive overhangs. The amplification protocol consisted of a 10 min denaturation at 94 °C followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s and 72 °C for 3 min. The amplified sdAb genes were gel-purified from agarose by high pure PCR product purification kit (Roche, Mannheim, Germany) digested with *Eco*RI and *Not*I, and purified again. These single domain antibody fragments coding regions were cloned into pET32a$^+$ vector. This vector is designed for expression of recombinant protein fused to the 109 amino acid thioredoxin (11.7 kDa), a 6 amino acid His-tag, and 15 amino acid S-tag sequences upstream of the cloning site. The fusion tags together can be removed from the recombinant target protein by protease cleavage using enterokinase.

*Electroporation*

Top10 *E. coli* strains were transformed by electroporation. Preparation of electrocompetent Top10 was performed as per supplier's instructions. Eighty microliters of electrocompetent cells was mixed with 1–3 μg of ligated plasmid in a 0.2 cm electroporation cuvette, incubated on ice for 2 min, and electroporated in an Eppendorf Multiporatore (Germany) with settings of 2500 V and 5 ms. After pulsing, 1.0 ml of ice-cold medium (LB medium supplemented with 0.01 M $MgCl_2$ and 0.02 M glucose) was added immediately to the cuvette and the cells were transferred to a sterile 15 ml culture tube. The tubes were incubated at 37 °C without shaking for 10 min, then 2.0 ml supplemented LB medium was added to the tube, and the cells were allowed to recover for 1 h at 37 °C at 250 rpm. Transformants were plated (10 or 100 μl) on LB plates containing 85 μg/ml ampicillin and grown at 37 °C to isolate ampicillin-resistant transformants. The selected transformants were checked by PCR and digestion using restriction enzymes (*Eco*RI and *Not*I). *E. coli* BL21(DE3) cells were transformed with positive recombinant plasmid and used for protein expression.

*Cultivation conditions*

*Escherichia coli* BL21(DE3) cells were transformed with selected recombined plasmids, RR-EB-ET and RR-ED-ET of *C. bactrianus* and *C. dromedarius*, respectively. The transformants were culture in 15 ml LB medium containing 70 μg/ml ampicillin and grown overnight at 37 °C and 250 rpm. These pre-inocula were then transferred to 100 ml TB medium containing ampicillin at the same concentration. The cultures were grown at 37 °C and 250 rpm until $OD_{600}$ of 0.7 was achieved. These cultures were aliqouted (2 ml) in sterile 45 ml culture tubes. The tubes were induced with different concentrations of IPTG (0.5, 1, and 2 mM) in various conditions of induction time and temperature (2, 4, and 8 h and 25, 30, and 37 °C).

*Preparation of cell lysates and comparison of various induction conditions*

The cells were harvested by centrifugation at 6000*g* and 4 °C for 20 min. The pellets containing the bacteria of each aliquot were suspended in 200 μl of 50 mM Tris–HCl buffer, pH 8.0, containing 100 mM NaCl and 1 mM EDTA (adsorption buffer). The protease inhibitor phenylmethylsulfonyl fluoride (PMSF) and lysozyme were added to final concentration of 1.0 mM and 1.0 mg/ml, respectively. The suspensions were incubated for 20 min at 4 °C with stirring, and then 0.04 mg of deoxycholic acid (Sigma) and 1 μl of 1 mg/ml DNase (Roche) were

added. The suspensions were incubated at 37 °C with stirring for 30 min, then Triton X-100 and RNase were added to the tube to a final concentration of 1% and 5 μg/ml, respectively, and continued the incubation with rocking for another 10 min at 4 °C. The lysed material was clarified by centrifugation at 14,000*g* for 30 min at 4 °C and the supernatant was collected. To confirm and compare the quality of recombinant protein expression, SDS–PAGE electrophoresis of the proteins was performed as described by Laemmli [23], using 12% acrylamide gels followed by staining with Coomassie brilliant blue and scanned on a densitometer. Biological activity of recombinant sdAb-tags and sdAbs was compared by ELISA.

*Purification*

The target proteins were purified by loading the clarified supernatant onto a 1 × 5 cm column, packed with 1.0 ml nickel–nitrilo-triacetic acid ($Ni^{+}$–NTA) resin (Qiagen, Valencia, CA).The resin was washed with 10 column volumes (CV) of adsorption buffer (see above). The adsorbed proteins were eluted from the column using an imidazole gradient (from 0 to 250 mM in 10 CV) in adsorption buffer. Fractions containing 1.0 CV of volume were collected and a flow rate of 0.5 ml/min was used during all the chromatographic steps. Fractions were assayed for total protein concentration according to the method presented by Bradford [24], and analyzed by SDS–PAGE [23]. The purified samples were dialyzed against adsorption buffer.

Alternatively, the target proteins were purified by immuno-affinity chromatography. This column was prepared by linking TSA-P1-24-BSA to CNBr-Sepharose 4B (Amersham Pharmacia Biotech) as described by the manufacturer. The cleared cell lysates were loaded onto these peptide–BSA–Sepharose column, equilibrated with phosphate-buffered saline (PBS) (0.39 g/L $NaH_2PO_4$, 0.89 g/L $Na_2HPO_4$, and 8.9 g/L NaCl, pH 7.0). After washing with PBS, the bound recombinant tagged sdAb-tags were eluted with 100 mM glycine–HCl buffer, pH 2.7. Fractions of 1 ml were collected, immediately neutralized with Tris buffer (1 M, pH 9.5), and dialyzed against phosphate buffer (10 mM, pH 7.0). The amounts of purified proteins were determined [24] and then analyzed by SDS–PAGE [23].

The recombinant sdAb was separated from the fusion tags by enterokinase proteolysis (1 U/μl per 10 μg of recombinant protein). The hydrolysis was performed at 25 °C for 14 h according to the protease supplier's instructions. The protease was inactivated by PMSF at 1 mM and the proteolysis products were analyzed by SDS–PAGE. The sample was dialyzed against adsorption buffer containing 10–20 mM imidazole to prepare for the next purification step.

The final purification step was performed using the same column resin mentioned above. The sample containing the sdAb and the cleaved fusion protein was loaded onto the column equilibrated with the same dialysis buffer. The flow through proteins (sdAb) were collected and washed out with 10 CV of the same buffer. Bound proteins were eluted (0.5 ml/min) with equilibration buffer containing 200 mM imidazole. The fractions containing 1 CV were collected and analyzed by SDS–PAGE, and their total soluble protein concentration was determined.

*Reactivity of recombinant sdAb towards synthetic peptide or purified MUC1 of various sources*

The synthetic peptide–BSA (50–1000 ng/well), D-HMFG (50–1000 ng/well) or purified native cancerous MUC1 (10–200 ng/well) were coated onto the wells of microtiter plates at 37 °C overnight. The same concentration of BSA or a 14 amino acid irrelevant peptide (LEEKKGNVVTDHC) conjugated to BSA was used as a negative control. The plates were washed and blocked with a 2% solution of BSA in PBS for 1 h at 37 °C. At the end of incubation time, wells were washed and added with diluted cell lysates, purified recombinant sdAb-tags, or sdAbs.

In this experiment, cell lysate of BL21(DE3) cell transformed with $pET32a^{+}$ (without insert) was used as non-specific binding (NSB). The contents of the wells were incubated at 37 °C for 2 h, washed, added with rabbit anti-camel conjugated to HRP, and incubated at 37 °C for 1 h. The rest of experiment was performed as explained before [15].

## Results

*Construction and transformation of the vectors*

The sequence of the anti-MUC1 sdAbs was modified by PCR at the 5′-end by introduction of an *Eco*RI site. The 0.4 kb inserts were ligated into the multiple cloning site region downstream of the Trx-Tag/His-Tag/S-Tag of $pET32a^{+}$ vector using the *Eco*RI/*Not*I restriction sites. The resulting plasmids (RR-EB-ET and RR-ED-ET) were transformed into the *E. coli* strain Top10. The $pET32a^{+}$ plasmid contains the ampicillin resistance gene for selection in *E. coli*. Plasmids carrying the insert were selected on LB plates containing ampicillin. The selected transformants were then confirmed by PCR and digestion using restriction enzymes (*Eco*RI and *Not*I) *E. coli* BL21(DE3).

*Comparison of cultivation conditions*

Dose dependence, temperature, and time course studies of the induction of the recombinant protein expression, analyzed by SDS–PAGE led to a IPTG concentration of 1 mM and induction time of 8 h at 30 °C. The expression of the fusion protein containing

the sdAb (sdAb-tags) was considered 48 and 19% of total soluble protein of RR-EB-ET and RR-ED-ET bacterial cell lysate, respectively.

*Purification of sdAb antibody fragments*

High quantities of relatively pure recombinant protein containing the Trx-Tag/His-Tag/S-Tag were recovered and purified from the lysate extract by using immobilized metal affinity chromatography (IMAC) (Fig. 1). The reduction of non-specific interactions between extract proteins and the matrix was done by washing the resin with high salt concentration, and low imidazole concentration buffers. The elution of the recombinant protein was performed using a gradient of imidazole concentration, a procedure that considerably improved the final protein purity. Elution fractions containing the recombinant protein with a high level of purity (analyzed by SDS–PAGE) were separated and combined as a pool for further purification steps.

To remove the N-terminal fusion tags from the sdAb-tags, a cleavage by enterokinase protease was performed. The fusion tags were used so as to improve the recombinant protein solubility, also allowing ease of detection and purification from the extract. The fusion tags were successfully removed from sdAb-tags by cleavage and few non-specific cleavages were noted. The analysis of the proteolysis products is shown in Fig. 1. Since the fusion tags contain higher molecular masses compared to recombinant sdAb fragments (without tags), they are seen on the gel as the separate bands. Final chromatography step on the IMAC resin was performed to separate the fragment containing the tags and the target protein. In this case, the purified recombinant sdAb (without tags) was collected in the flow through fractions (Fig. 1). SDS–PAGE analysis of enterokinase cleavage of tagged-protein exhibited only one band in the region of 12 kDa (no band in 25 kDa) which indicated 100% cleavage efficiency. The final amount of purified recombinant sdAb obtained was approximately 70 and 42 mg/L of initial RR-EB-ET and RR-ED-ET bacterial broth, respectively.

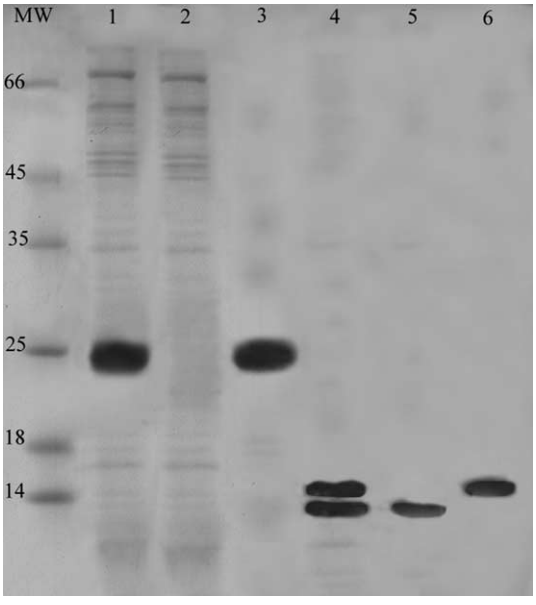


Fig. 1. SDS–PAGE analysis of the fractions collected during the purification steps. Lane MW, marker; lane 1, extract from bacterial lysis loaded onto the IMAC column; lane 2, flow through extract; lane 3: eluted fraction (200 mM imidazole), containing the recombinant fusion protein (sdAb-tags); lane 4: proteins resulted from enterokinase cleavage step. This fraction was loaded onto the IMAC column to promote final purification of the sdAb; lane 5: flow through fraction of final purification step containing the sdAb; lane 6: eluted fraction of final purifications step containing the fusion tags.

Alternatively, purification of sdAb-tags recombinant antibodies was performed by affinity chromatography on TSA-P1-24-BSA–Sepharose column. The fractions containing the pure sdAb-tags fragments were pooled. From the absorption measurement at 280 nm, a yield of 10 and 5 mg of purified protein per liter of initial RR-EB-ET and RR-ED-ET bacterial cultures was calculated. Each antibody preparation was evaluated for immunoreactivity by titration in ELISA wherein all of the purified antibodies showed high immunoreactivity. These results definitively pointed out that the immobilized metal affinity chromatography would be the choice for purification of these recombinant tagged antibodies.

*Reactivity of recombinant sdAb-tags and sdAbs*

The reactivity of sdAb-tags and sdAbs (RR-EB-ET and RR-ED-ET) with synthetic peptide conjugated to BSA (TSA-P1-BSA), D-HMFG (chemically synthesized cancerous MUC1), and the native cancerous MUC1 (purified from ascitic fluid of a patient with aggressive small-cell lung carcinoma) was tested using ELISA procedure (Fig. 2). All of the sdAb-tags and sdAbs showed high immunoreactivity towards MUC1-related peptide, chemically synthesized MUC1 and purified native cancerous MUC1, and almost no cross-reaction to non-specific proteins used in this study. However, the ELISA results which used single domain antibodies as capture antibody were the same as those of sdAb-tags when similar molar concentrations of antibodies were used. These results clearly indicated that digestion of sdAb-tags did not decrease the affinities and immuno-reactivity of sdAb towards the antigens.

The binding activity of these sub-cloned recombinant sdAb fragments (without tags) was compared with that of the parent sdAb fragments in ELISA, by registrating optical density values produced of different origins. We found that the sub-cloned sdAbs compare satisfactorily with the original sdAb fragments.

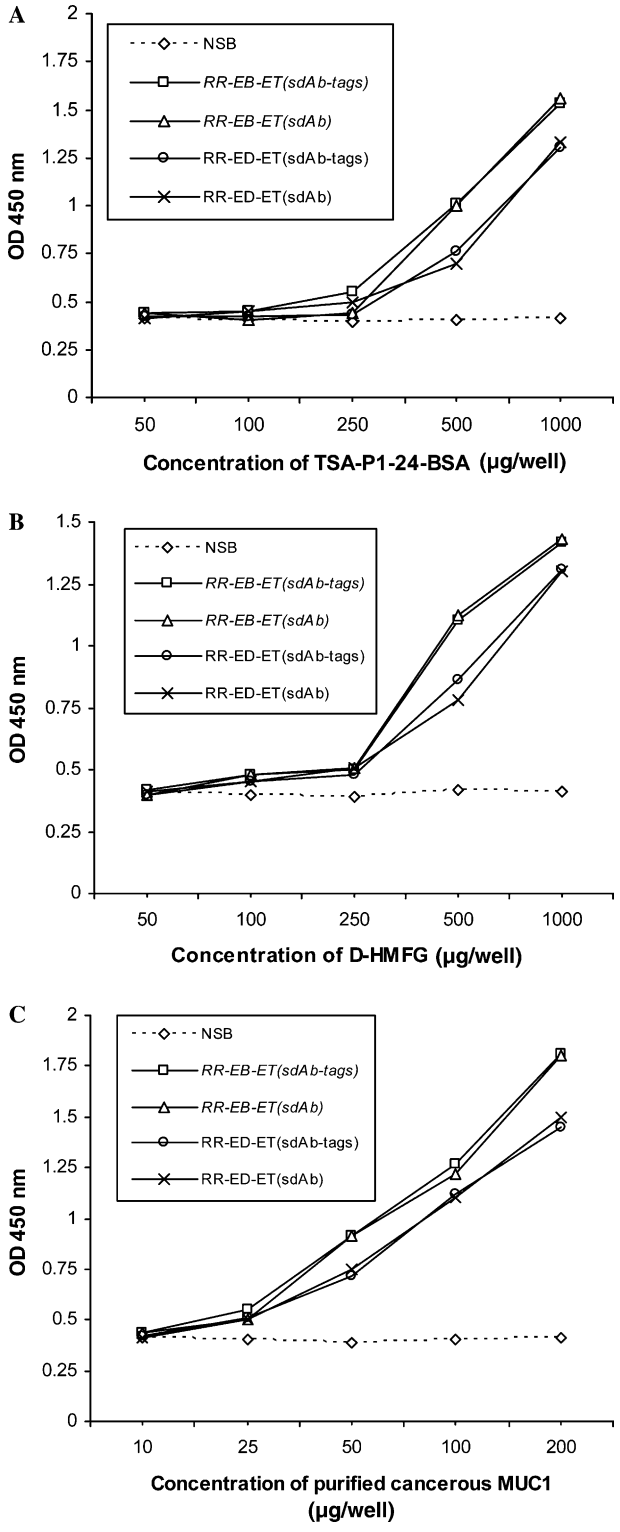


Fig. 2. Reactivity of purified sdAb-tags and sdAb antibody fragments (1 µg/well) with synthetic peptide–BSA (A), D-HMFG (B), and cancerous MUC1 purified from ascitic fluid (C). NSB was determined using cell lysate of BL21(DE3) cell transformed with pET32a$^+$ (without insert). The experiments were repeated four times and the results are expressed as mean value.

## Discussion

Antibodies and their derivatives fragments have long been used as tools in a variety of applications, in fundamental research work, biotechnology, diagnosis, and even human therapy. Not surprisingly, immunoglobulins constitute at least 25% of the proteins in clinical trials. Utilization of antibodies as drug delivery vehicles is a clearly successful application. For most applications, high-yield production, solubility, stability, and small size (when efficient biodistribution or reduced immunogenicity is required) are critical factors. Thus, many attempts to reduce the size of antibody, while retaining its antigen-binding properties, have been reported. This resulted in a series of antibody fragment constructs, such as Fvs [7], scFv [25], and even single domain VH [9], which can be expressed in *E. coli*, or yeast.

Camelid heavy-chain antibodies form a novel concept in both immunology and biotechnology. Their unique structural features include the absence of both immunoglobulin light chains and the CH1 constant domain. Antigens specific for the heavy-chain IgGs (VHH) are very stable, highly soluble, and react specifically and with high affinity to the antigens [10,11]. Recently, some of the investigators demonstrated that VHs from camelidae conventional antibodies like their VHHs, counterparts from heavy-chain antibodies, are excellent sdAbs and highly soluble, and exist as monomers [12,26]. Following the immunization of *C. dromedarius* [15,27], llamas [28], and *C. bactrianus* [15] recombinant antibody fragments (sdAbs) can be isolated, which consist of a single domain immunoglobulin only. Because of high production yield, the reduced size, improved solubility, and higher stability, the sdAbs are of special interest in biotechnological and medical applications. We have generated two sdAb libraries against MUC1-related peptide (from *C. dromedarius* and *C. bactrianus*) displayed on phage particles [15]. In this work, the RR-EB and RR-ED, encoding the VHs antibody fragments, were successfully cloned in the pET32a$^+$ expression vector and no difficulty was found to transform the *E. coli* BL21(DE3) strain for expression studies. IPTG dose dependence, temperature, and time course studies of induction of recombinant protein expression were analyzed and demonstrated the optimum condition for target proteins expression.

The sdAb anti-MUC1 antibody fragments were purified using two steps of affinity chromatography on $Ni^+$–NTA resin, and its purity and identity were verified by SDS–PAGE and ELISA. The recombinant sdAbs proved to specifically bind to MUC1-related synthetic peptide, chemically synthesized cancerous MUC1 (D-HMFG), and native cancerous MUC1 (purified from ascitic fluid). Our yield and specific activity results definitively demonstrate the new recombinants *E. coli* BL21(DE3) (pET32a$^+$) strain allow rapid, large-scale production of sdAb antibody fragments.

The camels' VHs described here are potentially more useful than camelized human VHs [12]. Their fragments should be less immunogenic in humans, making them more useful as therapeutics. The features of these single domain anti-MUC1 antibodies make them particularly valuable as tumor targeting reagents.

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