

Impact of base matrix types on the properties of affinity chromatography resins with ligands based on staphylococcal protein A B-domain multimers

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Experimental Section

Materials

Polymethacrylate beads UniGel-65NH₂ (70 μ mol (amino groups)/mL, Suzhou NanoMicro Technology, Suzhou, China) or epoxy-activated agarose Seplife 6FF matrix (6% cross-linked agarose beads, 31 μ mol (epoxy groups)/mL) from Sunresin (Xian, China) were used as a solid support (matrix) for the immobilization of BsrtA ligands. Immunoglobulins HUMIRA® (adalimumab from AbbVie, Ludwigshafen, Germany) were used for DBC₁₀ values testing of the affinity resins produced. All reagents from Panreac (Barcelona, Spain) were of analytical grade unless otherwise specified.

Pre-treatment of solid matrices before ligand immobilization

To provide primary amino groups on the surface, the epoxy-activated Seplife 6FF agarose matrix was supplemented with a Gly-Gly-EDA-Gly-Gly linker, flanked by -NH₂ groups. A significant excess of the linker in the reaction was used to prevent the formation of glycine bridges on the surface. The detailed protocols for the synthesis of the polyGly linker and its attachment to the Seplife 6FF matrix can be found in our previous publication [S1]. The final concentration of -NH₂ groups was estimated using the Cl⁻ ion titration method and was found to be 27 μ mol/mL.

Polymethacrylate beads UniGel-65NH₂ were used without further modification.

Preparation of recombinant proteins BsrtA

The construct [leader peptide]-[domain B of SpA protein]-n-[LPETGEAHHHHHH], where n (n=2÷4) represents the number of B-domains in the protein sequence, was used. In this construct, the LPETG peptide fragment is specifically recognized by the enzyme, while the His tag facilitates the isolation of the construct.

Escherichia coli cells producing a BsrtA-fragment containing hybrid protein were obtained by transforming an expression vector containing the coding sequence of pKanR-Par101_BDPA-2 into the BL21(DE3) pLysS strain using chemical transformation protocol. The cells were cultured and the protein expression was induced using isopropyl1-thio- β -D-galactopyranoside (IPTG) in a final concentration of 0.25 mM. Bacterial cells were collected by centrifugation at 5000xg for 20 min at 4 °C and lysed using an ultrasonic disintegrator (Elma, Schmidbauer GmbH, Singen, Germany) in 700 mL of 50 mM Tris-HCl and 5 mM EDTA at pH 8. Urea was then added to the lysate to a concentration of 4 M, followed by a subsequent dilution of the sample with buffer A (50 mM Tris-HCl, pH 8.0) to achieve a final urea concentration of 1 M.

The protein was purified using an XK 26/20 column (Cytiva, Life-Sciences, Marlborough, MA, USA) packed with DEAE Sepharose ion exchange sorbent, under gradient elution conditions ranging from 0 to 0.5 M NaCl in buffer A. Next, the IMAC metal chelate sorbent (SepFast BioToolomics, Consett Co., Durham DH8 6TJ, United Kingdom) was applied using an XK 26/20 column. The column was pre-equilibrated with buffer B (50 mM Tris, 0.3 M NaCl, pH 8.0). The target protein was eluted using 0.5 M imidazole in buffer B. Finally, the BsrtA solution was desalted using a Sephadex G-25 XK 50/100 column (Cytiva, Life-Sciences, Marlborough, MA, USA) with buffer A, yielding proteins with an electrophoretic purity and HPLC purity of >90% (see Fig. S1).

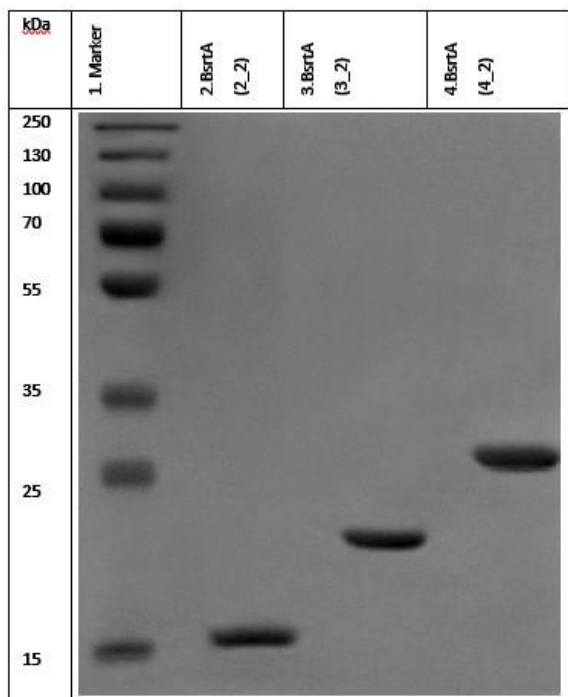


Figure S1. SDS-PAGE analysis of recombinant proteins BsrtA.

More details can be found in [S1, S2].

Preparation of Recombinant Sortases A (SrtA)

Recombinant calcium-dependent pentamutant SrtA enzyme was obtained following the protocol described in [S3, S4]. To generate a strain that produces the calcium-dependent SrtA pentamutant enzyme, the pSortase_SNAT expression plasmid DNA was created based on the pET23b_KanR-Par101 vector and a synthetic DNA sequence encoding for SrtA with an N-terminal hexahistidine tag. The pSortase_SNAT plasmid was used to transform *E. coli* BL21(DE3) pLysS cells.

After culturing and induction of SrtA expression for 4 hours, the bacterial pellet was separated by centrifugation (5000xg, 20 min, 4°C) and lysed using an ultrasonic disintegrator (Elma, Schmidbauer GmbH, Singen, Germany) in buffer C (50 mM Tris, 0.5 M NaCl, 10% glycerol, pH 7.5). After centrifugation, the resulting protein solution was applied to an XK 26/20 column packed with Ni-IMAC metal chelate resin (SepFast BioToolomics, Consett Co., Durham DH8 6TJ, United Kingdom), that had been pre-equilibrated with buffer C containing 20 mM imidazole. The column was then washed with

buffer C containing 50 mM imidazole and the target protein was eluted using buffer C containing 500 mM imidazole. Finally, the SrtA solution was purified using size exclusion chromatography on a Sephadex G-25 XK 50/100 column (Cytiva, Life-Sciences, USA), and eluted with buffer D (50 mM Tris, 150 mM NaCl, 10% glycerol, pH 7.0). This yielded up to 650 mg of calcium-dependent pentomutant SrtA enzyme with an electrophoretic purity of >95% (see Fig. S2).

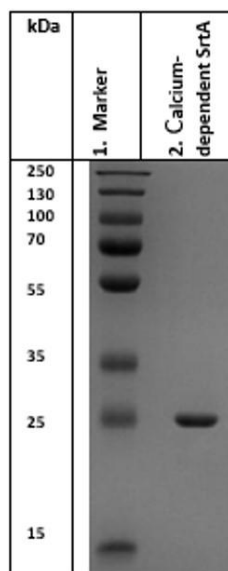


Figure S2. SDS-PAGE analysis of recombinant proteins Srt A

Enzymatic Immobilization of the BsrtA Proteins onto a Matrix Using Sortase A

The sample of solid matrix was washed sequentially on a glass filter using the following buffer solutions (100 mL of buffer per 10 mL of a matrix): 100 mM NaHCO₃ (pH 8.4), 50 mM Tris (pH 8.0), 100 mM sodium acetate (pH 4.5), and 50 mM Tris-HCl (pH 8.0).

A solution of BsrtA ligand (21 mg/mL, 9.5 mL) in 50 mM Tris-HCl buffer (pH 8.0) was added to a pre-prepared matrix. Then, 1.25 mL of 4 M NaCl, 1.25 mL of 0.2 M CaCl₂, and 2.8 mL of calcium-dependent pentomutant SrtA (5 mg/mL) were added, adjusting the total volume to 50 mL using 50 mM Tris buffer (pH 8.0). The mixture was incubated at 37°C for 4 hours with stirring. This protocol was used for ligands of all lengths (from dimers to tetramers).

The protein concentration in the samples was determined spectrophotometrically using a UV/visible spectrophotometer Ultrospec-1000 (Amersham, Pharmacia Biotech, Cambridge, UK). The extinction coefficients (ϵ_{280} , M-1cm-1) were calculated using the ProtParam tool (<https://web.expasy.org/protparam/>, accessed on 1 September 2022), and were 4470 for BsrtA(2_2), 5960 for BsrtA(3_2), 7450 for BsrtA(4_2), and 18910 for the calcium-dependent pentomutant SrtA enzyme, respectively.

In parallel, a negative control experiment was conducted without SrtA in the reaction mixture. The sample was incubated and then washed with various solutions (in accordance with the protocol) to remove any possible binding of BsrtA due to non-specific interactions with the matrix.

Dynamic Binding Capacity (DBC) Determination

The estimation of DBC₁₀ values was performed using a column C10/10 (Pharmacia, Bromma, Sweden, size 10 cm × 10 mm). The volume of the resin sample applied was 2 mL.

The linear flow velocity corresponding to a given residence time (RT) of 6 min was calculated using Formula 1:

$$v = (H/t) \times 60 \quad (1)$$

where t is the residence time of IgG1 with the resin (in min), H is the bed height of the resin in the column (in cm), and v is the linear flow velocity (in cm/h).

The flow rate was then calculated using Formula 2:

$$q = v \times S/60 \quad (2)$$

where q is the flow rate (in mL/min), v is the linear flow velocity (in cm/h), and S is the cross-sectional area of the column (in cm²).

The flow rate during the washing and elution steps was 2 mL/min. To determine the DBC₁₀ values, a working solution of immunoglobulin G₁ at 2 mg/mL in phosphate-buffered saline (PBS) with pH 7.4 was used. The maximum absorption of the IgG solution with a known concentration was measured using

a UV detector installed on the AKTA Pure instrument at a wavelength of 280 nm. After the resin had been equilibrated with 10 mL of PBS (pH 7.4), the IgG solution was applied to the resin until an optical density equal to 10% of the initial solution's optical density had been achieved. The dynamic binding capacity of the resin for a residence time of 3 minutes was calculated using the UNICORN Extension 7.3 SP1 software's Dynamic Binding Capacity calculation. Finally, the resin was washed with 10 mL of PBS (pH 7.4), and the bound IgG was eluted using 100 mM sodium acetate at pH 3.5. After elution, the resin was again washed with 10 mL of PBS and 20% ethanol.

References

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