MabSelect SuRe –studies on ligand toxicity, leakage, removal of leached ligand, and sanitization

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MabSelect SuRe–studies on ligand toxicity, leakage, removal of leached ligand, and sanitization

Key words: MabSelect SuRe · recombinant protein A · toxicity · ligand leakage · sanitization · monoclonal antibody

Summary

MabSelect SuRe™ is an affinity medium designed for purification of large volumes of monoclonal antibodies. The alkali-stabilized protein A-derived ligand allows extensive use of sodium hydroxide for cleaning- and sanitization-in-place. The ligand had no toxic effects at the high doses administered to rats described in this study. Leakage of the ligand from MabSelect SuRe was shown to be low and ligand leakage was effectively removed by ion exchange chromatography. Sanitization was accomplished in 15 min using 0.5 M NaOH.

Introduction

Protein A-based media are the industry standard for the large-scale capture of monoclonal antibodies (MAbs). These media offer high selectivity and yield a highly pure product in a single step. However, in most protein A affinity chromatography applications, small amounts of the protein A ligand are leached from the affinity medium into the eluate. In order to meet the regulatory criteria for the production of biopharmaceuticals, the presence of protein A impurities has to be reduced to acceptable levels, typically 10–12 ppm in the final product (1).

MabSelect SuRe is an affinity medium that offers low leakage of the ligand in process applications. The medium is ideal for purification of large amounts of MAbs and is resistant to the alkali treatment employed in cleaning-in-place (CIP) procedures. The novel MabSelect SuRe ligand was developed by protein engineering of one of the IgG binding domains of protein A. Amino acids particularly sensitive to alkali were identified and substituted for more stable ones. The final construct is a tetramer of the engineered domain with a C-terminal cysteine residue, which enables single-point attachment to the matrix (Fig 1).

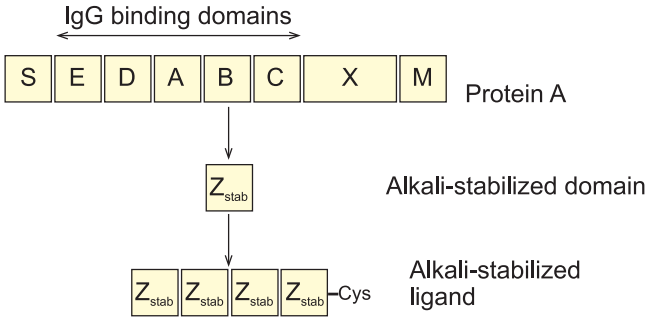

Fig 1. Site-specific mutagenesis of protein A yields an alkali-stabilized tetramer variant.

As the MabSelect SuRe ligand has a slightly different amino-acid sequence compared with conventional recombinant protein A (rProtein A), an investigation into its toxicity was undertaken in a rat study and mutagenic acitivity was assessed in a bacterial reverse mutation test. The leakage of the MabSelect SuRe ligand during elution was also investigated, as well as methods for removal of leached ligand.

The production of biopharmaceuticals for clinical applications is governed by regulations that impose very high standards on levels of purity. Sanitization is one method of reducing a population of microbial organisms to a level acceptable for production. Regulations require that the efficiency of sanitizing agents is evaluated. A microbial challenge test was performed to measure the efficiency of sanitization of MabSelect SuRe using sodium hydroxide.

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MabSelect SuRe ligand is nontoxic and nonmutagenic

Studies were performed to investigate whether the MabSelect SuRe ligand has any mutagenic activity in a bacterial reverse mutation test, or any toxic properties after a single dose administration to rats. The studies were designed to comply with the latest versions of the guidelines formulated by ICH and OECD and were performed in accordance with the current OECD Good Laboratory Practice guidelines.

For evaluation of mutagenicity, the MabSelect SuRe ligand was subjected to the Ames test designed in accordance with the OECD guideline "Bacterial Reverse Mutation Test", No. 471 (references 2–4). The test detects mutations caused by the test substance that revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. The bacterial cells were exposed to the test substance both in the presence and absence of an exogenous mammalian metabolic activation system.

For evaluation of toxicity, a single-dose intravenous injection study in the rat was performed. A sighting study was first performed to find appropriate dose levels. As no signs of toxicity were observed in this study, the highest practical dose was chosen for the main study. Five female and five male Wistar rats were injected with a single dose of 149 mg ligand protein/kg body weight. This dosage corresponds to a 10^9-fold higher level than can be expected in a worst-case scenario resulting from residual ligand in the end product. All animals were closely observed over a period of 14 days for signs of ill health and behavioral changes. At the end of the observation period, all rats were anesthetized and exsanguinated, after which a gross necroscopy examination was performed.

The Ames test showed no indication of mutagenic activity of the MabSelect SuRe ligand when tested to the limit concentration (5 mg/plate), with and without a metabolic activation system. In the single-dose toxicity study with intravenous administration of the MabSelect SuRe ligand up to 149 mg/kg body weight, no animals showed any abnormal behavior. The gross necroscopy examination did not reveal abnormalities in any of the animals.

Low ligand leakage

The level of leakage of the MabSelect SuRe ligand during elution is low. A normal range of leakage is estimated to be 5–20 ppm (5–20 ng ligand/mg IgG). However, leakage is affected by chromatography running conditions and the composition of the feedstock. For more data on the leakage of the MabSelect SuRe ligand, see data file 11-0011-65.

A laboratory-scale study was performed to evaluate the performance of MabSelect SuRe over 50 purification cycles in conjunction with CIP. A MAb was purified from a cell culture supernatant. A CIP step with 0.5 M NaOH was applied for each run for a total of 50 runs. Contact time of the MabSelect SuRe medium with the sodium hydroxide was 10 min for each run.

Numerous CIP cycles with 0.5 M NaOH had no effect on the elution profile of the MAb (Fig 2). Fractions collected from the purification were analyzed by ELISA. The data in Table 1 show the low leakage of the MabSelect SuRe ligand over numerous purification cycles. Ligand leakage throughout this study was well below the standard level of leakage expected with conventional rProtein A-based media.

Column:	Tricorn™ 5/50 (1-ml column packed with MabSelect SuRe)
Each cycle included the following steps:	
Equilibration:	PBS, 5 CV, 1 ml/min
Sample load:	90-ml cell culture supernatant (0.2 mg human MAb/ml), 0.33 ml/min
Wash:	PBS, 5 CV, 1 ml/min
Elution:	0.1 M glycine, pH 3.0, 0.5 ml/min
CIP:	0.5 M NaOH, 10 min contact time, 0.33 ml/min
Re-equilibration:	PBS, 5 CV, 1 ml/min

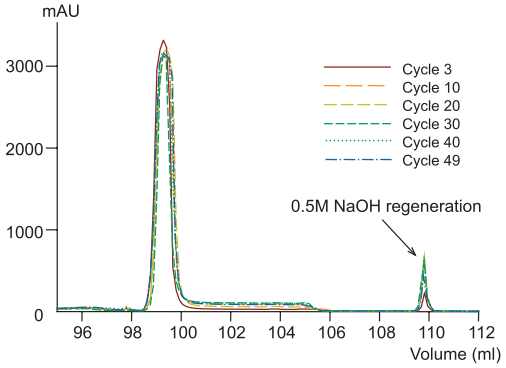

Fig 2. Affinity chromatography purification of a MAb after repeated CIP cycles. The Figure is a partial chromatogram showing only CIP and elution steps.

Table 1. Enzyme-linked immunosorbent assay (ELISA) showing leakage of the MabSelect SuRe ligand after repeated purifications including CIP with 0.5 M NaOH

Purification cycle	Leakage of MabSelect SuRe ligand (ng/ml)	Leakage of MabSelect SuRe ligand (ppm)
1*	3.70	–
5	12.44	3.11
10	5.21	1.30
15	6.28	1.57
25*	0.33	–
32	4.03	1.01
40	10.44	2.61
48	8.45	2.11
50*	0.34	–

* Cycle 1, 25, and 50 were blank cycles using 10 ml PBS instead of sample.

Peak fractions of the MAb from the affinity purification were analyzed by gel filtration (Fig 3). Host cell protein levels in the eluates were maintained below 1000 ppm throughout the study (Table 2).

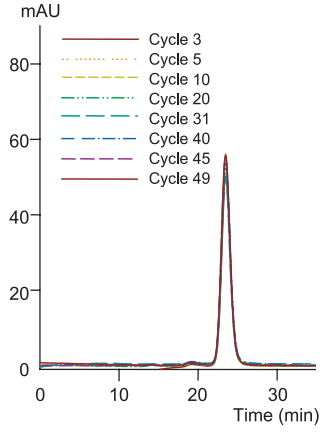

Fig 3. Peak fractions containing MAb (from Fig 2) analyzed by gel filtration using Superdex™ 200.

Table 2. Host cell protein levels in MabSelect SuRe eluates

Purification cycle	Host cell protein (ng/ml)	Host cell protein (ppm)
2	3025	756
3	2907	726
10	1872	468
24	1736	434
40	1924	481
49	1768	442

Removal of leakage by ion exchange chromatography

The binding and elution characteristics of conventional rProtein A and the MabSelect SuRe ligand were studied. Ligand leakage resulting from the protein A affinity capture step is typically reduced or eliminated in subsequent chromatography steps using, for example, ion exchange chromatography.

The similarity in binding and elution characteristics of the conventional rProtein A ligand and MabSelect SuRe ligand are shown in the ion exchange chromatography elution profiles (Figs 4 and 5).

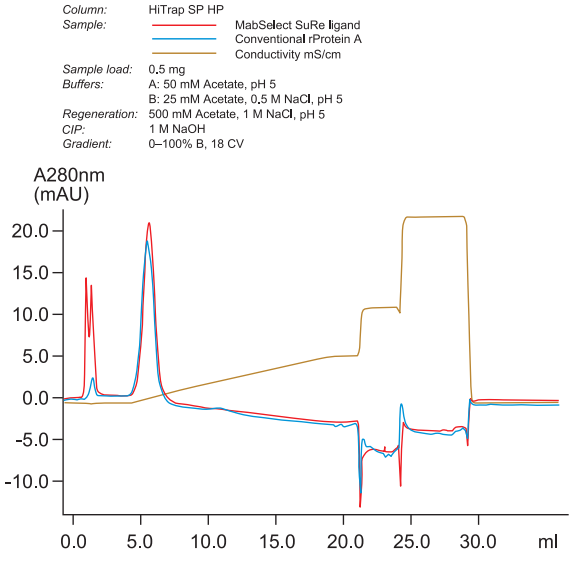

Fig 4. Elution profile of conventional rProtein A and the MabSelect SuRe ligand in cation exchange chromatography on HiTrap™ SP HP.

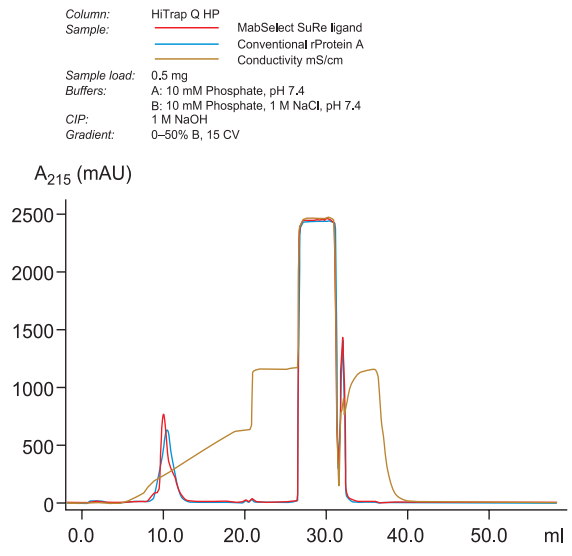

Fig 5. Elution profile of conventional rProtein A and the MabSelect SuRe ligand in anion exchange chromatography on HiTrap Q HP.

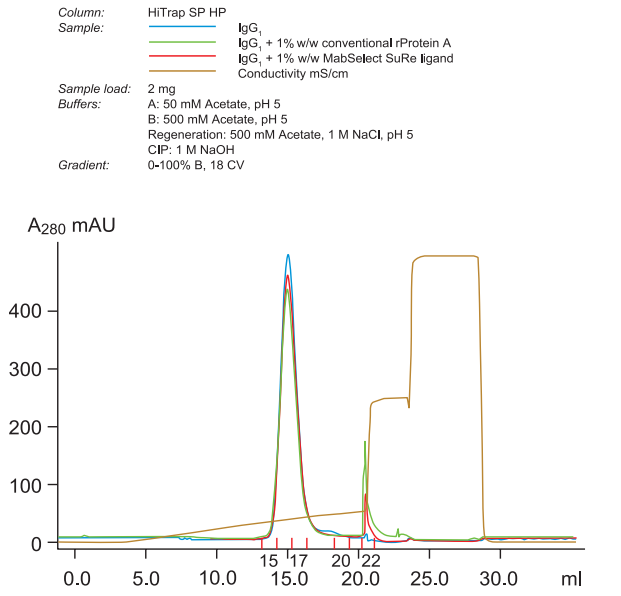

Fig 6. Removal of conventional rProtein A and the MabSelect SuRe ligand from IgG_1 by cation exchange chromatography. The fractions marked in red were analyzed by ELISA in order to determine the relative amounts of remaining ligand in the purified IgG_1 sample.

In a separate study, conventional rProtein A and the MabSelect SuRe ligand were spiked into an IgG_1 sample to a concentration of 10 000 ppm and subsequently removed by cation exchange chromatography. Figure 6 shows the removal of ligands from IgG_1.

To determine the amount of remaining rProtein A ligand in the peak fractions, ELISA was performed on fractions 15, 17, 20, and 22 from the run shown in Figure 6. Results from the ELISA analysis indicate that the spiked MabSelect SuRe ligand was removed by a factor of 10^2 from the IgG_1-containing peak (fraction 17, Table 3).

Table 3. Enzyme-linked immunosorbent assay (ELISA) of fractions from a cation exchange run of IgG_1 and IgG_1 spiked with ligands

Sample	Fractions (ng ligand/ml)†			
	15	17	20	22
IgG_1*	2	0.9	33	72
IgG_1 + conventional rProtein A ligand	29	725	> 900	> 900
IgG_1 + MabSelect SuRe ligand	29	74	> 900	> 900

*The humanized IgG_1 used in this study was purified by affinity chromatography using conventional protein A media.

† Fractions indicated in the Table correspond to fractions collected from the cation exchange run shown in Figure 6.

The same approach was taken for removal of rProtein A ligands from IgG_1 using anion exchange chromatography (Fig 7 and Table 4). After purification of IgG_1 under nonbinding conditions, selected fractions were analyzed by ELISA (Table 4). Results from the ELISA analysis indicate that fraction 4 contained almost no ligand while fraction 32 contained a ligand/IgG_1 complex. The amount of ligand was efficiently reduced in the IgG_1-containing fractions.

Table 4. Enzyme-linked immunosorbent assay (ELISA) of fractions from a anion exchange run of IgG_1 and IgG_1 spiked with ligands

Sample	Fractions (ng ligand/ml)†			
	4	12	26	32
IgG_1*	n.d.‡	n.d.‡	1.6	8
IgG_1 + conventional rProtein A ligand	1.5	n.d.‡	882	> 900
IgG_1 + MabSelect SuRe ligand	n.d.‡	n.d.‡	900	> 900

† Fractions indicated in the Table correspond to fractions collected from the anion exchange run shown in Figure 7.

‡ Not detected (less than 0.6 ng/ml).

* The humanized IgG_1 used in this study was purified by affinity chromatography using conventional protein A media.

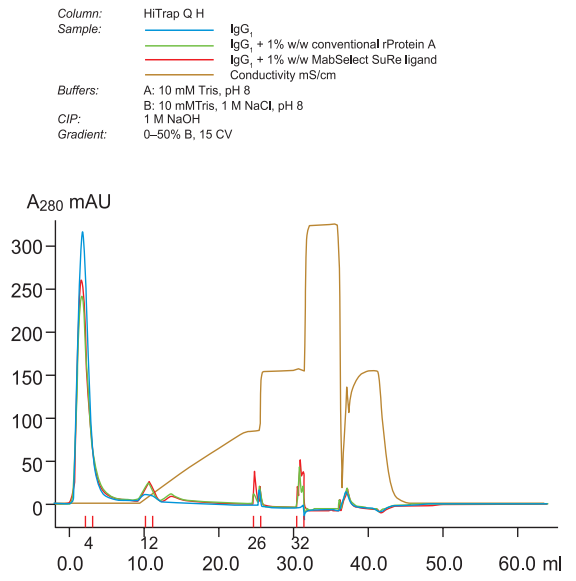

Fig 7. Removal of conventional rProtein A and the MabSelect SuRe ligand from IgG_1 by anion exchange chromatography. The fractions marked in red were analyzed by ELISA in order to determine the relative amounts of remaining ligand in the purified IgG_1 sample.

Sanitization of MabSelect SuRe

Sanitization with chemical reagents is commonly used in chromatography systems to minimize contamination of the product being processed. Regulations require that the efficiency of individual sanitization agents is evaluated. The microbial challenge test described here is a measure of this efficiency. The principle of microbial challenge tests is to introduce a high concentration of contaminants into the equipment or media and then treat it with an antimicrobial agent, in this case, sodium hydroxide. After a specified time, the number of surviving organisms is counted.

MabSelect SuRe was sanitized from gram-negative and gram-positive bacteria using different concentrations of sodium hydroxide. Suspensions of *Escherichia coli* and *Staphylococcus aureus* were prepared for the experiment. The microorganisms (5 ml, corresponding to 1×10^6 of each) were added to flasks containing a 50% slurry of MabSelect SuRe. Sodium hydroxide was added to give a final concentration of 0.1 M or 0.5 M NaOH, and the flasks were shaken at room temperature. Samples were taken at 15 min, 30 min, 1 h, 2 h, and 4 h. The number of remaining microorganisms was determined by viable count.

The results shown in Figures 8 and 9 indicate the efficiency of sanitization of MabSelect SuRe with 0.1 M and 0.5 M NaOH. Complete sanitization of MabSelect SuRe was achieved within 15 min using 0.5 M NaOH or 30 min using 0.1 M NaOH.

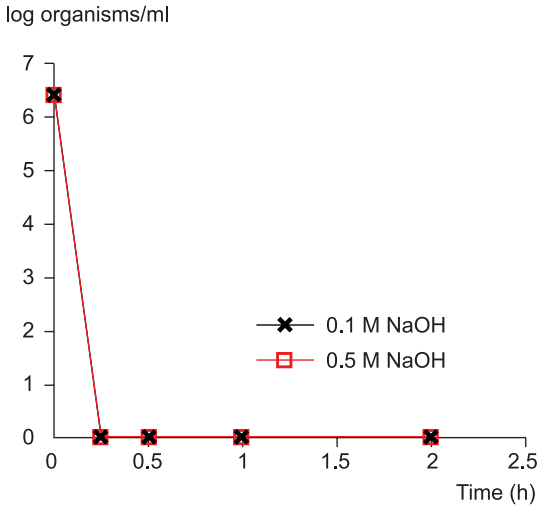

Fig 8. Sanitization of *E. coli* ATCC 8739 (gram-negative bacterium) from MabSelect SuRe using 0.1 M and 0.5 M NaOH.

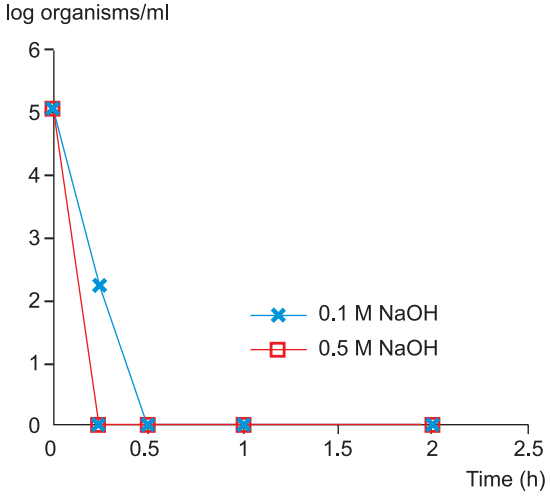

Fig 9. Sanitization of *S. aureus* ATCC 6538 (gram-positive bacterium) from MabSelect SuRe using 0.1 M and 0.5 M NaOH.

Conclusions

The toxicology of the MabSelect SuRe ligand was investigated in a single-dose intravenous toxicity study in rats. Although a high dose of the ligand was administered to the rats, no toxic effects of the ligand were observed. In the Ames test, the MabSelect SuRe ligand did not show any mutagenic activity.

Leakage of ligand from MabSelect SuRe was low. The level of leakage was maintained well below the standard level of leakage throughout the course of 50 purification/CIP cycles of a MAb. The purity and yield of the MAb were consistently high throughout the studies performed.

Ion exchange chromatography was used for removal of leached ligand. Efficient removal of leached ligand was accomplished using either cation or anion exchange chromatography. Furthermore, the MabSelect Sure ligand had similar binding and elution characteristics compared with a conventional rProtein A ligand. It should therefore be possible to use similar chromatographic conditions for removal of leached rProtein A and MabSelect SuRe ligand.

Elimination of gram-negative and gram-positive bacteria from MabSelect SuRe was rapidly accomplished using 0.1 M NaOH or 0.5 M NaOH.

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