GE Healthcare
Life Sciences

Protein L Reference List

1. Bjorck, L. **Protein L. A novel bacterial cell wall protein with affinity for Ig L chains.** *J. Immunol.* **140**, 1194-1197 (1988).

   A novel Ig-binding protein has been isolated from the surface of bacteria belonging to the anaerobic species *Peptococcus magnus*. To solubilize the protein, peptococci were treated with different proteolytic enzymes (papain, pepsin, and trypsin) or with mutanolysin, a bacteriolytic agent known to digest the cell walls of streptococci. Papain, trypsin, and mutanolysin all solubilized peptides showing affinity for radiolabeled human IgG in Western blot anal. Compared with papain and trypsin, mutanolysin liberated a more homogeneous material, which also had a higher mol. wt. This mutanolysin-solubilized protein (95 kilodaltons) was obtained highly purified by a single isolation step on IgG-Sepharose, and the mol. exhibited unique Ig-binding properties. Thus, in dot blots and in Western blots, human IgG, F(ab')2 and Fab fragments of IgG, and human κ and λ light (L) chains all showed affinity for the protein. Moreover, the mol. also bound human IgM and IgA, whereas no binding was recorded for IgG-Fc fragments or IgG heavy chains. Finally, the protein bound to human polyclonal Ig L chains immobilized on polyacrylamide beads. Thus, the isolated peptococcal protein binds Ig through L chain interaction. The name protein L is therefore suggested for this novel Ig-binding bacterial cell wall protein.

2. Nilson, B. H. *et al.* **Purification of antibodies using protein L-binding framework structures in the light chain variable domain.** *J. Immunol. Methods*, **164**, 33-40 (1993).

   Protein L from the bacterial species *Peptostreptococcus magnus* binds specifically to the variable domain of Ig light chains, without interfering with the antigen-binding site. In this work a genetically engineered fragment of protein L, including four of the repeated Ig-binding repeat units, was employed for the purification of Ig from various sources. Thus, IgG, IgM, and IgA were purified from human and mouse serum in a single step using protein L-Sepharose affinity chromatography. Moreover, human and mouse monoclonal IgG, IgM, and IgA, and human IgG Fab fragments, as well as a mouse/human chimeric recombinant antibody, could be purified from cultures of hybridoma cells or antibody-producing bacterial cells, with protein L-Sepharose. This was also the case with a humanized mouse antibody, in which mouse hypervariable antigen-binding regions had been introduced into a protein L-binding kappa subtype III human IgG. These experiments demonstrate that it is possible to engineer antibodies and antibody fragments (Fab, Fv) with protein L-binding framework regions, which can then be utilized in a protein L-based purification protocol.

3. De Chateau, M. *et al.* **On the interaction between protein L and immunoglobulins of various mammalian species.** *Scand. J. Immunol.* **37**, 399-405 (1993).

   Protein L, a cell wall molecule of certain strains of the anaerobic bacterial species *Peptostreptococcus magnus*, shows high affinity for human immunoglobulin (Ig) light chains. In the present study protein L was tested against a panel of human myeloma proteins of the IgG, IgM, IgA and IgE classes, and strong binding was seen with antibodies carrying kappa light chains. A high degree of specificity for Ig was demonstrated in binding experiments with human plasma proteins. Apart from human Ig, strong protein L-binding activity was also detected in the serum of 12 out of 23 tested additional mammalian species, including other primates and rodents. Subsequent analysis with purified Ig samples demonstrated the binding of protein L to Ig of important laboratory animal species such as the mouse, the rat and the rabbit. The affinity constants for the interactions between protein L and polyclonal IgG of these species were 2.6 × 10(9), 3.9 × 10(8) and 7.4 × 10(7), respectively. In non-human species, the binding of protein L was also found to be mediated through Ig light chains, and the results demonstrate the potential value of protein L as an immunochemical tool.

4. Akerstrom, B. and Bjorck, L. **Protein L: an immunoglobulin light chain-binding bacterial protein. Characterization of binding and physicochemical properties.** *J. Biol. Chem.* **264**, 19740-19746 (1989).

   Protein L, a cell wall molecule of the bacterial species *Peptostreptococcus magnus* with affinity for immunoglobulin (Ig) light chains, was isolated after solubilization of the bacterial cell walls with mutanolysin or from the culture medium by a single affinity chromatography step on human IgG-Sepharose. A major protein band with an apparent molecular weight of 95,000 was obtained from both sources. The protein from the growth medium was size heterogeneous. From 1 ml of packed bacteria was prepared 0.92 mg of the mutanolysin-solubilized protein L (73% yield), whereas 4.1 mg of spontaneously released protein L (49% yield) was purified from the corresponding culture medium. The Mr of protein L was estimated to 76,000 by gel chromatography in 6 M guanidine HCl. Using this Mr value, the Stokes radius and the frictional ratio of protein L were determined to 4.74 nm and 1.70, respectively, suggesting an elongated fibrous molecule. No disulfide bond or subunit structure could be shown. The amino-terminal amino acid sequences of the whole protein and two internal non-IgG-binding tryptic fragments were determined and found to be unique.

One of the tryptic fragments showed homology (40% identical residues) to a sequence within the cell wall-binding region of protein G, the Fc-binding protein of group C and G streptococci. The binding specificity of protein L was directed to the light chains of immunoglobulins; the affinity constant for polyacrylamide-coupled kappa-chains was 1.5 × 10(9) M-1 and for IgG, IgA, and IgM around 1 × 10(10) M-1. Maximal binding was achieved between pH 7 and 10. The binding to lambda-chains was too weak for determination of the affinity constant. 125I-Protein L was also shown to bind to mouse immunoglobulins. It could be used for detection of antigen-bound polyclonal and monoclonal antibodies in Western blots. This shows that the protein L/light chain reaction was not obstructed by occupation of the antigen-binding site. Finally, protein L and kappa-chains of human Ig formed precipitates upon double immunodiffusion analysis, an indication of at least two binding sites on protein L.

5. Isaksen, M. L. and FitzGerald, K. **Purification and analysis of antibody fragments using proteins L, A and LA.** In *Antibody Engineering* (Kontermann, R. and Duebel, S. eds.), Springer Verlag, Berlin, ch. 21 (2001).

Tag-based detection and purification of antibody fragments pose several disadvantages including the relatively low affinity and/or specificity for the appended tag that is typically exhibited by the detecting antibody as well as sensitivity of the tags to *E. coli* proteases. Some new affinity proteins, such as rProtein L and rProtein LA, have been shown to overcome the limitations of affinity tags. RProtein L is a recombinant form of the native protein L and contains only the four Ig binding domains. RProtein LA is a recombinant fusion protein consisting of four Ig-binding domains from protein L and four Ig-binding domains of protein A. RProtein L and rProtein LA, as well as protein A, have been found to be very useful reagents when working with antibody fragments derived from phage libraries. Providing that the antibody fragments bind to protein L, protein A or protein LA, the use of these affinity proteins greatly simplifies both detection and purification of antibody fragments.

6. Kouki, T. *et al.* **Separation method of IgG fragments using protein L.** *Immunol. Invest.* **26**, 399-408 (1997).

Protein L (IgG kappa-chain-binding bacterial protein) showed a precipitate line (pseudo-immuno-reaction) with IgG and F(ab')2 fragment, but did not show any line with the Fab fragment, the Fc fragment and free kappa-chains in the micro-Ouchterlony method. The IgG and Fab fraction obtained from pa-pain-digested IgG (from the sera of patients with chronic thyroiditis), followed by Protein A-Sepharose, were separated by Protein L-Sepharose affinity chromatography. The unbound fraction (UF) consisted of IgG (lambda) or Fab (lambda) and the bound fraction (BF) consisted of IgG (kappa) or Fab (kappa) were obtained. Anti-thyroglobulin and anti-thyroid peroxidase antibody activities were found equally in both the UF and the BF. When Fab (kappa) was reduced with dithiothreitol (DTT), the Fd fragment in the UF could be separated from the free kappa-chain and the unreduced Fab(kappa) in the BF with a Protein L-Sepharose column. A separation method of human IgG fragments such as free kappa-chain, combined forms of kappa-chain [Fab or F(ab')2], and the Fd region, using Protein L, is described.

7. Vola, R. *et al.* **Recombinant proteins L and LG: efficient tools for purification of murine immunoglobulin G fragments.** *J. Chromatogr. B.* **668**, 209-218 (1995).

In order to improve antibody purification methods, recombinant proteins L and LG were tested in the purification of murine monoclonal immunoglobulin G (IgG) and its fragments. After affinity constant evaluation in different buffer systems, high-performance affinity chromatographic columns were prepared by coupling the proteins to Affi-prep 10 resin and tested with eight different murine monoclonal antibodies and their fragments of different isotypes. Affinity chromatographic experiments confirmed radioimmunoassay results showing that protein L bound 75% of the tested antibody fragments whereas protein LG had affinity for all the tested fragments. These results demonstrate that protein LG is the most powerful Ig-binding tool so far described.

8. Devaux, C. *et al.* **Construction and functional evaluation of a single-chain antibody fragment that neutralizes toxin AahI from the venom of the scorpion *Androctonus australis hector*.** *Eur. J. Biochem.* **268**, 694-702 (2001).

9C2 is a murine monoclonal IgG that participates in the neutralization of Androctonus australis hector scorpion venom. It recognizes AahI and AahIII, two of the three main neurotoxins responsible for almost all the toxicity of the venom when injected into mammals. Using PCR the authors cloned the antibody variable region coding genes from 9C2 hybridoma cells and constructed a gene encoding a single-chain antibody variable fragment mol. (scFv). This scFv was produced in the periplasm of *Escherichia coli* in a sol. and functional form and purified in a single step using protein L-agarose beads yielding 1-2 mg·L-1 of bacterial culture. ScFv9C2 was predominantly monomeric but also tended to form dimeric and oligomeric structures, all capable of binding toxin AahI. The affinity of scFv and the parental mAb for toxin AahI and homologous toxin AahIII was of the same magnitude, in the nanomolar range. Similarly, purified forms of scFv9C2 completely inhibited the binding of toxin AahI to rat brain synaptosomes. Finally, scFv9C2 was efficient in protecting mice against the toxic effects of AahI after injection of the toxin and scFv to mice by the intracerebroventricular route in a molar ratio as low as 0.36: 1. Thus, the authors produced a recombinant scFv that reproduces the recognition properties of the parent antibody and neutralizes the scorpion neurotoxin AahI, thereby opening new prospects for the treatment of envenomation.

9. Kriangkum, J. *et al.* **Development and characterization of a bispecific single-chain antibody directed against T cells and ovarian carcinoma.** *Hybridoma,* **19**, 33-41 (2000).

Bispecific antibodies with specificity for tumor antigen and CD3 have been shown to redirect the cytotoxicity of T cells against relevant tumor. Our objective was to generate single-chain bispecific antibodies (bsSCA) that could retarget mouse cytotoxic T lymphocytes (CTL) to destroy human ovarian carcinoma in a xenogeneic setting. A bsSCA, 2C11 × B43.13, was constructed by genetic engineering and expressed in mammalian cells. Mol. characteristics, binding properties, and ability to retarget CTL were studied. Western blot analysis showed that the product is a 65-kDa protein. Purifn. of antibodies could be done by single-step affinity chromatography using protein L-agarose with an unoptimized yield of 200 µg/L. BsSCA 2C11 × B43.13 was capable of binding to mouse CD3 and human CA125 as detected by FACS anal. of EL4 and OVCAR Nu3H2 cells, resp. It could also bridge activated splenic T cells and human ovarian carcinoma as demonstrated by a bridge FACS assay. Redirected mouse CTL could mediate human target cell lysis in a 20-h 51Cr release assay despite that they are xenogeneic. Prolonged incubation of redirected CTL and tumor targets resulted in a dramatic reduction in tumor cell no. CD28 co-stimulation enhanced redirected CTL function in both types of assays. BsSCA 2C11 × B43.13 thus can be used as a preclinical immunotherapeutic model for human ovarian cancer in a xenogeneic setting.

10. Boes, A. *et al.* **Affinity purification of a framework 1 engineered mouse/human chimeric IgA2 antibody from tobacco.** *Biotechnol. Bioeng.* **108**, 2804-2814 (2011).

Complex multimeric proteins such as dimeric and secretory immunoglobulin A (IgA) can be difficult to produce in heterologous systems, although this has been achieved using several platforms including plants. As well as topical mucosal applications, dimeric IgA (dIgA), and secretory IgA (sIgA) can be used in tumor and anti-viral therapy, where their more potent cell-killing properties may increase their efficacy compared to current drugs based on IgG. However, the

development of therapeutic IgA formats is hampered by the need to co-express four different polypeptides, and the inability to purify such molecules using conventional protein A or protein G affinity chromatography. The light chain (LC)-specific affinity ligand protein L is a potential alternative, but it only recognizes certain kappa light chain (LC(κ)) subtypes. To overcome these limitations, we have adapted a framework-grafting approach to introduce LCs that bind protein L into any IgA. As a model, we used the chimeric anti-human chorionic gonadotropin (hCG) antibody cPIPP, since this contains a murine LC((κ)) subtype that does not bind protein L. Grafting was achieved by replacing selected framework region 1 (FR1) residues in the cPIPP LC(κ) variable domain with corresponding residues from LC(κ) subtypes that can bind protein L. The grafted antibody variants were successfully purified by protein L affinity chromatography. These modifications affected neither their antigen-binding properties nor the yields achieved by transient expression in tobacco plants. Our results therefore show that LC FR1 grafting can be used as generic strategy for the purification of IgA molecules.

11. Zahid, M. *et al.* **Design and reshaping of an scFv directed against human platelet glycoprotein VI with diagnostic potential.** *Anal. Biochem.* **417**, 274-282 (2011).

Blood platelets play a key role in physiological hemostasis and in thrombosis. As a consequence, platelet functional analysis is widely used in the diagnosis of hemorrhagic disorders as well as in the evaluation of thrombosis risks and of the efficacy of antithrombotics. Glycoprotein (GP) VI is a platelet-specific collagen-signaling receptor. Clinical studies suggest that increased GPVI expression is associated with a risk of arterial thrombosis. Conversely, GPVI deficiencies have been identified in patients with defective platelet responses to collagen. Currently, there is no standard test available for measuring GPVI expression, essentially because antibodies usually cross-link GPVI upon binding, leading to platelet activation and consecutive changes in GPVI expression. Here, we designed a recombinant monovalent antibody fragment (scFv) derived from an anti-GPVI monoclonal IgG, 3J24, with the characteristics required to analyze GPVI expression. Guided by in silico modeling and V-KAPPA chain analysis, a Protein L (PpL) recognition pattern was engineered in the scFv, making possible its purification and detection using PpL conjugates. The PpL affinity-purified scFv is functional. It retains GPVI-binding specificity and allows detection of platelet surface-expressed GPVI without inducing platelet activation. In conclusion, the reshaped scFv may be very useful in the development of diagnostic approaches.

12. Hu, W-G. *et al.* **Generation of a recombinant full-length human antibody binding to botulinum neurotoxin A.** *Appl. Biochem. Biotech.* **160**, 1206-1216 (2010).

In order to develop a recombinant full-length human anti-botulinum neurotoxin A (BoNT/A) antibody, human peripheral blood mononuclear cells (PBMC) were collected from three healthy volunteers and induced for BoNT/A-specific immune response by in vitro immunization. The genes encoding human Fd fragment, consisting of antibody heavy chain variable region and constant region 1 with the genes encoding antibody light chain, were cloned from the immunized PBMC. Afterwards, one combinatory human antigen-binding fragment (Fab) library was constructed using a lambda phage vector system. The size of the constructed library was approximately 10(5) *Escherichia coli* transformants. After screening the library by BoNT/A antigen using a plaque lifting with immunostaining approach, 55 clones were identified as positive. The Fab gene of the most reactive clone exhibiting particularly strong BoNT/A binding signal was further subcloned into a full-length human IgG1 antibody gene template in an adenoviral expression vector, in which the heavy and light chains were linked by a foot-and-mouth-disease virus-derived 2A self-cleavage peptide under a single promoter. After the full-length human IgG1 was expressed in mammalian cells and purified with protein L column, sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the heavy and light chains of the antibody were cleaved completely. The affinity expressed as the dissociation constant (K(d)) for the recombinant human antibody to bind to BoNT/A was determined by indirect enzyme-linked immunosorbent assay and results confirmed that the recombinant full-length human antibody retained BoNT/A-binding specificity with K(d) value of 10^{-7} M.

13. Coelho, V. *et al.* **Design, construction, and in vitro analysis of A33scFv::CDy, a recombinant fusion protein for antibody-directed enzyme prodrug therapy in colon cancer.** *Int. J. Oncol.* **31**, 951-957 (2007).

Antibody-directed enzyme-prodrug therapy (ADEPT) aims at improving the specificity of conventional chemotherapy by employing artificial antibody-enzyme constructs to convert a non-toxic prodrug into a cytotoxic agent specifically localized to the tumor site. The gpA33 antigen is a promising target for ADEPT in colon cancer, as it is expressed by >95% of human colon cancers, but is absent in all non-gastrointestinal tissues. We designed a recombinant fusion construct of a phage display-generated anti-gpA33 single chain fragment, A33scFv, with cytosine deaminase from yeast (CDy), which converts 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU). The resulting construct, A33scFv::CDy, was overexpressed in Pichia pastoris and secreted into culture supernatant. The fusion protein was purified by affinity chromatography on protein L. Silver-staining after SDS-polyacrylamide gel electrophoresis confirmed molecular mass and purity. Antibody binding and specificity were quantified by flow cytometry. The complete ADEPT system was applied in vitro on gpA33-positive LIM1215 cells, assessing cell survival by a fluorescein diacetate assay. Cytotoxicity of the prodrug 5-FC after A33scFv::CDy binding was equimolar to that of 5-FU, and this effect depended specifically on both antibody and enzyme function. These results demonstrate bifunctional activity of the heterogeneous Pichia-produced A33scFv::CDy fusion protein and proof of principle for the ADEPT system proposed herein.

14. Cossins, A. J. *et al.* **Recombinant production of a VL single domain antibody in *Escherichia coli* and analysis of its interaction with peptostreptococcal protein L.** *Protein Expres. Purif.* **51**, 253-259 (2007).

A kappa-light chain from a Fab expression system was truncated by the insertion of a stop codon in the gene sequence to produce a variable light (VL) single domain antibody (dAb). Here, we describe the expression of dAb in the periplasm of *Escherichia coli* through fermentation in a defined media. Immunoglobulin binding domains from peptostreptococcal protein L (PpL) have been shown to bind specifically to kappa-light chains. We have produced recombinant PpL, at high yield, and this was used to custom-produce PpL-Sepharose affinity columns. Here, we show that the affinity purification of VL dAb by this method is simple and efficient with no apparent loss in protein at any stage. The truncated dAb protein product was confirmed by electrospray mass spectrometry and N-terminal sequencing. When analyzed by SDS-PAGE it was shown to be over 95% pure and produced at yields of 35-65 mg/L of culture medium. The dAb protein produced was shown by NMR and CD to be a folded beta-sheet domain. This domain is bound by PpL with a Kd of approximately 50 nM as determined by stopped-flow fluorimetry.

15. Aubrey, N. *et al.* **Design and evaluation of a diabody to improve protection against a potent scorpion neurotoxin.** *Cell. Mol. Life. Sci.* **60**, 617-628 (2003).

Diabodies are recombinant, dimeric, antibody-based molecules composed of two non-covalently associated single-chain antibody fragments that bind to an antigen in a divalent manner. In an attempt to develop more effective therapeutic molecules against scorpion venoms, we designed a diabody derived from monoclonal antibody 9C2, which neutralizes the toxicity of scorpion neurotoxin AahI in mammals. The recombinant diabody produced in the periplasm of *Escherichia coli* was purified to homogeneity in a single step by protein L-agarose affinity chromatography. It was functional, and possessed a high

binding affinity to AahI (8 × 10(-11) M). The bivalence of the diabody was confirmed by size-exclusion chromatography, isoelectrofocusing and electron microscopic observations. Finally, the diabody showed high thermal stability in serum and demonstrated protective activity when injected intraperitoneally in mice experimentally envenomed with toxin AahI. In conclusion, the diabody format gives the 9C2 molecule advantageous properties that are particularly important for potential clinical applications in the treatment of envenomations.

16. Willems, A. *et al.* **Optimizing expression and purification from cell culture medium of trispecific recombinant antibody derivatives.** *J. Chromatogr. B.* **786**, 161-176 (2003).

Antibody fragments offer the possibility to build multifunctional manifolds tailored to meet a large variety of needs. We optimized the production of a manifold consisting of one (bibody) or two (tribody) single-chain variable fragments coupled to the C-terminus of Fab chains. Different strong mammalian promoters were compared and the influence of expression media on production and recovery was investigated. Since the physical and chemical nature of these molecules largely depends on the nature of the antibody building blocks incorporated, a generally applicable process for the purification of recombinant antibody derivatives from serum containing mammalian cell culture medium was designed. To this end we compared protein L, hydroxyapatite, immobilized metal affinity chromatography, cation-exchange chromatography and hydrophobic charge induction chromatography.

17. Kriangkum, J. *et al.* **Development and characterization of a bispecific single-chain antibody directed against T cells and ovarian carcinoma.** *Hybridoma,* **19**, 33-41 (2000).

Bispecific antibodies with specificity for tumor antigen and CD3 have been shown to redirect the cytotoxicity of T cells against relevant tumor. Our objective was to generate single-chain bispecific antibodies (bsSCA) that could retarget mouse cytotoxic T lymphocytes (CTL) to destroy human ovarian carcinoma in a xenogeneic setting. A bsSCA, 2C11 × B43.13, was constructed by genetic engineering and expressed in mammalian cells. Molecular characteristics, binding properties, and ability to retarget CTL were studied. Western blot analysis showed that the product is a 65-kDa protein. Purification of antibodies could be done by single-step affinity chromatography using protein L-agarose with an unoptimized yield of 200 microg/L. BsSCA 2C11 × B43.13 was capable of binding to mouse CD3 and human CA125 as detected by FACS analysis of EL4 and OVCAR Nu3H2 cells, respectively. It could also bridge activated splenic T cells and human ovarian carcinoma as demonstrated by a bridge FACS assay. Redirected mouse CTL could mediate human target cell lysis in a 20-h 51Cr release assay despite that they are xenogeneic. Prolonged incubation of redirected CTL and tumor targets resulted in a dramatic reduction in tumor cell number. CD28 co-stimulation enhanced redirected CTL function in both types of assays. BsSCA 2C11 × B43.13 thus can be used as a preclinical immunotherapeutic model for human ovarian cancer in a xenogeneic setting.

18. Svensson, H. G. *et al.* **Protein LA, a novel hybrid protein with unique single-chain Fv antibody- and Fab-binding properties.** *Eur. J. Biochem.* **258**, 890-896 (1998).

Existing Ig-binding proteins all suffer from limitations in their binding spectrum. In the pursuit of the ultimate, non-restricted, Ig-binding protein, we have constructed the hybrid protein LA, by fusing four of the Ig kappa light-chain-binding domains of peptostreptococcal protein L with four of the IgGFc- and Fab-binding regions of staphylococcal protein A. Ligand-blot experiments demonstrated that the L and the A components were both functional in the hybrid, as the protein was shown to bind purified kappa light chains and IgGFc. Protein LA bound human Ig of different classes and IgG from a wide range of mammalian species. IgG, IgM and IgA were purified from human serum and saliva by affinity chromatography on protein LA agarose. Similarly, single-chain Fv (scFv) antibodies carrying the kappa light-chain variable domain or expressing the V(H)III (variable domain of the heavy chain of Ig) determinant, were efficiently purified on immobilized protein LA. As judged by surface plasmon resonance (SPR), protein LA showed enhanced affinity for all tested ligands, including several scFv antibodies, compared with proteins L and A alone. SPR analysis also demonstrated that binding of a ligand to one of the components in protein LA did not affect the ability of the hybrid protein to interact simultaneously with a ligand for the other component. The antigen-binding capacity of a kappa-expressing scFv antibody was unaffected by the interaction with protein LA, whereas the binding of a V(H)III-expressing scFv antibody to its antigen was, unexpectedly, blocked by protein A and protein LA. Together, these data demonstrate that protein LA represents a highly versatile Ig-binding molecule.

19. Kihlberg, B-M. *et al.* **Protein LG: a hybrid molecule with unique immunoglobulin binding properties.** *J. Biol. Chem.* **267**, 25583-25588 (1992).

Immunoglobulin (Ig)-binding bacterial proteins have attracted theoretical interest for their role in molecular host-parasite interactions, and they are widely used as tools in immunology, biochemistry, medicine, and biotechnology. Protein L of the anaerobic bacterial species *Peptostreptococcus magnus* binds Ig light chains, whereas streptococcal protein G has affinity for the constant (Fc) region of IgG. In this report, Ig binding parts of protein L and protein G were combined to form a hybrid molecule, protein LG, which was found to bind a large majority of intact human Ig's as well as Fc and Fab fragments, and Ig light chains. Binding to Ig was specific, and the affinity constants of the reactions between protein LG and human IgG, IgGFc fragments, and kappa light chains, determined by Scatchard plots, were 5.9 × 10(9), 2.2 × 10(9), and 2.0 × 10(9) M-1, respectively. The binding properties of protein LG were more complete as compared with previously described Ig-binding proteins when also tested against mouse and rat Ig's. This hybrid protein thus represents a powerful tool for the binding, detection, and purification of antibodies and antibody fragments.

20. Roque, A. C. *et al.* **An artificial protein L for the purification of immunoglobulins and fab fragments by affinity chromatography.** *J. Chromatogr. A.* **1064**, 157-167 (2005).

The development and characterization of an artificial protein L (PpL) for the affinity purification of antibodies is described. Ligand 8/7, which emerged as the lead from a de novo designed combinatorial library of ligands, inhibits the interaction of PpL with IgG and Fab by competitive ELISA and shows negligible binding to Fc. The ligand 8/7 adsorbent (Ka approximately 10(4) M(-1)) compared well with PpL in binding to immunoglobulins from different classes and sources and, in addition, bound to IgG1 with K and lambda isotypes (92% and 100% of loaded protein) and polyclonal IgG from sheep, cow, goat and chicken. These properties were also reflected in the efficient isolation of immunoglobulins from crude samples.

21. Enokizono, J. *et al.* **NMR analysis of the interaction between protein L and Ig light chains.** *J. Mol. Biol.* **270**, 8-13 (1997).

Protein L is a cell wall protein expressed by some strains of the anaerobic bacterial species *Peptostreptococcus magnus*. It binds to immunoglobulin (Ig) light chains predominantly of the kappa subtype from a wide range of animal species. This binding is mediated by five highly homologous repeats designated as B1-B5, each of which comprises 72 to 76 amino acid residues. In the present study, we have identified the protein L-binding site of an Ig light chain by use of stable isotope-assisted NMR spectroscopy. The present NMR data, in combination with sequence comparisons between kappa light chains with and without protein L affinity, suggest that the amino acid substitutions at positions 9, 20, and/or 74 of the kappa light chains could crucially affect the interaction between protein L and the V(L) domain.

Antibody Fragment Reference list

1. Nelson, A. L. and Reichert, J. M. **Development trends for therapeutic antibody fragments.** *Nat. Biotechnol.* **27**, 331-337 (2009).

2. Nelson, A. L. **Antibody fragments: hope and hype.** *mAbs*, **2**, 77-83 (2010).

   The antibody molecule is modular and separate domains can be extracted through biochemical or genetic means. It is clear from review of the literature that a wave of novel, antigen-specific molecular forms may soon enter clinical evaluation. This report examines the developmental histories of therapeutics derived from antigen-specific fragments of antibodies produced by recombinant processes. Three general types of fragments were observed, antigen-binding fragments (Fab), single chain variable fragments (scFv) and "third generation" (3G), each representing a successive wave of antibody fragment technology. In parallel, drug developers have explored multi-specificity and conjugation with exogenous functional moieties in all three fragment types. Despite high hopes and an active pipeline, enthusiasm for differentiating performance of fragments should, perhaps, be tempered as there are yet few data that suggest these molecules have distinct clinical properties due only to their size.

3. Bird, R. E. and Walker, B. W. **Single chain antibody variable regions.** *Trends Biotechnol.* **9**, 132-137 (1991).

   A review, with 43 refs. The use of antibodies or antibody fragments for targeting tumors (either for tumor imaging or as carriers for drugs or toxins), has encountered problems of clearance, and non-specific or inefficient binding in clin. trials. A novel approach, linking 2 antibody variable fragments (Fvs), with a short peptide to generate a continuous polypeptide chain, may be able to overcome some of these problems. Since these single-chain antibody variable regions (scFvs), are transcribed from constructed genes, large-scale production in, for example, *Escherichia coli*, should be straightforward.

4. Holliger, P. and Hudson, P. J. **Engineered antibody fragments and the rise of single domains.** *Nat. Biotechnol,* **23**, 1126-1136 (2005).

   A review. With 18 monoclonal antibody (mAb) products currently on the market and more than 100 in clin. trials, it is clear that engineered antibodies have come of age as biopharmaceuticals. In fact, by 2008, engineered antibodies are predicted to account for >30% of all revenues in the biotechnol. market. Smaller recombinant antibody fragments (for example, classic monovalent antibody fragments (Fab, scFv)) and engineered variants (diabodies, triabodies, minibodies and single-domain antibodies) are now emerging as credible alternatives. These fragments retain the targeting specificity of whole mAbs but can be produced more economically and possess other unique and superior properties for a range of diagnostic and therapeutic applications. Antibody fragments have been forged into multivalent and multispecific reagents, linked to therapeutic payloads (such as radionuclides, toxins, enzymes, liposomes and viruses) and engineered for enhanced therapeutic efficacy. Recently, single antibody domains have been engineered and selected as targeting reagents against hitherto immunosilent cavities in enzymes, receptors and infectious agents. Single-domain antibodies are anticipated to significantly expand the repertoire of antibody-based reagents against the vast range of novel biomarkers being discovered through proteomics. As this review aims to show, there is tremendous potential for all antibody fragments either as robust diagnostic reagents (for example in biosensors), or as nonimmunogenic *in vivo* biopharmaceuticals with superior biodistribution and blood clearance properties.

5. Chapman, A. P. *et al.* **Therapeutic antibody fragments with prolonged *in vivo* half-lives.** *Nat. Biotechnol,* **17**, 780-783 (1999).

   Antibody fragments can be isolated rapidly using techniques such as phage display and can be expressed to high levels in microbial systems. However, to date such antibody fragments have been of limited use for many therapeutic applications because they are rapidly cleared from the body. We present a strategy for the site-specific chem. modification of antibody fragments with polyethylene glycol, which results in the production of antibody fragments with long *in vivo* half-lives and full retention of antigen-binding properties. This technol. should allow more rapid and economical production of therapeutic antibodies for chronic disease therapy.

6. Holt, L. J. *et al.* **Domain antibodies: proteins for therapy.** *Trends Biotechnol,* **21**, 484-490 (2003).

   Occurring naturally in 'heavy chain' immunoglobulins from camels, and now produced in fully human form, domain antibodies (dAbs) are the smallest known antigen- binding fragments of antibodies, ranging from 11 kDa to 15 kDa. dAbs are the robust variable regions of the heavy and light chains of immunoglobulins (VH and VL respectively). They are highly expressed in microbial cell culture, show favorable biophysical properties including solubility and temperature stability, and are well suited to selection and affinity maturation by in vitro selection systems such as phage display. dAbs are bioactive as monomers and, owing to their small size and inherent stability, can be formatted into larger molecules to create drugs with prolonged serum half-lives or other pharmacological activities.

Further information

Related literature	Code number
Data file: Capto™ L	29-0100-08
Application note: Capture of ScFv using Capto L	29-0144-56
Selection guide: Capture toolkit for antibody fragments	29-0164-33
Handbook: Antibody Purification, Principles and methods	18-1037-46

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UK

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Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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