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APPLICATION OF IMMUNOGLOBULIN-BINDING PROTEINS A, G, L IN THE AFFINITY CHROMATOGRAPHY

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Proteins A, G and L are native or recombinant proteins of microbial origin that bind to mammalian immunoglobulins. Preferably recombinant variants of proteins A, G, L are used in biotechnology for affinity sorbents production. Comparative characteristics of proteins A, G, L and affinity sorbents on the basis of them, advantages and disadvantages of these proteins application as ligands in the affinity chromatography are done. Analysis of proteins A, G, L properties is presented. Binding specificities and affinities of these proteins differ between species and antibody subclass. Protein A has high affinity to human IgG1, IgG2, IgG4, mouse IgG2a, IgG2b, IgG3, goat and sheep IgG2, dog, cat, guinea pig, rabbit IgG. Protein G binds strongly to human, mouse, cow, goat, sheep and rabbit IgG. Protein L has ability of strong binding to immunoglobulin kappa-chains of human, mouse, rat and pig. Expediency of application of affinity chromatography with usage of sorbents on the basis of immobilized proteins A, G, L are shown for isolation and purification of antibodies different classes. Previously mentioned method is used as an alternative to conventional methods of protein purification, such as ion-exchange, hydrophobic interactions, metal affinity chromatography, ethanol precipitation due to simplicity in usage, possibility of one-step purification process, obtaining of proteins high level purity, multiuse at maintenance of proper storage and usage conditions. Affinity sorbents on the basis of immobilized proteins A, G, L are used not only for antibodies purification, but also for extraction of different antibodies fractions from blood serum.

Key words: affinity chromatography, *Staphylococcus* protein A, peptostreptococcal protein L, protein G.

Nowadays antibodies are widely used in the medical biotechnology and fundamental research, purity level of antibodies has a great importance for their application. Combinations of separate methods, particularly, ethanol precipitation and different methods of chromatography (ion-exchange, hydrophobic interactions and metal affinity) are used for obtaining of great amounts of antibodies with high level of purity [1]. But these methods are nonspecific, application of them leads to the particular loss of antibodies functional activity. High productivity way for antibodies obtaining is affinity chromatography with application of sorbents on the basis of immobilized immunoglobulin binding proteins, particularly, recombinant protein A *Staphylococcus aureus,* protein G and peptostreptococcal protein L (PpL). This method is widely used for purified antibodies fractions obtaining from cultural and ascitic liquids, blood sera; and also for immunosorbtion of autoantibodies and immune complexes from blood plasma of ill people [2, 3].

Immobilization of protein ligands, for example proteins A, G, L, on the chemically activated matrices can lead to the loss of their functional activity. That's why creation of new affinity sorbents with high activity is very important and is very perspective area of research in pharmaceutical biotechnology. Protein A can be effective for application as ligand in the affinity chromatography due to the fact that each of its five domains can specifically interact with constant domains of antibodies; and this provides specific binding with IgG of different animal species and human [4]. Proteins G and L can be also successfully used as ligands in the affinity chromatography because protein G like protein A binds to the Fc domain of the human IgG, protein L binds through kappa light chain interactions without

interfering with an antibody's antigen-binding site [5, 6].

In the review main advantages of proteins A, G, L application as ligands in the affinity chromatography are determined, those are concerned with the possibility of one-step high level purification of antibodies different classes, or with different antibody fractions from blood serum extraction. Information, concerning affinity sorbents on the basis of immobilized proteins A, G, L and their application, is analyzed.

Properties of immunoglobulin-binding proteins

SPA structure and mechanism of interaction with immunoglobulins

Staphylococcus protein A (SPA) is 42-kDa a cell wall-associated protein of Staphylococcus aureus. SPA consists of five highly homologous domains E, D, A, B, and C (Fig. 1), all with IgG-binding activity, and region XM by means of which protein A is anchored to the cell wall, a signal sequence (S) processed during secretion of SPA (Fig. 2) [1, 7].

Each of the five domains in SPA is arranged in an antiparallel three α-helical bundle of approximately 58 amino acids and the three dimensional structure is stabilized through a hydrophobic core.

SPA possesses two distinct immunoglobulin-binding activities: each domain can bind the constant region Fc-region of IgG and variable fragment Fab-region that is responsible for antigen recognition. 11 amino acid residues of helix 1 and helix 2 of SPA interact with constant region of IgG molecule. D and E domains of SPA interact mainly with Fab domains of immunoglobulin and have very low affinity to its Fc domain, while A, B, C domains bind strongly to the immunoglobulin Fc domain [8]. SPA interacts strongly with human IgG1, IgG2 and IgG4, mouse IgG2a, IgG2b, IgG3, rabbit IgG, sheep IgG2 (Table 1).

Fig. 1. **Structure of domain B of SPA, homologous to domains A, C, D, E in ribbon presentation**

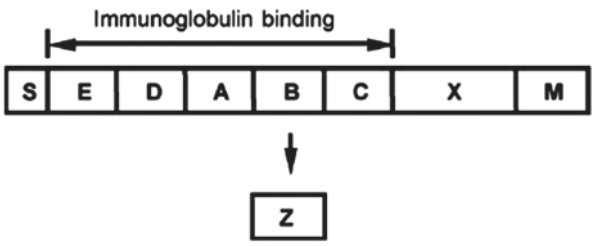

Fig. 2. **The schematic structure of SPA**

Binding site of immunoglobulins with SPA for most IgG subclasses is localized on the region of heavy chain which includes CH2 and CH3 domains [1, 9]. This property is widely used for antibodies purification. SPA can be used for immunoglobulins fractionation because it has different affinity degree for immunoglobulins different subclasses.

SPA stability. SPA has a high conformational stability, remarkable resistance to physicochemical stress and proteases action. SPA is stable in a wide pH range (2.0–11.0) and is able to refold after treatment with denaturing solutions such as urea and guanidine salts. The lack of cysteine residues allows SPA cleaning with reducing agents [10].

Protein G. Structure and mechanism of interaction with immunoglobulins

Protein G with molecular weight 30 000 Daltons is a cell-surface protein from *Streptococcus:* it contains in its structure multiple copies of two different small domains (COOH-terminal and NH_2-terminal domains) which can independently bind albumin and IgG. The COOH-terminal domain is responsible for IgG binding, whereas NH_2-terminal domain of the protein binds human serum albumin (HSA) [11, 12]. This property of protein G is used for extraction of albumin form blood serum. Protein G binds all the four subclasses of human IgG [12].

Protein G binds to the Fc fragment of immunoglobulins [13]. Protein G has three immunoglobulin-binding domains (C1, C2 and C3), each of 55 amino acid residues. It has been reported the X-ray crystallographic structure of the C2 fragment of protein G and the Fc domain of human IgG complex. The binding site of protein G is located on the interface between the CH2 and CH3 domains of the Fc domain of IgG (Fig. 3) [5, 12].

Protein G binds stronger than protein A to polyclonal IgGs from cow, horse, and sheep [5]. Protein G has a higher affinity for IgG

Table 1. **Binding of different antibodies to SPA**

Species	Subclasses	Binding to SPA	Species	Subclasses	Binding to SPA
Human	IgG1	Strong binding	Rat	IgG	Nonbinding
	IgG2	Strong binding		IgM	Nonbinding
	IgG3	Weak or nonbinding	Rabbit	IgG	Strong binding
	IgG4	Strong binding		IgM	Nonbinding
	IgA	Nonbinding	Sheep	IgG1	Nonbinding
	IgM	Nonbinding		IgG2	Strong binding
Mouse	IgG1	Weak or nonbinding		IgM	Nonbinding
	IgG2a	Strong binding	Goat	IgG1	Weak or nonbinding
	IgG2b	Strong binding		IgG2	Weak or nonbinding
	IgG3	Strong binding		IgM	Nonbinding
	IgM	Nonbinding			

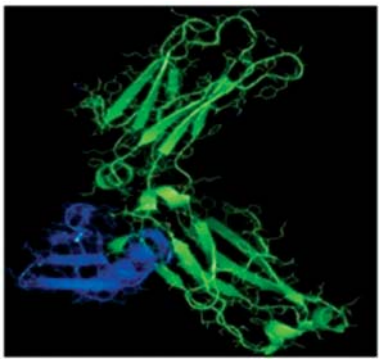

Fig. 3. **X-ray crystallographic structure of protein G C2 fragment (blue color) interacting with human IgG Fc domain (green color)**

than SPA, binding constants of SPA and G are $8{,}02 \cdot 10^3$ and $3{,}29 \cdot 10^4$ respectively. Protein G binds with greater capacity than SPA to several IgG subclasses such as human IgG3, mouse IgG1 and rat IgG2a [6]. Consequently, protein G can be effective in immunoglobulins purification when SPA cannot be used. Both SPA and protein G ligands are useful during antibodies purification and fractionation by affinity chromatography for different classes and subclasses of antibodies isolation [13].

PpL structure and mechanism of interaction with immunoglobulins

PpL is an immunoglobulin-binding protein that was originally derived from the bacteria *Peptostreptococcus magnus*, but now it is produced as recombinant protein. The structure of PpL is comprised of two antiparallel β-hairpins and one α-helix (Fig. 4). The two hairpins have similar length [14].

The 62-residue immunoglobulin-binding domain of PpL consists of central α-helix packed on four-stranded β-sheet formed by N- and C-terminal β-hairpins. The overall topology of the protein is quite symmetric: the β-hairpins have similar lengths [16].

PpL has the unique property to bind to kappa light chain without interaction with an antibody's antigen-binding site. PpL, unlike SPA and protein G, has the ability to bind a wider range of immunoglobulin classes and subclasses such as human IgG2, IgG4, human and mouse IgM, IgG1, IgG3, human IgA, human IgD, mouse and rat IgG2a, IgG2b, rat IgG2c, IgG1. In addition, PpL binds to single chain variable fragments (scFv) without interfering to antigen binding site. PpL binds kappa I, III and IV human light chains, but not to kappa II in human and kappa I on mouse. PpL recognizes 50% of human and more than 75% of murine immunoglobulins [6, 17, 18]. PpL binds weakly to rabbit immunoglobulins and does not bind to bovine, goat or sheep immunoglobulins [19]. All these properties make PpL an excellent one in application as ligand in the affinity chromatography, giving possibility of recombinant ScFv molecules purification, human and mouse IgM, IgG1, human IgD, IgA,

Fig. 4. **Three-dimensional of PpL structure, of four β-sheets (I, II, III, IV) and one α-helix V [15]**

rat IgG1 isolation from blood serum. So, affinity chromatography with application of sorbents on the basis of immobilized protein L has advantage over other ways of antibodies purification.

Application of SPA in the affinity chromatography

There are many papers concerning IgG classes and subclasses isolation using method of affinity chromatography with application of affinity sorbent with immobilized SPA on the Sepharose. In biotechnology affinity chromatography with application of sorbents on the basis of immobilized SPA is one of the best technique for the purification of monoclonal antibodies to homogeneity, due to its simplicity and high degree of antibody specificity, it is applied for immunosorption of antibodies from blood for diabetes mellitus, rheumatoid arthritis and other autoimmune diseases treatment.

SPA, immobilized on the Sepharose, provides oriented immobilization of IgG (Fig. 5).

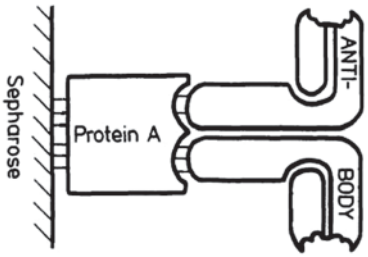

Fig. 5. **Schematic drawing of immunoglobulin G, oriented immobilization by usage of SPA, covalently attached to Sepharose:**
covalent bonds are shown as full lines

Today high performance immunoaffinity chromatography (HPIAC) is applied. SPA is used as a coating to either solid or controlled pore glass beads by cross linking with carbodiimide application. This attachment helps to orient the antigen receptors of the antibody toward the mobile phase of the column. SPA binds to Fc domains of immunoglobulins by means of hydrophobic interaction [1].

Affinity sorbents on the basis of an immobilized SPA

Affinity sorbents on the basis of the immobilized SPA are available from several commercial suppliers and vary with respect to the source of the SPA (natural wild type or recombinant protein), chemistry of immobilization, and beads' characteristics of a sorbent. The two leading manufacturers of affinity sorbents on the basis of the immobilized SPA are General Electric (GE) Healthcare and Millipore.

In affinity sorbent recombinant SPA is immobilized on the Sepharose. SPA is coupled via the C — terminal cysteine to the cyanogen bromide (CNBr) — activated Sepharose matrix through a single thioether linkage. Thioether coupling allows the ligand to extend farther into the mobile phase than would be possible for a laterally immobilized ligand, and this improves antibody binding [9]. In addition effective coupling of SPA molecule to the sorbent matrix provides absence of sorbent leaking, obtaining of pure antibodies fractions.

By means of genetic engineering techniques B domain of SPA was modified, and on its basis a new ligand was created in the form of a tetramer of four identical modified B domains. The absence of D and E domains of SPA in the given ligand also helps to eliminate variable region interactions, ligand interacts only with antibodies Fc domains. As a result antibodies binding heterogeneity is reduced, i.e. ligand binds only antibodies with high affinity to it. A newly developed sorbent, MabSelect SuRe, withstands strong alkaline conditions allowing the repeated use of 0.1–0.5 M NaOH for cleaning and sanitization.

The sorbent with the immobilized SPA on porous glass (ProSep A), on coated porous polystyrene materials (POROS) are also produced commercially.

Process of affinity chromatography with application of sorbents with the immobilized SPA provides 5- to 10-fold increase of the product concentration [10]. Antibodies purification with application of affinity sorbents on the basis of the immobilized SPA is effective due to its physicochemical stability and low operating expenses associated with sorbent cleaning and re-usage.

Antibodies purification procedure

A typical chromatogram of antibodies purification procedure by the way of usage of affinity chromatography with application of the sorbent on the basis of the immobilized SPA is shown in Fig. 5.

Loading/Binding. Most of the monoclonal antibodies currently being used or investigated for therapeutic applications are human molecules belonging to IgG classes 1, 2, or 4, all of which bind strongly to SPA. To the solution appointed for antibodies dilution before applying to the chromatographic

column packed by the sorbent with immobilized SPA, salt may be added for encouragement of monoclonal antibody binding to the SPA, and ethylene diamine tetraacetate to reduce proteolytic degradation, which leads to ligand loss. Monoclonal antibodies purification process runs several cycles on the above mentioned chromatographic column to purify a single batch. This reduces capital costs for such operation.

Washing procedure. For maximum removal of nonspecifically bound material, pH of washing solution must be low in order to untimely elution of antibodies does not begin. In such way buffer solutions with different combinations of salts and detergents, salts and solvents, salts and polymers, and high concentrations of Tris (hydroxymethylaminomethane) buffer can be used.

Elution. Elution pH is typically set at the highest possible value while maintaining high product yield. Urea is an effective hydrogen donor/acceptor that can outcompete hydrogen bonds, it could possibly be used at low concentrations to facilitate protein elution and keep product stability. Elution process on chromatographic columns packed with the sorbent with the immobilized SPA is conducted at low temperatures in order to avoid proteins aggregation.

Regeneration. The ability of the sorbent with the immobilized SPA to withstand a significant number of its reuse cycles is an important factor for high effective antibodies purification process for therapeutic and laboratory application in pharmaceutical biotechnology. Regeneration of the sorbent with the immobilized SPA is typically carried out with low NaOH concentrations (typically <100 mM) usage because native or recombinant SPA is stable in slightly alkaline conditions [10].

Thus, antibodies purification process with application of the sorbent on the basis of the immobilized SPA provides ease and simplicity of purification process development and performance, high degree of protein purity because of several cycles performance. Consequently, large purification factor value obtained from this process step helps to simplify the entire downstream process of antibodies purification. Such proteins purification process is also economically beneficial because it doesn't need equipment for antibodies additional purification by other types of chromatography.

Immunoadsorption on affinity sorbents with immobilized SPA application

SPA is used for autoimmune diseases treatment in clinical practice. Immunoadsorbtion (IA) with application of SPA sorbents columns enables to bind antibodies and immune complexes and causes modification

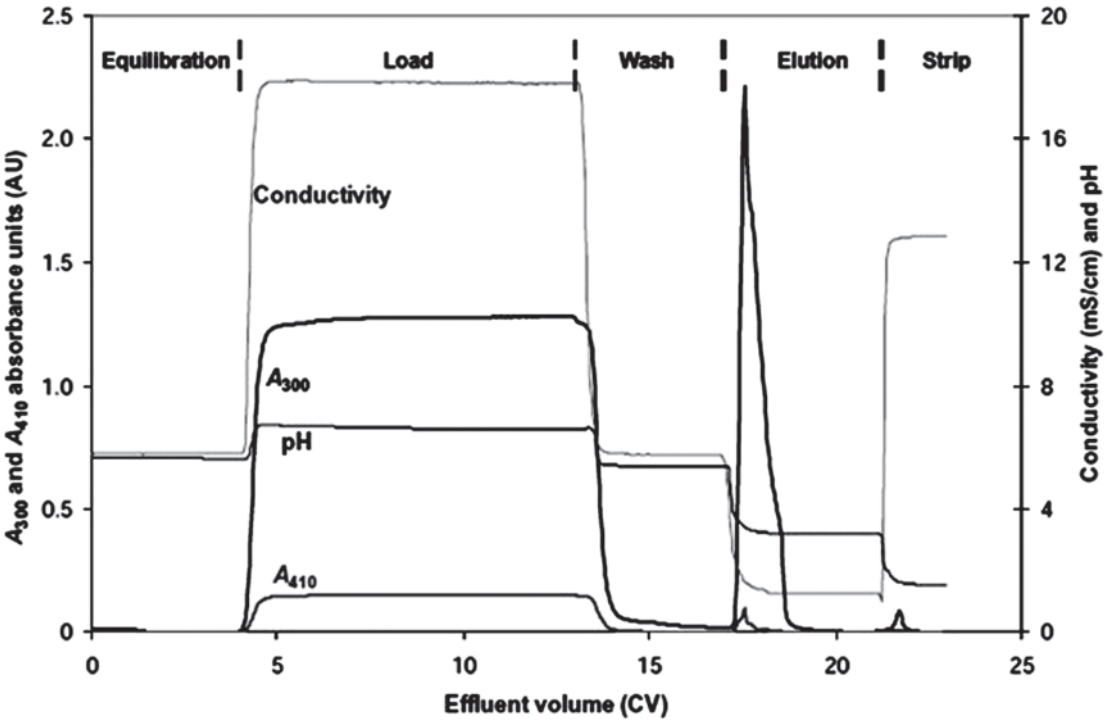

Fig. 6. **A chromatogram of antibodies purification procedure by affinity chromatography on the sorbent with immobilized SPA application**

of immune response. IA is effective for treatment of glomerulosclerosis, idiopatic thrombocytopenic purpura, rheumatoid arthritis, diabetes mellitus, and also used in kidney transplantation. According to the clinical studies during treatment the level of immune complexes decreases [20–22].

Dilated cardiomyopathy is a chronic myocardial disease which results in progressive ventricular enlargement, myocardial contractile dysfunction. It causes heart failure, consequently, necessity of heart transplantation arises. Diabetes mellitus is a metabolic disorder resulted in severe systemic consequences: increased levels of IgG, IgG3, significant cardiovascular morbidity and mortality. IA improved myocardial inflammation in patients and decreased antibodies level in blood. IA was conducted applying columns with SPA, immobilized on the agarose (Immunosorba, Fresenius Medical Care, Bad Homburg, Germany). Consequently, IgG level decreased from 10.7 g/L to 1.1 g/L (by 89.7%) and IgG3 from 0.6 g/L to 0.2 g/L (by 66.7%) [23, 24].

Such dangerous autoimmune disease as systemic lupus erythematosus causes autoantibodies production, for example, anti-double-stranded DNA antibodies, and circulating immune complexes. Clinical trial in 20 systemic lupus erythematosus patients (two groups with 10 patients in each) showed the efficacy of two different adsorption columns and an 80% clinical response after 1 month (Immusorba and Ig-Therasorb) [25].

Therefore, IA with SPA is very useful tool for diseases therapy, which is concerned with increased levels of antibodies in the blood. IA enables an efficient isolation of antibodies and immune complexes from patients' blood, thus improves their feeling, and prolongs clinical benefits.

Protein G application in affinity chromatography

In the affinity chromatography recombinant form of protein G, molecular weight 22 000 Da, is usually used. In this variant of protein G albumin- and cell surface binding sites have been eliminated in order to reduce nonspecific binding during purification of immunoglobulins. This advantage gives opportunity of recombinant protein G application for albumin separation from crude human immunoglobulin samples.

Immobilized protein G is most commonly used for the purification of mammalian monoclonal and polyclonal antibodies that do not bind strongly to SPA. Optimal binding for most immunoglobulins to protein G occurs at pH 5.0, although more neutral Tris-HCl or phosphate buffers for binding (pH 7.5) have been used in many studies.

Affinity sorbents with an immobilized protein G, packed into chromatographic columns, are available commercially today. The most popular manufacturers of sorbents on the basis of the immobilized protein G are Pierce and General Electric (GE) Healthcare.

Sorbents on the basis of the immobilized protein G of Pierce manufacturing the recombinant form of protein G immobilized to either 6% cross-linked beaded agarose or UltraLink Biosupport chromatographic matrix are applied. Both types of sorbents with the immobilized protein G are leach-resistant and obtained matrix provides a minimal nonspecific binding. Both affinity sorbents can be regenerated and reused multiple times when stored properly [17]. Such advantages of sorbents on the basis of the immobilized protein G provide obtaining pure immunoglobulin fractions without contaminant proteins, time expenses reduction and application of such inexpensive reagent as NaOH for the sorbent regeneration and cleaning.

GE Healthcare produces affinity sorbent on the basis of the recombinant protein G. Protein G is immobilized on the sepharose, activated by the cyanogens bromide (CNBr). Such oriented immobilization of protein G provides higher exposure of mobile phase to this ligand, which gives possibility of obtaining of high yield of separated IgG (Table 2).

The sorbent on the basis of the immobilized protein G from manufacturer GE Healthcare has such advantages: maintaining of the IgG binding capacity and recovery after storage in all commonly used aqueous buffers

Table 2. **The dynamic IgG capacity of the sorbent on the basis of the immobilized protein G on sepharose for various species (evaluation performed at GE Healthcare)**

Species	Total IgG capacity (mg/ml)
Human	17
Rat	7
Sheep	18
Rabbit	19
Goat	19
Guinea-pig	17
Cow	23
Mouse	6

and denaturants such as 6 M guanidine hydrochloride and 8 M urea, and chaotropic salts such as 3 M sodium isothiocyanate; binding of IgG over a wide pH range. The affinity sorbent may be sanitized by washing with 70% ethanol.

Some of the most important application areas for this affinity sorbent are the isolation and purification or the removal of IgG from serum, the purification of monoclonal antibodies. In cases when IgG binds more strongly to the protein G than to the SPA (or binds little or doesn't bind to SPA), the sorbent on the basis of the immobilized protein G becomes a valuable tool to increase yield of immunoglobulins.

Purification of IgG from serum can be carried out effectively in a single step with the sorbent on the basis of the immobilized protein G. IgG from human, cow, horse, sheep, guinea-pig, dog, rabbit, mouse, and rat can be successfully purified. Because of its binding characteristics, the affinity sorbent on the basis of the immobilized protein G (GE Healthcare) is a valuable tool for the separation of antibodies from biological liquids, cell cultures. In fact, affinity chromatography with application of this sorbent is suitable for separations from cell culture fluid, where it is necessary to purify rapidly large volumes [26].

There are many scientific articles in the field of pharmaceutical biotechnology that describe application of affinity chromatography using affinity sorbents on the basis of the immobilized protein G for antibodies purification.

The recombinant human anti-Rhesus D antibodies of IgG1-subtype, produced by a Chinese Hamster Ovary cell line, were purified using affinity sorbent on the basis of the immobilized protein G. Yield of antibodies was 57% [27].

Recombinant human monoclonal IgG1 antibodies produced by a transfected Chinese hamster ovary (CHO) cell line were purified by application of affinity sorbent "Poros G" on the basis of the immobilized protein G using elution buffer of composition 0.1 M acetic acid and 0.15 M sodium chloride at a pH of 2.5. Yield of antibodies was 4.64 mg/l [28–31]. Monoclonal antibodies IgG1 to progesterone, obtained from hybridoma cell line, were purified also using the affinity sorbent on the basis of the immobilized protein G [32].

Affinity chromatography with application of sorbents on the basis of the immobilized protein G for isolation of antibodies can be used in medical biotechnology, e. g. for isolation of autoantibodies to cytokines from human serum. Autoantibodies to cytokines can contribute to disease predisposition or pathogenesis. To describe the role of anti-cytokine autoantibodies in disease pathogenesis, it is important to quantitate accurately the levels of such autoantibodies in different patient groups. This can be made using enzyme-linked immunosorbent assay (ELISA). Isolation of IgG autoantibodies to tumor necrosis factor (TNF) from human serum was performed by the method of affinity chromatography with application of sorbent with the immobilized protein G on the Sepharose (GE Healthcare). Antibodies were eluted applying elution buffer (0.1 M Glycine–HCl, pH 2.5). Affinity chromatography with application of sorbents on the basis of the immobilized protein G enables to isolate of all four subclasses of immunoglobulin G to TNF and to conduct further analysis of their content applying ELISA [33–36].

Thus method of affinity chromatography with immobilized protein G has great number of advantages over other methods of proteins purification because it enables to purify antibodies for different purposes (e.g., in industrial biotechnology purification of recombinant antibodies from cell cultures, antibodies purification for application in medical diagnostics of diseases), high level of antibodies can be achieved without any particular losses.

Recently, high-performance magnetic affinity particles with immobilized genetically engineered fused protein A/G are applied for antibody purification. Such manufacturers as Thermo Scientific Pierce, BioVision etc. are produced such particles with immobilized Protein A/G. Protein A/G is a recombinant fusion protein which combines four Fc-binding domains from SPA and two domains from protein G. It can bind antibodies of a great variety from a wider range of animal species and a wider range of isotypes than either protein alone. These magnetic beads with the immobilized protein A/G have such advantages: low non-specific binding, sorbent losses elimination, obtaining of clean separate immunoglobulin fractions. They allow rapid antibody fractions obtaining of different classes and subclasses. Such method is very valuable for both fundamental research and laboratory clinical tests [37].

PpL application in affinity chromatography and immunoassays

PpL is very useful for purification of monoclonal antibodies containing kappa light chains from culture supernatant because it doesn't bind bovine immunoglobulins, which are present in the media serum supplement. Also, in contrast to proteins A and G, PpL is very effective at binding IgM. Although it binds to the Fab portion of the immunoglobulin monomer, PpL does not interfere with the antigen-binding site of the antibody, in contrast to SPA, which binds both Fab and Fc domains of immunoglobulin during purification procedure. Therefore, PpL is used in immunoprecipitation procedures and as a ligand in the affinity chromatography [17].

In 2007 Roque et al. described the application of an affinity sorbent on the basis of an immobilised PpL of trademark ImmunoPure for purification of goat serum and human serum. The percentages of human IgG and Fab fragments obtained from this affinity sorbent with the immobilized PpL were 75% and 70%, respectively. 0.1M glycine-HCl buffer, pH 2.0, was used [38].

PpL interacts mainly with region 1 (FR1) of kappa light chain without interfering with the antigen-binding site of the antibody. That's why, PpL could be used not only for the purification of human scFvs but also as a secondary reagent to detect antigen–scFv complexes in immunoassays. So this is one of the advantages of PpL that marks it out proteins A and G [39].

Thus in 2009 Muzard et al. reported a quick, easy, and efficient method that involves substituting a consensus sequence for the N-terminal sequence of the antibody VL domain that does not react with PpL. As this sequence mimics the antibody pattern recognized by PpL, so such substitution allows the detection and purification of murine label-free scFvs, obtained from *E. coli* culture, and the immunodetection of antigens using untagged antibody fragments [40].

Proteins scFvs were purified from periplasmic preparations extracted from bacterial culture onto an affinity sorbent with the immobilized PpL on the agarose (chromatographic column volume — 0.5 ml, Pierce Biotechnology, USA). During the purification process scFv molecules were eluted at pH 3.0. Preparations of purified scFvs were homogeneous, no contaminants were detected.

PpL is a potentially valuable tool for the purification, immobilization, and detection of unlabeled scFvs even when they are associated with their antigen [41]. The work of Muzard et al. shows that DNA sequence introduction of a PpL-binding site to the ScFv by means of genetic engineering methods does not alter antibody expression as a functional molecule in genetically modified bacteria of *Escherichia coli* HB2151. The sequence grafted onto the scFvs is an ideal affinity tag which allowed a single-step purification of each antibody fragment [42].

One else example of PpL application for antibodies purification is Cossins et. al. research. The last obtained recombinant PpL, at high yield, and this protein was used to produce the affinity sorbent with the immobilized PpL on the sepharose. PpL was shown to be over 95% pure after sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) analysis. PpL was coupled to a solid matrix at an efficiency of 94–98%. Typical yields of pure antibodies (>95% purity) were in the range of 35–65 mg/L [43, 44].

There is also one example of PpL application in the affinity chromatography but in fusion with protein G, particularly their DNA sequences of single light-chain binding domain of PpL and single Fc-binding domain of protein G. This study shows that despite a small size of the protein (molecular weight is 16.5 kDa) each domain behaves independently from each other with respect to the binding characteristics. The PpL domain was able to bind two equivalents of kappa light chains and the protein G domain binds to Fc domain of IgG. LG protein was expressed in *E. coli* cells with yield 75 mg/l.

In order to obtain an affinity sorbent with an immobilized protein LG it was immobilized onto thiopropyl-activated agarose through its C-terminal Cys residue and packed in 1 ml chromatographic column. This chromatographic column was used in experiments for purification of human Fc fragments, kappa chains, human Fc fragments combined with kappa chains, yields were released approximately 94.1% of bound human Fc fragments, 87.8% of bound kappa chains and 96.4% of combined bound human Fc + kappa chains. This fact confirms that the immobilized protein LG has the ability to bind both human Fc fragments and kappa chains simultaneously so it can be potential stable and multi-valent affinity ligand [45, 46]. Protein

LG ligand allows purification and isolation of immunoglobulins with high affinity to both protein G and PpL from blood serum, ascite fluids, and pure fractions obtaining of ScFv molecules due to their binding by kappa chains to PpL.

Due to its unique properties PpL has great advantages as affinity ligand, because protein L allows isolation of immunoglobulins wide row, including human and mouse IgM, IgG1, IgG3, human IgG2, IgG4, IgA, IgD, rat IgG2c, IgG1, mouse and rat IgG2a, IgG2b. What's more, PpL doesn't interact with antigen-binding site of antibodies, it interacts only with kappa light chains of immunoglobulins. Proteins A and G have not high affinity to human IgM, IgA, IgD, ScFv fragments, don't bind to mouse IgM, but PpL is successfully used to isolate and purify these antibodies.

Nowadays proteins A, G, L are used in the affinity chromatography as ligands and are applied for purification and isolation of immunoglobulin different classes.

SPA binds strongly to human IgG1, mouse IgG3, rabbit IgG, sheep IgG2. But both SPA and PpL interact strongly with human IgG2 and IgG4, mouse IgG2a, IgG2b. SPA is effective ligand for the affinity chromatography due to its high conformational stability, resistance to physicochemical stress and proteases action, stability in the wide pH range (pH 2,0–11,0). In addition, affinity sorbents with the immobilized SPA are applied in the procedure of immunosorption for autoimmune and other diseases therapy.

Protein G is also successfully used as ligand in the affinity chromatography due to presence in its structure multiple copies of two different small domains (COOH-terminal and NH_2-terminal domains). It can independently bind albumin and IgG, immunoglobulins Fc fragments. Protein G binds stronger than SPA to polyclonal IgGs from cow, horse, and sheep; to human IgG3, mouse IgG1 and rat IgG2a. It is active in wider pH range, in a wider variety of buffers than SPA. So, in some cases protein G can be used for antibody purification instead of SPA, for albumin isolation, for complex antibody mixtures division on several fractions. Affinity sorbents with immobilized protein G or PpL can be used for human IgG3, rat IgG2a purification, because the last have high affinity to these ligands.

PpL binds to kappa light chains of antibodies, that's why it is effectively used as ligand for affinity chromatography. In contrast to proteins A and G, PpL has affinity to wider range of immunoglobulin classes and subclasses (human and mouse IgM, IgG1, IgG3, human IgG2, IgG4, IgA, IgD, rat IgG2c, IgG1, mouse and rat IgG2a, IgG2b). Affinity chromatography with PpL is used for ScFv fragments purification, unlike SPA and protein G ligands, which cannot be used for this purpose.

So due to the different affinity of proteins A, G, L to immunoglobulin divergent classes and subclasses they are used as ligands in the affinity chromatography for diverse purification purposes.

In the area of pharmaceutical biotechnology the proteins A, G, L application is very useful and beneficial, it is fulfilled by means of immunoglobulin-binding proteins properties improving, genetic engineering fusion of DNA sequences encoded PpL and protein G, SPA and protein G. Affinity chromatography on the basis of immobilized proteins A, G, L can be used instead of conventional methods of protein purification as ethanol precipitation and different chromatography methods due to simplicity in usage, opportunity of purification in a single step, high level purity proteins receiving, multiuse at upholding of proper disposal conditions.

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ВИКОРИСТАННЯ ІМУНОГЛОБУЛІНЗВ'ЯЗУВАЛЬНИХ ПРОТЕЇНІВ A, G, L В АФІННІЙ ХРОМАТОГРАФІЇ

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Протеїни A, G, L — це нативні та рекомбінантні протеїни бактеріального походження, що зв'язуються з імуноглобулінами ссавців. У біотехнології для виробництва афінних сорбентів використовують переважно рекомбінантні варіанти протеїнів A, G та L. Наведено порівняльну характеристику цих протеїнів, афінних сорбентів на їхній основі, переваги та недоліки застосування як лігандів в афінній хроматографії. Подано аналіз властивостей протеїнів A, G та L. Специфічність зв'язування й афінність цих протеїнів розрізняються залежно від виду тварин та підкласу антитіл. Протеїн A має високу афінність до IgG1, IgG2, IgG4 людини, IgG2a, IgG2b, IgG3 миші, IgG2 кози та вівці, IgG собаки, кота, кролика, морської свинки. Протеїн G міцно зв'язується з IgG людини, миші, корови, кози, вівці та кролика. Протеїн L має здатність до міцного зв'язування з легкими каппа-ланцюгами імуноглобулінів людини, миші, щура та свині. Показано доцільність застосування афінної хроматографії з використанням сорбентів на основі іммобілізованих протеїнів A, G і L для виділення та очищення антитіл різних класів. Вищезазначений метод застосовують як альтернативу традиційним методам очищення протеїнів, таким як іонообмінна, гідрофобних взаємодій, метало-афінна хроматографія, осадження етанолом, завдяки простоті, можливості одностадійного процесу очищення, одержання протеїнів з високим рівнем чистоти, багаторазового використання за дотримання правильних умов зберігання та експлуатації. Афінні сорбенти на основі іммобілізованих протеїнів A, G та L застосовують не лише для очищення антитіл, але й для виділення різних їхніх фракцій із сироватки крові.

Ключові слова: афінна хроматографія, стафілококовий протеїн A, пептострептококовий протеїн L, протеїн G.

ИСПОЛЬЗОВАНИЕ ИММУНОГЛОБУЛИНСВЯЗЫВАЮЩИХ ПРОТЕИНОВ A, G, L В АФФИННОЙ ХРОМАТОГРАФИИ

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Протеины A, G, L — это нативные и рекомбинантные протеины бактериального происхождения, которые связываются с иммуноглобулинами млекопитающих. В биотехнологии для производства аффинных сорбентов используют преимущественно рекомбинантные варианты протеинов A, G и L. Приведена сравнительная характеристика этих протеинов, аффинных сорбентов на их основе, преимущества и недостатки использования в качестве лигандов в аффинной хроматографии. Представлен анализ свойств протеинов A, G и L. Специфичность связывания и аффинность данных протеинов различаются в зависимости от вида животных и подкласса антител. Протеин A имеет высокую аффинность к IgG1, IgG2, IgG4 человека, IgG2a, IgG2b, IgG3 мыши, IgG2 козы и овцы, IgG собаки, кота, кролика, морской свинки. Протеин G прочно связывается с IgG человека, мыши, коровы, козы, овцы и кролика. Протеин L обладает способностью к прочному связыванию с легкими каппа-цепями иммуноглобулинов человека, мыши, крысы и свиньи. Показана целесообразность применения аффинной хроматографии с использованием сорбентов на основе иммобилизованных протеинов A, G и L для выделения и очистки антител разных классов. Вышеуказанный метод используется как альтернатива традиционным методам очистки протеинов, таким как ионообменная, гидрофобных взаимодействий, металло-аффинная хроматография, осаждение этанолом, благодаря простоте, возможности одностадийного процесса очистки, получения протеинов с высоким уровнем чистоты, многократного применения при соблюдении правильных условий хранения и эксплуатации. Аффинные сорбенты на основе иммобилизованных протеинов A, G и L используют не только для очистки антител, но и для выделения различных их фракций из сыворотки крови.

Ключевые слова: аффинная хроматография, стафилококковый протеин A, пептострептококковый протеин L, протеин G.