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Histidine Pseudobioaffinity Separation Technology: a Powerful Tool for the Separation of Antibodies

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Abstract

Vital substances derived from blood are extracted, a great deal by procedures of fractionation. The vast progress in biotechnology and molecular biology presented a vast range of possibilities to improve these procedures. One of the major targets of biotechnologies applied to blood plasma is to produce valuable therapeutic proteins. This is done either by purification or by synthesis through microorganisms or animals. Nowadays, the procedures of purification revolve around the one developed by Cohn. Several chromatographic methods, which are based on certain physico-chemical parameters were developped and suggested. Nevertheless, the majority of these methods had limitations such as, the purity of the final product, the cost, the toxicity, the selectivity, the recovery rate following separation in order to obtain the final desired product. Introducing histidine as a ligand, in pseudobioaffinity chromatography eliminated these limitations and presented a reliable and efficient mean of separation of several molecules intended for therapeutic and medical use. This review focuses on the separation of antibodies by pseudobioaffinity separation technology on histidine grafted to different activated matrix.

INTRODUCTION

It is obvious that modern biology has been profoundly marked by discoveries and spectacular progress in biotechnology. These progresses have enriched our understanding of the molecular basis of vital and processes and widened the horizon of new domains of research. Far from underestimating the importance of physical chemistry and the chemistry of proteins, the progress of molecular genetics, the production and clonage of new combination of genes, together with the discovery of monoclonal antibodies, render genetic and cellular engineering indispensable for the out come of biotechnology. Based on these two branches of biotechnology, we are able to develop systems allowing the production of drugs derived from blood [1]. However, this necessitates evidently the purification of these molecules in order to obtain good quality products. This necessity has opened new horizons in the domain of extraction and separation of proteins.

The techniques of affinity chromatography are valuable tools for efficient separation due to their vis-a-vis specificity to a given molecule in a crude

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mixture. However, such specificity has critical disadvantages such as its limited range of utilization and decline in specificity with time due to changes in proteins structure. Furthermore, although constituting a powerful technique, the utilization of immobilized antibodies (immunoaffinity) remains hindered by factors such as its running cost, ligand availability as well as the necessity of specific adjustment of the technique depending on the protein that needs to be purified [2]. In addition, the strong affinity exists between the antigen and antibody has a consequence, the utilization of harsh desorption condition, an acidic pH, urea (8 M) or guanidine [3]. Ligands of moderate affinity are therefore obviously needed for an efficient exploitation of the affinity technique at an industrial scale. For that purpose some available ligands are simple molecules with well know structure and possessing a large spectrum of specificity depending on suitable environmental conditions (pH, temperature, ionic force). These ligands known as "general ligands" or pseudobioaffinity ligands, may be biological molecules such as amino acids. This category of pseudobioaffinity ligands bears a particular interest for the recovery of molecules having therapeutic uses (antibodies, serum proteins). Since 1980, research has demonstrated that aromatic amino acids [4], or heterocyclic ones (histidine), can be utilized as chromatographic ligands for the separation of proteins and peptides [5]. Amino acids have been used at large as pseudobiospecific ligand namely lysine, arginine and tryptophan which were coupled to supports such as sepharose for the separation of proteins [6-8]. An attempt at using histidine as a pseudobiospecific ligand were performed by Kanoun et al 1986 while purifying IgG_1 subclass from placental serum and for the separation of several biological molecules from different sources, using conventional chromatographic matrices such as sepharose and membranes [9-12]. In the present paper, we reviewed the use of histidine pseudobioaffinity separation technology for the separation of monoclonal antibodies from culture supernatant, of subclasses IgG from human serum, monoclonal antibodies to cyclosporine, catalytic and anti-DNA autoantibodies from sera of patients suffering from autoimmune diseases. These different antibodies were separated by using two sorbents namely, histidyl-aminohexyl-sepharose and histidyl-bisoxirane-sepharose. The structures of the sorbents are illustrated in Figure 1 and Figure 2.

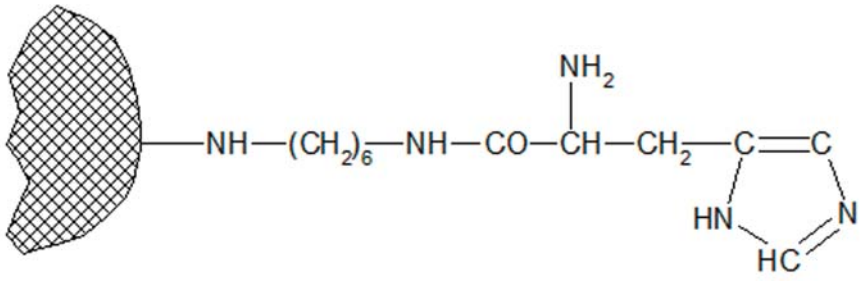

Figure 1. Structure of the histidyl-aminohexyl-sepharose gel.

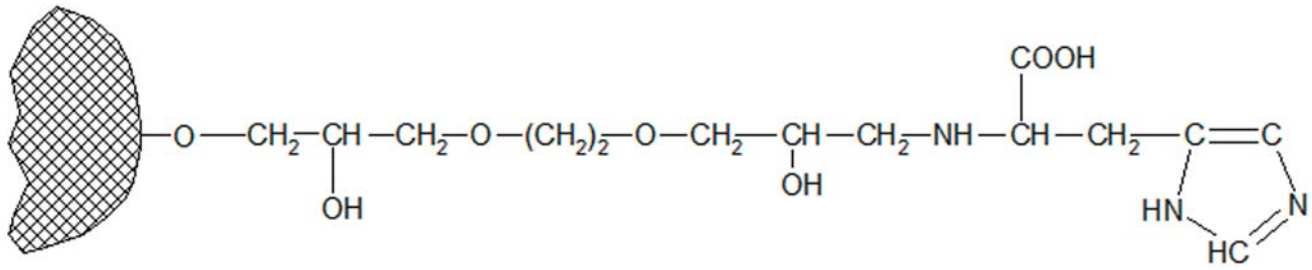

Figure 2. Structure of the gel histidyl-bisoxirane-sepharose gel.

Choice of histidine

Histidine, an organic compound, one of the 22 α-amino acids is commonly found in animal proteins. Histidine has been chosen for its peculiar properties, such as its moderate hydrophobicity, the wide range of its pK_a, its lateral chain (imidazole ring) and other functional groups (NH_2 or COOH). In fact, histidine contains an imidazole aromatic hydrophobic ring which is favorable for electronic transfer and nucleophilic attacks due to nitrogen 3 of imidazole. The presence of amine and carboxylic groups attribute to the histidine its zwitterionic nature. These different properties, as well as its role in the acid-base system, allow histidine to interact via different pathways depending on its microenvironment : neighbouring amino acid, pH, ionic strength and temperature. These different properties emphasize the importance in the interaction of histidine and its neighbouring atoms. Histidine residues in proteins are involved in the interactions in immobilized metal affinity chromatography systems, and sometimes in the dye-ligand system, but histidine can also be immobilized and act as a ligand to adsorb proteins [13].The interaction of histidine with proteins can vary with respect to pH, so the choice of the buffer and the pH is of primordial importance.

Separation of mouse monoclonal antibodies

The advent of the Köhler-Milstein method in 1975 for the biosynthesis of monoclonal antibodies [14] opened the door to important developments in immunology and many fields of biology and technology [15]. Monoclonal antibodies can be used as reagents in immuno-diagnostics [16], therapeutics [16], or purification tools [17]. In this part, we describe the purification of mouse monoclonal antibodies IgG_1 and IgG_{2b} from culture supernatant, and ascites fluid, respectively, using histidyl-bisoxirane-sepharose, and histidyl-amino-hexyl-sepharose.

Preparation of monoclonal antibodies

Hybridoma cell lines (VO 208) were grown in RPMI 1640 medium + 10% FCS. A 200 mL aliquot of culture supernatant was centrifuged than filtered through 0.45µm filter. The harvested monoclonal antibodies were then precipitated with 50% ammonium sulfate at 4°C for 1 h with gentle stirring for 1 h. The solution was then centrifuged and the precipitate was dissolved in 25 mM Tris-HCl buffer pH 7.4, and dialysed for 18 h against three 2 L changes of the same buffer.

Mouse ascites fluid containing monoclonal antibodies IgG_{2b} to Clostridium Tyrobutyricum was centrifuged to remove cells, than filtered through 0.45 µm filter. A 9 mL aliquot of filtrate was diluted in 25 mM Tris-HCl buffer pH 7.4, precipitated with 50% ammonium sulfate at 4°C for 1 h and then centrifuged. The precipitate was dissolved in 25 mM Tris-HCl buffer pH 7.4 and dialysed against the same buffer.

Chromatography and analysis

Separation was performed using a column containing the gels mentioned above. All chromatographic procedures were carried at 4°C. Three mL of each of the dissolved and dialyzed precipitates were injected into the column and equilibrated with 25 mM Tris-HCl buffer pH 7.4, and elution was performed at the same buffer containing increasing amount of sodium chloride. Determination of antibody was done by solid-phase enzyme linked- immunosorbent assay (ELISA). The purity of the proteins separated was analyzed by SDS-PAGE.

RESULTS

Two histidyl-coupled adsorbents were compared for the separation of mouse monoclonal antibodies from culture supernatant and ascites fluid. The

separation of mouse monoclonal antibodies from culture supernatant occurred in the fraction eluted with 25 mM Tris-HCl buffer + 0.2 M NaCl at pH7.4, together with albumin and transferrin; the recovery was reduced. The reduced antibody recovery is due to the repulsion forces between these antibodies and the histidine's charged free COOH groups of the gel. In an attempt to increase the efficiency of the adsorbent we coupled the histidine to aminohexyl-sepharose through COOH group. The latter gel was then used to separate monoclonal antibodies IgG_1 from culture supernatant and IgG_{2b} from ascites fluid. Monoclonal antibodies IgG_1 and IgG_{2b} were separated at high purity and 90% for IgG_1 and 94% for IgG_{2b}. Chromatography on histidine-free aminohexyl-sepharose gel was performed in order to check whether the adsorption of monoclonal antibodies (IgG_{2b}) is due to aminohexyl sepharose or to histidine with results confirming the latter as the adsorbent in a histidyl-aminohexyl-sepharose gel (Table 1). These finding clearly proved that histidine pseudobioaffinity separation technology is a successful method for separation of mouse monoclonal antibodies with protein elution achieved under mild conditions [18].

Separation of IgG subclasses from human serum

The immunoglobulins IgG are a major class of serum glycoproteins that confer humoral immunity in humans, with a serum concentration ranging from 6.7 to 15.5 mg/mL [19]. IgG is routinely fractionated from large donor pools of normal plasma, where a broad spectrum of antibodies is available. Intravenous immunoglobulin G (IVIG) is primarily used in the treatment of primary humoral immunodeficiencies and in the prevention and attenuation of infectious diseases [20,21]. Plasma fractionation involves Cohn [22]/Oncley [23] precipitation, which utilizes tightly controlled process conditions, including those of temperature (≤ 0°C), pH, ionic strength and protein concentration. In addition, further precipitation steps can be employed using other organic precipitants such as polyethylene glycol [24]. The precipitation methods result in the separation of plasma proteins into crude fractions, albeit with a typical loss of 50% in recovery. We were able to develop a separation method to improve, if not maximize, the recovery rate of human IgG_1 and IgG_2 from an ethanol precipitate (Cohn fraction II) of human plasma containing 95% IgG and < 0.5% albumin. Separation was performed on histidyl-aminohexyl-sepharose gel by pseudobiospecific recognition whereby both IgG subclasses were bound to the histidine ligand at pH 7.4 and eluted as a pure fractions by adding with sodium chloride to the starting buffer 25 mM Tris-HCl at the same pH.

Human gamma globulin

The sample containing human IgG (163 mg/mL), as precipitated by ethanol from human plasma was kindly supplied by Institut Mérieux (Lyon-France).

Chromatography and analysis

Histidyl-aminohexyl-sepharose was packed into a column (6.4 x 1.0 cm I.D.) to give a bed volume of 5 mL. All chromatographic experiments were carried out at 4°C. A linear flowrate of 45.0 cm/h was maintained and 3 mL fractions were collected. The 245 µL aliquot containing 40 mg of human IgG was injected into the column. The 25 mM Tris-HCl buffer

Table 1. Comparative efficiencies and capacities of histidyl-aminohexyl-sepharose and aminohexyl-sepharose for mouse McAbs (IgG_{2b}) purification.

Gel using	Yield (%)	Capacity of mouse McAbs (IgG_{2b}) per ml of gel (mg)
Histidyl-aminohexyl-sepharose	94	3.82
Aminohexyl-sepharose	2	0.05

Table 2. Comparison of isoelectric point of human IgG subclasses adsorbed on histidyl-bisoxirane-sepharose and histidyl-aminohexyl-sepharose.

	Isoelectric point	
	Fraction eluted with 0.2 M NaCl	Fraction eluted with 1 M NaCl
Histidyl-bisoxirane-sepharose	7.9-8.2	-
Histidyl-aminohexyl-sepharose	8.1-8.2	6.8-7.2

at pH 7.4 was used to equilibrate the column. Elution was carried out using the same buffer containing increasing amounts of sodium chloride (0.2 and 1 M). Obtained fractions, following affinity chromatography on histidyl-aminohexyl-sepharose, were tested for specificity by immunoelectrophoresis in 1% agarose gel according to Grabar and Williams [25], using sheep anti-human IgG_1 and sheep anti-human IgG_2 obtained from Janssen Biochimica (Beerse, Belgium). Radial immunodiffusion assays according to Mancini [26], were realized with immunoplates containing a monospecific antiserum in agarose (Janssen Biochimica, Beerse, Belgium), by using 100 µL aliquot of fractions eluted with 0.2 and 1 M NaCl. Each well was filled with 5 µL of those diluted fractions. Immunoprecipitation diameters were read after a diffusion of 48 h at room temperature. The amounts of IgG_1, IgG_2, were derived from a standard curve.

RESULTS

Histidyl-aminohexyl-sepharose was used for the separation of two human IgG subclasses, IgG_1 and IgG_2 from human plasma (Cohn fraction II). The elution profile showed that IgG_1 and IgG_2 were separated in two fractions following elution with 25 mM Tris-HCl buffer pH 7.4 containing 0.2 M and 1 M NaCl respectively. Immunoelectrophoresis with anti-human IgG_1 and anti-human IgG_2 revealed precipitation zones showing the presence of IgG_1 and IgG_2 in fraction eluted with 0.2 M NaCl, whereas, the one eluted with 1 M NaCl contained only IgG_2. Determination of the levels of the two subclasses separated was performed by radial immunodiffusion technique. A total recovery of 80% for IgG_1 and 13% for IgG_2 was calculated. Isoelectric focusing in polyacrylamide and agarose gels were performed to determine the isoelectric points (pI) distribution of the total IgG-Cohn-II fraction as well as of the retained and non-retained fraction on histidyl-epoxy-sepharose and histidyl-aminohexyl-sepharose gels. The IgG-Cohn-II fraction (material before injection onto histidyl-aminohexyl-sepharose) had a pI distribution ranging between from 4.76 and 10.21. The pI values for the retained eluted IgG_1 fraction with 0.2 M NaCl on histidyl-epoxy-sepharose and histidyl-aminohexyl-sepharose gels ranged between 7.9 to 8.2. Those values were between 6.8 to 7.2 for the retained then eluted IgG_2 fraction with 1 M NaCl on histidyl-aminohexyl-sepharose only (Table2). Moreover, the unbound IgG fraction as well as IgG fraction the histidyl-aminohexyl-sepharose bound IgG_1 and IgG_2 fractions were rechromatographed to confirm the adsorptive specificity for IgG_1 and IgG_2 subclasses. Once dialyzed and concentrated, these fractions were injected again onto histidyl-aminohexyl-sepharose gel. Following purification, the totality of unbound IgG was released from the sorbent while IgG_1 and IgG_2 fractions were retained and eluted with 0.2 and 1 M, respectively similarly to the previous chromatographic run. This successful ability of histidyl-aminohexyl-sepharose gel to specifically bind adsorb IgG_1 and IgG_2 from human plasma was at pH 7.4 at 4°C, considered to be mild conditions. Thus, pseudobioaffinity separation

technology is reported to be quite a reliable method for IgG_1 and IgG_2 separation from human plasma [27].

Adsorption study of monoclonal antibodies to cyclosporine

The cyclic undecapeptide cyclosporine Cs (Sand-Immune®) is a powerful immunosuppressive drug commonly used for preventing graft rejection and in the treatment of autoimmune diseases. More than 180 monoclonal antibodies to Cs have been generated [28] and the fine specificity of 63 of them has been elucidated. The latter was acheived by testing their ability to discriminate Cs derivatives modified singly at each of the 11 residues of the Cs molecules while still presenting a preserved peptide ring conformation [29,30]. In view of the rigid ring structure of Cs established by crystallographic and NMR studies [31,32], the existence of several hundred synthetic and natural Cs derivatives [33] and the availability of a large number of monoclonal antibodies, Cs constitutes a unique model for a detailed study of the molecular basis of antibody-antigen interaction. The following is an assay where we tested the adsorption of 7 types of monoclonal antibodies to cyclosporine having different CDR regions on histidyl-aminohexyl-sepharose.

Monoclonal antibodies

The monoclonal antibodies to Cs, namely A (R14-85-1), B (R45-200-7), C (R45-45-11), D (V45-271-10), E (78-299-4), F (V14-203) and G (R45-109-22) obtained from the (Department of Biotehnology, Sandoz Ltd, Switzerland) were produced as described by (Quesniaux et al., 1987). Monoclonal antibodies A and F were obtained from two separate fusions performed with spleen cells from the same mouse 14; monoclonal antibodies B, C and G were obtained from one fusion with spleen cells of mouse 45 while monoclonal antibodies D and E were from another fusion with spleen cells from the same mouse 45. Hybridomas were grown in serum free medium. Amino acid sequencing, solid tumor production, RNA and genomic DNA extraction from frozen cells, southern blot, cloning , DNA and mRNA sequencing, and light and heavy chain sequences determination of the monoclonal antibodies were performed as described by Schmitter et al [34]. Tables 3 and 4 show the amino acid variability sequence in the three hypervariable regions namely CDR1, CDR2 and CDR3 of the light and the heavy chain of the 7 tested monoclonal antibodies.

Chromatography and results

We chromatographed separately, and under the same conditions each one of 7 monoclonal antibodies (A, B, C, D, E, F, G) on histidyl-aminohexyl-sepharose. Adsorption was performed in 25 mM Tris-HCl buffer pH 7.4 at 4°C. Elution was done with the same buffer containing 0.2M NaCl. Figure 3. shows the chromatogram of the separation of the 7 monoclonal antibodies on the gel. As we have mentioned above, histidine residues are involved in substrate binding by many enzymes. The active histidine residue may act as a proton acceptor or donor in a protein-ligand interaction, in charge-relays interactions between amino acids at the active site, in conformational changes associated with substrate binding or oligomerization of proteins chains. Several examples emphasize the important role of histidine in bilological systems. For instance, the enzyme ribonuclease contains in its catalytic site three histidine (His 12, His 119, His 105) and one lysine (Lys 41). Histidine 12 and 119, play an important role because each of these two amino acids can be protonated and deprotonated. On the other hand, and on the basis of interaction between histidine and lysine, lysine 41 contributes in substrate immobilization of the due to its cationic charge. In the case of chymotrypsin enzyme, the catalytic site contains aspartic acid, histidine and serine. In the mechanism of action of this enzyme, histidine obtains a proton from serine and bears a nucleophilic characters [35,36].

The variable domains of both light and heavy immunoglobulins chains contain three hypervariable loops, or complementarity determining regions (CDRs). The loops of the VL and VH domains, cooperate in constructing a specific antigen binding site surface indicate, that the CDR1, and CDR2 regions are involved in antigen binding, while the CDR3 is responsible for its recognition. Due to their diversity and the large number of amino acids residues, in the six CDR regions of a single antibody, molecule. It is difficult to determine which ones of them are involved in the interaction between anti-cyclosporine antibodies (anti-Cs) and histidine. A detailed analysis of the amino acids distribution in the antibodies CDR1, CDR2, CDR3 regions reveals that the six of them contain a large quantity of serine, aspartic acid and lysine (Table 3 and 4). The identification of these 3 amino acids specifically in CDR1 and CDR2 regions suggest 2 possible means for anti-Cs adsorption on histidyl-amino hexyl-sepharose gel.

First, the interaction between histidine and anti-Cs antibodies involves the residue serine, aspartic acid and lysine in a mechanism similar to that of the enzymes ribonuclease and chymotrypsin. A nucleophilic attack would occur as due to the nitrogen number 3 of the imidazole ring of histidine. In addition, a hydrogen bond is formed between histidine and aspartic acid.

Second, the difference in retention of these antibodies on the gel, is simply due to the fact that these antibodies belong to the different subclasses (A, D, E, G : IgG_1), (B, C : IgG_{2a}), and (F : IgG_3).

Separation of catalytic and DNA-hydrolyzing autoantibodies from patients sera with autoimmune diseases.

Normally the immune system distinguishes the self from the non self. It spares the organism, and attacks foreign agents. Nevertheless, this system can turn against the self causing severe diseases

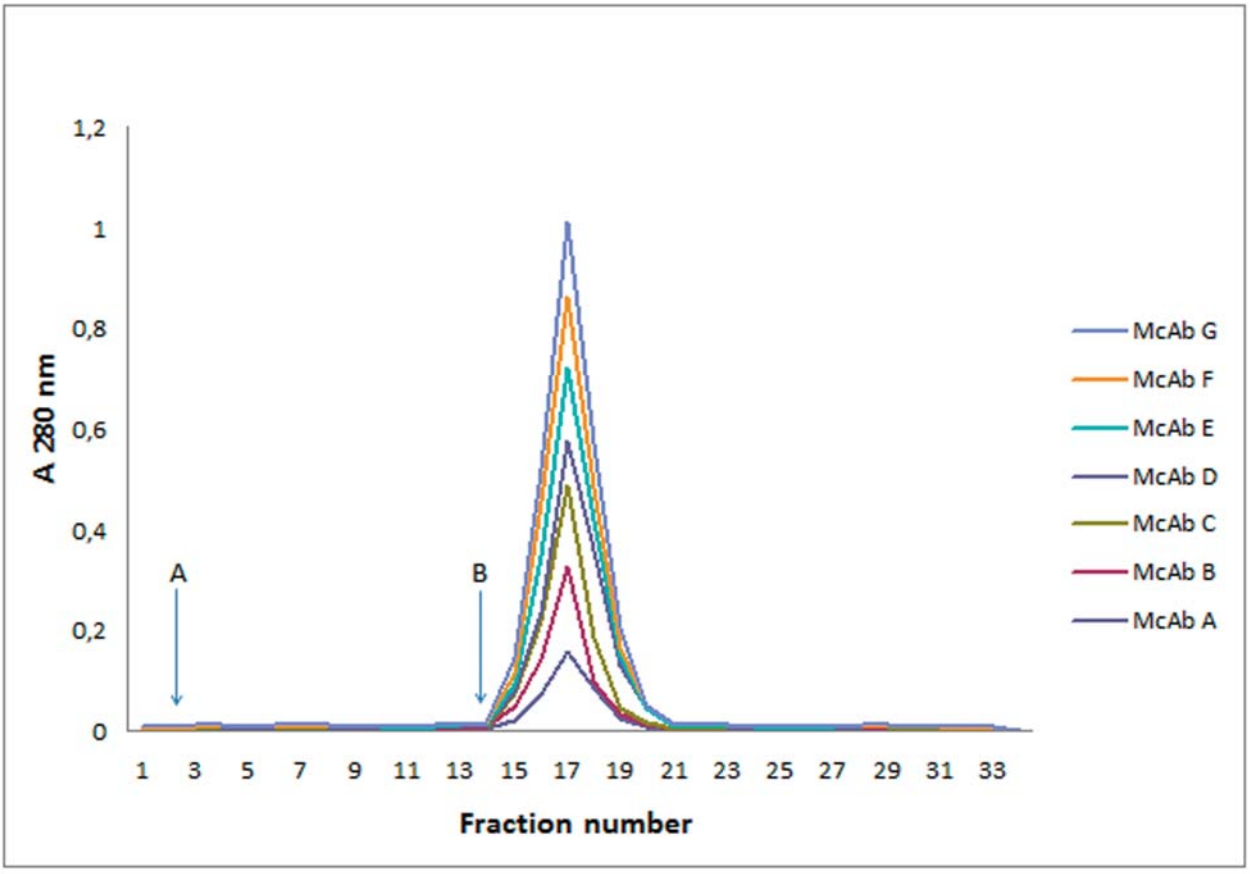

Figure 3. Typical chromatogram adsorption of the 7 McAbs to Cs on histidyl-aminohexyl-sepharose gel. A: Tris-HCl pH 7.4; B: Tris-HCl + 0.2M NaCl pH 7.4.

Table 3. Amino acid sequence alignement of the VH regions of the 7 McAbs to Cs. The symbol (-) corresponds to the same amino acid as in sequence.

Amino acid \ Monoclonal antibody	VH							
	A	B	C	D	E	F	G	
Aspartic acid	-	-	1	1	-	-	-	
Serine	3	2	-	1	1	1	1	CDR1
Methionine	1	1	1	1	1	1	1	
Tyrosine	1	1	3	3	1	1	2	
Isoleucine	1	1	2	2	2	2	1	
Serine	3	4	2	2	1	-	2	
Asparagine	1	-	1	1	3	2	-	
Glycine	3	3	4	4	2	1	2	CDR2
Tyrosine	3	3	1	1	1	3	3	
Aspartic acid	1	1	1	1	1	1	2	
Lysine	1	1	1	1	1	1	1	
Tyrosine	1	1	2	2	4	3	-	
Glycine	1	1	1	1	1	1	2	
Aspartic acid	1	2	1	1	1	1	-	CDR3
Serine	1	2	-	-	2	-	1	

characterized by the appearance in the plasma of the patients of an abnormally high levels of autoantibodies. Antiphospholipid antibodies (aPL) [37], including anticardiolipin antibodies (aCL) have been associated with arterial and venous thrombotic diseases, recurrent fetal and thrombocytopenia with systemic lupus erythematosus (SLE), and also with antiphospholipid syndrome (APS) [38-41]. Various catalytically active IgG antibodies have been recently detected in the sera of patients with autoimmune pathologies, where their presence is most probably associated with spontaneous induction of anti-idiotypic antibodies. The physiological role of catalytic antibodies is not very clear. The autoimmune antibodies are conventionally purified using protein A/G affinity chromatography [42,43]. In both purification methods, the drastic elution conditions of IgG might induce conformational alterations and structural modifications due to denaturation [44]. Therefore, the observed catalytic activity of the purified antibodies may not reflect the actual catalytic efficiency of the native antibodies. In this part we studied the separation of catalytic and DNA-hydrolyzing autoantibodies from the total autoimmune serum of 5 patients with antiphospholipid syndrome by using histidyl-aminohexyl-sepharose gel. Following separation, the DNA-hydrolyzing activity of the purified autoantibodies was determined by using pUC19 plasmid DNA as the substrate as reported by Gabibov [45] while Pro-Phe-Arg-7-amido-4-methylcoumarin was the substrate for catalytic activity, as reported by Sarath [46].

Chromatography

Histidyl-aminohexyl-sepharose was packed into a column (3.2 x 1 cm I.D.). All chromatographic procedures were carried out at room temperatures at a flow rate of 1 mL min^{-1}. A 400 µL aliquot of

Table 4. Amino acid sequence alignement of the VL regions of the 7 McAbs to Cs. The symbol (-) corresponds to the same amino acid as in sequence.

	VL							
Monoclonal antibody / Amino acid	A	B	C	D	E	F	G	
Serine	5	5	2	2	5	5	3	
Tyrosine	2	2	1	1	-	3	3	
Aspartic acid	1	-	1	1	1	1	1	
Arginine	1	1	1	1	1	1	1	CDR1
Leucine	3	3	1	1	-	1	3	
Glycine	1	1	-	1	1	1	1	
Lysine	1	1	-	1	1	1	1	
Serine	2	2	2	2	2	2	-	
Arginine	1	1	2	2	-	-	-	CDR2
Leucine	1	1	1	1	-	2	2	
Glutamine	1	1	2	2	1	1	1	
Alanine	1	1	1	-	1	2	1	
Leucine	2	1	-	1	-	1	2	CDR3
Tryptophane	1	1	-	-	1	-	1	
Serine	-	1	1	1	1	1	-	

centrifuged patients sera was diluted (1:20) in 25 mM MOPS buffer at pH 7.4 and then injected into the column. Elution was carried out at the same flow rate with the equilibrating buffer containing 0.2 M NaCl. The absorbance of the pooled retained and non retained fractions was measured at 280 nm, and 3 mL fractions were collected.

RESULTS

Separation of IgG from plasma of patients

Patients sera, sera from healthy donors and the commercially available human IgG were all chromatographed on histidyl-aminohexyl-sepharose gel at pH 7.4. Results failed to show any difference in the chromatograms of different patients. The IgG autoantibodies were negatively separated in the non retained fraction which contained pure IgG as seen by SDS-PAGE.

Nephelometric determination of IgG

The concentration of IgG in the non retained fractions of all patients was determined nephelometrically. The results obtained shows 90% IgG recovery for patient 1.93% IgG recovery for patient 2.98% IgG recovery for patient 3.91% IgG recovery for patient 4.93% IgG recovery for patient 5.

DNA-hydrolyzing autoantibodies

The non retained fractions has been analysed for their activity using plasmid (pUC19) DNA as a substrate. The reaction mixture was analysed by electrphoresis in 1% agarose gel. The DNA-hydrolyzing activity of all the separated IgG autoantibodies is shown in Table 5. All purified IgG has shown DNA-hydrolyzing activity but in some cases the activity was very high. In fact, patients 1 and 3 both suffering from systemic lupus erythematosus and lupus anticoagulant have shown very high DNA-hydrolyzing activity.

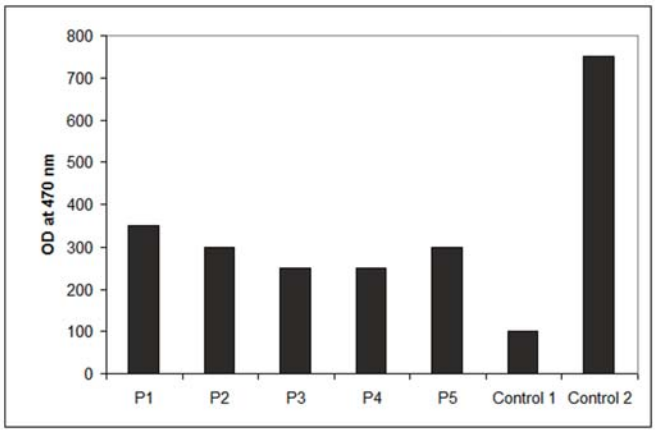

Figure 4. Hydrolysis of peptide substrate Pro-Phe-Arg-methylcoumarin by the purified autoantibodies from patients (P1 to P5).

Peptide cleaving catalytic activity

The peptide cleaving activity for the purified IgG autoantibodies was also measured. The average OD for the three catalysis assays following an incubation period of 24 h at 37°C are shown in Figure 4. All patients showed a catalytic activity but the higher one was observed in patient 2.

Distribution of autoantibodies subclasses

The distribution of the different subclasses of IgG in the different separated fractions was detected by ELISA. Using subclasses of specific antibodies. IgG subclasses on the used gel depend on the buffer nature. Comparison was carried out between the MOPS and Tris-HCl at the same chromatographic conditions. In MOPS buffer for patient 2 and patient 3 suffering from primary antiphospholipid sundrome IgG_1, IgG_2, and IgG_3 were detected only in the non retained fraction. For patient 1, patient 4 and patient 5 suffering from systemic lupus erythematosus, IgG_1 and IgG_3 were detected in the non retained fraction. Where as in Tris-HCl buffer only IgG_1 and IgG_2 were selectively retained and separated in two fractions eluted respectively with 0.2 M and 0.4 M NaCl in Tris-HCl buffer pH 7.4.

In the present part a simple one-step separation procedure allowed the separation of IgG from serum of patients with antiphospholipid syndrome. We have succeeded in separating two subclasses of IgG_1 and IgG_2 autoantibodies, while using Tris-HCl buffer at pH 7.4. However, while using MOPS buffer, we have succeeded in separating three of the four subclasses of autoantibodies, that is IgG_1, IgG_2, and IgG_3. The purified autoantibodies from patients with (aPL) sera show DNA-degrading activity of the plamid pUC19 DNA and catalytic activity in hydrolyzing the peptide substrate Pro-Phe-Arg-7-amido-4-methylcoumarin [47].

General conclusion

New protein-based therapeutics such as intravenous immunoglobulin G, monoclonal

Table 5. Hydrolysis of sc pUC19 plasmid DNA by the separated autoantibodies IgG from different patients.

+ + + + : maximum activity; + + +: high activity; + +: medium activity; -: no hydrolyzing activity detected; Control 1 and 2 : healthy blood donor

	DNA hydrolyzing activity of purified autoantibodies on histidyl-aminohexyl-sepharose
Patient 1 : Systemic lupus erythematosus	+ + +
Patient 2 : Primary antiphospholipid syndrome	+ + +
Patient 3 : Primary antiphospholipid syndrome	+ + + +
Patient 4 : Systemic lupus erythematosus	+ +
Patient 5 : Systemic lupus erythematosus	+ +
Control 1 : Healthy donor	-
Control 2 : Human IgG commercially available	-

antibodies, monoclonal-based vaccines, growth factors and plasma proteins implies the need to study, characterize, and purify. The separation step is likely to be a bottleneck and cost-effective technology will be needed to rectify it. Currently prevalent matrices for separation of immunoglobulins are based on protein A/G matrices. Affinity chromatography using Protein A/G is specific. They display excellent selectivity and specificity, but the cost is very high. The conditions used to elute the retained antibody are harsh and require acidic pH. This can have an impact on the biological activity of the separating antibody. These drawbacks preclude the use of biological ligands in practical applications and has prompted many researchers to turn their attention to the development of new ligands like dyes, amino acids, and other. These ligands show comparable affinities, and specificitiy to those of immunoadsorption.

In histidine pseudobioaffinity separation technology, the amino acid histidine can be coupled to solid matrices like sepharose, or hollow-fibre membranes, either through its NH_2 or COOH functional groups with or without a spacer arm, the nature of the buffer's ions can influence adsorption capacity and subclasses of IgG adsorbed. In this review, we have demonstrated the efficiency of histidine pseudobioaffinity separation technology in the separation of monoclonal antibodies, subclasses of human IgG, monoclonal antibodies to cyclosporine, DNA-hydrolyzing and catalytic autoantibodies from sera of patients suffering from autoimmune diseases. The results obtained show that, this class of ligand is of particular interest for the isolation of molecules aimed at therapeutic ends. The advantage of gentle elution, non toxicity of histidine, good specificity for subclasses, good stability, as well as its low cost make histidine pseudobioaffinity separation technology a powerful tool for separation of antibodies and removal of autoantibodies from the plasma of patients in future clinical applications.

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