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Surface modification of polystyrene particles for specific antibody adsorption

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Abstract

Biospecific interactions between biological molecules such as antibodies and polymer particles bearing the chemical groups capable of mimicking natural bioactive sites were investigated. Polystyrene particles were substituted by various amino-acids and exposed to antiviral antibodies directed against two different enveloped viruses related to the Arbovirus group. Functionalization yields of polystyrene particles were found to depend on the nature of the amino-acid. The interactions between the functionalized latexes and the antiviral antibodies were systematically compared to the interactions with the 'non-antiviral' antibodies. Results indicated that the adsorption of antiviral antibodies depends on the chemical composition of the polystyrene particles surface, i.e. substituted amino acid, the amount of substitution and the surface charge density of the polymer particles. These differences are illustrated by variation in the immunoglobulin adsorption capacities and in the affinity constants. Therefore amongst the assessed polystyrene derivatives, some precise compositions were shown to display specificity to one antiviral antibody whereas other compositions displayed specificity to both antiviral antibodies but with different affinities.

Keywords: Polystyrene particle; Amino-acid substitution; Antibody adsorption

1. Introduction

Many studies have shown that biospecific interactions can be developed between biological compounds and functionalized polystyrene particles with suitable chemical groups [1]. Indeed, random substitution of macromolecular chains with appropriate chemical groups was suggested to allow the creation of active sites able to interact specifically with proteins like immunoglobulins. The probability of appearance of these sites during the synthesis process depends on the nature, number and distribution of the chemical groups. This has been explored for polymer particles substituted with different functional groups that display various biological activities. For example, polystyrene particles substituted with phosphoester groups exhibit a strong affinity for anti-DNA antibodies detected in the sera of Systemic Lupus Erythematosus patients [2]. It has been demonstrated that the adsorption of anti-DNA antibodies onto latex particles involves their Fab fragment and is maximal, with an affinity constant $K_{aff} = 10^8\ M^{-1}$, for 20% substitution in phosphoester groups [3]. Polymer particles exhibiting the same antigenic characteristics as the coagulation factor VIII were obtained by functionalization of polystyrene particles with L-tyrosine methyl ester. The affinity constant of anti-FVIII antibodies is maximal, $K_{aff} = 4 \times 10^9\ M^{-1}$, for latexes containing 15 and 25% of functional groups [4,5]. These modified latexes were shown to allow epuration of 90% of anti-FVIII antibodies from haemophilic plasma in an extracorporal circulation system. Various affinity-type particulate adsorbents have been used for the selective removal of the IgG class of antibodies from human plasma or for the purification of monoclonal

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antibodies. The most common are protein A and protein G-based adsorbents because of its high affinity for the Fc region of immunoglobulin, but these techniques cannot distinguish between antibodies with different specificities. Peptides have also been used as ligands in affinity purification of antibodies [6–8]. However, in some cases refinement of the peptide was required to make it useful as an affinity matrix.

The Arbovirus group, including flavivirus and alphavirus genus's, causes haemorrhagic fever in humans with outbreaks spreading around the world from foci of infection in intertropical zones. The flaviviruses comprise members including yellow fever, dengue, West Nile, tick-borne encephalitis virus (TBE) and Japanese encephalitis viruses (JE) [9]. The dominant antigen of flaviviruses is the envelope (E) glycoprotein that binds cellular receptors, mediates cell membrane fusion, and contains an array of epitopes that elicit virus-neutralizing and non-neutralizing antibodies [10–12]. Thus, RGD sequence found in the envelope glycoprotein of some flavivirus was shown to be implicated in host-cell adsorption [13,14]. Furthermore, mutagenesis studies performed on the envelope glycoprotein of Japanese encephalitis virus have demonstrated the importance of precise amino acid residues: arginine/lysine at position 52, glutamic acid at position 138, phenylalanine and serine at positions 364 and 365, asparagines and leucine at positions 367 and 368 [15]. Alphaviruses are extensively studied because (i) they are basically simple enveloped viruses, (ii) they display high yield of propagation, and finally (iii) they mostly produce unapparent or mild symptoms in human hosts compared with the frequently severe effects of flavivirus infections. Well-characterized members of alphavirus are semliki forest virus and sindbis virus. In contrast to the smooth surface of flaviviruses, the alphaviruses have prominent spikes that project from the bilayer membrane [16]. Infection of cells by alphaviruses is a receptor-specific event mediated by the viral envelope glycoproteins, E1 and E2, associated in trimers of heterodimers [17,18]. Studies of sindbis virulence have shown that arginine at position 144 [19], valine at positions 75 and 237, and glycine at position 313 [20] in the E2 glycoprotein are involved in host-cell adsorption.

The present paper describes the synthesis of functionalized polystyrene particles able to strongly interact with antiviral antibodies. We have studied polyclonal antibodies obtained from immunized mice with babanki virus (an alphavirus from *togaviridae* family) and zika virus (a flavivirus from *flaviviridae* family), two enveloped viruses belonging to the Arbovirus group. The surface modification of polystyrene particles by chemical grafting of the most representative amino acids involved in the virulence of the studied viruses was achieved in order to develop specific interactions between the latex particles and the antiviral antibodies.

2. Experimental section

2.1. Materials

Crosslinked polystyrene (PS) particles (35–75 μm in diameter), BioBeads SX3 (Bio-Rad Laboratories, Richmond, CA, USA) were washed successively with 1 M NaOH and 1 M HCl. The polystyrene latexes were then thoroughly washed with distilled water and dried at 60 °C under vacuum. Elemental analysis was carried out at the Service Centrale d'Analyse CNRS (Gif sur Yvette, France).

Reagents and solvents (dichloromethane, monochlorosulfonic acid, triethylamine, acetone) were supplied by Carlo Erba (Rueil-Malmaison, France).

Amino acid methylester (AAOMe), bovine serum albumin (BSA) and mouse IgG were purchased from Sigma (La Verpillère, France).

Ascitic fluids containing polyclonal anti-babanki or anti-zika antibodies were produced at the 'Centre de Recherche du Service de Santé des Armées' (La Tronche, France) by injection of Babanki virus (*Togaviridae*) or Zika virus (*Flaviviridae*) in combination with Freund adjuvent and lymphoma cells, into the peritoneal cavity of mice.

Protein G-sepharose column (Mab Trap G II Kit) and PD-10 G 25 M column were supplied by Pharmacia Biotech (Saint-Quentin-en-Yvelines, France), and ultrafiltration unit of 100,000 kDa cut-off (Biomax-100) by Millipore (Saint-Quentin-en-Yvelines, France).

Viruses were grown in Vero cells (from green monkey kidney) obtained from the American Type Culture Collection (ATCC). Cells were cultured at 37 °C in a humidified CO_2 incubator (95% air/5% CO_2), in culture medium M199 with L-glutamine supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, and 100 μG/ml streptomycin (all from Gibco, Cergy-Pontoise, France).

Phosphate buffer saline (PBS) was purchased from Gibco. ^{125}I–Na (14.5 mCi/mg) was supplied by Amersham (Les Ulis, France), and chloramine T by Merck-Schuchard. An automatic γ counter 1470 Wizrad from Wallac was used to measure the radioactivity of the polystyrene particles.

2.2. Functionalization of polystyrene particles

Crosslinked polystyrene particles functionalized by various amino acids were prepared in three steps, according to Fougnot et al. method [21,22]. First, phenyl groups of polystyrene (PS) were chlorosulfonated by reaction with 10 molar excess monochlorosulfonic acid (HSO_3Cl) in dichloromethane (CH_2Cl_2) for 1 h at room temperature. The resulting poly(para-chlorosulfonyl)styrene (PS-SO_2Cl) particles were quickly washed with CH_2Cl_2, with acetone and then with CH_2Cl_2.

Second, PS-SO_2Cl was condensed with various amino acid methyl esters in order to obtain the different functionalized particles, i.e. arginine sulfamide polystyrene (PS-SO_2R), aspartic acid sulfamide polystyrene (PS-SO_2D),

leucine sulfamide polystyrene (PS-SO_2L), phenylalanine sulfamide polystyrene (PS-SO_2F), tyrosine sulfamide polystyrene (PS-SO_2Y). Reactions were performed in CH_2Cl_2 by adding various amounts of amino acid methylester in the presence of an equimolar amount of triethylamine (Et_3N), at room temperature for 48 h. Then, particles were washed with 10^{-2} M NaOH, and dried at 50 °C under vacuum.

Third, ester groups were gently hydrolyzed by successive washing of the particles with different aqueous solutions of NaOH (10^{-2}, 10^{-1}, 1, 2, 10^{-1}, 10^{-2} M). Functionalized polystyrene particles were dried at 50 °C under vacuum and were characterized by elemental analysis.

2.3. Purification of antiviral antibodies from ascitic fluid

Purification of IgG from ascitic fluid was performed by affinity chromatography using a protein G-sepharose column. Fractions of 1 ml were collected and their optical density was measured at 595 nm in order to plot the chromatographic profile of the ascitic sample. IgG were recovered in fractions 13, 14 and 15. These fractions were pooled, desalted with PBS 1× and concentrated to a final volume of 1 ml using ultrafiltration unit of 100,000 Da cut-off. The purity of the IgG fraction was checked on SDS-PAGE stained with silver nitrate.

The concentration of the IgG fraction was determined by ELISA (Enzyme Linked ImmunoSorbent Assay).

2.4. Seroneutralization test

The biological activity of the purified IgG from anti-Babanki ascitic fluid was examined by evaluating the neutralizing effect against the virus and was compared to the neutralizing activity of the ascitic fluid. Briefly, various dilutions of purified IgG sample, or of ascitic fluid, were incubated volume to volume with a suspension of Babanki virus at one constant titer (4.3 log $TCID_{50}$/ml) for 1 h at 37 °C. Then, the infectious titer of each mixture was determined as the 50% tissue culture infective dose per ml ($TCID_{50}$/ml) by the Reed and Muench statistical method, on Vero cells (ATCC). The seroneutralization titer corresponds to the dilution giving at least a decrease of 0.6 log $TCID_{50}$/ml compared to infectious titer of the viral suspension without antiviral antibodies.

2.5. Radiolabeling of IgG

Commercially available mouse IgG, further called 'non-specific IgG', and purified IgG from anti-Babanki and anti-Zika ascitic fluids were radiolabeled with ^{125}I–Na using the chloramine T method. The iodination reaction was carried out at room temperature according to the following procedure: 10 µl of ^{125}I–Na (1 mCi) were added to a mixture containing 10 µl of chloramine T (25 µg), 60 µl of PBS 0.04 M and 100 µl of IgG (200 µg). The reaction was mixed 45 s with vortex mixed, and stopped by adding 10 µl of sodium metabisulfite (of 5 mg/ml). The mixture was then loaded on a PD-10 G 25 M column equilibrated with ammonium acetate 0.1 M pH 7.4, and 0.5 ml fractions were collected. The specific activity of the radiolabeled IgG was determined by γ-counting and was about 3 mCi/mg. Radiolabeled IgG were stored at 4 °C.

2.6. Adsorption of radiolabeled IgG onto functionalized polystyrene particles

Prior to incubation with radiolabeled IgG, functionalized latexes were albumin preadsorbed using bovine serum albumin (BSA) at 1 g/l for 1 h at room temperature. Then 10 mg of BSA preadsorbed-particles were incubated with 100 µl of ^{125}I–IgG solution at various concentrations in PBS–BSA buffer, pH 7.4 (BSA 1 g/l). The mixture was shaken gently for 1 h at room temperature. Particles were recovered by centrifugation and washed three times with PBS–BSA buffer. The radioactivity of the pellet (= particles) was measured using the γ-counter.

3. Results and discussion

3.1. Polymer particles functionalization

Amino acid functionalized polystyrene particles (35–75 µm in diameter) were prepared in three steps, as described in Section 2. The chemical characterization of the latexes was performed by elemental analysis of nitrogen (N) and sulphur (S). The amounts of the different monomeric units of the polymers, i.e. styrene (S), styrene sulfonate (S-SO_3Na) and styrene amino acid sulfamide (S-SO_2AA) are expressed in molar percentages. Chemical compositions of the amino-acid substituted polystyrene particles are reported in Table 1. A wide range of substitution rate was obtained by varying the amount of amino acid in the reaction mixture. Because amino acid grafting depends on the rate of reactive chlorosulfonyl groups substituted on particles, a maximum yield of 60% substitution was reached, except for tyrosine with a yield less than 31%. This can be due to the lower solubility of tyrosine methyl ester in the reaction solvent (dichloromethane) as compared to the solvent solubility of the other amino acids. However, in order to compare the different functionalizations of the particles, the molar ratio $COO^-/(COO^- + SO_3^-)$ was defined, indicating the polymer charge brought by the different substituted amino acids. The COO^- value corresponded to the amount of phenyl groups substituted with an amino acid, and the SO_3^- value corresponded to the amount of styrene sulfonate unit.

3.2. Purification of antiviral antibodies from ascitic fluid

The purification of IgG from ascitic fluids anti-babanki and anti-zika was performed by affinity chromatography

Table 1
Chemical composition of functionalized particles with phenylalamine (PS-SO_2F), arginine (PS-SO_2R), leucine (PS-SO_2L), aspartic acid (PS-SO_2D) and tyrosine (PS-SO_2Y)

Polymers	Elemental analysis		% of units			Molar ratio
	N (meq/g)	*S* (meq/g)	S	S-SO_3Na	S-SO_2AA	$COO^-/(COO^- + SO_3^-)$
PS-SO_2F_{10}	0.51	3.79	27	63	10	0.14
PS-SO_2F_{14}	0.70	3.84	21	65	14	0.18
PS-SO_2F_{21}	1.03	3.27	34	45	21	0.32
PS-SO_2F_{30}	1.38	3.18	30	40	30	0.43
PS-SO_2F_{36}	1.62	3.31	27	37	36	0.49
PS-SO_2F_{49}	1.20	3.01	23	28	49	0.64
PS-SO_2F_{63}	2.30	2.77	24	13	63	0.85
PS-SO_2R_4	0.24	3.74	33	63	4	0.06
PS-SO_2R_{13}	0.67	3.50	32	55	13	0.19
PS-SO_2R_{20}	0.99	3.40	28	52	20	0.28
PS-SO_2R_{38}	1.57	3.10	26	36	38	0.51
PS-SO_2R_{63}	2.25	2.89	23	17	60	0.78
PS-SO_2L_{17}	0.84	3.68	28	36	17	0.32
PS-SO_2L_{36}	1.68	3.08	35	30	36	0.55
PS-SO_2L_{39}	1.74	3.38	23	37	40	0.52
PS-SO_2L_{46}	1.93	3.31	22	32	46	0.59
PS-SO_2L_{58}	2.36	3.00	16	27	58	0.68
PS-SO_2D_5	0.23	4.00	25	70	5	0.13
PS-SO_2D_8	0.41	3.77	16	87	8	0.16
PS-SO_2D_{11}	0.55	3.34	23	66	11	0.25
PS-SO_2D_{22}	1.05	3.59	23	54	22	0.45
PS-SO_2D_{29}	1.32	2.10	24	47	29	0.55
PS-SO_2D_{42}	1.8	3.00	33	25	42	0.77
PS-SO_2D_{60}	2.25	2.89	23	17	60	0.88
PS-SO_2Y_2	0.10	4.39	11	87	2	0.02
PS-SO_2Y_8	0.41	3.69	17	75	8	0.10
PS-SO_2Y_{12}	0.57	3.33	20	68	12	0.15
PS-SO_2Y_{16}	1.07	3.43	23	61	16	0.21
PS-SO_2Y_{31}	1.25	2.52	5	64	31	0.33

using a protein G-sepharose column. Chromatograms obtained are plotted in Fig. 1. IgG were recovered in fractions 13, 14 and 15 as confirmed by SDS-PAGE analysis (data not shown). The purification yield of IgG was calculated by comparison between concentrations of IgG (determined by ELISA) before and after affinity chromatography. It was around 85% with a purity level of 100%.

Biological activity of purified IgG from anti-babanki ascitic fluid was evaluated by seroneutralization test and compared to the one of the ascitic fluid prior IgG purification (Fig. 2). Results show a seroneutralization titer of 1/400 in both cases indicating that purified IgG retained their neutralizing power. Seroneutralization tests cannot be done with IgG purified from anti-zika ascitic fluid because it was not enable to grow zika virus in our laboratory. However, taking into account data obtained with anti-babanki antibodies, it was assumed that anti-zika antibodies, like anti-babanki antibodies, retained their biological activity after purification from ascitic fluid.

3.3. Adsorption of 'non-specific IgG' onto polystyrene derivatives

'Non-specific IgG' are commercially available mouse immunoglobulins non-directed against viral antigens. Adsorption isotherms were obtained by incubation of functionalized polystyrene particles with various concentrations of radiolabeled mouse IgG. As shown in Fig. 3, the plot $1/B$ versus $1/F$, where 'B' is the bound part of IgG to latex and 'F' is the free part of IgG in solution, gives a straight line indicating that the adsorption of IgG onto particles follows a Langmuir law. The corresponding Langmuir plot led to the adsorption parameters, pKd = $\log(Ka)$ and B_{max}, summarized in Table 2. The apparent affinity constant (Ka) is around $10^5\ M^{-1}$ for all polymers. Indeed, the mean value of pKd is around 5.14 ± 0.35. These results indicate that the adsorption of mouse IgG non-directed against viral antigens onto functionalized

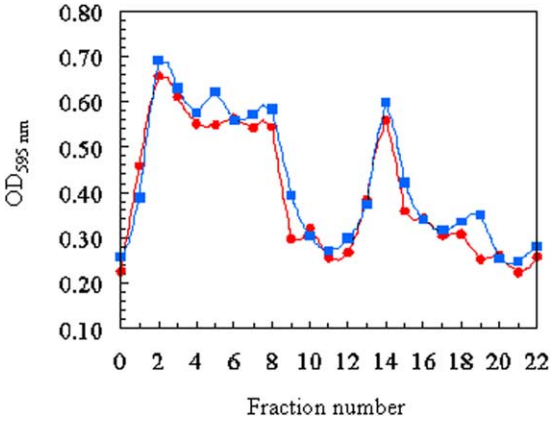

Fig. 1. Chromatograms of the purification of IgG from anti-babanki ascitic fluid (circles) and antizika ascitic fluid (squares). Purified IgG are recovered in fractions 13, 14 and 15.

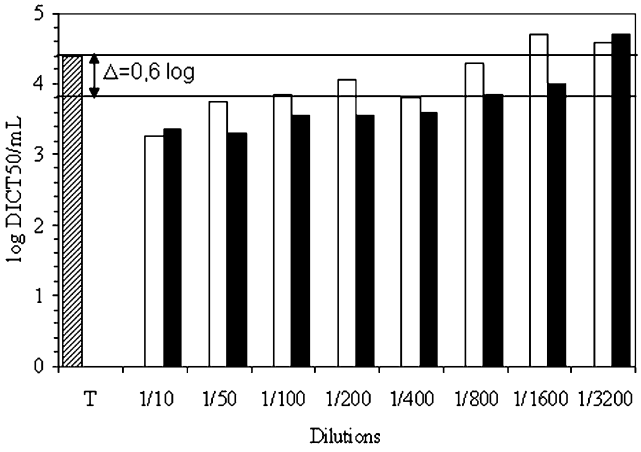

Fig. 2. Seroneutralization test of IgG purified from anti-babanki ascitic fluid (white bar) and the whole anti-babanki ascitic fluid (black bar) compared to the infectious titer of babanki virus (T).

polystyrene particles is non-specific and is not polymer composition dependent.

On another hand, B_{max} values show significant variations with the chemical composition of latexes. The highest variations are obtained for phenylalanine-substituted polystyrene particles (PS-SO_2F) and the lowest ones for

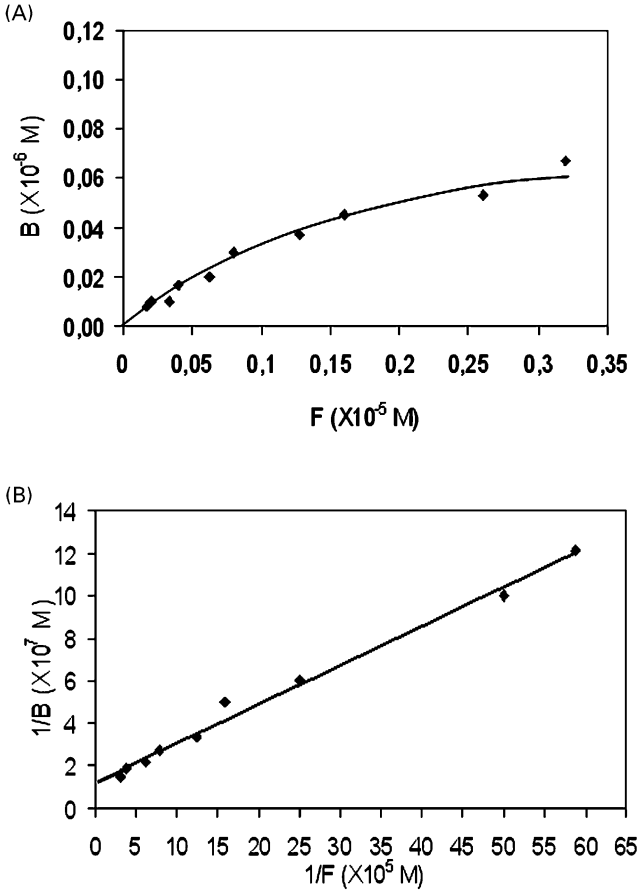

Fig. 3. Adsorption of 'non-specific' IgG onto polystyrene derivatives. Isotherm (plot A) and Langmuir plot (plot B).

Table 2
Adsorption parameters of 'non-specific' IgG onto polystyrene derivatives

Polymers	pKd = −log *Ka*	B_{max} ($\times 10^{-12}$ M/mg)
PS-SO_2F_{10}	5.46	3.08
PS-SO_2F_{14}	4.90	14.20
PS-SO_2F_{21}	5.17	4.17
PS-SO_2F_{30}	5.34	4.54
PS-SO_2F_{36}	4.78	16.01
PS-SO_2F_{49}	5.47	3.44
PS-SO_2F_{63}	5.46	2.49
Mean value	5.22 ± 0.30	
PS-SO_2R_4	5.60	2.40
PS-SO_2R_{13}	5.91	4.32
PS-SO_2R_{20}	5.77	4.28
PS-SO_2R_{38}	5.77	4.87
PS-SO_2R_{63}	5.74	1.82
Mean value	5.76 ± 0.11	
PS-SO_2L_{17}	5.11	8.1
PS-SO_2L_{36}	4.70	24.6
PS-SO_2L_{39}	4.87	16.7
PS-SO_2L_{46}	4.95	16.2
PS-SO_2L_{58}	4.76	21.0
Mean value	4.87 ± 0.16	
PS-SO_2D_5	4.81	4.25
PS-SO_2D_8	4.92	3.22
PS-SO_2D_{11}	4.86	3.50
PS-SO_2D_{22}	4.57	4.80
PS-SO_2D_{29}	4.81	3.09
PS-SO_2D_{42}	5.04	2.00
PS-SO_2D_{60}	5.04	1.51
Mean value	4.86 ± 0.16	
PS-SO_2Y_2	5.14	7.00
PS-SO_2Y_8	5.14	6.80
PS-SO_2Y_{12}	5.14	6.40
PS-SO_2Y_{16}	5.11	6.20
PS-SO_2Y_{31}	5.08	4.60
Mean value	5.12 ± 0.02	

tyrosine-substituted particles (PS-SO_2Y). In the case of non-specific adsorption, B_{max} data is related to two parameters: the swelling rate of the particle and the surface characteristic of the modified polymer particle. For instance, depending on the amino acid substituted and on the substitution yield, functionalized polystyrene particles exhibit swelling abilities in PBS–BSA buffer varying from 10 to 170% (data not shown). Moreover, surface heterogeneities of functionalized polystyrene particles were observed by scanning electron microscopy, probably due to the harshness of the elaboration process. Consequently, functional groups are randomly distributed on the interfacial macromolecular chain, then the surface chemical composition is statistically the same for a given functionalized particles batch.

3.4. Adsorption of antiviral antibodies onto functionalized polystyrene particles

Adsorption experiments of IgG purified from anti-babanki and anti-zika ascitic fluids were carried out as previously described for the adsorption of 'non-specific' IgG. However, adsorption isotherm plot indicated that it was impossible to reach the surface saturation (B_{max}) because concentrations of antiviral IgG samples were too low: around 1.5 mg/ml available for antiviral IgG versus 2.5 mg/ml needed to reach B_{max} value for 'non-specific' IgG. Indeed, as shown in Fig. 4 (plot A), for low equilibrium concentrations of IgG data are distributed on a straight line which corresponds to the slope of the adsorption isotherm (Fig. 4: plot B). Taking into account the adsorption results of 'non-specific' IgG onto functionalized particles, adsorption plots of antiviral IgG were analyzed according to the Langmuir adsorption law. The equation of the adsorption isotherm is:

$$B = (KaB_{max}F)/(1 + KaF)$$

It can be transformed to:

$$B/[(B_{max} - B)F] = Ka$$

At the beginning of the isotherm, conditions are $B \ll B_{max}$; therefore the equation of the adsorption isotherm in these conditions can be transformed to:

$$B = (KaB_{max})F$$

To evaluate the adsorption of anti-babanki and anti-zika antibodies for the functionalized particles, the ratio *R* is

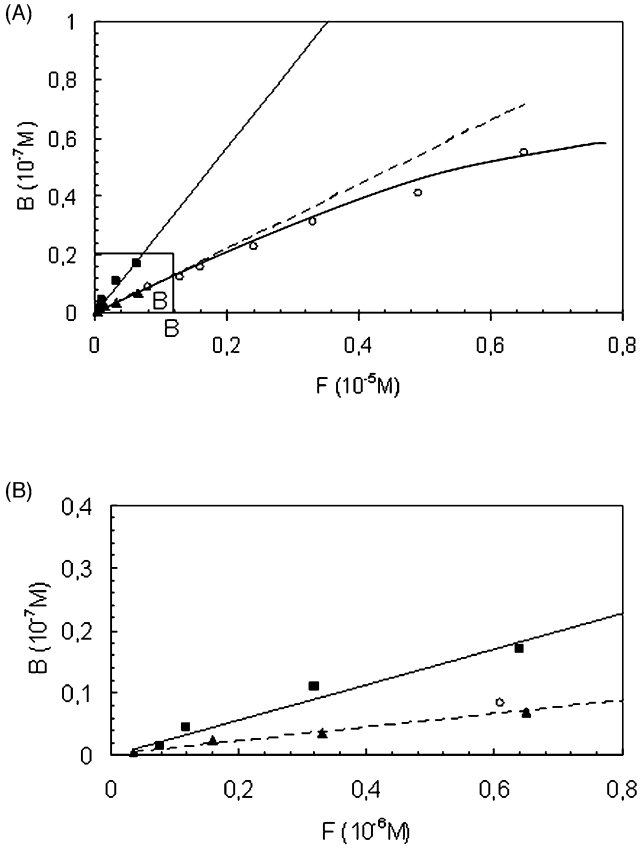

Fig. 4. Plot A: Adsorption of 'non-specific' IgG (open circles), anti-babanki IgG (black squares) and anti-zika IgG (black triangles) onto latexes substituted with 11% aspartic acid (PS-SO2D11); Plot B is an enlargement of the B part of plot A.

calculated as follows:

R = [(KaB_{max}) of antiviral IgG]/[(KaB_{max}) of non − specific IgG]

A ratio $R=1$ indicates that antiviral IgG display the same adsorption rate than 'non-specific' IgG for the used polymer particles. So the latter does not exhibit specificity for antiviral antibodies. A ratio $R>1$ underlines that the adsorption rate of antiviral IgG for the particle is higher than 'non-specific' IgG. The higher the ratio R, the best is the adsorption rate. Such polymer particles are suggested to present on their surface, active sites able to interact specifically with antiviral antibodies. These active sites are made of precise arrangement of chemical groups substituted onto the macromolecular chain.

Results obtained for IgG purified from anti-babanki ascitic fluid indicate that the adsorption of anti-babanki antibodies (related to ratio R) is dependent on the chemical composition of the particles (Fig. 5). Indeed, two different behaviours of functionalized latexes are observed:

1. The adsorption of anti-babanki antibodies, with a ratio $R>1$, varies with the chemical composition of the particles. This is the case for aspartic acid- and phenylalanine-substituted particles, PS-SO_2D and PS-SO_2F. Moreover, these latexes present adsorption maxima for given compositions of the particles. Concerning PS-SO_2D latexes, two maxima were obtained for molar ratio $COO^-/(COO^- + SO_3^-)$ equal to 0.16 (PS-SO_2D_8) and to 0.55 (PS-SO_2D_{29}). The rates of adsorption are respectively $R=4.2$ and $R=6$, indicating that PS-SO_2D_{29} adsorbs six times more anti-babanki antibodies than the 'non-specific' IgG. PS-SO_2F latexes show one maximum adsorption rate for the molar ratio $COO^-/(COO^- + SO_3^-)=0.32$ (PS-SO_2F_{21}) corresponding to $R=4$. These results suggest that for these precise compositions of the particles, specific interactions are developed between anti-babanki antibodies and bioactive sites present at the surface of the functionalized polystyrene particles.
2. The adsorption rate of anti-babanki antibodies onto arginine-, leucine- and tyrosine-substituted particles is almost constant whatever the chemical composition of the polymer suggesting no specificity towards antiviral antibodies. Moreover, PS-SO_2Y latexes showed a ratio value $R=1$ indicating that interactions of anti-babanki antibodies with these particles occurred in the same way than 'non-specific' IgG.

The adsorption of IgG purified from anti-zika ascitic fluid onto functionalized particles displays also two different behaviours as anti-babanki antibodies (Fig. 6). Thus, the adsorption rate of anti-zika antibodies varies with the chemical composition of aspartic acid-, phenylalanine-, arginine- and tyrosine-substituted particles, with a ratio $R>1$; whereas the adsorption rate of anti-zika antibodies is almost constant onto leucine-substituted particles, with a R value closed to 1. Indeed, PS-SO_2D latexes present two maxima of adsorption at molar ratios $COO^-/(COO^- + SO_3^-)$ equal to 0.16 (PS-SO_2D_8) and 0.55 (PS-SO_2D_{29}). The corresponding R values are respectively equal to 2.2 and 2.7. Furthermore, onto PS-SO_2D_{60} ($COO^-/(COO^- + SO_3^-)=0.88$) a higher rate of adsorption ($R=6$) of anti-zika antibodies is obtained. PS-SO_2F latexes show one adsorption peak ($R=6$) at molar ratio $COO^-/(COO^- + SO_3^-)$ equal to 0.32 (PS-SO_2F_{21}), and a high adsorption rate ($R=6$) onto PS-SO_2F_{10} ($COO^-/(COO^- + SO_3^-)=0.14$). PS-$SO_2$Y derivatives display one maximum of adsorption ($R=2$) at a molar ratio $COO^-/(COO^- + SO_3^-)$ equal to 0.10 (PS-SO_2Y_8). At least, anti-zika antibodies highly adsorb onto PS-SO_2R_4 ($COO^-/(COO^- + SO_3^-)=0.06$) with a R value equal to 5.

It is worth noting that anti-babanki and anti-zika

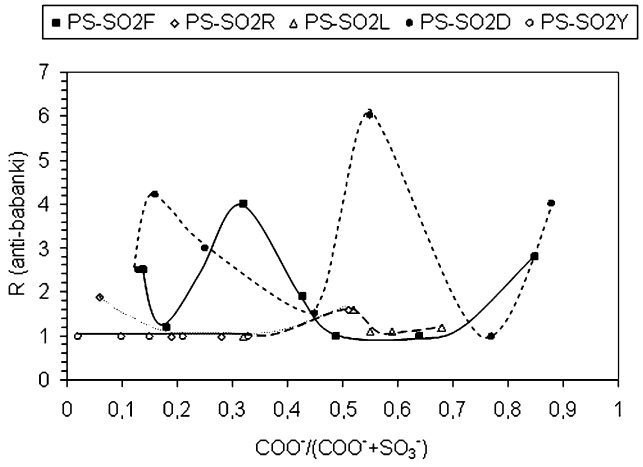

Fig. 5. Adsorption of IgG purified from anti-babanki ascitic fluid compared with adsorption of 'non-specific' IgG (ratio R) versus the chemical composition of particles (molar ratio $COO^-/(COO^- + SO_3^-)$).

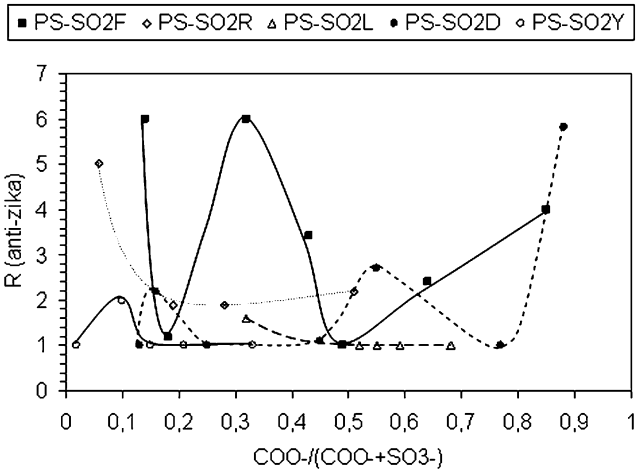

Fig. 6. Adsorption of IgG purified from anti-zika ascitic fluid compared with adsorption of 'non-specific' IgG (ratio R) versus the chemical composition of particles (molar ratio $COO^-/(COO^- + SO_3^-)$).

antibodies show the same adsorption peaks onto aspartic acid- and phenylalanine-substituted particles, more precisely for PS-SO_2D_8, PS-SO_2D_{29} and PS-SO_2F_{21}. However, the adsorption rate on PS-SO_2F_{21} of anti-zika antibodies is higher ($R=6$) than anti-babanki antibodies ($R=4$). On aspartic acid-substituted particles it is exactly the opposite: anti-babanki antibodies adsorb with a higher level onto PS-SO_2D_8 ($R=4.2$) and PS-SO_2D_{29} ($R=6$) than anti-zika antibodies ($R=2.2$ and $R=2.7$, respectively). These results suggest that the distribution of functional groups, such as aspartic acid and sulfonate for PS-SO_2D_8 and PS-SO_2D_{29}, phenylalanine and sulfonate for PS-SO_2F_{21}, enabled the creation of active sites that were able to strongly interact with both antiviral antibodies. We assumed that these active sites were the same for both antiviral antibodies. Indeed, previous studies demonstrated that random substitution of polystyrene particles by suitable chemical groups allows the creation of active sites along the macromolecular chain able to interact with biological components [1]. These bioactive sites correspond to precise distributions of the different functionalized monomer units of the polymer. The probability of occurrence of bioactive sites depends on the nature, number, and distribution of chemical groups, as well as the overall composition of the polymer. This probability displays a maximal value for a given chemical composition of the polymer. Thus, obtained results indicate that the probability of occurrence of active sites allowing specific interactions with anti-babanki and anti-zika antibodies presents a maximum for PS-SO_2D_8, PS-SO_2D_{29} and PS-SO_2F_{21} suggesting that antiviral antibodies studied recognize the same sites on these particles but with variable affinities, the adsorption rates being different. In fact, these antiviral antibodies were polyclonal antibodies directed against two viruses belonging to two close families of the Arbovirus group, togaviridae (babanki virus) and flaviviridae (zika virus). Indeed, alphaviruses (togaviridae) have a number of structural and functional similarities to flaviviruses, suggesting a common origin of some of their components although their genomes differ significantly [23,24]. The structure of a large fragment of the ectodomain of E1 protein of sindbis virus has been determined and was suggested to be homologous to the E glycoprotein found in flaviviruses [25]. It is likely that babanki virus and zika virus contain similar antigenic domains which part of them was mimicked on the particles. Previous study performed on the interactions between functionalized latexes and viral particles, i.e. babanki virus (Togaviridae) and kedougou virus (Flaviviridae), have shown that both viruses presented a maximum of adsorption onto PS-SO_2R_{20} and PS-SO_2D_{42} corresponding to molar ratios equal to 0.29 and 0.75, respectively [26]. These results indicated that their envelope glycoproteins contained similar domains allowing the recognition of the same chemical sites on functionalized particles. This is in agreement with the results presented above suggesting that these similar domains could be antigenic domains. Moreover, for babanki virus it is noteworthy that the chemical compositions of particles corresponding to maximum of adsorption of viruses correspond to minimum of adsorption of respective antiviral antibodies, and the chemical compositions of particles corresponding to maximum of adsorption of anti-viral antibodies correspond to minimum of adsorption of respective viruses. Comparison of both studies underlines that interactions developed between functionalized polystyrene particles and viruses/or antiviral antibodies, are driven by multiple factors:

– charge of the interaction sites
complementarity of the chemical groups involved in both parts,
– distribution and spatial arrangement of chemical groups constituting the interaction site onto the particle surface.

Mutational studies have indicated that some amino acids, like Glu, Arg, Phe, Ser, Lys, Asn, at various positions in E-glycoprotein are implicated in cell–virus interactions [15,27,28]. RGD (arginin-glycine-aspartic acid) sequences, or RGD structural mimicry, known to bind cellular integrins, were also found to be involved in the process of viral infection [14,29]. It was showed that highly sulfated heparan sulfate molecules present on the target cell surface mediate flavivirus binding [29,30,31].

4. Conclusion

In this study, the interactions between IgG purified from anti-babanki and anti-zika ascitic fluids, and polystyrene functionalized particles with various amino acids were examined. Results showed that the adsorption rate of the antiviral antibodies was dependent on the surface chemical composition of the particles, characterized by the amino acid substituted, its substitution percentage and the molar ratio $COO^-/(COO^- + SO_3^-)$. Indeed, among the various polystyrene derivatives, only some precise compositions displayed maximal adsorption level for both antiviral antibodies. It is the case for PS-SO_2D_8, PS-SO_2D_{29} and PS-SO_2F_{21} but the observed different adsorption rates suggest that anti-babanki and anti-zika antibodies probably recognized the same active sites on the interfacial polymer chains with different affinities. Although antiviral IgG displayed higher affinity than ‘non-specific’ IgG towards functionalized latexes, the specificity or the biospecificity of the interaction was not demonstrated. Indeed, IgG can adsorb onto polystyrene particles via its Fab part (specific interaction) or via its Fc fragment (non-specific interaction).

On another hand, many polymers interact strongly with one specific antiviral IgG and weakly with the other one suggesting that the chemical sites created on the macromolecular chain are more or less better recognized depending on the antiviral IgG. For example, onto PS-SO_2D_{11} ($COO^-/(COO^- + SO_3^-) = 0.25$) the adsorption

rate of anti-babanki IgG is higher ($R=3$) than of anti-zika IgG ($R=1$) displaying the same adsorption as 'non-specific' IgG. Adsorption experiments were performed under 'phys-physiological conditions' (PBS 1X-BSA pH 7.4), so the effect of salt or pH under adsorption process was not studied.

Work is now in progress to evaluate the specificity of this interaction. Polymers developing biospecific interactions with antiviral IgG could be used in vaccinal approach as 'virus-like' polymers or as affinity matrix for the purification of antiviral antibodies.

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