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ARTICLE

A novel high-capacity immunoadsorbent with PAMAM dendritic spacer arms by click chemistry

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Polyamidoamine (PAMAM) dendrimers, bearing multiple peripheral end groups that can be used as clickable modules, make it possible to bind a large number of small-molecule ligands via click chemistry to prepare high-capacity immunoadsorbents. Thus, an immunoadsorbent with PAMAM dendritic spacer arms possessing pseudo-biospecific affinity for IgG from human plasma, Sep-PAMAM-AA, was designed and prepared by click chemistry using sepharose gel as a support and the amino acids His, Phe and Trp as ligands; two sepharose-based control samples, Sep-triazole-His and Sep-PA, with linear spacer arms were prepared using L-histidine and protein A as ligands, respectively. The ligand density and IgG adsorption performance of Sep-PAMAM-AA from human plasma were measured and evaluated. The influences of the structure and generation number of the PAMAM spacer arms on the performances of the products were also investigated. The results indicate that the immunoadsorbent using PAMAM G3 as a spacer arm and His as a ligand, Sep-G3-His, is the best among the prepared immunoadsorbents. Its ligand density reaches 1.58 mmol/g sepharose gel, almost 5-fold higher than that of Sep-triazole-His; its IgG adsorption capacity is 28.43 mg/g, which is higher than those of Sep-triazole-His and Sep-PA. Moreover, Sep-G3-His has a relatively low level of non-specific adsorption, which indicates that the immunoadsorbents using PAMAM as a spacer arm and His as a ligand are expected to have great application prospects in the field of blood purification.

Introduction

In the conventional preparation of immunoadsorbents, if the ligand is coupled directly to the support, steric hindrance between the support surface and the target molecule will occur.[1] Therefore, the ligand that is critical for selectively absorbing the target molecule should be sufficiently distant from the solid support, which can be achieved by binding the ligand at the end of a long chain or "spacer arm".[2] The employment of a spacer arm may set the ligand away from the solid matrix to make it more accessible to the target molecules.

It is known that the length and chemical structure of spacer arms have direct effects on the adsorption performance of an immunoadsorbent.[3-5] An increase of the length of spacer arms, to a certain extent, will correspond to an increase of its rotational flexibility, which can endow it with sufficient mobility to allow it come in proximity with the target molecules.[6-9] In addition, the chemical structure of the spacer arm is another important factor that affects the performance of the immunoadsorbent.[10, 11] Commonly used spacer arms, bridge chains between the reactive group on the support surface and the ligand, are linear molecular chains of compounds.[3, 4, 10] Generally speaking, one spacer arm with a linear molecular structure can only conjugate one ligand molecule. The performance of the corresponding immunoadsorbent may be unsatisfactory, especially when the ligand is a small molecule with only one active site for adsorption. For this reason, it is difficult to enhance the ligand density of an immunoadsorbent using a linear molecular chain as a spacer arm. Nevertheless, employing dendrimers[12-14] as spacer arms can overcome this disadvantage. As the most widely studied dendrimer, polyamidoamine (PAMAM), which can be synthesized by the repetition of a given set of reactions using divergent strategies, possesses a highly branched, regular and well-defined architecture.[15-18] For this reason, an

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advantage of using PAMAM as a spacer arm is that its multiple peripheral end groups can covalently bind more ligands than the corresponding linear spacer arm with only one end group. Thus, the immunoadsorbent containing PAMAM spacer arms is expected to show increased adsorption capacity for the target molecules.

On the other hand, the Cu(I)-catalysed Huisgen 1,3-dipolar cycloaddition reaction[19-22] between an azide and an alkyne generating 1,2,3-triazole or click chemistry, developed by Sharpless,[22-24] appears to offer a simple, reliable, and fruitful method of preparing immunoadsorbents.[25-28] Because of the high yields and lack of by-products provided by the click chemistry for coupling ligands and supports,[29] it can be readily adopted for the preparation of immunoadsorbents.

In this study, a novel immunoadsorbent with PAMAM dendritic spacer arms was prepared by click chemistry, and its preparation principle is shown in Figure 1. Sepharose (Sep) was used as the support for the immunoadsorbents, and the pseudo-biospecific[30-33] amino acid ligands with molecular recognition and selective adsorption for the target molecules were bonded to the amine-terminated groups of the PAMAM spacer arms. Finally, the PAMAM-ligand complexes with terminal alkynes were coupled with the azide groups of the functionalized support through click chemistry. For purposes of comparison, protein A immunoadsorbent with linear spacer arms, Sep-PA, prepared using the same sepharose as a support and protein A as a ligand, was used as a control in this work.

With respect to the preparation process, the synthesis of the novel immunoadsorbent with PAMAM dendritic spacer arms designed in this work is completely different from that of Sep-PA in the key technical aspects involved. Protein ligands are difficult to immobilize on the support matrix in the proper orientation[34]. Moreover, protein ligands possess some drawbacks that need be taken seriously, such as potential immunogenicity, safety concerns caused by protein ligands detached from the support matrix, sensitivity to harsh sanitization procedures, and high cost. Accordingly, compared with Sep-PA, the novel immunoadsorbent using amino acids as ligands will possess high stability and low cost and be easy to store and handle, thereby having advantages over Sep-PA in cost and use security.

The immunoglobulin-G (IgG) adsorption performance of the prepared immunoadsorbents from human blood was evaluated to estimate their application prospects in treating various autoimmune diseases by means of blood purification, and the influences of the structure and generation number of the PAMAM spacer arms on the performance of the products were also investigated.

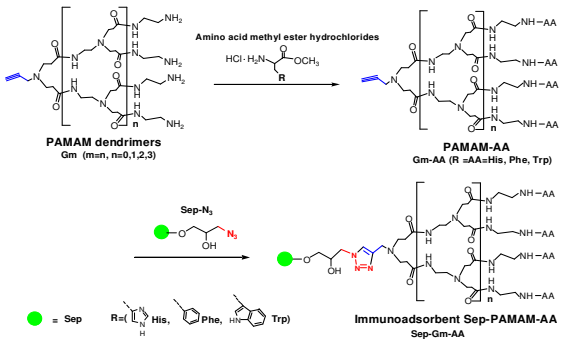

Figure 1. Schematic of the preparation principle of immunoadsorbents using PAMAM as spacer arms.

Results and discussion

Structure characterization of PAMAM-AA

To examine whether the amino acid ligand was functionalized with terminal alkynes, the structure of the produced PAMAM-AA, such as PAMAM G3-His, was analysed by ^{1}H NMR spectrum in D_2O, as shown in Figure 2. From the ^{1}H NMR spectra, besides the original characteristic peaks of PAMAM G3 (a ~ j), there appeared new peaks of histidine units at 3.97 ppm (k), 3.39 ppm (l), 7.81 ppm (m), and 7.00 ppm (n) in PAMAM G3-His, and the peak of methyl in His-OMe·2HCl disappeared here. All of these demonstrated that His ligands were successfully bonded to the PAMAM spacer arms. According to the ^{1}H NMR spectra of PAMAM G3-His in Figure 2., the area of each peak m, n, k and l is 6, 6, 6 and 12 times larger than the area of peak a, respectively; and the number of protons in the corresponding positions, m, n, k, l and a, in the molecular structure of PAMAM G3 is 1, 1, 1, 2 and 1, respectively. From the above data, it was then calculated that 6 histidine units were conjugated to the terminal amines of PAMAM G3. This number of conjugated units means that 75% of the terminal amines reacted with His as a ligand.

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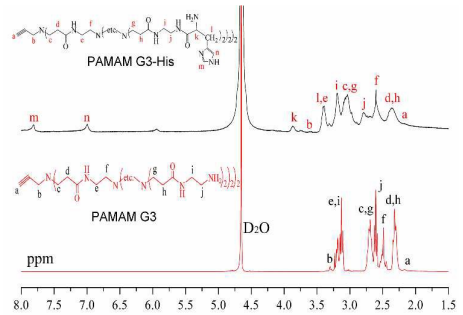

Figure 2. The ^{1}H NMR spectra of PAMAM G3 and PAMAM G3-His.

The number of amino acid units conjugated with other generations was calculated by the same method as PAMAM G3-His, which was shown in Figure 2. According to the number of $–NH_2$ in different generations of PAMAM dendrimers, PAMAM G1, G2, G3 and G4 can theoretically combine 2, 4, 8 and 16 amino acid units, respectively. From the results listed in Table 1, 100% of the terminal amines at the outer sphere in PAMAM G1 and PAMAM G2 reacted with amino acid His, while only 75% and 37.5% of the terminal amines for PAMAM G3 and PAMAM G4 reacted with His, respectively, although both PAMAM G3 and G4 can conjugate 6 His ligands. This implies that as the generation number increases, the number of terminal groups increases exponentially, until steric crowding at the surface occurs. Thus, the number of ligands conjugated to PAMAM is lower than the theoretical value. Obviously, when more ligands are conjugated to PAMAM, the prepared immunoadsorbent will possess more active sites and higher adsorption capacity for the target molecules, which is the advantage of choosing PAMAM dendrimers as the spacer arms. Compared with PAMAM G4, PAMAM G3 can conjugate as many ligands as PAMAM G4, but its preparation is less complicated than that of G4. Therefore, PAMAM G3 is the optimal spacer arm. Consequently, the molecular masses of PAMAM-AA (M_{Gm}-AA) were obtained, as listed in Table 1.

Table 1 The analysis of PAMAM-AA

Sample	Number of $–NH_2$ in Gm	Number of His in Gm-AA	Efficiency of conjugation (%)	M_{Gm}-AA (g/mol)
G1-His		2	100	557.65
G1-Phe	2	2	100	577.71
G1-Trp		2	100	655.79
G2-His		4	100	1288.51
G2-Phe	4	4	100	1328.63
G2-Trp		4	100	1484.79
G3-His		6	75	2475.96
G3-Phe	8	6	75	2536.14
G3-Trp		6	75	2770.38
G4-His		3	19	3890.87
G4-Phe	16	6	75	4362.47
G4-Trp		6	37.5	4596.71

Structure characterization of Sep-PAMAM-AA

The FTIR spectra of Sep-PAMAM-His, Sep-PAMAM-Phe and Sep-PAMAM-Trp are presented in Figure 3. Compared to the FTIR spectrum of Sep-N_3, the peak at 2100 cm^{-1}, attributed to azide groups, disappeared almost completely in the FTIR spectra of Sep-PAMAM-AA. This implies that the azide groups of Sep-N_3 have been consumed completely in the course of the click reaction of Sep-N_3 and PAMAM-AA, which clearly exhibited that the coupling efficiency of the support and the ligand with spacer arms was nearly 100%. For this reason, the ligand density could be evaluated according to the number of amino acid units conjugated to PAMAM. As the azide content in Sep-N_3 was calculated to be 751.4 μmol/g gel, according to the elemental analysis data, the ligand density of Sep-PAMAM-AA, D_{Ligand}, was then calculated and listed in Table 2. Compared to the immunoadsorbent Sep-triazole-His with linear spacer arms, the immunoadsorbents using PAMAM dendrimers as the spacer arms exhibited greatly enhanced ligand density, especially when the spacer arm was G2 or G3. Among them, the ligand density of Sep-G3-His reaches 1.58 mmol/g sepharose gel, almost 5-fold as high as that of Sep-triazole-His.

In addition to the above method, the ligand density may be calculated from the data on the N content of Sep-N_3 and Sep-PAMAM-AA, which can be determined by elemental analysis, as well as the N content of PAMAM, which can be calculated from its molecular structure. Unfortunately, this way is complicated and has low accuracy in practice, so that it is rarely adopted.

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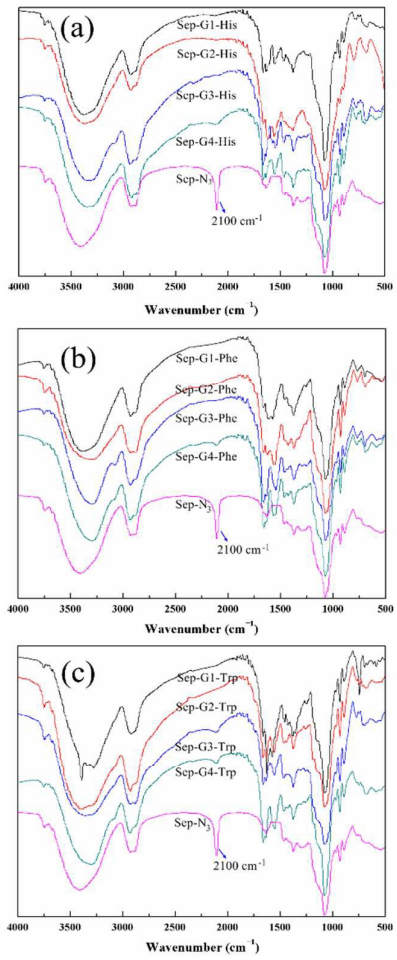

Figure 3. The FTIR spectra of Sep-PAMAM-AA. (a) Sep-PAMAM-His; (b) Sep-PAMAM-Phe; (c) Sep-PAMAM-Trp.

Table 2 The ligand density of Sep-PAMAM-AA

Spacer arm	D_{Ligand} (mmol/g)		
	Sep-Gm-His	Sep-Gm-Phe	Sep-Gm-Trp
G1	1.06	1.05	1.01
G2	1.53	1.50	1.42
G3	1.58	1.55	1.46
G4	0.57	1.05	1.01

Adsorption capacity of IgG from human plasma

The IgG adsorption capacity of Sep-PAMAM-AA from human plasma was determined and compared with Sep-PA and Sep-triazole-His, as shown in Table 3. It is observed that immunoadsorbents with PAMAM as the spacer arms had a higher IgG adsorption capacity than those with linear spacer arms, and they were comparable to Sep-PA. Strictly speaking, the immunoadsorbents with His as the ligand showed remarkable IgG adsorption capacity, except for Sep-G4-His, which was poor in ligand density. Furthermore, the IgG adsorption capacity of the immunoadsorbents with His ligand was higher than those with Phe and Trp ligands when the spacer arms were the same. This indicates that His is better than Phe and Trp to act as a pseudo-biospecific ligand. Moreover, the immunoadsorbents with PAMAM G2 or PAMAM G3 spacer arms were relatively preferable in adsorption capacity, which was consistent with the ligand density.

Table 3 The adsorption capacity of Sep-PAMAM-AA

Sample	V (ml)	m (g)	A_{280}	Q (mg /g)
Sep-PA	6.002	3.635	18.782	22.97
Sep-triazole-His	6.035	0.685	2.527	16.49
Sep-G1-His	6.004	0.662	3.153	20.72
Sep-G2-His	6.110	0.661	3.656	24.49
Sep-G3-His	6.015	0.652	4.252	28.43
Sep-G4-His	6.006	0.690	3.306	20.85
Sep-G1-Phe	6.013	0.690	3.085	19.48
Sep-G2-Phe	6.005	0.692	3.655	22.98
Sep-G3-Phe	6.997	0.620	3.302	27.00
Sep-G4-Phe	6.010	0.689	3.831	24.22
Sep-G 1-Trp	6.008	0.683	2.590	16.51
Sep-G2-Trp	6.022	0.680	3.147	20.20
Sep-G3-Trp	6.001	0.656	3.523	23.35
Sep-G4-Trp	6.005	0.680	3.414	21.85

Based on the above results, Sep-G3-His exhibits the highest adsorption capacity for IgG from human plasma. Thus, the adsorption performance of Sep-G3-His was studied intensively. In the static adsorption experiment using Sep-G3-His as an adsorbent, the IgG concentration in the human plasma before and after adsorption was measured via the standardized immunoturbidimetric assay. The obtained results and analysis are shown in Table 4. From the difference in IgG concentration between the initial plasma and the plasma after the adsorption test, the adsorption ratio or the yield of the static adsorption process can be calculated to be 44%, while the amount of adsorbed IgG by Sep-G3-His is calculated to be 28.99 mg/g. This value is considered to be the accurate and true IgG adsorption capacity of Sep-G3-His, and the IgG adsorption capacity of Sep-G3-His given in Table 3 (28.43 mg/g) is actually the amount of IgG eluted from the adsorbent

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Sep-G3-His used in the static adsorption experiment, which indirectly reflects the IgG adsorption capacity of Sep-G3-His. Accordingly, the efficiency of the IgG desorption is calculated to be 98%.

Table 4 IgG concentration in the human plasma before and after adsorption using Sep-G3-His as an immunoadsorbent.

Sample	IgG concentration (mg/ml)	IgG adsorption capacity (mg/g)	Adsorption ratio (%)
Plasma before adsorption	7.11	———	———
Plasma after adsorption	3.96	28.99	44

Adsorption selectivity of IgG from human plasma

As the immunoadsorbents with G2 or G3 spacer arms are preferable in terms of ligand density and adsorption capacity, their adsorption selectivity was further evaluated by analysing the purity of the adsorbed molecules in the eluate from the adsorption experiment through SDS–PAGE. The result is presented in Figure 4. As observed in Figure 4, the band corresponding to the molecule (150 kDa) of IgG can be found in the eluates from all of the immunoadsorbents. Moreover, the light chain (25 kDa) and the heavy chain (50 kDa) of IgG can be observed in the eluates from both Sep-G2-His and Sep-G3-His (Lane 2 and Lane 3). In addition to IgG, traces of several other proteins are faintly visible in the eluate from the latter, while there are some impurities in the eluates from the immunoadsorbents with Phe or Trp as the ligand, which means that Phe and Trp are inferior to His in acting as a pseudo-biospecific ligand. Furthermore, Sep-G3-His, with G3 spacer arms and His as the ligand, is capable of highly selective adsorption for IgG from human plasma, and its non-specific adsorption is almost negligible.

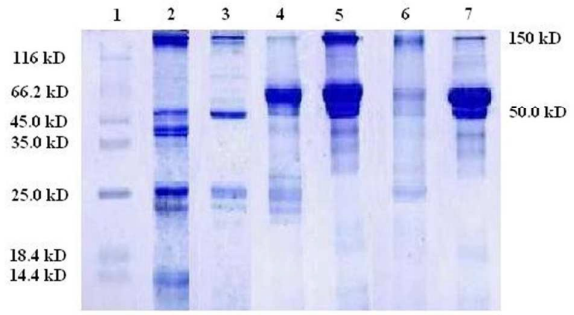

Figure 4. SDS-PAGE analysis of fractions eluted from Sep-G2-AA and Sep-G3-AA. Lane 1: protein markers; Lane 2: eluate from Sep-G2-His; Lane 3: eluate from Sep-G3-His; Lane 4: eluate from Sep-G2-Phe; Lane 5: eluate from Sep-G3-Phe; Lane 6: eluate from Sep-G2-Trp; Lane 7: eluate from Sep-G3-Trp.

Theoretically speaking, the adsorption selectivity of an immunoadsorbent mainly depends on its ligand. The above qualitative analysis obtained by SDS-PAGE shows that among three small-molecule ligands His, Phe and Trp, His gives the best performance. The immunoadsorbent using His as the ligand, Sep-G3-His, indeed exhibits high adsorption selectivity for IgG, which is consistent with the result obtained by MALDI-TOF MS in our previous work.[35] On the other hand, the non-specific adsorption of Sep-G3-His and Sep-PA as a control was evaluated by quantitative detection to further study the adsorption selectivity of the prepared immunoadsorbents from another aspect. The total protein and albumin concentration in the human plasma before and after the static adsorption experiment were measured via the standardized biuret and coagulation methods, respectively; the obtained results and analysis are shown in Table 5. Based on the data in Table 5, Sep-G3-His exhibits a slightly higher adsorption ratio for the total protein and albumin compared to Sep-PA, which are 9% and 6%, respectively. Therefore, the non-specific adsorption of Sep-G3-His is close to that of Sep-PA. That indicates that the dendrimers of PAMAM as spacer arms do not cause high non-specific adsorption, thus indirectly confirming the adsorption selectivity of Sep-G3-His for IgG from human plasma. Moreover, the blood purification mode of both the prepared adsorbents with PAMAM dendritic spacer arms is plasma adsorption. Therefore, the negative effects from the PAMAM dendritic spacer arms of the adsorbent are negligible, and the adsorbent cannot damage blood cells.

Table 5 Total protein and albumin concentration in the human plasma before and after adsorption using Sep-G3-His and Sep-PA as the immunoadsorbent, respectively.

Sample	Total protein concentration (mg/ml)	Total protein adsorption ratio (%)	Albumin concentration (mg/ml)	Albumin adsorption ratio (%)
Plasma before adsorption	57.4	———	36.1	———
Plasma after adsorption by Sep-G3-His	52.2	9	34.0	6
Plasma after adsorption by Sep-PA	53.3	7	34.5	4

Adsorption stability over repeated use

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As Sep-G3-His possesses the best IgG adsorption performance, the adsorption-desorption cycle was repeated 10 times using Sep-G3-His to investigate its reusability. The data on the IgG adsorption capacity with the times of reuse are listed in Table 6. It is observed that the IgG adsorption capacity declines slowly from the initial value of 28.43 mg/g to 24.97 mg/g during 10 cycles of usage, which means that Sep-G3-His can be repeatedly used without a noticeable loss in its IgG adsorption capacity to a certain extent.

Table 6 Adsorption stability over repeated use of Sep-G3-His.

Times of reuse	1	2	3	4	5	6	7	8	9	10
IgG Adsorption Capacity (mg/g)	28.43	27.55	27.81	26.81	27.09	26.13	25.71	25.22	25.46	24.97

Experimental

Reagents

Sepharose 6FF (Sep) was purchased from Pharmacia (Sweden). Azidated sepharose (Sep-N_3) was prepared in our laboratory. Human plasma was obtained from the Guangzhou blood station donated by volunteers, related experiments were performed in compliance with the relevant laws and institutional guidelines. Human immunoglobulin G (IgG, 98%) was purchased from Sigma (USA). L-Histidine (His), L-Phenylalanine (Phe), L-Tryptophan (Trp), propargylamine, methyl acrylate (MA, 98%), 1,2-ethylenediamine (EDA, 98%) and trimethyl chlorosilane (TMSCl) were purchased from Jingchun (Shanghai) and were used as received. Methanol (MeOH), triethylamine (TEA), sodium ascorbate, copper sulphate pentahydrate and ethylene diamine tetraacetic acid (EDTA) were all of analytical purity and were used without further purification. Throughout this study only double-distilled water was used.

Synthesis of PAMAM Dendrimers

PAMAM dendrimers were synthesized by the divergent approach using propargylamine as the core.[36-38] This methodology involves typical stepwise and iterative two-step reaction sequences, consisting of Michael addition of primary amines with MA and amidation of methyl ester groups with a large excess of EDA to produce amine terminations. The completion of this two-step process gives a full generation (G = 1); if only one step is finished, then a half generation (G = 0.5) is obtained. Repeating this two-step reaction sequence together with purification at each step leads to the second (G = 2), third (G = 3) and fourth generations (G = 4), depending on the numbers of repetition. The specific steps of preparing generation 1 ~ 4 PAMAM dendrimers (G1 ~ G4) are described in the ESI. The structures of the synthesized PAMAM G1 ~ PAMAM G4 were characterized by FTIR, ^{1}H and ^{13}C NMR analyses, as depicted in the ESI.

Synthesis of Amino Acid Methyl Ester Hydrochlorides

Amino acid methyl ester hydrochlorides were synthesized according to the procedures described in Figure 5. The carboxyl group of amino acids should be converted into highly reactive methyl ester groups, thereby generating amino acid methyl ester hydrochlorides. TMSCl (50 mmol) was added to a solution of amino acid (10 mmol) in methanol (30 ml) under stirring, and the reaction mixture was refluxed until the solution turn clear. The volatiles were removed under reduced pressure on a rotary evaporator, yielding the desired product. The structure of amino acid methyl ester hydrochlorides was confirmed by FTIR and ^{1}H NMR analyses, as depicted in the ESI.

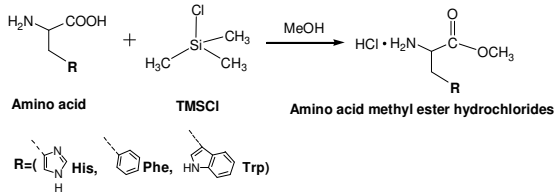

Figure 5. The route to preparing amino acid methyl ester hydrochlorides.

Synthesis of Amino Acid Modified PAMAM (PAMAM-AA)

Amino acids, His, Phe and Trp, were chosen as small molecular ligands. The prepared PAMAM dendrimers (G1 ~ G4) bearing symmetrical end groups were end-capped with amino acids,[39] thus creating complexes of spacer arm and ligand, PAMAM-AA. TEA was added to an aqueous solution of amino acid methyl ester hydrochloride. After mixing to uniformity, an aqueous solution of PAMAM was added. The resulting solution was stirred at 60 ˚C for 48 h. (Ashford et al., 2002) After dialyzing and freeze drying, the product PAMAM-AA was obtained. Here, the molar ratio of the amine end groups of the PAMAM to TEA was 1:6 and to AA-OMe·HCl was 1:2.5.

Preparation of azidated sepharose (Sep-N_3)

Azidated sepharose, Sep-N_3, can be prepared *via* two reaction steps as depicted in our previous work.[35] Firstly, sepharose was activated

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through its hydroxy with epichlorohydrin to prepare epoxidized sepharose; then, a nucleophilic displacement reaction was carried out in which an azide ion attacks the epoxide, resulting in the opening of the ring to obtain azidated sepharose. The detailed experimental data can be observed in the ESI.

Preparation of immunoadsorbent Sep-PAMAM-AA by click chemistry

Immunoadsorbents Sep-PAMAM-AA were prepared by coupling the complexes PAMAM-AA to the "clickable" support Sep-N_3 *via* click chemistry. The azidated sepharose (3 g, in 5 ml water), $CuSO_4.5H_2O$ (50 mg, 0.2 mmol, in 2.5 ml water) and sodium ascorbate (79 mg, 0.4 mmol, in 2.5 ml water) were added to the solution of PAMAM-AA (10 mmol, in 10 ml water). The mixture was stirred at room temperature for 24 h and then washed sequentially with H_2O, 0.1 M EDTA and H_2O to purify the prepared immunoadsorbents Sep-PAMAM-His, Sep-PAMAM-Phe and Sep-PAMAM-Trp. The ligand density of the immunoadsorbent was evaluated according to the number of amino acid units conjugated to PAMAM, which was calculated based on the ^{1}H NMR data.

For comparison purposes, protein A immunoadsorbent with linear spacer arms using the same sepharose as a support and protein A as a ligand, Sep-PA, was also prepared in our laboratory[35]. To evaluate the effectiveness of PAMAM spacer arm, the immunoadsorbent with linear spacer arms, Sep-triazole-His, was prepared as depicted in our previous study.[35]

Instrumental analyses

^{1}H NMR spectra were recorded on a 300 MHz NMR spectrometer (Mercury-Plus 300, Varian, USA). The peaks of different shapes in the ^{1}H NMR spectra were expressed by the following abbreviations, respectively: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet. FTIR spectra were performed on an FTIR spectrophotometer (Nexus Por Euro, Nicolet, USA). The absorbance of the eluate at 280 nm was determined on an ultraviolet-visible spectrophotometer (UV-2450, Shimadzu, Japan). The determination of different compositions in human plasma such as IgG, total protein and albumin were carried out on an automatic biochemistry analyser (P800, Roche, Switzerland) by immunoturbidimetric assay, biuret method and coagulation method, respectively.

IgG adsorption capacity from human plasma

The IgG adsorption capacity of Sep-PAMAM-AA was measured by static adsorption experiments in a batch system. The human plasma was thawed for 1 h at 37 ˚C prior to use and the prepared immunoadsorbent Sep-PAMAM-AA was equilibrated with 0.02 M PBS buffer at pH 7.0 before being added to the human plasma. The static adsorption experiments were conducted at 25 ˚C and at a stirring rate of 100 rpm for 2 h. Then, the Sep-PAMAM-AA was washed by 0.02 M PBS buffer at pH 7.0 to remove those proteins or impurities not fully adsorbed by the adsorbent,[40] followed by suction-drying. Afterwards, the adsorbed IgG by the Sep-PAMAM-AA was eluted with 0.02 M citric acid buffer at pH 2.5. IgG concentration was determined by measuring the absorbance of the eluate at 280 nm according to the Beer–Lambert law:

$$A_{280} = \varepsilon c l \qquad (1)$$

Where A_{280} is the absorbance of the eluate at 280 nm; ε is the molar absorptivity of IgG, which is 1.35 ml/mg·cm according to the standard curve in Fig. S3, ESI; c is the IgG concentration of the eluate (mg/ml); and the path length l is 1.0 cm in the UV cell. The amount of adsorbed IgG was then calculated from the following equation:

$$Q = cV/m \qquad (2)$$

Where Q is the amount of IgG adsorbed onto a unit mass of immunoadsorbent (mg/g); c is the IgG concentration of the eluate (mg/ml); V is the volume of the eluate (ml), and m is the mass of the immunoadsorbent used in the experiment (g).

To facilitate comparative analysis, the IgG concentration in the human plasma before and after static adsorption experiment were further measured using a standardized commercial immunoturbidimetric assay by KingMed Diagnostics in Guangzhou, China.

Adsorption selectivity of IgG from human plasma

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to analyse the adsorption selectivity of Sep-PAMAM-AA for IgG from human plasma. The concentration of separation gel was 15% Tris–HCl, whereas stacking gel was 5% Tris–HCl. Samples were mixed in a 1:1 ratio with loading buffer and incubated for 5 min at 100 ˚C. The loading sample and molecular weight marker was 10 μL, stained with Coomassie Brilliant Blue R-250.

To further verify the adsorption selectivity of the immunoadsorbents Sep-PAMAM-AA for IgG, the total protein

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and albumin concentration in the human plasma before and after static adsorption experiment were measured using standardized commercial biuret and coagulation methods, respectively, by KingMed Diagnostics in Guangzhou, China.

Repeated use study

To evaluate the reusability of Sep-PAMAM-AA, a series of 10 adsorption cycles of IgG from human plasma were performed as described above. Desorption testing was achieved with 0.02 M citric acid buffer at pH 2.5, and then re-equilibrated with 0.02 M PBS buffer at pH 7.0. IgG binding capacity was measured for each cycle.

Conclusions

In this study, immunoadsorbents with PAMAM dendritic spacer arms possessing pseudo-biospecific affinity for IgG from human plasma, Sep-PAMAM-AA, were designed and prepared by click chemistry using sepharose gel as a support and using amino acids, His, Phe and Trp, as ligands. The as-prepared immunoadsorbents using PAMAM G3 as a spacer arm and His as a ligand, Sep-G3-His, possessed the best IgG adsorption performance among the prepared immunoadsorbents. Its ligand density reaches 1.58 mmol/g sepharose gel, almost 5 times higher than that of Sep-triazole-His; its IgG adsorption capacity is 28.43 mg/g, higher than those of Sep-triazole-His and Sep-PA. Moreover, Sep-G3-His shows a relatively low level of non-specific adsorption, which is of significance for the immunoadsorbent to be applied to remove autoantibodies from human plasma to treat various autoimmune diseases. Therefore, the immunoadsorbents using PAMAM as a spacer arm and His as a ligand are expected to have great application prospects in the field of blood purification.

Based on these findings, choosing PAMAM dendrimers as the spacer arms can greatly increase the ligand density of the immunoadsorbents, so leading to a marked increase in their adsorption capacity. There is no doubt that a dendritic spacer arm will enable the immunoadsorbent to efficiently bond more small-molecule ligands, thereby opening a new route for developing high-capacity immunoadsorbents using safe small molecules as ligands.

Conflicts of interest

There are no conflicts to declare

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The acknowledgements come at the end of an article after the conclusions and before the notes and references.

Notes and references

1 C. R. Lowe and P. D. G. Dean, Affinity Chromatography, Wiley-Interscience: London, 1974.

2 G. T. Hermanson, A. K. Mallia and P. K. Smith, Immobilized Affinity Ligand Techniques, Academic Press: California 1992, 137–158.

3 E. M. Martín del Valle and M. A. Galán, *Ind. Eng. Chem. Res.*, 2002, **41**, 2296–2304.

4 P. DePhillips, I. Lagerlund, J. Färenmark and A. M. Lenhoff, *Anal. Chem.*, 2004, **76**, 5816–5822.

5 C. L. Heldt, P. V. Gurgel, L. A. Jaykus and R. G. Carbonell, *Biotechnol. Prog.*, 2009, **25**, 1411–1418.

6 V. Busini, D. Moiani, D. Moscatelli, L. Zamolo and C. Cavallotti, *J. Phys. Chem. B*, 2006, **110**, 23564–23577.

7 L. Zamolo, M. Salvalaglio, C. Cavallotti, B. Galarza, C. Sadler, S. Williams, S. Hofer, J. Horak and W. Linder, *J. Phys. Chem. B*, 2010, **114**, 9367–9380.

8 D. Q. Lin, H. F. Tong, H. Y. Wang, and S. J. Yao, *J. Phys. Chem. B*, 2012, **116**, 1393–1400.

9 H. Yang, W. Liang, N. He, Y. Deng and Z. Li, *ACS Appl. Mater. Interfaces*, 2015, **7**, 774–781.

10 T. Tamura, T. Terada and A. Tanaka, *Bioconjugate Chem.*, 2003, **14**, 1222–1230.

11 E. Boschetti, *Trends Biotechnol.*, 2002, **20**, 333–337.

12 M. S. Urdea and T. Horn, *Science*, 1993, **261**, 534.

13 M. A. Mintzer and M. W. Grinstaff, *Chem. Soc. Rev.*, 2011, 40, 173–190.

14 C. Ornelas, *Macromol. Chem. Phys.*, 2016, **217**, 149–174.

15 P. Kesharwani, S. Banerjee, U. Gupta, M. C. I. M. Amin, S. Padhye, F. H. Sarkar and A. K. Iyer, *Mater. Today*, 2015, **18**, 565–572.

16 S. Mignani, J. Rodrigue, H. Tomas, M. Zablocka, X.Shi, A. Caminade and J.Majoral, *Chem. Soc. Rev.*, 2018, **47**, 514–532.

View Article Online
DOI: 10.1039/C8NJ02142B

17 R. Rajesh, S. S. Kumar, and R. Venkatesan, *New J. Chem.*, 2014, **38**, 1551–1558.

18 E. B. Bahadır and M. K. Sezgintürk, *Talanta*, 2016, **148**, 427–438.

19 R. Huisgen, *Pure Appl. Chem.*, 1989, **61**, 613–628.

20 Y. Shi, X. Cao and H. Gao, *Nanoscale*, 2016, **8**, 4864–4881.

21 V. K. Tiwari, B. B. Mishra, K. B. Mishra, N. Mishra, A. S. Singh and X. Chen, *Chem. Rev.*, 2016, **116**, 3086–3240.

22 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, **41**, 2596–2599.

23 H. C. Kolb and K. B. Sharpless, *Drug Discov. Today*, 2003, **8**, 1128–1137.

24 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2001, **40**, 2004–2021.

25 S. Punna, E. Kaltgrad and M. G. Finn, *Bioconjugate Chem.*, 2005, **16**, 1536–1541.

26 J. Horak, S. Hofer and W. Lindner, *J. Chromatogr. B*, 2010, **878**, 3382–3394.

27 J. Horak, S. Hofer, C. Sadler, S. Williams and W. Lindner, *Anal. Bioanal. Chem.*, 2011, **400**, 2349–2359.

28 A. Marechal, R. El-Debs, V. Dugas and C. Demesmay, *J. Sep. Sci.*, 2013, **36**, 2049–2062.

29 J. E. Moses and A. D. Moorhouse, *Chem. Soc. Rev.*, 2007, **36**, 1249–1262.

30 S. Ghose, B. Hubbard and S. M. Cramer, *J. Chromatogr. A*, 2006, **1122**, 144–152.

31 L. Niu, C. Guo, Z. Hao, J. Yuan and Z. Li, *J. Immunol. Methods*, 2011, **372**, 14–21.

32 G. Lagoumintzis, P. Zisimopoulou, N. Trakas, E. G, K. Poulas and S. J. Tzartos, *J. Neuroimmunol.*, 2014, **267**, 1–6.

33 B. Chen, Q. Ye, K. Zhou and Y. Wang, *J. Chromatogr. B*, 2016, **1017**, 174–181.

34 S. Özkara, H. Yavuz, S. Patır, M. Y. Arıca and A. Denizli, *Sep. Sci. Technol.*, 2002, **37**, 717–731.

35 X. Hu, G. Li and E. Huang, *J. Chromatogr. B*, 2012, **899**, 96–102.

36 J. W. Lee, B. K. Kim, J. H. Kim, S.C. Han, W. S. Shin and S. H. Jin, *Macromolecules*, 2006, **39**, 2418–2422.

37 J. W. Lee, H. J. Kim, S. C. Han, J. H. Kim and S. H. Jin, *J. Polym. Sci.: Part A: Polym. Chem.*, 2008, **46**, 1083–1097.

38 J. W. Lee, J. H. Kim, H. J. Kim, S. C. Han, J. H. Kim, W. S. Shin and S. H. Jin, *Bioconjugate Chem.*, 2007, **18**, 579–584.

39 S. W. Ashford, K. E. Henegar, A. M. Anderson and P. G. M. Wuts, *J. Org. Chem.*, 2002, **67**, 7147–7150.

40 L. Jia, L. Yang, H. Zou, Y. Zhang, J. Zhao, C. Fan and L. Sha, *Biomed. Chromatogr.*, 1999, **13**, 472–477.

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A novel high-capacity immunoadsorbent with PAMAM dendritic spacer arms by click chemistry

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A novel immunoadsorbent with polyamidoamine dendritic spacer arms was prepared. Click chemistry can improve the reaction selectivity between the ligands and support matrix under mild reaction conditions. The designed and prepared immunoadsorbent exhibits excellent adsorption for IgG. The IgG adsorption capacity of Sep-G3-His is superior to those of Sep-triazole-His and protein A immunoadsorbent.