SCIENCE CHINA
Chemistry

• ARTICLES • February 2011 Vol.54 No.2: 375–379
• SPECIAL ISSUE • In Honor of the 80th Birthdays of Professors SHEN JiaCong, SHEN ZhiQuan and ZHUO RenXi doi: 10.1007/s11426-010-4193-7

Affinity adsorption mechanism studies of adsorbents C1-Zn(II) for uremic middle molecular peptides containing Asp-Phe-Leu-Ala-Glu sequence

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Received September 9, 2010; accepted October 8, 2010

To exploit efficient adsorbents for removing middle molecular peptides containing DFLAE (DE5, a typical peptide sequence accumulated in uremic serum) sequence by hemoperfusion, we designed and synthesized three affinity adsorbents (C1-Zn^{2+}, C2-Zn^{2+} and C3-Zn^{2+}) that could have high affinity to DE5. Subsequently, we evaluated the corresponding adsorption ability of each adsorbent by static adsorption experiments and isothermal titration calorimetry (ITC). The results showed that C1-Zn^{2+} had the best adsorption ability to DE5-containing peptides and the adsorption capacity for DE5 was 8.52 mg/g. By changing the adsorption conditions, the adsorption mechanism was elucidated. The main driving force of the adsorption is metal-carboxyl coordination and the hydrophobic force affords the cooperative effect. It is expected that our present work can provide basic understanding for the design of adsorbents with high affinity and selectivity towards oligopeptides.

affinity adsorbent, adsorption mechanism, middle molecular peptides

1 Introduction

Affinity adsorbents are effective materials for purification or separation of proteins and peptides owning to their high affinity and low cost [1, 2]. For example, they have been used in blood purification for selective elimination of toxin in patients [3].

Effective affinity adsorbent was usually obtained by screening the candidates using affinity chromatography or equilibrium binding experiments [4–6]. This process is time consuming and usually require comparatively large quantities of samples. Thus, designing effective adsorbent for given target is a challenge, and basic understanding of the adsorption mechanism is needed.

Uremia is a common syndrome occurring at the end-stage of renal failure. In general, it will lead to the accumulation of the toxins and further disorder organ functions. According to the middle molecule hypothesis [7–10], middle molecular weight toxins ranging from 500–5000 Da are the main toxic substances that accumulated in uremic patients, and middle molecular peptides have been assumed to be one type of major uremic toxins. As a complement to pharmaceutical therapy for uremia, hemoperfusion is an effective method since it can eliminate many toxins in the blood of patients. Kaplan *et al.* reported that, there are 38 middle molecular peptides isolated from the serums of uremic patients containing DE5 sequences [11]. It seems that DE5 is quite related to the uremia. So, It will be much helpful for the treatment of uremia if there are some efficient adsorbents to eliminate DE5-containing peptides by hemoperfusion.

In this study, we synthesized three polyacrylamide (PAM)-based adsorbents (Scheme 1, C1–C3) bearing functional monomers which could have high affinity to peptide DE5. Then the static adsorption experiments were carried out, and the adsorption mechanism was studied.

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2 Experimental

2.1 Materials

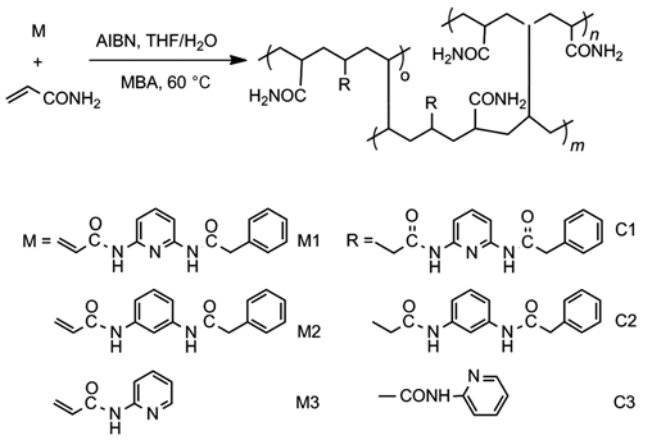
Tetrahydrofuran (THF) was dried over sodium/benzophenone and distilled immediately before use. Acrylamide (AM) was recrystallized from acetone, 2,2′-azobisisobutyronitrile (AIBN) was recrystallized from ethanol, and 2,6-diaminopyridine was recrystallized from toluene. Phenylacetyl chloride and 3-phenylpropanoyl chloride were prepared by reaction of thionyl chloride with the corresponding carboxylic acid. Acryloyl chloride was prepared by reaction of benzoyl chloride with acrylic acid. Peptides were purchased from GL Biochem (Shanghai) Ltd. 2-Aminopyridine was purchased from Shanghai Chemicals Co. and recrystallized in petroleum ether. Other reagents and solvents were commercial available and used without purification.

2.2 Synthesis of functional monomers

The functional monomers M1, M2, M3 were synthesized from the corresponding acylchloride by the reaction with 2,6-diaminopyridine in tetrahydrofuran (THF) at −10 °C under nitrogen atmosphere. Typical synthetic procedures are according to the method in our previous work [12, 13]. The corresponding acylchloride in tetrahydrofuran was added dropwise to a solution of 2,6-diaminopyridine and triethylamine in tetrahydrofuran (150 mL) at −10 °C over night. Then, triethylamine was added to the mixture and acryloyl chloride was added dropwise, and stirred overnight. After filtration and decoloration, the residue was purified by column chromatography, eluting with 8:1 dichloromethane/acetic ether.

2.3 Synthesis of the adsorbents (C1, C2, C3) and the liner polymers (P1, P2, P3)

The functional monomer (80% molar fraction), acrylamide (10% molar fraction), crosslinking agent *N,N*′-methylenebisacrylamide (10% molar fraction), AIBN (w = 3%) and THF (concentration of monomer: 0.1 g/mL) were placed in a round-bottom flask under nitrogen atmosphere with a magnetic stir bar. The reaction was carried out at 60 °C in an oil bath for 24 h. The yields of three adsorbents were 77.1%, 98.0% and 90.9% respectively. The obtained adsorbents were extracted in a Soxhlet extractor successively by acetone and distilled water for 48 h, respectively. The final adsorbents were grinded and sieved (80–100 meshes).



Scheme 1 Synthesis route of the adsorbents.

The linear polymers were synthesized and purified as reported method earlier [12]. The mole fraction of the functional monomer in **P1, P2,** and **P3** was 2.7%, 3.6% and 8.6% respectively as determined by ^{1}H NMR.

2.4 Adsorption experiments

The static adsorption experiment was carried out according to the method in ref. [14]. Briefly, the dry adsorbent (0.1 g) was incubated with $ZnCl_2$ solution (0.66 mM, 10 mL) and shook at 30 °C for 24 h. The peptide (0.2 mM) was then added with further shake. The peptide adsorption was determined by a Unico UV-4802 spectrometer at 220 nm. The control experiments were carried out under the same condition. The adsorption capacity was calculated by the equation:

$$A_c = \frac{(c_1 - c_2)MV}{m}$$

where A_c is the adsorption capacity, c_1 and c_2 are respectively the concentration of the target peptides before and after the adsorption, M is the molar mass of the target peptides, V is the volume of solution used in adsorption, and m is the mass of the dry adsorbent.

2.5 ITC experiments

ITC was used to evaluate the affinity constants between the adsorbents and the object peptides. As our earlier study has demonstrated, linear polymers bearing the same functional groups can be used as adsorbent models to investigate the affinity constants [15, 16]. In the present work, the linear co-polymers (P1, P2, P3) of functional monomers and acrylamide were used as the adsorbent models.

The ITC titration was performed on a VP-ITC microcalorimeter (MicoCal Inc., Northampton, MA). In general, a solution of the peptide was injected in portions to a solution of the polymer or polymer-Zn^{2+} under different conditions. Dilution effects were eliminated by the subtraction of the data from blank experiment.

3 Results and discussion

3.1 The design of functional monomers

In the present study, peptide DE5 is selected as the target

peptide. Since DE5 is a peptide with multiple carboxyl groups (Asp and Glu) in the ends and a hydrophobic groups in the middle (Scheme 2), thus, the designed functional monomers contain either coordination group (M3 in scheme 1) or hydrophobic group (M2) or both (M1).

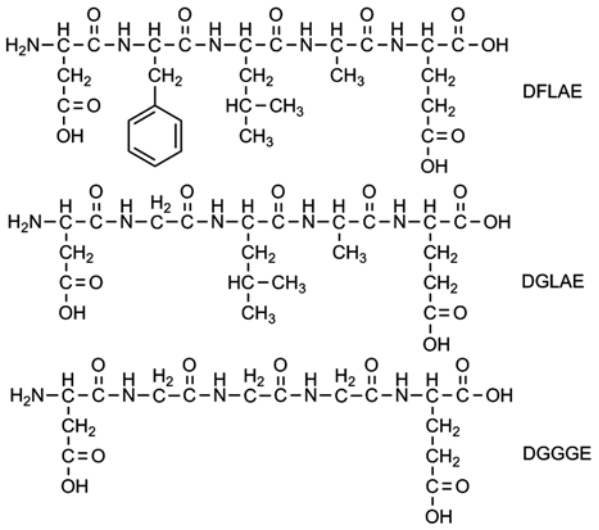
3.2 Adsorption capacities for DE5

We assessed the adsorption capacities of C1-Zn^{2+}, C2-Zn^{2+} and C3-Zn^{2+} for DE5 at room temperature by static adsorption experiments. As shown in Table 1, C1-Zn^{2+} had optimal adsorption to DE5, which was almost six times more than that of C2-Zn^{2+} or C3-Zn^{2+}. It has been found that cross-linked polyacylamide showed little adsorption to DE5 (< 0.1 mg/g), so the adsorption of DE5 onto the adsorbents should be attributed to the functional groups of adsorbents. The elemental analyses showed that C1, C2 and C3 had the similar content of functional groups, which was 29.2%, 22.7% and 32.7%, respectively. Compared with the great difference in their adsorption capacities, the difference in functional group content could be neglected.

To investigate the adsorption affinity constants, ITC was used to quantify the interaction between the ligands and DE5. However, the designed functional monomers (ligands) are all water-insoluble molecules. Taking the advantages of copolymerization with acrylamide, it is realizable to introduce hydrophobic functional groups into aqueous solution for further interaction study. [12] Thus, it is convenient to study the adsorption mechanism between functional ligands and the target peptide by PAM-based copolymer (P1, P2, P3). Affinity constants obtained from ITC were summarized in Figure 1. Consistent with the adsorption results, C1-Zn^{2+} showed high affinity to peptide DE5, while C2-Zn^{2+} and C3-Zn^{2+} exhibited much weaker binding affinity under the same conditions. The agreement of adsorption results with affinity constant further suggested that the different adsorption capacities of the adsorbents were mainly determined by the affinity between functional ligands and DE5.

3.3 Adsorption selectivity toward DE5 and DE5-containing peptides

To examine the adsorption selectivity of C1-Zn^{2+} to peptides containing DE5 sequence, another five peptides were selected (Table 1). DE8, EV11 and DG12 were middle molecular peptides containing DE5 sequence [9]. SH5 is another penta-peptide with double acidic residues but without the hydrophobic residue. WF4 has multiple hydrophobic



Scheme 2 The amino sequence of the three peptides with different hydrophobic abilityies.

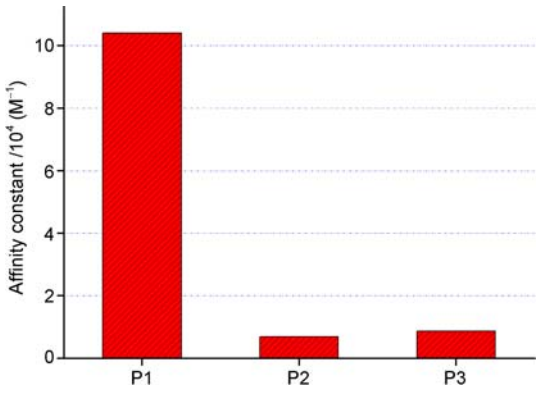


Figure 1 Affinity constants between the linear polymers and DE5 at 30 °C ($n = 3$).

Table 1 The adsorption capacity of three adsorbents to DE5 containing peptides (mg/g)

Peptides	Amino acid sequence	C1-Zn^{2+}	C2-Zn^{2+}	C3-Zn^{2+}
DE5	Asp-Phe-Leu-Ala-Glu	8.52	0.49	1.34
DG8	Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly	6.64	0.24	0.77
EV11	Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val	7.17	0.72	1.21
DG12	Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly	6.99	0.69	0.83
SH5	Ser-Glu-Ala-Asp-His	1.02	–	–
WF4	Trp-Met-Asp-Phe	2.90	–	–

residues but only one acidic residue. We tested the adsorption capacity of C1-Zn^{2+} to these peptides by static adsorption experiments. As shown in Table 1, C1-Zn^{2+} exhibited good adsorption ability to the peptides containing DE5 sequence (DE5, DE8, EV11 and DG12). In contrast, C1-Zn^{2+} exhibited much weaker affinity to the peptides without DE5 sequence (SH5, WF4), about one eighth to one half the capacities of peptides containing DE5. The adsorption results indicated that C1-Zn^{2+} could provide selective adsorption to DE5-containing peptides.

Considering the difference in the structure of the three adsorbents, it seems that the collaboration of coordination and hydrophobic interactions led to the good adsorption ability selectivity of C1-Zn^{2+}. Next, our main job is to confirm this deduction.

3.4 Investigation of the adsorption mechanism

The adsorbents with different structures were expected to generate coordination and hydrophobic interactions with the DE5 containing peptides. To verify this idea, further adsorption experiments were designed to study the adsorption mechanism between the adsorbents (C1-Zn^{2+}, C2-Zn^{2+} and C3-Zn^{2+}) and DE5 containing peptides.

As is known, metal ions play a crucial role in coordination interaction. To understanding the role of metal ions (or coordination interaction) in the adsorption, the adsorption of DE5 containing peptides by C1 in the presence or absence of Zn^{2+} was investigated. Figure 2 shows that, without the complex-ion, the adsorption capacity of C1 to four DE5 containing peptides significantly decreased by 86.9% (DE5), 72.0% (DG8), 70.3% (EV11) and 71.8% (DG12) respectively. Thus, it could be seen that the coordination interaction indeed plays crucial role in the adsorption of DE5-containing peptides onto C1, and it was the main driving force for the adsorption of the DE5-containing peptides.

To confirm the conclusion above, the adsorption capacity of C1-Zn^{2+} for DE5 under different pH was also studied. As can be seen from Figure 3, the adsorption capacity of C1-Zn^{2+} for DE5 dropped significantly with the pH value

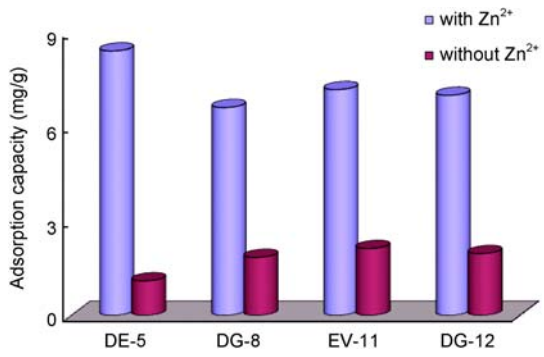


Figure 2 The effect of Zn^{2+} to adsorption capacity of C1 for the targeted peptides.

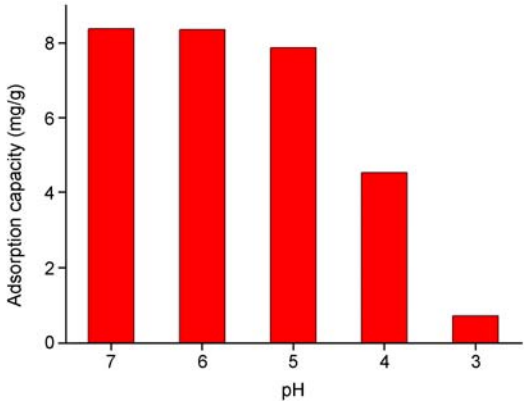


Figure 3 Adsorption capacity of DE5 onto C1-Zn^{2+} under different pH values.

decreasing. This phenomenon is mainly due to the protonation of N atoms in the functional ligand of C1 as the acidity increased, thus the effective N atoms involved in the coordination reduced, finally leading to the reduced the binding ability of adsorbents.

To determine the coordination site, the adsorption of peptides by C1-Zn^{2+} and C2-Zn^{2+} was compared. Although the only difference between C1 and C2 is the pyridine-N, there was almost no adsorption of the four DE5-containing peptides onto C2-Zn^{2+}, which indicated that the pyridine-N is the main site for the coordination. In addition, considering the obvious difference in the adsorption of DE5 containing peptides and WF4, multi-carboxyl structure on the target peptides probably play another key role in the coordination interaction. In WF4, there is only one acidic amino acid residue, so it could not provide the effective coordination interaction, and thus the adsorption amount is much lower than that of DE5-containing peptides. Hence, we could conclude that both the structure of adsorbent and peptide could affect the coordination interaction and thus the adsorption process.

In a word, coordination interaction is one of the main driving force for the adsorption of DE5-containing peptides onto C1-Zn^{2+}, and the pyridine-N of C1 as well as the carboxyl groups of the peptides are the main binding sites.

The role of hydrophobic interaction in the adsorption was also studied. Similarly, comparing C1 with C3, the benzene ring is the main difference. Seen from Table 1, the adsorption capacity of C3-Zn^{2+} for the four DE5-containing peptides is very low, which is only 10%–20% of that of C1-Zn^{2+} for corresponding peptides. The results indicated that hydrophobic interaction is also an important driving force during this adsorption process and the benzene ring is the main binding site.

Adsorption of peptides with different hydrophobic properies (Scheme 2) using C1-Zn^{2+} was utilized to ellucidate the hydrophobic interaction in adsorption. The hydrophobicity of the peptides is DFLAE > DGLAE > DGGGE. The adsorption capacities of C1-Zn^{2+} to DFLAE, DGGGE and

DGLAE were 8.52, 4.20 and 5.34 mg/g, respectively, which is correlated well with the hydrophobic property of the peptides.

4 Conclusions

In this work, three affinity adsorbents were designed and prepared for eliminating DE5-containing peptides in serums of uremic patients. The results showed that C1-Zn^{2+} has the best adsorption capacity and adsorption selectivity for targeted peptides. Meanwhile, adsorption mechanism study revealed that coordination and hydrophobic interactions were the main drive forces in the adsorption process.

This work was supported by the National Natural Science Foundation of China (20634030 & 51003048), the Specialized Research Fund for the Doctoral Program of Higher Education (20090031120020) and the Fundamental Research Funds for the Central Universities.

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