# Adsorption Mechanism at the Molecular Level between Polymers and Uremic Octapeptide by the 2D $^1$H NMR Technique

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To remove uremic octapeptide from the blood stream of uremic patients, various modified polyacylamide cross-linked absorbents were prepared. Adsorption experiments showed these absorbents have significant differences in adsorption capacity to the target peptide. In this paper, two-dimension proton nuclear magnetic resonance (2D $^1$H NMR) spectroscopy was used to investigate the interaction mechanism between the peptide and the adsorbents. Because of the insolubility of the absorbent, some soluble linear polymers with the same functional groups as the absorbents were employed as the model adsorbents in 2D $^1$H NMR. The preferred binding site for the peptide and polymers was identified to be at the C-terminal carboxyl group of the octapeptide via chemical shift perturbation effects. In this study, we found that hydrogen bonding, electrostatic, and hydrophobic interactions all play a role in the interaction force but had different contributions. Especially, the great chemical shift changes of the aromatic amino acid residues (Trp) during the interaction between butyl-modified polyacrylamide and octapeptide suggested the hydrophobic interaction, incorporated with the electrostatic force, played an important role in the binding reaction in aqueous solutions. This information not only rationally explained the results of the adsorption experiments, but also identified the effective binding site and mechanism, and shall provide a structural basis for designing better affinity-type adsorbents for the target peptide.

## 1. Introduction

In contrast to traditional hemodialysis, hemoperfusion is a complementary therapy to remove macromolecular toxins, such as proteins and peptides, which were not effectively eliminated by hemodialysis.[1−3] The study of novel adsorbents for hemoperfusion has attracted much interest for their efficiency and cost-effectiveness.[4−9] To date, there are mainly two approaches to improving the selectivity of the adsorbents: bioconjugation and organic molecule modification.[10−13] Between the two methods, bioconjugation directly immobilizes bioactive substances on polymer surfaces. Thus, the molecular recognition ability of the bioactive substances is transferred to the bioconjugate polymers. Yet this approach is limited by two factors. One is that biomolecules for specific targets do not always exist or are difficult to purify. In addition, biomolecules such as enzymes or antibodies may lack long-term stability. Another method, organic molecule modification, provides a versatile approach for the synthesis of polymers with the ability to recognize target molecules. For example, an amphiphilic adsorbent for eliminating low-density lipoproteins prepared by this method has been reported by Yu and his co-works.[14,15] Besides the ligand, the chemical property of the matrix is also a factor that could not be neglected because it will affect the interaction between the target and the adsorbents. Yet the adsorbent is usually a highly cross-linked networks with a rigid structure, so it is difficult to study the adsorption mechanism at the molecular level. Conventionally, proper adsorbent should be selected after numerous adsorption experiments. For this reason, attempts to rationalize model adsorbents are highly needed to improve our understanding of the interaction mechanism between the polymers and the targets. This knowledge is used to design and test the novel adsorbents that we have synthesized.

In this study, the target peptide is an octapeptide that was isolated from serum of uremic patients reported in our previous work.[16,17] To our knowledge, hydrophobic interactions, ionic (or electrostatic) attraction, hydrogen bonding, and van der Waals interactions are responsible for adsorbents, such as charcoal and resins, being able to adsorb peptide toxins. Yet which of them plays the vital role in the adsorption is not yet clear. According to the recognition principle from artificial receptor, the electrostatic force and hydrophobic interaction are important in aqueous solution. For this reason, various modified polyacrylamide cross-linked adsorbents with potential cation and hydrophobic functional groups were prepared to eliminate the peptide in hemoperfusion. Aqueous adsorption experiments were preformed and examined. The soluble linear polymers with the same functional groups as the adsorbents were then employed as the model adsorbents. The binding of the octapeptide to linear polymers was investigated by 2D $^1$H NMR spectroscopy via chemical shift perturbation effects.[18] The results provided a structural basis for designing better affinity-type adsorbents for the target peptide.

## 2. Materials and Method

Acrylamide was purchased from Shanghai chemical Co. and recrystallized in acetone before use. Potassium *tert*-butoxide, sodium hypochlorite, and *n*-butyraldehyde were purchased from Sigma and used as received. The other reagents and solvents were commercial available and used without purification.

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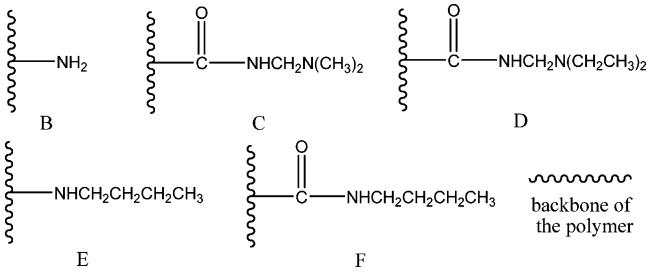


**Figure 1.** The schematic diagram of the main modified group structures of linear polyacrylamide derivants.

**Table 1.** Assignment of the Proton Signals of the Octapeptide

| residues | NH | αH | βH | others |
|---|---|---|---|---|
| $Val_1$ | 8.82 | 4.10 | 1.99 | $\gamma CH_3$ 0.97, 0.94 |
| $Val_2$ | 8.72 | 3.78 | 2.13 | $\gamma CH_3$ 0.97, 0.94 |
| $Arg_3$ | 8.85 | 4.21 | 1.89, 1.79 | $\gamma CH_2$ 1.70, $\delta CH_2$ 3.32 NH 7.72, 6.62 |
| $Gly_4$ | 8.72 | 3.85 | | |
| $Cys_5$ | 8.43 | 4.32 | 3.28, 2.96 | |
| $Thr_6$ | 8.36 | 4.08 | 4.22 | $\gamma CH_3$ 1.23 |
| $Trp_7$ | 7.93 | 4.56 | 3.32, 3.19 | 2H 7.24; 4H 7.65; 5H 7.17; 6H 7.24; 7H 7.54; NH 10.22 |
| $Trp_8$ | 8.02 | 4.56 | 3.32, 3.19 | 2H 7.24; 4H 7.65; 5H 7.17; 6H 7.24; 7H 7.54; NH 10.22 |

**2.1. Preparation of Octapeptide.** The uremic octapeptide samples were isolated from the serum of uremic patients by a multistep chromatographic procedure, including gel permeation, ion exchange, and reversed-phase high performance liquid chromatography as described in our previous papers.[16,17]

**2.2. Preparation of Linear Polymers.** *2.2.1. Preparation of Polyacrylamide.* Polyacrylamide (PAM) with a weight average molecular weight (Mw, determined by GPC) of $2.5 \times 10^4$ was synthesized by precipitation polymerization. Briefly, a solution of acrylamide (10 g) in anhydrous ethanol (200 mL) was heated to 60 °C, and then AIBN (0.2 g) was added under $N_2$ protection. The solution was stirred at 60 °C for 4 h. The polymer was then filtered and washed thoroughly with anhydrous ethanol and finally dried under vacuum at room temperature.

*2.2.2. Preparation of the Derivatives of Polyacrylamide.* The PAM prepared above was modified to produce some PAM derivants (B−F in Figure 1).

The polyvinylamine (B, PVM) was synthesized by the Hoffman degradation reaction according to the literature.[19] At the end of the reaction, the pH of the mixture was adjusted to approximately 8 by HCl (37%), and the mixture was dialyzed against distilled water until no chloride ion was observed in the dialyzate. The solution of the polyvinylamine was then condensed by vacuum evaporation, and the product was obtained by lyophilization.

The synthesis of the dialkylamine-modified PAMs (C and D in Figure 1) was described below. PAM (6 g, 85 mmol) was dissolved in distilled water (200 mL) by stirring at 50 °C. Next, 36% formaldehyde aqueous solution (6 mL, 85 mmol) and dialkylamine (dimethylamine or diethylamine, 85 mmol) were added. The Mannich reaction was allowed to proceed at 50 °C for 4 h. The reaction mixture was condensed by vacuum evaporation. The dialkylamine-modified PAM was precipitated into acetone, filtered, rinsed with acetone, and finally dried under vacuum at 30 °C for 48 h.

The butyl-modified PVM (E) was synthesized by Schiff-base reaction and sodium borohydride reduction. The polyvinylamine (1 g, approximately 23 mmol of vinylamine) prepared above was dissolved in distilled water (100 mL) at room temperature. Butyraldehyde (1.4 mL, 23 mmol) was added with vigorous stirring, and the pH of the reaction mixture was adjusted to 8 with an aqueous solution of NaOH (0.1 M). The mixture was stirred at room temperature for 4 h, cooled to 4 °C, and $NaBH_4$ (0.88 g, 23 mmol) was added. While being maintained at this temperature, the mixture was stirred for 15 h, and finally acetone was added to quench the excessive $NaBH_4$. The resulting solution was dialyzed against distilled water for 1 week and condensed by vacuum evaporation. The polymer was obtained by lyophilization as a white solid.

**Table 2.** Average Loading of Modified Groups on the Adsorbents

| adsorbents | modified group | average loadings of modified group (%) |
|---|---|---|
| (A) polyacrylamide (PAM) | —[a] | —[a] |
| (B) Hofmann degradation of PAM | vinylamine | 28.35 |
| (C) dimethylamine-modified PAM | dimethylaminomethyl | 7.69 |
| (D) diethylamine-modified PAM | diethylaminomethyl | 5.98 |
| (E) butyl-modified PAM | *N*-butylvinylamine | 8.4 |

[a] Without any modified group.

*N*-Butyl-modified PAM (F) was prepared according to the method from Deguchi and Lindman.[20] Butyl chloride was used to modify PAM in DMSO solution.

**2.3. Preparation of Cross-Linked Adsorbents.** The cross-linked PAM beads were prepared by reverse suspension polymerization using a procedure described previously.[21] The beads were extracted in a Soxhlet extractor successively by ethanol and distilled water for 24 h, respectively. The crosslinking degree of the PAM beads was 5%. A similar modification (mentioned in section 2.2.2) of cross-linked PAM beads was carried out, and a series of adsorbents B−E were obtained as shown in Table 2. All of the adsorbents were filtered and washed with alcohol and distilled water thoroughly until no absorbance was detected at 281 nm for the washing solutions. Finally, the modified PAM beads were freeze-dried for 48 h.

**2.4. Characterization of Polymers.** $^1H$ NMR spectra of linear polymers were obtained using a Bruck 300 MHz spectrometer. $D_2O$ was used as the solvent.

The adsorbents were swelled thoroughly in $D_2O$ and then analyzed on a Varian UNITYplus-400 MHz spectrometer equipped with a solid resonance probe head.

**2.5. Adsorption Experiments.** 1.0 mL of wet cross-linked adsorbents was incubated with 3 mL of octapeptide solution (0.25 mg/mL) and oscillated at 37 °C for 30 min. The adsorbance of the octapeptide solution was determined with a Shimazu UV-2101 spectrometer at 281 nm. A series of octapeptide solutions with different concentrations were utilized to make a calibration curve, and the concentration of each sample was determined by comparison with the calibration curve. The data obtained were processed by software automatically. Adsorption capacity was calculated according to the following equation:

$$\mathrm{Ac} = \frac{(c_1 - c_2)V}{m}$$

where Ac stands for the adsorption capacity, $c_1$ and $c_2$ stand for the concentration before and after adsorption, respectively, $V$ stands for the volume of solution used in adsorption, and $m$ is the mass of the wet adsorbent.

**2.6. NMR Experiments.** NMR samples of the peptide were prepared by dissolving 4 mg of the octapeptide in 0.6 mL of a mixture of $H_2O$ and $D_2O$ (90/10, v/v) and 100% $D_2O$, respectively. The pH of $H_2O$/$D_2O$ solution was adjusted to 5.0. The NMR sample of interaction contained 4 mg of the octapeptide and 1 mg of the linear polymer. Homonuclear 2D $^1H$ NMR spectra (COSY, TOCSY, and NOESY) of the samples were recorded on a Varian UNITYplus-400 MHz spectrometer at room temperature. For all samples, the mixing times were 80 and 300 ms for the TOCSY and ROESY spectra, respectively. NMR experiments were performed at room temperature. The spectral widths of the $F1$ and $F2$ dimensions were 2873.6 and 2873.6 Hz, respectively. Each collected data set contained 256 ($t1$) × 1024 ($t2$) data points. The data sets were linearly predicted to be 1024 × 1024 data points.

**2.7. CD Spectroscopy.** Static CD measurements were taken with a JASCO (Easton, MD) J-715 spectropolarimeter with thermoelectric temperature control. Samples were allowed to equilibrate at 20 °C for

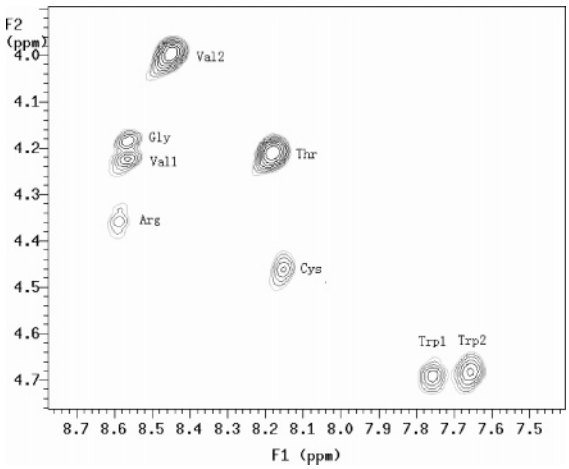


**Figure 2.** 2D $^1$H NMR COSY spectrum of the fingerprint region of the octapeptide (90% $H_2O$/10% $D_2O$ solution at pH 4.0).

5 min. Spectra were recorded at 20 °C in distilled $H_2O$. The peptide concentration was 0.2 mg/mL. Ellipticity scans were collected in triplicate, at 0.1 nm intervals, over the wavelength range 190−250 nm, with a light path of 1 mm and a 1 s response time in a 1.0 cm quartz cell. Final spectra representing the plots of mean residue ellipticity ([$\theta$]) in deg cm$^2$ dmol$^{-1}$ versus wavelength ($\lambda$) in nanometers were smoothed over an interval of five data points prior to plotting. The percentage helical content was estimated by the method of Yang.[22]

## 3. Results and Discussion

**3.1. NMR Assignment of the Octapeptide.** According to the "middle molecule" hypothesis, middle molecular peptides have been assumed to be one type of the major uremic toxins. Therefore, our study focuses on the selective clearance of uremic peptides. It is reported that arginine and aromatic residues, such as tryptophan and phenylalanine, are the main contribution to the protein−protein interaction. Also, Titani et al. had reported the key role of Trp on peptide interactions with chromatography media.[23,24] To our interest, these amino acid residues were also observed in most of the uremic peptides. Hence, the investigation of these peptides with such residues could help us have a better understanding of the interaction between the peptides and adsorbents, and then design novel adsorbents. In our previous paper, a peptide was isolated from uremic peptide sera, and its primary sequence was identified as $Val_1-Val_2-Arg_3-Gly_4-Cys_5-Thr_6-Trp_7-Trp_8$ by MALDI TOF MS and LC/ESI MS/MS.[17] There were six types of amino acid residues including arginine and tryptophin in this octapeptide. For this reason, it was chosen as the target molecule in the adsorption process.

The chemical shift values of the typical amino acid residue protons were taken from the Biomagresbank site at Biomagresbank (http://www.bmrb.wisc.edu/ref_info/statsel.htm). Unlike the 1D $^1$H NMR spectra, 2D $^1$H NMR spectra usually give well-separated cross-peaks, which provide more detailed information for the studied systems. Spin systems for amino acid residues have been readily identified on the basis of the coupling characteristic of different systems. According to the "fingerprint" region in the COSY spectrum of the octapeptide (90% $H_2O$/10% $D_2O$ solution, pH 4.0, Figure 2), the $^1$H NMR peaks of all amino acid residues in the octapeptide were completely assigned, and the results are shown in Table 1.

**3.2. Synthesis and Characterization of Cross-Linked Adsorbents.** Polyacrylamide (PAM) has been extensively used in recent years for protein separations. Moreover, it has good biocompatibility, stability in the environment, and can be easily modified to provide diverse functional groups. On the other hand, cross-linked PAM beads were easy to produce by reverse phase polymerization. So polyacrylamide was selected as a matrix of the adsorbents.

Because peptides are usually charged in aqueous solution, the charged surface of adsorbents could improve their biocompatibility and enhance the electrostatic interaction between the peptide and protein. Therefore, we attempted to increase the adsorption capacity of PAM beads by introduction of a tertiary amine group, which was produced by Mannich reaction, and two types of dialkylamine-modified PAM (C and D) were obtained. Polyvinylamine (PVM) is another polymer likely to be charged in aqueous solution and was prepared through degradation of PAM. Because of the occurrence of hydrophobic amino acid residues in the octapeptide, another hydrophobic adsorbent (E) was prepared by Schiff-base reaction and sodium borohydride reduction from PVM.

The structures and average loadings of modified groups on the adsorbents were determined via solid NMR, and the results are shown in Table 2.

**3.3. Synthesis and Characterization of Linear Polymers.** To investigate the interaction between the peptide and polymers by solution NMR, the polymers must have good water solubility. Obviously, the cross-linked adsorbents could not be used for this purpose. The substitution of these adsorbents was required. The linear PAM and its derivants with the same modified group as that of the adsorbents mentioned above could be the model for the cross-linked adsorbent, as they were soluble and had similar chemical structures.

The structures of prepared linear polymers were confirmed via $^1$H NMR and IR. The results are shown in Table 3.

**3.4. Adsorption Experiments.** The adsorption capacities of various adsorbents were studied by static adsorption experiment. The results (Figure 3) showed that the adsorption capacity of

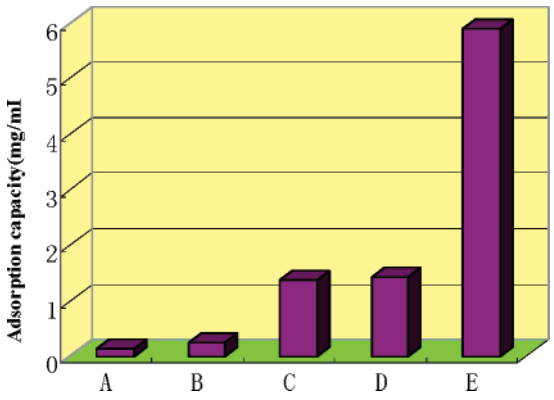


**Figure 3.** Adsorption of the octapeptide by different adsorbents: (A) polyacrylamide; (B) polyvinylamine; (C) dimethylamine-modified polyacrylamide; (D) diethylamine-modified polyacrylamide; and (E) butyl-modified polyvinylamine.

the diethylamine-modified adsorbent (D) was slightly higher than that of the dimethylamine-modified adsorbent (C), while the adsorption capacity of the butyl-modified adsorbent (E) for the octapeptide was significantly higher than that of the other adsorbents. The adsorption capacity of E is about 20 times that of A and B, and 5 times that of C and D. Because adsorbent E is more hydrophobic than the others, as is clearly seen from their structures, it seems that the hydrophobic interaction plays an important role in the adsorption of the octapeptide.

**3.5. The Binding Interaction Site in the Octapeptide.** Although the adsorption experiment revealed the interaction mode between the octapeptide and the adsorbents to some

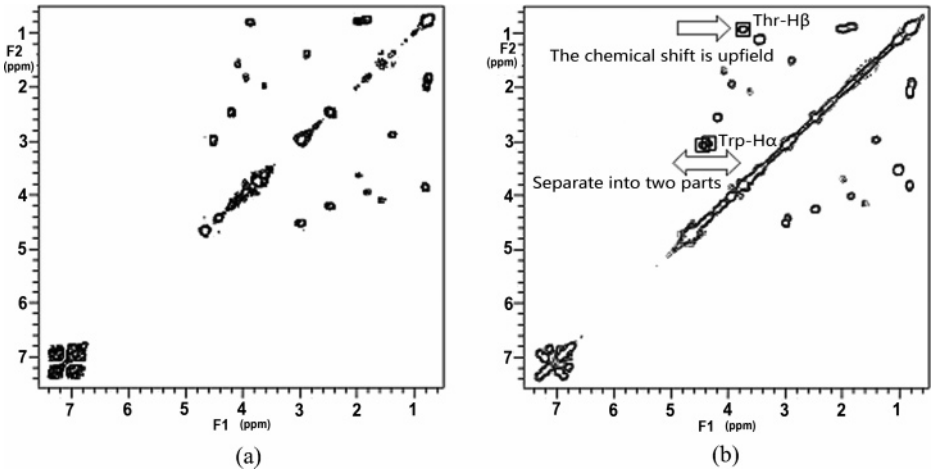


**Figure 4.** COSY spectra: (a) the octapeptide; and (b) octapeptide−polyvinylamine (structure B).

**Table 3.** The Characterization of Linear Polymers

| linear polymer | NMR value (ppm) and IR value ($cm^{-1}$) |
|---|---|
| (A) polyacrylamide (PAM) | $CH_2$ (b, 1.4 ppm), CH (b, 2.0 ppm), $-CONH_2$ (3197.45 $cm^{-1}$, 3332.56 $cm^{-1}$), $-CH_2-$ (2865.76 $cm^{-1}$, 2936.12 $cm^{-1}$), $-CONH_2$ (1668.45 $cm^{-1}$) |
| (B) Hofmann degradation of PAM (PVM) | $CH_2$ (b, 1.4 ppm), C−CH−N (b, 3.6 ppm), $-NH_2$ (3300 $cm^{-1}$, 771.66 $cm^{-1}$), $-CONH_2$ (1668.45 $cm^{-1}$) decreased |
| (C) dimethylamine-modified PAM | $CH_2$ (b, 1.4 ppm), CH (b, 2.0 ppm), $CH_3$ (s, 2.1 ppm), $N-CH_2-N$ (b, 3.8 ppm), $NH-CH_2$ (1512.34 $cm^{-1}$) |
| (D) diethylamine-modified PAM | $CH_2$ (b, 1.4 ppm), CH (b, 2.0 ppm), $CH_3$ (s, 1.0 ppm), $N-CH_2-N$ (b, 4.0 ppm), $N-CH_2-C$ (b, 2.4 ppm), $NH-CH_2$ (1508.12 $cm^{-1}$) |
| (E) butyl-modified PVM | $CH_3$ (m, 0.9 ppm), −NH− (3326.34 $cm^{-1}$) |
| (F) *N*-butyl-modified PAM | $CH_3$ (m, 0.8 ppm), $-CH_2-$ (m, 1.2 ppm), $N-CH_2-$ (3.0 ppm), $NH-CH_2$ (1534.32 $cm^{-1}$) |

extent, the interaction mechanism and sites between them were not adequately clear. Using 2D $^1H$ NMR, the interaction site between the peptide and the polymers could be identified by examining the cross-peaks of those amino acid residues that displayed significant chemical shift changes upon interaction with a series of polymeric species (A−E). To efficiently observe the specific binding, 1 mg of polymeric sample was added in the octapeptide solution. For each polymeric sample examined, only a few of amino acid residues' cross-peaks were found to be perturbed upon binding, indicating the presence of a preferred interaction site. The results for polyvinylamine were discussed in detail as an example.

By comparing the COSY spectrum of the free octapeptide and that of the octapeptide−polyvinylamine system, obvious chemical shift changes were observed for the side chain signals of the terminal $Trp_8$ and the side chain signals of Thr. As shown in Figure 4, the original overlap cross-peaks for the $Trp_7-Trp_8$ ($\alpha CH-\beta CH$) residue were separated, with one keeping its original position and the other (correlating with the terminal $Trp_8$) shifted upfield. In addition, the addition of polyvinylamine induced a slight change of the chemical shift of $\beta CH-\gamma CH$ cross-peaks for Thr residue, indicating the presence of a high shielding effect on the $\beta$ proton (from 3.96 to 3.78 ppm). This phenomenon might be caused by the formation of the hydrogen bond between the $\beta$ hydroxyl group in the Thr residue and the amino group in polyvinylamine. The formation of the hydrogen bonding (O···H−N) weakens the electron-withdrawing inductive effects of the oxygen in the hydroxyl group. At the same time, the shielding effect of the methylene connected with the hydroxyl group was strengthened, so that a downfield chemical shift was observed.

The octapeptide is a relatively flexible oligopeptide, so from a theoretical point of view, each residue has the opportunity to interact with the polymer without sterical hiding. Yet only the signals from the terminal Trp and Thr were affected by the polyvinylamine, while no other signals were observed to move significantly. The high specificity of the observed spectral change permitted these two amino acid residues to be the polyvinylamine binding site.

On the basis of the analysis of the octapeptide−polyvinylamine system, the binding site of other polymeric samples on the octapeptide was also investigated. The COSY spectra of octapeptide−polyacrylamide (not shown) showed that the chemical shifts of the octapeptide residues were hardly affected by addition of the polyacrylamide. This result indicated there was no specific binding site between the octapeptide and polyacrylamide.

The COSY spectra of octapeptide-C and octapeptide-D are similar to that of octapeptide-B except for the Thr residue. As shown in Figure 5, when C was added in octapeptide solution, the two overlap cross-peaks of $Trp_7-Trp_8$ ($\alpha CH-\beta CH$) were still separated, and only the signal from the terminal $Trp_8$ was effected by C. On the basis of the results shown in Table 4, we could conclude that the interaction between diethylamine-modified polyacrylamide and octapeptide is stronger than that between the dimethylamine-modified polyacrylamide and the octapeptide.

The interaction between the octapeptide and the butyl-modified polyvinylamine (E) is the most remarkable one in comparison with the other systems. As shown in the COSY spectrum of octapeptide-E (see Figure 6), the cross-peaks of Trp-$\alpha$CH and Trp-$\beta$CH disappeared, and meanwhile two new cross-peaks appeared in the upfield (the inset in Figure 6). Combining the results from the COSY, TOCSY, and ROESY spectra, it is confirmed that both the $Trp_7$ and the $Trp_8$ (not only the terminal $Trp_8$) were effected by E. That is, a strong

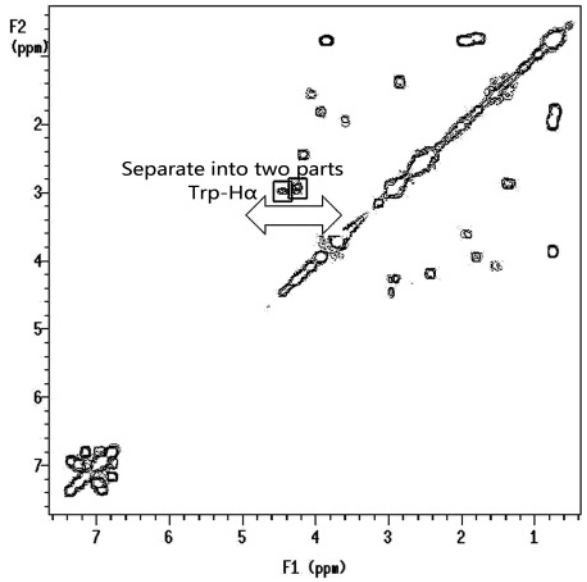


**Figure 5.** COSY spectrum of octapeptide−dimethylamine-modified polyacrylamide (structure C).

**Table 4.** (Trp-Hα, H$\beta$) Chemical Shift of the Octapeptide Terminal Group in Different Systems[a]

| system | Hα chemical shift (ppm) | H$\beta$ chemical shift (ppm) |
|---|---|---|
| octapeptide | 4.56 | 3.32 |
| octapeptide−DMA-modified PAM | 4.44 | 3.01 |
| octapeptide−DEA-modified PAM | 4.31 | 2.99 |

[a] DMA, dimethylamine; DEA, diethylamine.

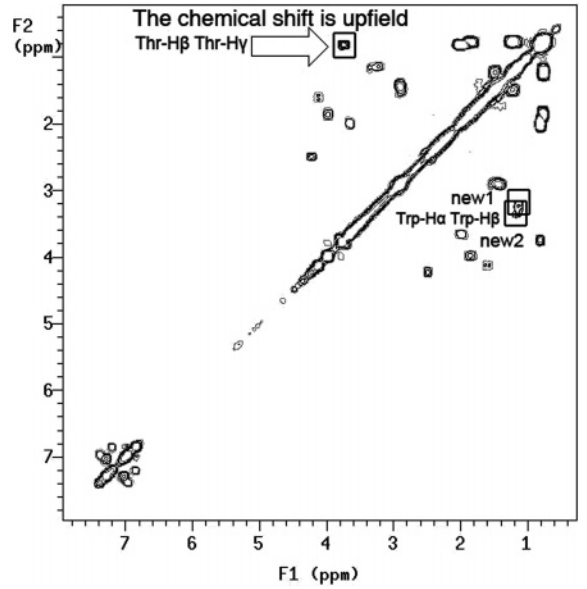


**Figure 6.** COSY spectrum of octapeptide−butyl-modified polyvinylamine at pH 7.0 (structure E).

interaction happened between the butyl-modified polyvinylamine and $Trp_7$−$Trp_8$ sequence in the octapeptide. Moreover, just like those in the polyvinylamine−octapeptide system, the cross-peaks of Thr-H$\beta$ and Thr-H$\gamma$ moved upfield.

On the basis of the above analysis, the signals of $Trp_8$ and Thr residues were feasible to be perturbed by the addition of polymers, so they were identified as the main binding sites in the octapeptide. Hence, it is likely that the C-terminal portion is responsible for the interaction with the polymers.

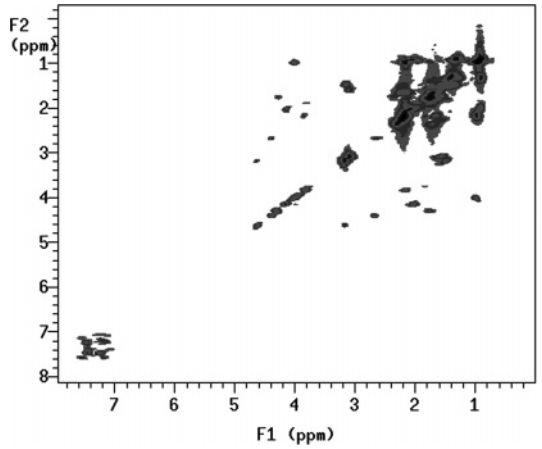


**Figure 7.** COSY spectrum of octapeptide−*N*-butyl-modified PAM (structure F).

**3.6. The Interaction Force between the Octapeptide and the Polymers.** With the confirmation of the binding site, the interaction mechanism was further studied by comparing the chemical properties of the polymers investigated in the present work. It is clear that all of the polymers used could be protonated in aqueous solution except polyacylamide. For example, polyvinylamine with the primary amino group could easily turn into amino cation in aqueous solution with proton. Dialkyl-modified PAM and butyl-modified polyvinylamine have a tertiary amine group and a secondary amine group, respectively. These groups may accept a proton and form cations, too. To our interest, the polyacrylamide that could not be charged did not have obvious interaction with the octapeptide. This implicates that the electrostatic interaction may be the driving force in the interaction between the charged polymers and the octapeptide, although the hydrophobic interaction was considered to play an important role in the adsorption of the octapeptide according to the results of adsorption experiments. To prove this hypothesis, we prepared an uncharged polymer, *N*-butyl PAM (F), which had the same hydrophobic group as butyl-modified polyvinylamine (E). The interaction between the octapeptide and F was investigated, and the COSY spectrum of this system was shown in Figure 7. In contrast to the remarkable chemical shift changes of αCH−$\beta$CH cross-peaks for $Trp_7$−$Trp_8$ residues in the COSY spectrum of octapeptide-E, there were no obvious changes in this spectrum. This result suggested that *N*-butyl polyacrylamide did not interact with the octapeptide. The main difference between F and E was that there was a potential cation N in polymer E. Because it was proven that the hydrophobic groups in the *N*-butyl polyacrylamide did not result in a strong interaction between this polymer and the octapeptide as we expected, it implicated that the electrostatic interaction was essential for the adsorption of the octapeptide, only when the hydrophobic interaction could happen (E). The cooperation of electrostatic interaction with hydrophobic interaction attributed to the great chemical shift changes of $Trp_7$−$Trp_8$ and the highest adsorption capacity of adsorbent E.

The COSY spectrum of octapeptide−dimethylamine-modified polyacrylamide at pH 4.0 also supported this hypothesis. In this spectrum (not shown), the two overlap cross-peaks of Trp (αCH−$\beta$CH) were not divided in contrast to those observed at pH 7.0 (Figure 4), indicating that the main interaction between the octapeptide and the dialkylamine-modified polyacrylamide was mainly due to the electrostatic force. On the basis of this hypothesis, the reason for the lowest adsorption capacity of PAM adsorbents could be explained rationally. Although there may be hydrogen bonding between the polyvinylamine and the

**Table 5.** The Secondary Chemical Shift and the Chemical Shift Indexes for Each Amino Acid Residue in the Octapeptide[a]

| residue | $^1H_\alpha$ in random coil (ppm) | $\delta$-$^1H\alpha$[b] in $D_2O$ (ppm) | $\delta$-$^1H_\alpha$ in B (ppm) | $\delta$-$^1H_\alpha$ in C (ppm) | $\delta$-$^1H\alpha$ in D (ppm) | $\delta$-$^1H\alpha$ in E (ppm) | chemical shift index |
|---|---|---|---|---|---|---|---|
| Val-1 | 3.95 ± 0.10 | +0.05 | +0.05 | +0.05 | +0.05 | +0.05 | 0 |
| Val-2 | 3.95 ± 0.10 | −0.17 | −0.17 | −0.17 | −0.17 | −0.17 | −1 |
| Arg-3 | 4.38 ± 0.10 | −0.17 | −0.17 | −0.17 | −0.17 | −0.17 | −1 |
| Gly-4 | 3.97 ± 0.10 | −0.12 | −0.12 | −0.12 | −0.12 | −0.12 | −1 |
| Cys-5 | 4.65 ± 0.10 | −0.33 | −0.33 | −0.33 | −0.33 | −0.33 | −1 |
| Thr-6 | 4.35 ± 0.10 | −0.27 | −0.27 | −0.27 | −0.27 | −0.27 | −1 |
| Trp-7 | 4.70 ± 0.10 | −0.14 | −0.14 | −0.14 | −0.14 | −3.50 | −1 |
| Trp-8 | 4.70 ± 0.10 | −0.14 | −0.18 | −0.26 | −0.39 | −3.50 | −1 |

[a] B, polyvinylamine−octapeptide $D_2O$ solution; C, dimethylamine-modified polyacrylamide $D_2O$ solution; D, diethylamine-modified polyacrylamide $D_2O$ solution; E, butyl-modified polyvinylamine $D_2O$ solution. [b] $\Delta\delta = \delta_{measured} - \delta_{random\ coil}$.

octapeptide, the strength of the hydrogen bonding decreased rapidly by polar solvent molecules in aqueous solution. Hence, the lower adsorption capacity of the polyvinylamine adsorbent than that of dialkylamine-modified PAM may be a result from its primary amine group, which has weaker charged capacity than that of tertiary amine group.

In summary, three types of weak interactions between the octapeptides and the modified polymers were observed. The polar side groups of the polymers interact with the octapeptide by hydrogen bonds and electrostatic interactions, while the hydrophobic side groups of the polymers interacted with the octapeptide by hydrophobic interactions. Among them, the electrostatic interaction was essential for the interaction between the polymers and the octapeptide, while the binding strength increased significantly by the introduction of the hydrophobic side groups cooperating with the electrostatic interaction.

**3.7. The Computer Model of the Interaction between the Octapeptide and Butyl-Modified Polyvinylamine.** The combination of experimental structural approach with computational modeling could offer more detailed information for the interaction between the target octapeptide and the polymers at the atomic level. In this study, the molecular DOCK method was used to investigate the interaction between the octapeptide and butyl-modified polyvinylamine. The lowest energy conformation of the octapeptide was minimized by simulated annealing. The lowest energy conformation of the complex of the peptide and the polymer was shown in Figure 8. The hydrogen bondings between the side chain of the polymer and the Arg-3, Cys-5, and Trp-7 were observed, respectively. The hydrophobic interaction between the indole group of the Trp residues and the butyl group on the side chain of the polymer was considered to have occurred due to the proper position between them. The cooperation of the various weak interactions force was confirmed again by the computer model.

**3.8. Conformation of the Octapeptide.** Octapeptide may retain a certain conformation in aqueous solution although it is an oligopeptide. So its advanced structure was investigated by 2D solution NMR. The chemical shift index (CSI) is a quick and robust way of deriving secondary structure information from chemical shift data.[25] The chemical shifts of αCH can be compared to their random coil values ±0.1 ppm and then assigned with an index of 1 (greater than), 0 (within), or −1 (less than). According to the rules of chemical shift index, a group of three or more "1s" not interrupted by a "−1" indicates a $\beta$-strand, and a group of four or more "−1s" not interrupted by a "1" indicates a helix. All other regions are designated as coil. The chemical shift indexes of αCH for each amino acid residue in the octapeptide were calculated by this method and shown in Table 5. The results indicated that the octapeptide chains mainly adopted α-helix conformations. This structure was also proven by the CD spectrum (Figure 9) with two negative peaks at 209 and 222 nm.

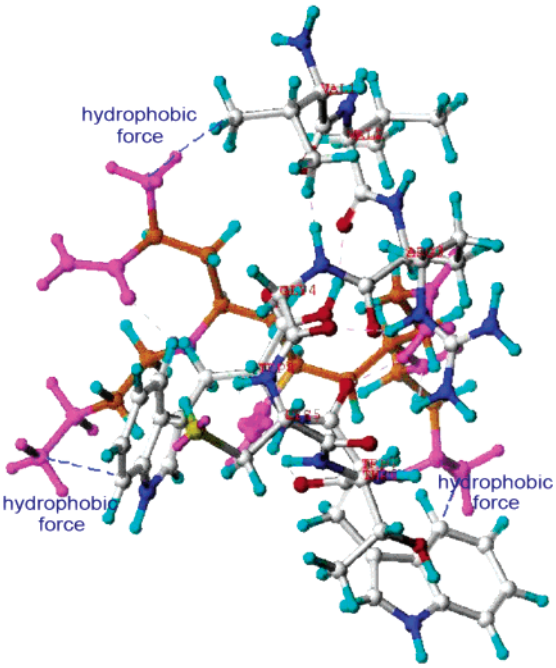


**Figure 8.** The interaction between octapeptide and butyl-modified PVM.

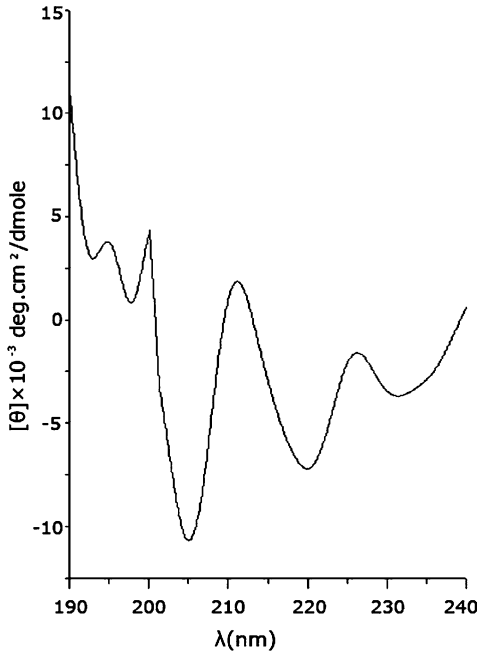


**Figure 9.** CD spectra of octapeptide in distilled $H_2O$ at 20 °C.

It is well known the change of conformation will result in denaturation of protein. Although octapeptide only has eight amino acid residues, the introduction of polymer may also

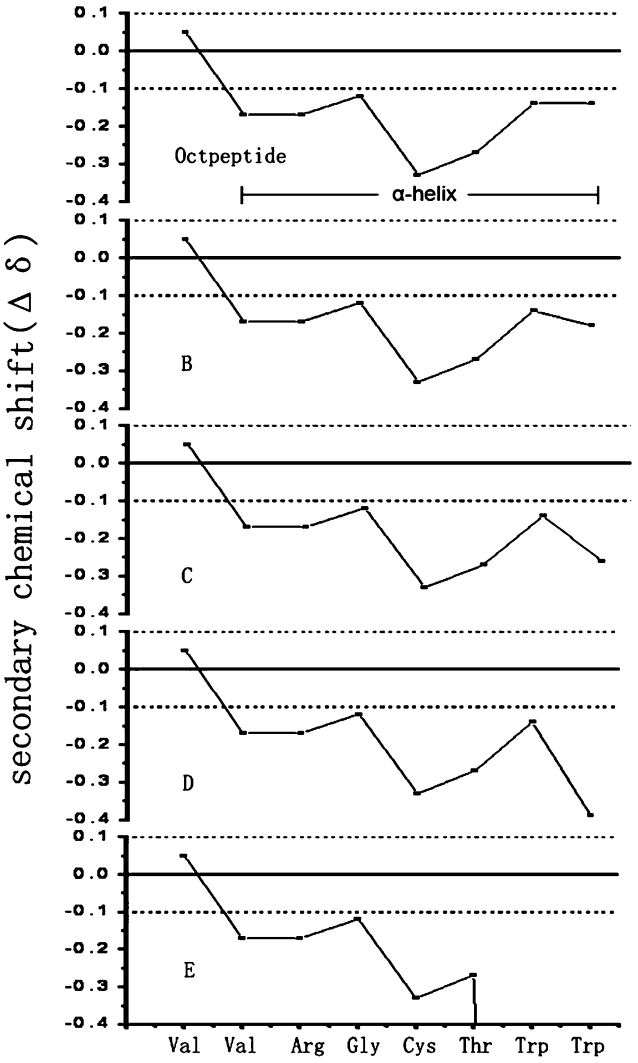


**Figure 10.** The secondary chemical shift and the secondary structure of octapeptide; (B) polyvinylamine; (C) dimethylamine-modified polyacrylamide; (D) diethylamine-modified polyacrylamide; and (E) butyl-modified polyvinylamine.

change the secondary structure of the peptide. This hypothesis was investigated by chemical shift indexes of αCH in the octapeptide when the interaction was investigated via NMR (Figure 10). In Table 5, the secondary chemical shift of octapeptide with different polymers showed that the signal of C-terminated amino acid residue was affected by polymers, but the conformation of the octapeptide did not change obviously.

## 4. Conclusion

With a model for adsorbents, it became realizable to investigate the interaction mechanism between adsorbents and a target, such as a peptide, by the 2D $^1$H NMR technique. The results suggested the hydrophobic interaction cooperating with electrostatic force played an important role in their binding with the peptide in aqueous solution. It rationally explains the results of the adsorption experiment and thus could be used in designing new adsorbents. Considering the fact that the amounts of many target compounds derived from living organisms are often too low to afford the traditional screening experiments for adsorbents, 2D $^1$H NMR analysis of a simulation system, with its high sensitivity, makes it an invaluable method for designing highly effective adsorbents in the future.

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## References and Notes

(1) Hörl, W. H. Hemodialysis membranes: interleukins, biocompatibility, and middle molecules. *J. Am. Soc. Nephrol.* **2002**, *13*, S62−S71.
(2) Aguilera, A.; Codoceo, R.; Selgas, R.; Garcia, P.; Picornell, M.; Diaz, C.; Sanchez, C.; Bajo, M. Anorexigen (TNF-alpha, cholecystokinin) and orexigen (neuropeptide Y) plasma levels in peritoneal dialysis (PD) patients: their relationship with nutritional parameters. *Nephrol., Dial., Transplant.* **1998**, *13*, 1476−1483.
(3) Haag-Weber, M.; Mai, B.; Hörl, W. H. Isolation of a granulocyte inhibitory protein from uraemic patients with homology of beta 2-microglobulin. *Nephrol., Dial., Transplant.* **1994**, *9*, 382−388.
(4) Winchester, J. F.; Ronco, C.; Brady, J. A.; Brendolan, A.; Davankov, V.; Tsyurupa, M.; Pavlova, L.; Pavlov, M.; Clemmer, J.; Polaschegg, H. D.; Muller, T. E.; La Greca, G.; Levin, N. W. Rationale for combined hemoperfusion/hemodialysis in uremia. *Contrib. Nephrol.* **2001**, *133*, 174−179.
(5) Peterson, R. G.; Peterson, L. N. Cleansing the blood. Hemodialysis, peritoneal dialysis, exchange transfusion, charcoal hemoperfusion, forced diuresis. *Pediatr. Clin. North Am.* **1986**, *33*, 675−689.
(6) Winchester, J. F. Sorbent hemoperfusion in end-stage renal disease: an in-depth review. *Adv. Ren. Replace Th.* **2002**, *9*, 19−25.
(7) Winchester, J. F.; Salsberg, J.; Yousha, E. Removal of middle molecules with sorbents. *Artif. Cells, Blood Substitutes, and Biotechnol.* **2002**, *30*, 547−554.
(8) Winchester, J. F.; Brady, J. A.; Ronco, C.; Yousha, E.; Smith, A.; Clemmer, J.; Muller, T. E.; La Greca, G.; Levin, N. W. The role of middle molecules in uremia—preliminary report of an international survey of nephrologists and scientists. Dialysis, Dialyzers and Sorbents. Where Are We Going? *Contrib. Nephrol.* **2001**, *133*, 23−27.
(9) Davankov, V.; Pavlova, L.; Tsyurupa, M.; Brady, J.; Balsamo, M.; Yousha, E. Polymeric adsorbent for removing toxic proteins from blood of patients with kidney failure. *J. Chromatogr., B* **2000**, *739*, 73−80.
(10) Hoffman, A. S.; Stayton, P. S.; Bulmus, V.; Chen, G.; Chen, J.; Cheung, C.; Chilkoti, A.; Ding, Z.; Dong, L.; Fong, R.; Lackey, C. A.; Long, C. J.; Miura, M.; Morris, J. E.; Murthy, N.; Nabeshima, Y.; Park, T. G.; Miura, M.; Morris, J. E.; Murthy, N.; Nabeshima, Y.; Park, T. G.; Press, O. W.; Shimoboji, T.; Shoemaker, S.; Yang, H. J.; Monji, N.; Nowinski, R. C.; Cole, C. A.; Priest, J. H.; Harris, J. M.; Nakamae K.; Nishino, T.; Miyata, T. Really smart bioconjugates of smart polymers and receptor proteins. *J. Biomed. Mater. Res.* **2000**, *52*, 577−586.
(11) Baba, Y.; Tsuhako, M.; Sawa, T.; Akashi, M.; Yashima, E. Specific base recognition of oligodeoxynucleotides by capillary affinity gel electrophoresis using polyacrylamide-poly(9-vinyladenine) conjugated gel. *Anal. Chem.* **1992**, *64*, 1920−1925.
(12) Yang, Z.; Mesiano, A. J.; Venkatasubramanian, S.; Gross, S. H.; Harris, J. M.; Russell, A. J. Activity and stability of enzymes incorporated into acrylic polymers. *J. Am. Chem. Soc.* **1995**, *117*, 4843−4850.
(13) Lu, Z. R.; Kopeckova, P.; Kopecek, J. Polymerizable Fab′ antibody fragments for targeting of anticancer drugs. *Nat. Biotechnol.* **1999**, *17*, 1101−1104.
(14) Wang, Sh. Q.; Yu, Y. T.; Cui, T.; Cheng, Y. A novel amphiphilic adsorbent for the removal of low-density lipoprotein. *Biomaterials* **2003**, *24*, 2799−2802.
(15) Cheng, Y.; Wang, Sh. Q.; Yu, Y. T.; Yuan, Y. In vitro, in vivo studies of a new amphiphilic adsorbent for the removal of low-density lipoprotein. *Biomaterials* **2003**, *24*, 2189−2194.
(16) Chu, J. G.; Yuan, Zh.; Liu, X. H.; Wu, Q.; Mi, H. F.; He, B. L. Separation of six uremic middle molecular compounds by high performance liquid chromatography and analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin. Chim. Acta* **2001**, *311*, 95−107.
(17) Li, G. H.; Chu, J.; Liu, X. H.; Yuan, Zh. Separation, identification of uremic middle molecules, and preliminary study on their toxicity. *Clin. Chim. Acta* **2004**, *350*, 89−98.

(18) Furness, E. L.; Ross, A.; Davis, T. P.; King, G. C. A hydrophobic interaction site for lysozyme binding to poly(ethylene glycol) and model contact lens polymers. *Biomaterials* **1998**, *19*, 1361−
(19) qj1369.
(20) Elachari, A.; Coqueret, X.; Lablache-Combier, A.; Loucheux, C. Preparation of polyvinylamine from polyacrylamide: a reinvestigation of the hofmann reaction. *Makromol. Chem.* **1993**, *194*, 1879−1891.
(21) Wu, Sh. H.; Shanks, R. A. Synthesis and characterisation of hydrophobic modified polyacrylamide. *Polym. Int.* **2004**, *53*, 1821−1830.
(22) Yuan, Zh.; Yang, D. F.; Shen, L. H.; He, B. L. Hofmann degradation of macroporous polyacrylamide resin. *Chin. Sci. Bull.* **1996**, *41*, 1957−1959.
(23) Yang, J. T.; Wu, C. S.; Martinez, H. M. Calculation of protein conformation from circular dichroism. *Methods Enzymol.* **1986**, *130*, 208−269.
(24) Sasagawa, T.; Titani, K.; Walsh, K. A. Selective isolation of tryptophan-containing peptides by hydrophobicity modulation. *Anal. Biochem.* **1983**, *134*, 224−229.
(25) Sasagawa, T.; Ericsson, L. H.; Teller, D. C.; Titani, K.; Walsh, K. A. Separation of peptides on a polystyrene resin column. *J. Chromatogr.* **1984**, *307*, 29−38.
(26) Wishart, D. S.; Sykes, B. D. Chemical shifts as a tool for structure determination. *Methods Enzymol.* **1994**, *239*, 363−392.

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