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Correlation study between molecular structure of sesquiterpene lactones and the selective adsorption performance of molecularly imprinted polymers

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ABSTRACT

We preliminarily report that the structure of template molecules and target components correlates with the selective adsorption performance of molecularly imprinted polymer (MIPs) in sesquiterpene lactones. Template molecules involved three categories of sesquiterpene lactones with distinct ring systems: 5-mem lactone ring atractylenolide III, 7-mem lactone ring dehydrocostus lactone, and 10-mem lactone ring costunolide lactone, of which the conformations were verified by variable-temperature ^{1}H NMR spectroscopy. Reciprocal MIPs were prepared by precipitation polymerization and employed as selective sorbents in the columns of solid phase extraction (SPE). These columns were further used for enriching the mixed adsorption solution of sesquiterpene lactone ingredients and reference components. Finally, the extract of *Radix Aucklandiae*, a Chinese medicine herb, was used to verify the efficiency of this method. Our results demonstrate that the steric conformational stability of molecules is associated with the selective adsorption of their corresponding MIPs. We have further observed that the maximum adsorption capacity occurs when the target molecule conformation is consistent with that of the template molecule. The addition of more hydrophilic groups correlates with weaker adsorption of MIPs. Our findings provide important information to help guide the selection of appropriate template molecules for synthesis of MIPs with specific adsorption.

1. Introduction

Molecularly imprinted polymers (MIPs) are polymers that have been processed using a molecular imprinting technique which leaves cavities in the polymer matrix that have affinity to a chosen "template" molecule. Due to their high selectivity, stability and efficiency, MIPs have been applied to many fields, such as environmental analysis, immunoassays biosensors, catalysts and chiral separation of drugs [1–5]. In recent years, molecular imprinting technique (MIT) has been applied to the directional separation of active ingredients in traditional Chinese medicines [6–11]. MIT has been mostly focused on investigating the preparation, characterization, adsorption properties and performance evaluation of MIPs [12–17], but further research regarding MIPs is rare. Several key questions about the application of MIT to Chinese medicine preparation remain unanswered: (1) Which structures in active ingredients are suitable as a template molecules to prepare MIPs? (2) What is the difference between the selective adsorption performances of MIPs for template molecules and for analogous structural compounds? (3) What variables exist between the structure of template molecules or target components and the adsorption performance of MIPs? Elucidating the answer to one or several of these questions would make a significant contribution to the field of active ingredient separation in traditional Chinese medicine.

In our recent research, more than 20 kinds of active ingredients in traditional Chinese medicines were chosen as template molecules to prepare the corresponding MIPs by precipitation polymerization. The experimental results showed that not all compounds were suitable for the MIPs preparation, and that the selective adsorption performance of MIPs prepared by some template molecules was poor. It was noted that the selective

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adsorption performance of MIPs prepared by lipophilic template molecules was significantly better than MIPs prepared by hydrophilic template molecules. This may be because the supramolecular interactions between the template molecule and the functional monomer were not easily damaged. Similarly, the binding sites with strong affinity to the target molecules on the molecular cavities of synthetic MIPs still remained during the process of polymerization. A question as of yet unanswered is whether the lipophilic compounds are suitable as template molecules.

In this study some lipophilic compounds were used as template molecules, and the correlation between template molecule structures, target molecule structures and the selective adsorption performance of the corresponding MIPs was explored. Three types of sesquiterpene lactones with different structures and activities were selected as template molecules to prepare the corresponding MIPs by precipitation polymerization and then used to prepare MIP-SPE columns. These columns were used for enrichment and separation of the adsorption test solutions mixed with three similar sesquiterpene lactones ingredients and one reference compound. The stability of template molecule conformation was tested by variable-temperature ^{1}H NMR spectroscopy. The relationship between MIPs structure and its selective adsorption performance was explored using the Chinese medicine *Radix Aucklandiae* extract. Furthermore, the difference of the adsorption performance for sesquiterpene lactones between MIP-SPE columns and C_{18}-SPE columns was detected by the adsorption test solutions. This study offers important references for effectively designing and synthesizing MIPs separation materials with acceptable selective adsorption properties.

2. Experimental

2.1. Materials and apparatus

All reagents were of analytical grade or better. Atractylenolide III, costunolide lactone and dehydrocostus lactone were purchased from Zelang Pharmaceutical Co., Ltd. (Nanjing, China, more than 99% purity). 1-Vinylimidazole (1-viny) and 4-vinylbenzoic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ethylene glycol dimethacrylate (EDMA) and trimethylolpropane trimethacrylate (TRIM) were purchased from TCI Development Co., Ltd. (Shanghai, China). 2′,2-Azo-bis-isobutyronitrile (AIBN) was Shanghai No. 4 Reagent & H.V. Chemical Co., Ltd. (Shanghai, China) product. Acetonitrile and methanol of HPLC grade were purchased from Merck (Darmstadt, Germany). Water was doubly distilled.

Isofraxidin, istanbulin A and lasianthuslactone A were separated by our laboratory from the herbal medicine *Chloranthus henryi* Hemsl, and their purities were 98.2%, 98.6% and 98.0%, respectively.

The UV spectrometer used was UV-1750 (Shimadzu, Japan). The HPLC system was an Agilent-1200 (Agilent, USA). ^{1}H NMR spectra were recorded on a Varian VNMRS-500 spectrometer. The incubation oscillator was a vortex genie 2 (Scientific Industries, USA).

2.2. Synthesis of sesquiterpene lactones MIPs

A specific amount of atractylenolide III was dissolved in acetonitrile and then mixed in a 250 mL round bottom flask with the functional monomer (1-viny) and the cross-linker (EDMA) according to the molar ratio of 1:5:8. Next, 23 mg of AIBN was added into the mixed solution. The mixture was sparged with oxygen-free nitrogen for 15 min and sealed under vacuum. The polymerization was carried out in a water bath at 60 °C for 24 h. After the reaction, the obtained polymers were collected by centrifugation at 10,000 rpm for 10 min. Methanol–acetic acid (9:1, v v^{-1}) was used to remove the andrographolide using the Soxhlet extraction until there was no template molecule to be detected by UV (at 220 nm) in the eluate. The polymers were then eluted by methanol to remove the remaining acetic acid and then dried under vacuum for 2 h.

The costunolide lactone MIPs and the dehydrocostus lactone MIPs were prepared by the same protocols as the atractylenolide III MIPs, with specific conditions listed in Table 1. The flowchart of MIP preparation can be seen in Fig. 1.

2.3. Preparation of molecularly imprinted solid phase extraction column

Next, 100 mg of atractylenolide III, costunolide lactone and dehydrocostus lactone were packed into a solid phase extraction cartridge. At first, the polymers were pre-equilibrated with methanol and then dried. After the preparation of MIP-SPE columns, the steps of loading, washing and elution vary slightly according to the different experimental conditions (see Sections 2.4, 2.6 and 2.7). The process of MIP-SPE preparation is illustrated in Fig. 2.

2.4. Determination of maximum loading capacity and enrichment factor of SPE columns

The standard solutions of atractylenolide III, costunolide lactone and dehydrocostus lactone were respectively prepared by methanol as a solvent with a concentration of 30 μg mL^{-1}. A 5 mL atractylenolide III standard solution was added gradually into the atractylenolide III MIP-SPE column, costunolide lactone MIP-SPE column, dehydrocostus lactone MIP-SPE column and C_{18}-SPE column, respectively. The flow rate was set at 0.2 mL min^{-1} and the filtrate was collected. The SPE columns were eluted by 5 mL methanol and the eluate was collected and then the eluant was filtered by 0.22 μm filter for HPLC analysis. Standard solutions of costunolide lactone and dehydrocostus lactone were dealt with by costunolide lactone MIP-SPE columns and dehydrocostus lactone MIP-SPE columns, respectively, and the remaining steps follow the same protocol as above.

2.5. The competitive adsorption experiments

MIP-SPE column was pre-treated with the solution of target components. One is template molecule, the other is reference component. The reference component is also a competitive compound. The composition and concentration of solutions that flow through each MIP-SPE column are shown in Table 3. Five milliliters of mixed solutions were added into the corresponding MIP-SPE columns. The flow rate is 0.2 mL min^{-1} and the MIP-SPE columns are eluted with 5 mL of methanol. Then, collect and filter the eluent, respectively, by 0.22 μm filter membrane for HPLC analysis.

2.6. Selective adsorption experiments

Next, 10 mg standard samples of atractylenolide III, isofraxidin, istanbulin A and lasianthuslactone A were weighed and placed in 10 mL flasks, respectively, and prepared by a stock solution of methanol with a concentration of 1 mg mL^{-1}. Then, 50 μL of each of the four stock solutions was mixed and diluted with methanol to 50 mL. The adsorption test solutions were prepared and used for the following experiments.

A 1 mL adsorption test solution was added into the atractylenolide III MIP-SPE column, the costunolide lactone MIP-SPE column, the dehydrocostus lactone MIP-SPE column and the C_{18} column in turn. The flow rate was set at 0.2 mL min^{-1}. After the sample solution passing through the SPE column, the SPE column was washed by 3 mL of 20% methanol. Then, 5 mL of methanol was used to elute