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Investigation of enantiomer recognition of molecularly imprinted polymeric monoliths in pressurized capillary electrochromatography screening the amino acids and their derivatives

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ABSTRACT

Molecularly imprinted monolithic columns were prepared for chiral separation of tyrosine and its amino acid derivatives by in situ therm-initiated copolymerization of methacrylic acid, 4-vinylpyridine and ethylene glycol dimethacrylate. The enantiomers were rapidly separated on monolithic columns in less than 10 min by pressurized capillary electrochromatography (pCEC). The influences of several parameters such as the content of cross-linking monomer on the composition of the pre-polymerization mixture were systematically investigated. The influence of the pCEC conditions including the composition of the mobile phase was also optimized to obtain the good enantioseparation. It was found that in addition to molecularly imprinted recognition, chromatographic retention and electrophoretic migration play important roles in the retention and chiral recognition of molecularly imprinted polymer (MIP) columns. The cross-selectivity for similar amino acids and its derivatives were systematical investigated for understanding the recognition mechanism on the MIP monolithic columns. The results indicated that molecularly imprinted polymer recognizes the template molecule by its molecular shape defined binding cavity.

1. Introduction

Molecularly imprinted polymers (MIPs) with a memory for the template have the potential to be powerful materials in the separation of chiral compounds, predicting not only the recognition ability but also the elution order [1–6]. Applications of molecularly imprinted polymer as separation media in liquid chromatography (LC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) for chiral separation have been extensively investigated [7–9]. CEC is considered to combine the advantages of the high separation efficiency of CE and the various retention mechanisms and selectivity offered by HPLC [10]. The MIP monolith format of CEC leads to a minimal consumption of chemicals, especially the imprinted molecule. So, CEC-based MIP monoliths are a promising tool for the research of the recognition properties of MIPs [11–13].

Understanding the underlying principles governing the formation of binding sites and rebinding behavior of MIPs is important for predicting the performance of template-targeted binding and cross-reactivity. In recent years, many attempts have been made to research the interaction mechanisms between the cross-linked polymer and the template, or similar molecule both theoretically and experimentally [14–17]. The origins of molecular recognition in MIPs are generally acknowledged to arise from pre-organization of functional groups within a shape selective cavity [18–20]. Also, the retention processes were interplay of multiple mechanisms of ion-exchange, electrophoresis and molecular imprinting, in which the molecular recognition is more profound. However, there are very few papers that report systematic cross-selectivity studies, which attempt to identify the key parameters in the imprinting process.

Amino acid and its derivatives were found to be suitable as the molecular imprinting template due to its molecular structure and functional group. In recent years, reports about the amino acids and its derivatives imprinted chiral monolithic column were abundant [21,22]. However, few reports have systematically investigated similar amino acids and its derivatives on the MIP monolith and research the interaction mechanisms of specific rebinding of the template [23], most investigations of MIPs concentrated on enantiomeric recognition, whereas investigations concerning the various structures of ligands have not been focused on to the same degree. In our study, five kinds of amino acid derivatives (for structures, see Fig. 1) were chosen and comparisons of their retentions and separations by different MIPs in pCEC in order to reveal recognition mechanism on the MIP monolithic columns were made.

The objective of this study was to investigate the properties of MIP monolithic column, and study the interactions between the template and functional monomers in the recognition process by the pCEC-based MIP monoliths. The cross-selectivity, the influence

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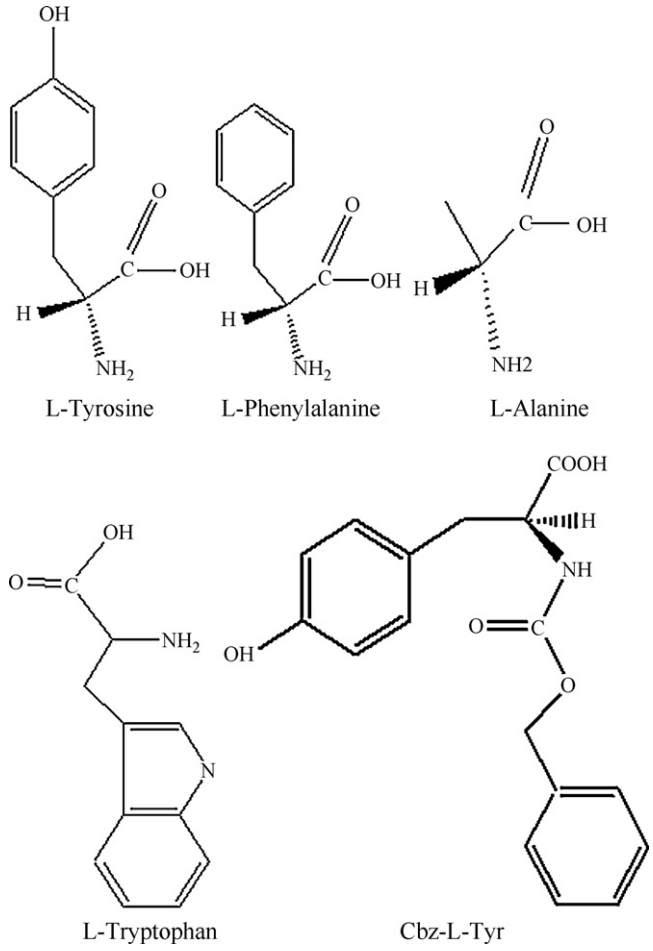

Fig. 1. Structures of template molecules of L-tyrosine (L-Tyr), L-phenylalanine (L-Phe), L-alanine (L-Ala), L-tryptophan (L-Trp), Cbz-L-tyrosine (Cbz-L-Tyr).

of several parameters about preparation of polymeric monolithic columns and the pCEC conditions including the composition of the mobile phase to obtain the good enantioseparation were systematical survey for understanding the recognition mechanism on the MIP monolithic columns. Under the optimized conditions, the recognition ability was improved and the peak-broadening and peak-tailing has been overcome to some extent.

2. Experimental

2.1. Instrumentation

pCEC was carried out on a Trisep 2100GV CEC system (UnimicroTechnologies, Pleasanton, CA, USA) which comprised a high-voltage power supply (+30 and −30 kV), a variable wavelength UV/vis detector, a solvent gradient delivery module (0.01–3.0 mL/min), a micro fluid manipulation module with a six-port injector and a data acquisition module, as described in literature [24]. Voltage was applied to the outlet of column, and the inlet of column was connected to the split valve and grounded. A supplementary pressure was applied to the column inlet during the separation. The μ-HPLC system was that the pCEC instrumental system without the application of voltage. In this experiment, the isocratic elution system was used. An HPLC pump was used to flush monolithic columns. Scanning electron microscopy (SEM) of the silica monolith was carried out on an XL30 scanning electron microscope (Philips, Eindhoven, The Netherlands).

2.2. Chemicals and materials

L-Ala, DL-Ala, L-Trp, DL-Trp, L-Phe, DL-Phe, L-Tyr, DL-Tyr, N-benzyloxycarbonyl-L-tyrosine (Cbz-L-Tyr), Cbz-DL-Tyr, ethylene dimethacrylate (EDMA), 3-trimethoxysilyl-propyl methacrylate (γ-MAPS), 4-vinylpyridine (4-VPY), and methacrylic acid (MAA) were purchased from Acros (New Jersey, USA). Azobisisobutyronitrile (AIBN) was obtained from the Fourth Chemical reagent Plant (Shanghai, China). Toluene and dodecanol were purchased from Tianjin Chemical Plant (Tianjin, China). HPLC-grade methanol and acetonitrile (ACN) were purchased from Chemical Reagent Corporation (Shanghai, China). The water used throughout all experiments was purified with a Millipore Milli-Q purification system (Milford, MA, USA). The fused-silica capillaries with a dimension of 100 μm I.D. (375 μm O.D.) were purchased from the Yongnian Optic Fiber Plant (Hebei, China).

2.3. Single-step preparation of polymeric monolithic columns

In order to covalently anchor the polymer to the capillary wall, the capillary was treated with a vinyl silanizing agent. Firstly, the capillary was rinsed with 1 M NaOH solution for 30 min, and then, with purified water until the pH value of the outlet solution reached 7.0. After subsequent flushing with methanol for 10 min, it was dried by the passage of nitrogen in a GC-oven at 60 °C. Then γ-MAPS/MeOH (1:1, v/v) solution was injected into the capillary with a syringe. It was then kept at 60 °C in a thermostatic bath overnight with both ends sealed with rubber. Finally, the capillary was rinsed with MeOH to flush out the residual reagents. The template (L-Tyr), functional monomer (MAA) and (4-VPY), cross-linker (EDMA), and initiator (AIBN) were dissolved into toluene–dodecanol composed as described in Table 1. The pre-polymerization mixture was sonicated for 15 min and sparged with nitrogen for 10 min to obtain a homogeneous solution and remove dissolved gases, and then injected into the pretreated capillary. The capillary was plugged at both ends with GC septa and submerged into a thermostatic bath for 12 h. The resultant monolithic capillary column was flushed with methanol–acetic acid (9:1, v/v) for about 1 h using an HPLC pump to remove the template molecule, porogens and unreacted monomers. A detection window was created at the end of the continuous polymer bed by burning out 1–2 mm segment of the polyimide outer coating. The other MIP monolithic columns were prepared according to the procedures described above. A blank capillary column without imprint molecule was prepared in the same way.

2.4. Capillary electrochromatography

The mobile phase was a mixture of acetonitrile and different ratios of buffer with different pH. All acetate buffers were filtered with 0.22 μm membrane. Applied voltage was operated at −10 kV. UV detection was performed at 230 nm.

Retention factor, k, was calculated by using the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of the analyte and the void marker (acetone), respectively. The enantioseparation factor, α is defined as the ratio of the retention factor of the lately eluted to the early eluted enantiomer, $\alpha = k_2/k_1$. The resolution is calculated from the equation $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times of the first and second eluted enantiomers, respectively, and w_1 and w_2 are the baseline peak widths of the first and second eluted enantiomers, respectively.