Molecular Imprinting in Hydrogen Bonding Networks of Polyamide Nylon for Recongnition of Amino Acids

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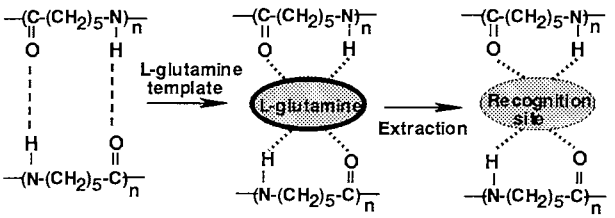
Molecularly imprinted Nylon-6 was prepared from the polymer solution of formic acid having L-glutamine template. The recognition of amino acids by L-glutamine imprinted-polymer was evaluated by binding experiments for L-glutamine and its analogues. It was revealed that the recognition was effective for L-glutamine by the imprinted polymer as compared with its racemate D-glutamine and L- or D-glutamic acid.

There are numerous reports and reviews documented on selective recognition of small molecules by molecularly imprinted polymers (MIPs).[1-4] These polymer materials have been used for biological and chemical processes involving chromatography,[5,6] solid phase extraction,[7] and separation membranes.[8-11] Since these show specific recognition characteristics to template molecule through hydrogen bonding, the unique characteristics of MIPs are that the tailored selectivity can be applicable for each specific use. For uses of protein, which is a kind of polyamide, there are some reports about molecular imprinting.[12-14] Using synthetic polyamide such as Nylon for MIPs, however, little is known. It is worth noting that Nylon-6 has inter- and intra-molecular hydrogen bonding through its amide group (-NH---O=C-) (Scheme 1). Such polyamide which is commercially available polymer has high strength nature and possesses desired functionality in practical applications.[15] Thus, we here tried to prepare imprinting polymer using Nylon-6. In this case, the ability of hydrogen bonding in polyamide is capable of using for MIP. The interactions of hydrogen bonding between the L-glutamine and active sites of amide groups in Nylon-6 can subsequently drive the specific molecular recognition process.

In this letter, we showed preliminary results of new type of molecularly imprinted Nylon-6 for recognition of L-glutamine and determined their recognition properties with various amino acids. Using formic acid solution of Nylon-6 and L-glutamine template, imprinted polymers were prepared by phase inversion method.[16] For the amino acid recognition by the L-glutamine imprinted Nylon-6, L-glutamine, D-glutamine, L-glutamic acid, and D-glutamic acid were selected as substrates to examine the imprinting ability. For amino acids binding to the imprinted Nylon-6, batch experiments were carried out at 30°C; known amounts of the polymer were taken in 10 μM solution of each above amino acid and the amino acid concentration at different times was determined by HPLC.[16]

Figure 1 shows the amounts (μmol/g-polymer) of the substrate taken by the imprinted Nylon-6 at different times. It is clearly indicated that the binding amounts of amino acids by the L-glutamine imprinted polymer increased with time and after 24 h the binding amounts became almost constant. At the equilibrium condition the amino acid recognition (μmol/g-polymer) was



Scheme 1. Schematic presentation of hydrogen bonding interactions between Nylons and amino acid.

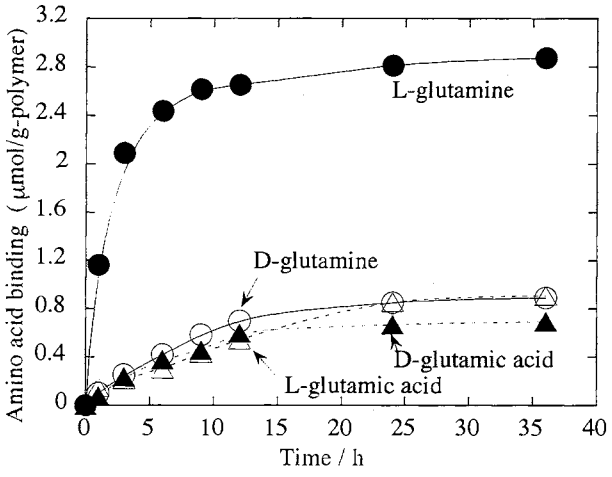


Figure 1. Amino acids binding by L-glutamine-imprinted Nylon-6.

2.8 for L-glutamine, 0.85 for D-glutamine, 0.86 for L-glutamic acid, and 0.67 for D-glutamic acid. This showed that the recognition of the L-glutamine by the imprinted Nylon-6 was substantially high as compared to that for other amino acids. Imprinted data also indicate that the chiral recognition by the Nylon-6 was possible. This may be explained by the specific interactions between the polymer and L-glutamine template. In order to examine the interaction of template with the polymer, FT-IR measurements were carried out. Figure 2 shows FT-IR spectra for the imprinted polymer before and after the template extraction.[17] The absorption of free amide group in Nylon-6 was clearly observed as a shoulder at 3450 cm^{-1} in the spectra.[18] It was noted that there was high absorption of the shoulder peak at that wavenumber after extraction of L-glutamine from the imprinted Nylon-6. This suggests that the hydrogen bonding of the free amide group may be exclusively responsible for the selectivity of the amino acids with respect to the L-glutamine template; the template molecule may interact through hydrogen

bonding in the Nylon-6 hydrogen bonding networks. For the Nylon-6 prepared without the template molecule, the FT-IR spectrum was the same as that shown in Fig. 2b). This indicated

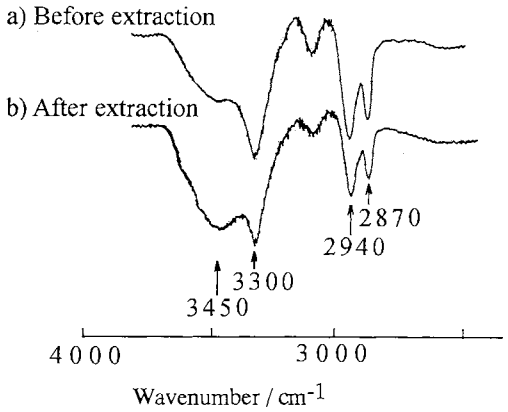


Figure 2. FT-IR spectral analysis of L-glutamine-imprinted Nylon-6.

that, although the free amide groups was present in the polymer, the binding experiment gave the evidence for no amino acid recognition by the polymer. Thus, the sites can not recognize L-glutamine molecules without molecularly imprinted process. The detailed properties of Nylon-6 imprinting for amino acids recognition will be published elsewhere.

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16 Nylon-6 was a product of Mitsubishi Chemical Co. having 2×10^4 molecular weight. The polymer solution for the method was contained 20 wt% of Nylon-6 and 8 wt% of the template, L-glutamine, dissolved in formic acid at 50 °C for 24 h. The polymer solution casted on a glass plate at 50 °C with 100 μm thickness and then immersed in distilled water bath at 21 °C. After coagulation of the casted solution in water, the polymer was well washed with distilled water to remove excess solvent and also template. The template molecule was subsequently removed from the solidified polymer by washing it with 2 wt% acetic acid solution at 40 °C over 30 days. The template and formic acid removal was subjected by FT-IR measurement of the polymer and aiso HPLC analysis for no template leak from the polymer into the acetic acid solution. Concentration of the solutes was determined with HPLC (TOSOH CCPS with ODS-80Ts) monitored by the absorption at 210 nm using UV8020 detector. The amide I and II peaks[17] observed in FT-IR spectra were also checked to make sure the absence of formic acid remained in resulting polymer .
17 IR spectra were measured using Shimadzu FT-IR-8200, in (KBr) 3450 cm^{-1}, 3300 cm^{-1} (NH) hydrogen bonding, 2940 cm^{-1}, 2870 cm^{-1} (CH_2) stretching, 1690 cm^{-1} (C=O), 1660 cm^{-1} (amide I), 1560 cm^{-1} (amide II), 1474 cm^{-1} (CH_2) (N), 1460 cm^{-1} (CH_2) and (C-N) stretch, 1435 cm^{-1} (CH_2) (N, CO), 1275 cm^{-1} (amide III), 690 cm^{-1} (amide IV), and 580 cm^{-1} (amide V).
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