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Novel strategy for molecular imprinting of phenolic compounds utilizing disulfide templates

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

A molecularly imprinted polymer was synthesized by using allyl phenyl disulfide as a template. The mixture of allyl phenyl disulfide, divinylbenzene, and 2,2′-azobis(isobutyronitrile) in chloroform was polymerized by UV irradiation for 24 h at 5 °C and further 3 h at 80 °C. The disulfide bonds of the resulting polymer were reductively cleaved by $NaBH_4$ in methanol to give thiol groups in the binding sites. The polymer selectively recognized phenol rather than thiophenol. In chromatographic study using polymer-packed columns, the retention factor of the IP for phenol was 5.60 and that of a reference polymer was 4.20. The higher retention for phenol was supported by ab initio calculation.

Keywords: Molecularly imprinted polymer; Disulfide; Thiol; Ab initio

1. Introduction

Molecular imprinting [1–6] is a useful and simple method for the preparation of tailor-made recognition materials. The polymer that has high recognition affinity only to a target molecule can be made by the use of the complexation between the target and functional monomer(s). For the complexation, a non-covalent bond and a covalent bond are used. In general, non-covalent interactions such as hydrogen-bondings and electrostatic interactions are best used, because non-covalent complexes are simply generated only by mixing of a template molecule and functional monomer(s) in pre-polymerization mixture. Since equilibrium exists in the formation of non-covalent bond complexes, the complexes formed during polymerization have various forms and, as a result, the affinity of recognition sites is widely distributed. This is clear by a spread peak of a chromatogram at the time of filling up a column with imprinted polymer [7,8], by Scatchard analysis [9], and by

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affinity distribution analysis [10,11]. On the other hand, the complexes are formed by strong bond(s) in covalently imprinted polymers, in which the homogeneity of the recognition ability is high. However, since the covalent bond(s) need to be cleaved after polymerization, covalent bonds that can be used are limited. If stronger covalent bonds like ester and amide are used, the covalent bond in the polymer cannot be hydrolyzed completely and the proportion of the recognition sites generated could decrease. Therefore, only comparatively weak covalent bonds such as boronic acid [12,13], carbonate ester [14], Schiff base [15,16], acetal/ketal [17,18], and coordination bonds [19–21] can be used for the effective formation of imprinted polymers.

We now report on a novel strategy for molecular imprinting, in which disulfide bond is used for imprinting process and, after polymerization, the disulfide bonds are reductively cleaved to yield thiol groups for the recognition of a target molecule. The interconversion of disulfide and thiol is relatively facile among covalent bonds, and it plays an important role in the structure maintenance of protein and in the reaction of enzyme; however, the interconversion is never applied to the synthesis of molecularly imprinted polymers.

2. Experimental

2.1. General

Chloroform and divinylbenzene (mixture of *m*- and *p*-) were distilled prior to use. Other reagents were purchased and used without further purification. *N*-(Phenylthio)succinimide was synthesized according to the method given in the literature [22].

Chromatographic studies were conducted with an HPLC system consisting of 322 pump, UV/Vis-152 detector, 234 auto injector (Gilson), and 503 degasser (M & S Instruments).

2.2. Synthesis of allyl phenyl disulfide (*1*)

A solution of *N*-(phenylthio)succinimide (0.424 g, 2.0 mmol) and allyl mercaptan (0.148 g, 2.0 mmol) in benzene (10 ml) was heated at 60 °C over night. After filtration, the filtrate was purified by silicagel chromatography (Wako gel C-200, benzene–hexane, 2:1, v/v) to give **1** as a colorless oil. The purity checked by NMR was sufficient to be used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 3.33$ (d, $J = 7$ Hz, 2H), 5.09–5.15 (m, 2H), 5.73–5.87 (m, 1H), 7.16–7.55 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 41.7$, 118.9, 126.7, 127.7, 128.8, 132.5, 137.2; IR (NaCl) 3073, 2909, 1580, 1477, 1437, 1219, 1071, 1024, 987, 921, 740, 680 cm^{-1}.

2.3. Synthesis of polymers

The imprinted polymer was synthesized as follows; the prepolymerization mixture of **1** (0.94 g, 5.2 mmol) as a template, divinylbenzene (12.35 g, 95 mmol) as a crosslinking agent, and 2,2′-azobis(isobutyronitrile) (360 mg, 2.2 mmol) as an initiator in chloroform (10 ml) was degassed with nitrogen for 5 min, and was polymerized by UV light irradiation (254 nm) for 24 h at 5 °C and further 3 h at 80 °C. The resulting polymer was ground and sieved to yield the polymer particles (26–32 μm). The reference polymer (RP) was synthesized by the use of allyl mercaptan instead of **1** and the blank polymer (BP) was synthesized as the same manner without addition of **1**. The particles (2.0 g) of IP were suspended in methanol (50 ml) and were cleaved by $NaBH_4$ for 12 h. This cleavage procedure was repeated further two times.

2.4. Chromatographic study

Three kinds of the polymer particles were independently slurried in methanol and packed in stainless steel columns (150 × 4.6 mm, i.d.). The columns were washed successively with methanol–acetic acid (7:3, v/v, 100 ml), ethyl acetate (100 ml), and hexane (100 ml). The retention factors, k', of phenol, thiophenol, aniline, and pyridine were measured by the use of these columns. Hexane

was used as eluent at a flow rate of 1.0 ml min^{-1}. The injection volume was 20 μl (10 mmol l^{-1}) and the detection was carried out at 263 nm. Retention factors were calculated by the equation, $k' = (t_R - t_0)/t_0$, where t_R is the retention time of a sample and t_0 is the time to elute a marker (toluene).

2.5. Ab initio calculation

The hydrogen-bonding ability of samples (phenol, thiophenol, aniline, and pyridine) with methyl mercaptan, which is used in the place of thiol group at binding site, was estimated by ab initio calculation with GAUSSIAN 98W [23] based on Hartree–Fock/6-31G(d). The hydrogen-bonding energy, ΔE_{HB}, can be expressed as:

$$\Delta E_{HB} = E(A{:}B) - [E(A{:}\beta) + E(\alpha{:}B)] \quad (1)$$

This equation means the stabilization energy between the two molecules at the geometries optimized for the supermolecule A:B. In the calculations, the basis set superposition error (BSSE), which often affects substantially the calculated stabilization energies, was corrected by means of the counterpoise method [24]. In this equation, E(A:B) indicates the total energy optimized for the supermolecule A:B. The $E(A{:}\beta)$ means the total energy of molecule A with additional basis sets β put on the position of molecule B, and $E(\alpha{:}B)$ for molecule B with additional basis sets α put on the position of molecule A.

3. Results and discussion

The retention factors of three imprinted polymers for phenol, thiophenol, aniline, and pyridine are shown in Table 1. The imprinted polymer hardly retained thiophenol in spite of covalently thiophenol-imprinting, while phenol was significantly retained. This result indicates that the thiol group in the binding sites can hardly recognize thiol group of thiophenol due to the weaker hydrogen-bonding ability of thiol group but can recognize the hydroxyl group of phenol for relatively strong hydrogen-bonding.

The hydrogen-bonding ability of thiol with phenol and thiophenol was estimated by ab initio calculation. As shown in Table 2, the hydrogen-bonding strength of phenol with methyl mercaptan is higher than that of thiophenol, supporting the higher retention of phenol to IP. Although the reference polymer having thiol group, RP, has also showed the retention for phenol, the retention factor of IP (5.64) is higher than that of RP (4.20), indicating that the increment of the retention factor is caused by the imprinting effect. If the imprint effect by using allyl phenyl mercaptan is absence, the retention factors of IP and RP should be the same. The deference of the retention factors exactly derives from the imprint effect, that is, the cavity suitable to phenyl group can be constructed by the phenyl group of allyl phenyl disulfide. After the reductive elimination of thiophenol by $NaBH_4$, the binding cavity generated can be complementary to phenyl group (perhaps by π–π interaction between phenyl rings of **1** and divinylbenzene). The blank polymer having no thiol groups, BP, has slightly retained phenol. Therefore, this strategy can be applicable to the imprinting of phenolic compounds by the use of thiophenol derivatives instead of the phenolic compounds. Despite the hydrogen-bonding ability of aniline and pyridine is

Table 1
Retention factors, k', of imprinted, reference, and blank polymers

	IP	RP	BP
Phenol	5.64	4.20	1.80
Thiophenol	0.84	0.76	0.89
Aniline	0.84	0.97	1.63
Pyridine	1.01	1.14	1.59

$k' = (t_R - t_0)/t_0$, where t_R is the retention time of a sample and t_0 is the time to elute a marker (toluene). Eluent, hexane; flow rate, 1.0 ml min^{-1}; sample concentration, 10 mmol l^{-1}.

Table 2
Ab initio calculation of hydrogen-bonding energy of the complexation with methyl mercaptan

	ΔE_{AB} (kcal mol^{-1})
Phenol	−2.67
Thiophenol	−0.82
Aniline	−1.56
Pyridine	−1.57

expected to be comparable to that of phenol from the above calculation, their retentions for IP were significantly lower. These results are thought not to be lower hydrogen-bonding ability of amino group and pyridine with thiol group, but to be lower suitability of aniline and pyridine in the binding cavities. The shape of binding cavities may be formed to be suitable for the formation of hydrogen-bonding between phenolic hydroxyl group and thiol group in the binding sites, so aniline and pyridine could not be fitted to the direction of thiol groups for the formation of favorable hydrogen-bonding in the binding cavities (Fig. 1).

4. Conclusions

We developed the novel system for the synthesis of phenolic compounds—imprinted polymers, in which the imprinting by disulfide bond and the recognition by hydrogen-bondings based on thiol group were used. In general, it is difficult to prepare molecularly imprinted polymers for small molecules. Even though the further improvement of the recognition ability will be needed, our strategy for molecularly imprinted polymers using disulfide-imprinting/thiol-recognition system is effective for the imprinting of small molecules.

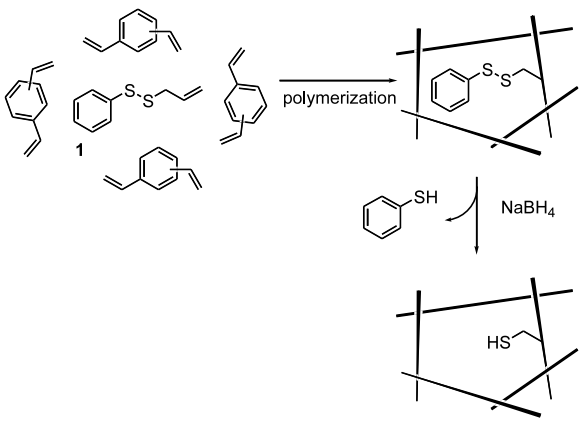

Fig. 1. Schematic representation of molecular imprinting using allyl phenyl disulfide (**1**) as a template and divinylbenzene as a crosslinking agent. After photopolymerization, a reductive cleavage of disulfide bonds was performed by $NaBH_4$ to give the IP having thiol groups in the binding sites.

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