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Molecular imprinting of bisphenol A and alkylphenols using amylose as a host matrix

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A novel molecularly imprinted polymer has been created by a radical polymerisation of acryloylamylose in the presence of a template.

Environmental pollution is an essential problem which has to be solved for human beings' well being in the future. A recently recognized issue is the endocrine disrupting activity of chemical substances such as bisphenol A (BisA) and alkylphenols.[1] For the recovery of these pollutants from the environment, it is necessary to develop materials by which the pollutants are efficiently captured. Here, we present a novel molecular imprinting strategy to obtain molecular recognition materials targeting BisA and alkylphenols. Amylose, a (1 → 4) α-D-glucan has a unique nature to form helical structures, and hydrophobic molecules are encapsulated within the cavity of the helix.[2] The helical pitch is changeable depending on the bulkiness of the encapsulated molecule.[3] If the helical conformation is fixed by a crosslinking reaction, it is expectable that the resultant polymer retains a higher-order structure suitable for the rebinding of the originally encapsulated molecule (template). Shinkai *et al.*[4] and Wulff and coworkers[5] had independently studied a similar idea; in their studies amylose-template inclusion complexes are crosslinked by utilising the reaction between a crosslinker (cyanuric chloride or epichlorohydrin) and hydroxyl groups in amylose. Their method had been proved to be an effective method to obtain molecularly imprinted polymers. However, a serious limitation exists in their method, *i.e.* it is not applicable to templates having hydroxyl groups such as BisA and alkylphenols because hydroxyl groups in the templates could also react with the crosslinkers. To solve this drawback, we have been developing a novel molecular imprinting strategy utilising a radical polymerisation process. Fig. 1 shows the outline of the method. First, amylose is modified with vinyl groups, then the modified amylose (acryloylamylose) is complexed with a template and then co-polymerised with a crosslinker. After thoroughly washing the resultant polymer to remove the template, the binding affinity of the imprinted polymer is examined.

Acryloylamylose was obtained by reacting native amylose (M_n = 16,000) with acryloyl chloride in DMF containing LiCl and trimethylamine at 25 °C for 3 h. The degree of substitution (DS[6]) was controlled in the range of 0.16–0.28 depending on the reactant ratio of acryloyl chloride to amylose. The complexation between acryloylamylose and a template was carried out by dispersing the solid template in an aqueous acryloylamylose solution and stirring it at 25 °C for 12 h. After the separation of the supernatant solution by centrifugation and filtration, the amount of complexed templates was estimated by measuring the solubility increase of the templates (Table 1). The number of anhydroglucose units per complexed template was, in practice, in the range 27.0–65.0. From examination using CPK models, we estimated that about ten and seven anhydroglucose units are enough to encapsulate one molecule of BisA and alkylphenols, respectively. It is believed that an amylose chain adopts a random coil conformation in the absence of guest molecules, and then changes to an interrupted helix by complexation with a hydrophobic guest.[7] Hence, significant numbers of anhydroglucose units must not be involved in the complexation with templates. Supposing that one molecule of BisA and alkylphenols interact with ten and seven anhydroglucose units, respectively, one can calculate that 37% (BisA), 11% (thymol) and 25% (*m*TBPh) of anhydroglucose units are involved in the complexation with each template.

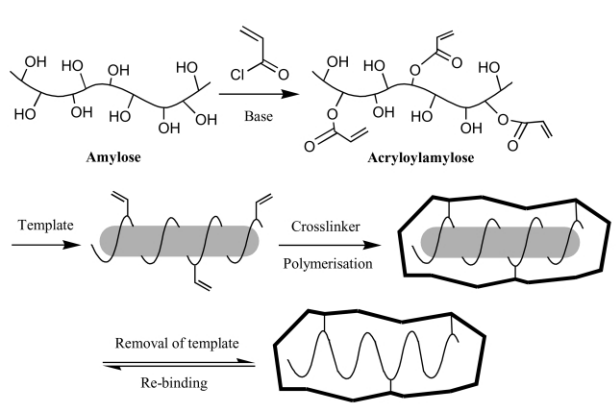

Fig. 1 Outline of molecular imprinting using amylose.

Polymerisation was carried out as follows: to an aqueous solution (2 mL) containing acryloylamylose (50 mg) and the template (saturated) was added crosslinker (*N*,*N*′-methylenebisacrylamide, 10–50 mg) and initiator [2,2′-azobis(2-amidinopropane) dihydrochloride, 2 mg]. Then UV light (365 nm) was irradiated at 25 °C for 2 h. To remove the template from the obtained polymer, it was successively immersed into water and acetone for longer than one day. We confirmed that the templates are completely removed from the imprinted polymers by calculating the mass balances based on HPLC analysis of template concentrations in the washing solvents. The yields of the imprinted polymers were higher than 90% and the compositions of the polymers determined by elemental analysis closely agreed with the feed compositions.

The rebinding abilities of the imprinted polymers toward BisA are shown in Fig. 2. BisA-imprinted polymers exhibited much higher binding affinities than non-imprinted polymers. This fact clearly indicates that BisA used as a template created a binding site suitable for the rebinding of BisA. The binding ratios for BisA-imprinted polymers increases with raising DS, while those for non-imprinted polymers remain constant. It is obvious from this fact that the higher the crosslinking density, the stronger the memory of the imprinted binding site.

Table 2 shows the effect of crosslinker content on the rebinding characteristics of the imprinted polymers. The

Table 1 Complexation of templates with acryloylamylose[a]

Template	Solubility in water/mM	Solubility in acryloylamylose solution/mM	[anhydroglucose unit]/[complexed template]
BisA	1.12	6.44	27.0
Thymol	3.76	5.96	65.0
*m*TBPh	5.07	10.21	27.8

[a] DS = 0.24.

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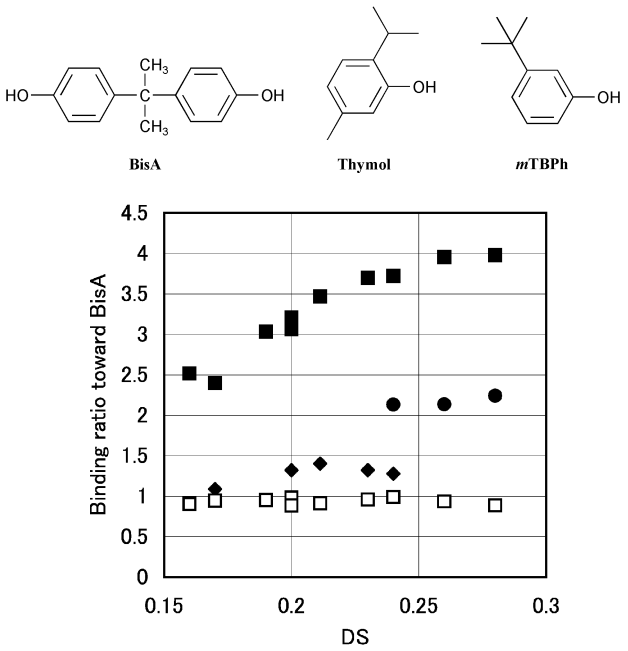

Fig. 2 Binding ratios[8] of imprinted polymers toward BisA: (■) BisA-imprinted, (●) *m*TBPh-imprinted, (◆) thymol-imprinted, (□) non-imprinted. Acryloylamylose/crosslinker = 5:2 (w/w).

Table 2 Influence of crosslinker content on the binding ratio toward BisA and imprinting efficiency

	Binding ratio		
Acryloylamylose[a]:crosslinker (w/w)	BisA-imprinted	Non-imprinted	Imprinting efficiency
5:1	3.05	1.07	2.85
5:2	3.02	0.97	3.10
5:5	1.75	0.63	2.78

[a] DS = 0.21.

binding ratios for both BisA-imprinted and non-imprinted polymers tend to decrease with increasing crosslinker content. This supports the view that the amylose portion is mainly responsible for the rebinding of BisA. The imprinting efficiency[9] shows the maximum value at a ratio between acryloylamylose and crosslinker of 5:2 (w/w). With increasing crosslinker content, the rigidity of the imprinted binding site becomes stronger, while a non-specific adsorption to the crosslinker portion becomes not negligible. Therefore, a moderate amount of crosslinker is optrimal for obtaining the highest imprinting efficiency. Thus, we fixed the acryloylamylos/crosslinker ratio at 5:2 in further experiments.

We also examined the rebinding selectivity[10] of imprinted polymers in which BisA or alkylphenols had been imprinted (Table 3). When the rebinding selectivity between BisA and thymol is compared, BisA-imprinted and thymol-imprinted polymers, respectively, show the highest and lowest selectivity

Table 3 Rebinding selectivity of imprinted polymers[a]

	Rebinding selectivity			
Analytes	BisA-imprinted	Thymol-imprinted	*m*TBPh-imprinted	Non-imprinted
BisA/thymol	9.87	4.76	6.28	6.41
BisA/mTBPh	8.32	5.23	4.86	6.71
Thymol/mTBPh	0.84	1.10	0.77	1.05

[a] DS = 0.24.

toward BisA, while non-imprinted and *m*TBPh-imprinted polymers show intermediate selectivity. A similar trend is seen in the other two couples of analytes, *i.e.* an imprinted polymer shows the highest selectivity toward the analyte used as the template. These observations are the clear evidence that the binding site suitable for the rebinding of the template molecule is created through the imprinting process.

In conclusion, the present work has demonstrated a novel molecular imprinting strategy to create amylose-based polymers having high and selective rebinding affinity toward bisphenol A and alkylphenols through a radical polymerisation process.

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8 Measurement of binding ratio: an imprinted polymer (10 mg) was stirred in 1 mL of an aqueous analyte solution (0.1 mM) at 25 °C for 12 h. After centrifugation (10,000 rpm, 5 min), analyte concentration remaining in the supernatant solution was determined by HPLC measurement. Binding ratio is defined as follows: binding ratio = [adsorbed analyte]/[analyte remaining in solution].

9 Imprinting efficiency was calculated as follows: imprinting efficiency = [binding ratio for BisA-imprinted polymer]/[binding ratio for non-imprinted polymer].

10 Rebinding selectivity of an imprinted polymer was calculated by dividing the binding ratios of different analytes.