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Preparation of molecularly imprinted polymers for organophosphates and their application to the recognition of organophosphorus compounds and phosphopeptides

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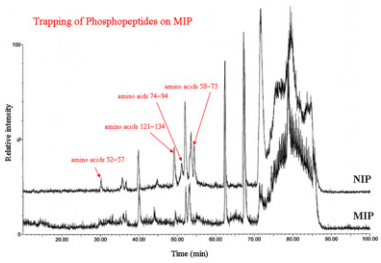
HIGHLIGHTS

- Monodisperse MIPs for organophosphates by multi-step swelling and polymerization.
- Application of MIPs to the separation of adenosine phosphates.
- Application of MIPs to the trapping of phosphopeptides in tryptic protein-digests.

GRAPHICAL ABSTRACT



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ABSTRACT

Monodisperse molecularly imprinted polymers (MIPs) for diphenyl phosphate (DPP) and 1-naphthyl phosphate (1-NapP) have been prepared by a multi-step swelling and polymerization method using 4-vinylpyridine as a functional monomer, glycerol dimethacrylate as a crosslinker and cyclohexanol or 1-hexanol as a porogen. The retention and molecular-recognition properties of these MIPs for organophosphorus compounds were evaluated by HPLC using a mixture of phosphate buffer and acetonitrile as an eluent. In addition to shape recognition, hydrogen bonding and hydrophobic interactions could play an important role in the retention and molecular recognition of DPP and 1-NapP. Furthermore, the MIPs were applied to the separation of adenosine and adenosine phosphates (AMP, ADP and ATP). These phosphates were retained on the MIPs according to the number of phosphate groups in the molecule and were well separated from one another. Hydrogen bonding and hydrophobic interactions seemed to affect the retention and recognition of adenosine phosphates in low acetonitrile content, while hydrophilic interactions affected these properties in high acetonitrile content. Finally, the MIPs were applied to the trapping of phosphopeptides. The MIPs non-selectively trapped phosphopeptides, which have phosphorylated tyrosine, serine or threonine in the sequences, and successfully trapped four phosphopeptides in tryptic digests of bovine α-casein.

1. Introduction

Organophosphates are the most important class of biochemicals. The organophosphates include DNA, RNA and cofactors; also, they encompass nerve agents and insecticides, which can cause several neurotoxic disorders in humans [1,2]. In addition, protein phosphorylation is a key regulating mechanism of biological processes and is one of the most significant posttranslational modifications in eukaryotes [3–5]. To extract or detect organophosphorus compounds or phosphorylated peptides selectively in complex matrices, it is important to develop materials that are highly selective for them.

A molecular imprinting technique is attractive because specific recognition sites for a target molecule can be easily molded into

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synthetic polymer networks. Such molecularly imprinted polymers (MIPs) have been utilized as chromatographic media, sensors, artificial antibodies and catalysts [6–8]. MIPs for organophosphates or organophosphonates have been prepared using functional monomers that were commercially available [9–14] or synthesized [15–19]. For instance, MIPs for pinacolyl methylphosphonate, ethyl methylphosphonate and methylphosphonic acid were prepared using methacrylic acid (MAA) as a functional monomer and trimethylolpropane trimethacrylate (TRIM) as a crosslinker [9]. The MIPs could recognize not only the template molecules but also the degradation products of the other nerve agents because of their cross-reactivity. A MIP that selectively recognized diphenyl phosphate (DPP) was prepared using a structural analog, ditolyl phosphate, as a template molecule, 2-vinylpyridine (2-VPY) or MAA as a functional monomer and ethylene glycol dimethacrylate (EDMA) as a crosslinker [10,11]. DPP was adsorbed onto the basic MIP (2-VPY-MIP), while the use of acidic functional monomer, MAA, resulted in no difference in selectivity between MIP and non-imprinted polymer (NIP). Commercially available amine-based functional monomers were surveyed for use in MIPs with carboxylate and phosphonate templates [12]. The best selectivity was found for the pyridine-based monomers, 2-VPY and 4-vinylpyridine (4-VPY), while the highest affinity was found for 2-(dimethylamino)ethyl methacrylate. The choice of 2-VPY or 4-VPY was template-specific [12]. In another example, MIPs for diisopropyl methylphosphonate (DIMP) and tributyl phosphate (TBP) were prepared using 4-VPY and TRIM as a functional monomer and a crosslinker, respectively [13]. Only DIMP was retained on the MIP for DIMP, while both DIMP and TBP were retained on the MIP for TBP. This result could be ascribable to steric exclusion of the larger TBP molecule from the DIMP imprint site. A MIP for adenosine-5′-monophosphate (AMP) was also prepared: the template used was a vinylphenylboronate diester of adenosine on which a 5′-free hydroxide is protected by a *tert*-butyldimethylsilyl group instead of a phosphate moiety. Acrylamide and EDMA were used as a functional monomer and a crosslinker, respectively. The MIP was selective for AMP over other nucleotides and deoxy analogs [14].

MIPs for phosphate and phosphonate esters, which contain a single hydrogen bond acceptor site, i.e., the P═O functionality, have been prepared using 1,3-diarylurea as a synthesized functional monomer [15]. Despite the weak hydrogen bonding interactions, the templates and their structural analogs were retained on these MIPs. MIPs that selectively recognized phosphotyrosine (pY) were prepared using an *N*,*O*-protected pY dianion template and a urea-based functional monomer [16]. The obtained MIPs displayed good binding affinities for the pY template and recognized tyrosine-phosphorylated peptides in the presence of an excess of non-phosphorylated peptides or serine-phosphorylated peptides. Furthermore, MIPs for phenyl phosphonate and DPP were prepared using 1-allyl-2-thiourea [17–19] and *N*-methyl-*N*′-(4-vinylphenyl) thiourea [17] as synthesized functional monomers; these MIPs had high binding affinities for phosphate ions in aqueous media or acetonitrile solution, respectively.

In this study, we sought to make monodisperse MIPs for DPP or 1-naphthyl phosphate (1-NapP) using 4-VPY as a functional monomer, EDMA or glycerol dimethacrylate (GDMA) as a crosslinker and cyclohexanol or 1-hexanol as a porogen by multi-step swelling and polymerization. The retention and molecular-recognition properties of the MIPs were evaluated using a mixture of phosphate buffer and acetonitrile as an eluent in HPLC. Furthermore, the MIPs were applied to the separation of adenosine and adenosine phosphates (AMP, ADP and ATP) and the non-selective trapping of phosphopeptides containing phosphorylated tyrosine, serine and threonine and phosphopeptides in tryptic digests of bovine α-casein.

2. Experimental

2.1. Materials

EDMA and 4-VPY were purchased from Tokyo Kasei (Tokyo, Japan). GDMA was a gift from Fuso (Osaka, Japan). Polyvinyl alcohol (degree of polymerization = 500, saponification value = 86.5 – 89 mol%) was purchased from Nacalai Tesque (Kyoto, Japan). Also, 2,2′-azobis(2,4-dimethyl valeronitrile) (ADVN) was purchased from Wako (Osaka, Japan). DPP and 1-NapP were purchased from Sigma–Aldrich, Japan (Tokyo, Japan). Synthetic enolase phosphopeptides (T18 1P, T19 1P and T43 1P) were purchased from Waters (Milford, MA, USA). Bovine α-casein was from Sigma–Aldrich, Japan. Trifluoroacetic acid (TFA), iodoacetamide (IAA) and dithiothreitol (DTT) were purchased from Nacalai Tesque. Modified trypsin of sequencing grade was obtained from Promega (Madison, WI, USA).

Water, acetonitrile and methanol of LC–MS grade were obtained from Wako. Other reagents and solvents were of analytical-reagent grade and were used without further purification. Water purified with a Purelab Ultra (Organo, Tokyo, Japan) was used to prepare eluents and sample solutions.

2.2. Preparation of MIPs

MIPs and NIPs were prepared by a multi-step swelling and polymerization method as reported previously [20,21]. The MIPs were prepared according to Table 1, and the corresponding NIPs were prepared similarly but without a template molecule; thus, NIP3 and NIP4 are the same polymer. Briefly, a water dispersion of uniformly sized polystyrene seed particles (0.497 g mL^{-1}) was mixed with a microemulsion prepared from dibutyl phthalate as an activating solvent, 0.02 g of sodium dodecyl sulfate and 10 mL of water by sonication. This first swelling step was carried out at room temperature for 15 h with stirring at 125 rpm until the microdrops of oil completely disappeared. Next, a dispersion of 0.1875 g of ADVN as an initiator, 2.5 mL of cyclohexanol or 1-hexanol as a porogen, 10 mL of 4.8% polyvinyl alcohol aqueous solution as a dispersion stabilizer, and 12.5 mL of water was added to the dispersion of swollen

Table 1
Template molecule, functional monomer, crosslinker and porogen used for the preparation of MIPs in this study.

Polymer	Template molecule (mmol)	Functional monomer (mmol)	Crosslinker (mmol)	Porogen
MIP1	DPP[a] (1.0)	4-VPY[c] (1.0)	EDMA[d] (25)	Cyclohexanol
MIP2	DPP (1.0)	4-VPY (2.0)	GDMA[e] (25)	Cyclohexanol
MIP3	DPP (1.0)	4-VPY (2.0)	GDMA (25)	1-Hexanol
MIP4	1-NapP[b] (1.0)	4-VPY (2.0)	GDMA (25)	1-Hexanol

[a] Diphenyl phosphate.
[b] 1-Naphthyl phosphate.
[c] 4-Vinylpyridine.
[d] Ethylene glycol dimethacrylate.
[e] Glycerol dimethacrylate.