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Molecularly imprinted polymer for glutathione by modified precipitation polymerization and its application to determination of glutathione in supplements

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ABSTRACT

Molecularly imprinted polymers (MIP) particles for glutathione (GSH) with a narrow particle size distribution were prepared by modified precipitation polymerization using methacrylic acid as a functional monomer, divinylbenzene as a crosslinker and water as a co-solvent. The particle diameters of the MIP and non-imprinted polymer (NIP) prepared under the optimum conditions were 3.81 ± 0.95 (average $\pm$ standard deviation) and 3.39 ± 1.22 μm, respectively. The retention and molecular-recognition properties of the prepared MIP were evaluated using a mixture of acetonitrile and water as a mobile phase in hydrophilic interaction chromatography. With an increase of acetonitrile content, the retention factor of GSH was increased on the MIP. In addition to shape recognition, hydrophilic interactions seem to work for the recognition of GSH on the MIP. The MIP had a specific molecular-recognition ability for GSH, while glutathione disulfide, L-Glu, L-Cys, Gly-Gly and L-Cys-Gly could not be retained or recognized on the MIP. The effect of column temperature revealed that the separation of GSH on the MIP was entropically driven. Binding experiments and Scatchard analyses revealed that one binding sites were formed on both the MIP and NIP, while the MIP gave higher affinity and capacity for GSH than the NIP. Furthermore, the MIP was successfully applied for determination of GSH in the supplements.

1. Introduction

Glutathione (GSH), L-γ-glutamyl-L-cysteinylglycine, is the most abundant intracellular antioxidant present in living organisms and plays an important role in detoxification against poisons, drugs or reactive metabolites and cellular defense against oxidative damages [1–3]. Therefore, it is believed that GSH could be related to a lot of diseases including influenza, acquired immune deficiency syndrome, cancers, diabetes and so on [4–7]. Furthermore, GSH is mainly present in its reduced form, and is readily oxidized to GSH disulfide (GSSG) under the oxidative stress. GSH is an indicator of cellular health, with GSH constituting up to 98% of cellular GSH under normal conditions [8]. The GSH to GSSG ratio is used for evaluating the oxidative stress in biological systems [8–10].

A lot of separation methods such as liquid chromatography (LC) and capillary electrophoresis (CE) have been developed for the determination of GSH in pharmaceutical formulations, biological fluids and wine samples. In LC, UV detection at 215 nm was used for GSH in pharmaceutical formulations [7], and GSH in cell lines was detected at 280 nm after derivatization with 5,5′-dithiobis(2-nitrobenzoic acid)(DTNB) [11]. Fluorescence detection after precolumn derivatization with 2,3-naphtalenedialdehyde [12] and 4-chloro-3,5-dinitrobenzotrifluoride [13], respectively, was used for the determination of GSH in wine samples and biological fluids. Postcolumn derivatization with o-phthalaldehyde was used for fluorometric detection of GSH in wine samples [14]. GSH in brain and liver of rats was electrochemically determined [15]. Furthermore, GSH derivatized with *N*-ethylmaleimide [16] or DTNB [17] and GSSG were simultaneously determined by LC–MS/MS.

In CE, GSH in baker's yeast was determined at 285 nm after derivatization with methyl propiolate [18]. Laser-induced fluorescence detection after derivatization with eosin-5-maleimide [19] and 5-iodoacetamidofluorescein [20], respectively, was applied to the determination of GSH in biological samples and cell lines. The determination of GSH in cell lines and human whole blood extract,

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respectively, was performed using electrochemiluminescent [21] and electrochemical [22] detection. Furthermore, γ-glutamyl peptides including GSH and GSSG were determined by CE-ESI-TOF MS [23].

Recently, molecularly imprinted polymers (MIPs) have been prepared and used for the selective recognition and/or extraction of small and large molecules in complex matrices [24,25] An MIP for GSH was prepared using methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and a crosslinker, respectively, by bulk polymerization [26]. The MIP was applied to extract GSH in yeast cell samples. MIP microspheres for GSH were prepared by reversed-phase suspension polymerization using acrylamide (AM) and *N*-vinyl pyrolidone as functional monomers, and *N*,*N*′-methylenebisacrylamide (MBAM) and dimethyl diallyl ammonium chloride as crosslinkers [27]. Song et al. [28–30] prepared three MIP particles for GSH. The first MIPs were synthesized through iniferter-controlled living radical precipitation polymerization using 4-vinylpyridine and EDMA as a functional monomer and crosslinker, respectively [28]. The second MIPs had the porous imprinted layers prepared AM and MBAM as a functional monomer and crosslinker, respectively [29]. The third MIPs were water-compatible as they had a hydrophilic surface-grafted poly(MAA) or poly(*N*-isopropylacrylamide) layer [30]. Those three MIPs could be applied to extract GSH in complex matrices such as plasma and urine as solid-phase extraction media.

Recently, we prepared monodisperse MIPs for hydrophilic compounds such as creatinine [31] and chlorogenic acid [32] by a modified precipitation polymerization method. In the method, hydrophilic compounds were first dissolved in a small portion of water, dimethyl sulfoxide or methanol and then their MIPs were prepared by precipitation polymerization. In this study, MIP particles for GSH were prepared using modified precipitation polymerization by first dissolving GSH in water. The retention and molecular-recognition properties of the MIP were evaluated using a mixture of acetonitrile and water as a mobile phase in hydrophilic interaction chromatography (HILIC). Effect of column temperature on the retention of GSH on the MIP was also evaluated. The binding properties of the prepared MIP were examined by binding experiments and Scatchard analyses. Furthermore, the MIP for GSH was successfully applied for the determination of GSH in supplements.

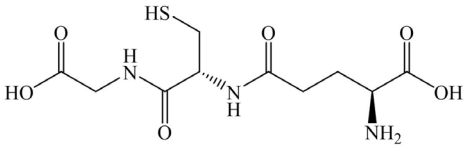
2. Experimental

2.1. Materials

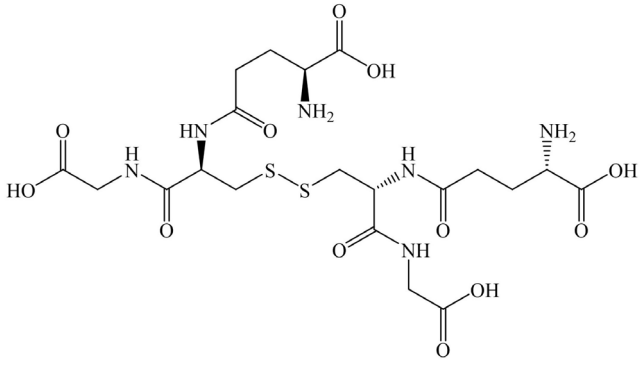
MAA, divinylbenzene (DVB), which is a mixture of 1,3- and 1,4-DVB, and 2,2′-azobis(isobutyronitrile) (AIBN) were purchased from Wako (Osaka, Japan). GSH and GSSG were purchased from Nacalai Tesque (Kyoto, Japan). L-Glu, L-Cys and Gly-Gly were purchased from Peptide Institute (Ibaraki, Japan). L-Cys-Gly was purchased from Sigma-Aldrich Japan (Tokyo, Japan). The supplement, Glutathione Reduced, was purchased from Jarrow Formulas (Los Angeles, CA, USA). Other reagents and solvents of analytical-reagent grade were used without further purification. Water purified with a Purelab Ultra system (Organo, Tokyo, Japan) was used to prepare mobile phases and sample solutions. The structures of GSH and GSSG are illustrated in Fig. 1.

2.2. Preparation of MIPs by modified precipitation polymerization

MIPs for GSH and non-imprinted polymers (NIPs) were prepared by a modified precipitation polymerization method as previously reported [31,32], as shown in Table 1. Briefly, GSH (0.75–3.0 mmol) dissolved in 8.0 mL of water as a template molecule, MAA (1.5–6.0 mmol) as a functional monomer, DVB (28.8 mmol) as a crosslinker and AIBN (1.9 mmol) as an initiator were added in 128 mL of acetonitrile–toluene (3/1, v/v). The mixture was degassed with argon gas for 15 min, and then rotated slowly at 16 rpm using a Shellspin VS-60 rotor (Taitec, Tokyo, Japan). Reaction temperature was increased from 25 to 60 °C for 2 h and then kept at 60 °C for a further 2–48 h. The corresponding NIPs were prepared similarly but without a template molecule. After polymerization, a dispersion of polymerized particles was poured into 200 mL of methanol and the supernatant was discarded after sedimentation of the particles. The obtained polymers were redispersed into methanol, and this procedure was repeated three times in methanol, once in water and twice in tetrahydrofuran. The resulting μm-sized polymer particles were collected using a membrane filter, washed with tetrahydrofuran and dried at room temperature.



Glutathione (GSH)



Glutathione disulfide (GSSG)

Fig. 1. Structures of GSH and GSSG.

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

Polymer	Template Molecule (mmol)	Functional monomer (mmol)	Crosslinker (mmol)	Initiator (mmol)
MIP1	GSH (0.75)	MAA (6.0)	DVB (28.8)	AIBN (1.9)
MIP2	GSH (1.5)	MAA (6.0)	DVB (28.8)	AIBN (1.9)
MIP3	GSH (3.0)	MAA (6.0)	DVB (28.8)	AIBN (1.9)
MIP4	GSH (3.0)	MAA (3.0)	DVB (28.8)	AIBN (1.9)
MIP5	GSH (3.0)	MAA (1.5)	DVB (28.8)	AIBN (1.9)
NIP1	–	MAA (6.0)	DVB (28.8)	AIBN (1.9)
NIP2	–	MAA (6.0)	DVB (28.8)	AIBN (1.9)
NIP3	–	MAA (6.0)	DVB (28.8)	AIBN (1.9)
NIP4	–	MAA (3.0)	DVB (28.8)	AIBN (1.9)
NIP5	–	MAA (1.5)	DVB (28.8)	AIBN (1.9)

2.3. Porosity measurements

Surface areas and porosity of MIPs and NIPs were measured by nitrogen sorption porosimetry using a TriStar surface area and porosity analyzer (Micrometrics Instruments, Norcross, GA, USA). Prior to measurement, 200 mg of the particles were heated at 80 °C for 5 h in vacuo. The specific surface areas were calculated using