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“Thiol-ene” click chemistry: A facile and versatile route to functionalization of porous polymer monoliths

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Abstract

The preparation of porous polymer monoliths with dodecyl and zwitterionic functionalities via the “thiol-ene” click chemistry of thiol-containing monoliths with both hydrophobic and polar methacrylate “ene” monomers has been demonstrated. Selected separations confirmed the excellent potential of these monoliths in chromatography.

Introduction

Monolithic columns prepared from rigid porous polymers featuring large through-pores with high permeability to flow were introduced in the early 1990s.¹ The motivation for their development was to (i) circumvent slow diffusional mass transport in the stagnant mobile phase within the pores of traditional columns packings, (ii) use fast convection instead of the diffusion, (iii) enable rapid separations of large molecules at high flow rates, and (iv) profit from the variability of chemistries of the polymer matrix. The control of chemistry of these early monoliths was achieved in a single step by direct copolymerization of selected monomers. While simple, this approach often required tedious re-optimization of the polymerization conditions for each new monomer that was used. Therefore, we have developed an alternative two-step process including the copolymerization of monomers bearing reactive groups followed by their chemical modification.² Due to the wide variety of potential applications of monoliths, it is desirable to explore new efficient approaches enabling their functionalization.

The copper(I) catalyzed (3+2) azide-alkyne cycloaddition (CuAAC) affording a 1,4-disubstituted triazole ring, a basic element of the current “click chemistry”, was first described in 1967 and advanced by Sharpless.³ The broad utility of this reaction promoted interest in other processes that also exhibit features of the original “click” reaction. For example, Diels–Alder reactions, metal-free dipolar cycloadditions, and approaches including thiol groups such as the thiol-ene, thiol-yne, thiol-isocyanate, thiol-p-fluorostyrene, and thiol-bromo reactions were studied.⁴ In contrast to the numerous examples of application of CuAAC in the preparation and functionalization of separation media for chromatography,⁵ the use of the click reactions involving thiols has been significantly less frequent. For example, Lindner's group pioneered the use of thiol-ene chemistry for the preparation of particulate silica-based chiral stationary phases.⁶ Similar support was later used for the

preparation of stationary phases for hydrophilic interaction chromatography (HILIC).⁷ The thiol-ene coupling was also used for the modification of monoliths. Thus, silica monoliths functionalized with vinyl groups were used for click immobilization of trypsin through its free thiols.⁸ To our best knowledge, all other applications concerned columns for capillary electrochromatography.⁹ Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (GMA-EDMA) monoliths with thiol groups were modified with quinine or phosphonic acid derivatives and the columns were used for enantioseparations.¹⁰ In a different implementation, the columns were prepared from a poly(*N*-acryloylsuccinimide-*co*-EDMA) monolith reacted with allylamine, followed by reactions with thiol-terminated PEG or octadecanethiol.¹¹

Our unrelated research concerning attachment of gold nanoparticles onto the pore surface of organic polymer-based monoliths required the preparation of monolithic structures with thiol functionalities.¹² Consequently, we realized that these monoliths are an excellent substrate for functionalization via the thiol-ene click reaction. Present report describes the use of this versatile reaction for the preparation of monolithic capillary columns suitable for the chromatographic separations in reversed phase and HILIC modes.

Materials and methods

Materials

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), lauryl methacrylate (LMA), and [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl) ammonium betaine (SPE) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and purified by passing them through an aluminum oxide column for removal of inhibitor. Azobisisobutyronitrile (AIBN), 2,2-dimethyl-2-phenylacetophenone (DMPA), benzophenone (BP), 3-(trimethoxysilyl)propyl methacrylate, cyclohexanol, 1-dodecanol, hydrochloric acid, sodium hydroxide, acetic acid, formic acid, trifluoroacetic acid, triethylamine, phosphoric acid, ammonium formate, cystamine dihydrochloride, ethanolamine, propylamine, tris(2-carboxylethyl)phosphine hydrochloride (TCEP) solution (0.5 mol/L, pH=7 adjusted with ammonium hydroxide), ribonuclease A (bovine heart), cytochrome C (bovine pancreas), myoglobin (horse skeletal muscle), Phe-Gly-Phe-Gly, Val-Try-Val, Gly-Leu, Gly-Try, Lys-Val, Gly-Gly-Gly, adenosine, cytidine, guanosine, uracil, benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene, and HPLC-grade solvents (acetonitrile, methanol, acetone) were purchased from Sigma-Aldrich and used as received. Polyimide and UV transparent fluorocarbon polymer coated 100 μ m i.d. fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Instrumentation

A syringe pump (Kd Scientific, New Hope, PA) was utilized for the modifications of monolithic columns with cystamine, TCEP, and ene monomers. A Dionex Ultimate 3000 HPLC system (Sunnyvale, CA, USA) equipped with a 3 nL UV detection cell and an external micro-valve injector with a 10 nL inner sampling loop (Valco, Houston, USA) was used for the chromatographic evaluation. Scanning electron micrographs and energy dispersive X-ray spectra of monoliths were obtained using a Zeiss Gemini Ultra Field-Emission Scanning Electron Microscope (Peabody, MA, USA) integrated with an energy dispersive X-ray spectrometer (Thermo Electron, USA).

Preparation of thiol-containing monolithic capillary columns

Thiol-containing monolithic capillary columns were prepared using the procedure developed previously^{12c}. Briefly, generic monoliths were prepared in vinylized capillaries via in situ polymerization of a mixture comprising 24% glycidyl methacrylate, 16% ethylene

dimethacrylate, 30% cyclohexanol, 30% 1-dodecanol, and 1% AIBN initiator (with respect to monomers) (all wt.%). After sonication for 15 min and degassed by purging with nitrogen for 5 min, the mixture was then introduced into the vinylized capillary. The capillary was sealed at both ends with a rubber septum and immersed in a thermostated water bath at 60°C for 24 h. After the polymerization reaction was completed, a few centimeters from both ends of the capillary were cut to liberate the virgin structure, and the monolith was flushed with acetonitrile to remove unreacted components.

Surface modification of generic poly(glycidyl methacrylate-ethylene dimethacrylate) monolith with cystamine was carried out by first pumping a 1.0 mol/L cystamine dihydrochloride in 2.0 mol/L aqueous sodium hydroxide at room temperature at a flow rate of 0.5 μ L/min for 1 h. The column was then sealed with rubber septa at both ends, and the reaction was carried out in a thermostated water bath at 50°C for 1 h. This modification procedure was repeated twice to achieve a higher conversion. While the sulfur content was 2.6 at% after the first run, it increased to 3.7 at% after repeating the modification procedure in the second cycle. The capillary column was then flushed with water until the pH of the eluent was neutral, followed by capping unreacted epoxy groups with 1.0 mol/L propylamine for hydrophobic monolith or 1.0 mol/L ethanolamine for hydrophilic monolith using the same conditions described above.

Thiol-containing monolith was prepared by pumping 0.25 mol/L TCEP solution through the monolith at room temperature at a flow rate of 0.25 μ L/min for 2 h to reduce the disulfide bonds. The column was then washed with water.

Functionalization of thiol-containing monolithic capillary columns via thiol-ene click chemistry

A solution of “ene” monomer and initiator was first pumped through the monolithic column at room temperature at a flow rate of 0.5 μ L/min for 1 h. LMA in methanol (1.0 mmol/L) was used for preparation of hydrophobic monolith, while zwitterionic SPE in mixture of acetone and water (1.0 mmol/L) was applied to obtain hydrophilic monolith. Thermally initiation of monolith was conducted in the presence of AIBN at 80°C. Photoinitiating reaction was performed at room temperature by irradiation the monolith for 30 min with a UV-lamp emitting nominally at 360 nm and a light intensity of 12 mW/cm² in the presence of DMPA or BP photoinitiators. After reaction, the monolith was flushed with methanol or water to remove unreacted components.

Results and discussion

The entire reaction scheme is shown in Fig. 1. Epoxy groups of the generic GMA-EDMA monolith react first with cystamine followed by snipping the disulfide bond using tris(2-carboxylethyl)phosphine (TCEP) that liberates the desired thiol groups. Energy-dispersive X-ray spectroscopy reveals about 3.7 at.% of sulfur in both cystamine-modified and TCEP-cleaved monoliths. These values indicate that most of the cystamine reacts through both amine functionalities. This high content of thiol functionalities significantly exceeds that obtained previously in modifications of generic GMA-EDMA monolith using sodium hydrogen sulfide and cysteamine.^{10a;12a,b} Use of cystamine promises to deliver a more substantial degree of functionalization. Another advantage of our approach is that it includes the wealth of compounds containing a carbon-carbon double bond compared to the number of readily available, frequently malodorous thiols. We used two monomers with completely different polarity and three initiators to map the functionalization of our monoliths using thiol-ene reaction.

The first demonstration includes a reaction with lauryl methacrylate (LMA). The monomer LMA and photoinitiator 2,2-dimethyl-2-phenylacetophenone (DMPA) were dissolved in methanol and the “clicking” was initiated at room temperature by irradiation with a UV-lamp at 360 nm for 30 min.¹³ The baseline separation of three proteins achieved in less than 8 min confirms the efficient surface grafting with hydrophobic dodecyl chains (Fig. 2). Another proof of the successful functionalization with long alkyl chains was the measurement of the difference in the retention factor for a small hydrophobic probe. While the retention factor for amylbenzene was 0.6 for the generic monolith, it increased significantly to 9.3 after the functionalization with LMA.

A similar experiment was carried out with monolithic column M-3, which was prepared using benzophenone (BP) as the photoinitiator. Clearly, this reaction is more complex and the grafting less efficient compared to that achieved with DMPA. This effect results from a different initiation mechanism of the photografting using BP. The lower retention factor for amylbenzene also indicates less efficient grafting. Nevertheless, the retention factor for amylbenzene increased from 0.6 to 8.8 after the successful functionalization. Simultaneous to the grafting of LMA to the monolith, we also carried out control experiments under the same conditions except for elimination of LMA from the mixture. As shown in Table 1, columns M-2 and M-4 prepared under these conditions exhibited a lower back pressure, similar to that of the untreated generic monolith M-0. The increase in the back pressure observed for clicked monoliths also confirms the presence of hydrophobic chains in the pores of the photografted monoliths.

We also used AIBN initiator, the decomposition of which is affected thermally.^{4,13b} The column containing monolith M-5 exhibited a high normalized back pressure of 153.5 MPa/m, a three-fold increase compared to 50.3 MPa/m found for the control monolith M-10. The linear polymers formed from LMA before their capture by the thiol functionality most likely partially fill the pores of the monolith. This inference is supported by SEM micrographs shown in Fig. 3. While the morphology of the generic monolith is characterized by small surface features, these are less pronounced in monolith M-6 due to their coverage with the grafted layer. Fig. 2 shows the excellent performance of the clicked monolithic column M-1 and M-6 in the separation of three proteins, ribonuclease A, cytochrome C, and myoglobin. The elution order follows the hydrophobicity of the proteins. We knew from our previous work that both generic monoliths and monoliths functionalized with cystamine and treated with TCEP do not enable separation of these proteins.^{12c} The slightly longer retention times for the thermally grafted monolith are due to use of slower flow rate that has to be used to avoid excessive back pressure in the system.

Table 1 confirms that the excessive polymerization of LMA can be controlled via kinetic parameters, i.e. concentration of the initiator and reaction time. Indeed, a decrease in both parameters produces monoliths with smaller back pressure indicating that the pores are less plugged with the formed polymer. The penalty paid for the reduction in back pressure is the decrease in retention. The grafted polymer is solvated in the mobile phase that includes tetrahydrofuran, and forms a gel-like layer at the pore surface. Presence of this layer has recently been found to improve separation performance of monolithic columns for small molecules.¹⁴ Indeed, Fig. 4. compares the separation of alkylbenzenes using generic and LMA grafted columns. While the separation in generic monolithic columns is very poor and the retention time is less than 7 min, a baseline separation is achieved using the LMA clicked monolith. The latter also exhibits a significant retention signaling the presence of the polymerized LMA. The column efficiency of monolith M-6 for small molecules is enhanced from 4,000 and 3,000 to 30,000 and 20,000 plates/m for uracil and amylbenzene, respectively.

To demonstrate versatility of the thiol-ene click chemistry, the thiols-containing monoliths were also clicked with zwitterionic [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)ammonium betaine (SPE) monomer. Both UV and thermal initiation were used for the initiation. This reaction, shown in Fig. 1, provides a monolith with a highly hydrophilic surface chemistry suitable for the separation in HILIC. In contrast to grafting with LMA that readily polymerizes and fills the pores thus decreasing the permeability to flow, clicking SPE using both photoinitiated and thermally-initiated techniques affords monoliths with small changes in permeability. The values of normalized back pressure, even for the thermally-initiated clicking of SPE, are very similar to those found for the monoliths prepared by the photoinitiated reaction of LMA.

Although monolith with the SPE chemistry were prepared by both direct copolymerization and UV initiated direct grafting by Viklund et al. in the past, their separation performance in HILIC mode has not been demonstrated.¹⁵ In contrast, monolith prepared in Smith's group using direct copolymerization of SPE and EDMA exhibited a column efficiency of 15,000 plates/m in HILIC mode.¹⁶ The left chromatogram of Fig. 5 shows the gradient elution of six peptides using thermally clicked monolith M-11 achieved in the mobile phase with a decreasing percentage of acetonitrile. The right chromatogram shows the fast baseline isocratic separation of nucleotides with an efficiency of 19,000 plates/m found for guanosine.

Conclusions

We used the thiol-ene click reaction to prepare monoliths with hydrophobic and zwitterionic functionalities and demonstrated the applications of these functionalized monoliths with chromatographic separations of proteins and alkylbenzenes in reversed phase as well as with peptides and nucleotides in HILIC modes. Our current modified monolithic columns do not offer very high efficiency. This results from the fact that the surface area of our monoliths is one order of magnitude less than that of silica-based monolithic columns. However, we have developed hypercrosslinking approach that lead to styrene-based monoliths with large surface area and demonstrated their good performance in reversed phase mode. The future direction is to prepare hypercrosslinked monoliths with reactive functionalities enabling the click reaction and extend the number of applications. For example, we envision the use of the thiol-ene reaction in the preparation of monoliths for other separation modes such as affinity chromatography, for immobilization of organic and biological catalysts, and the preparation of selective sorbents for fishing out certain compounds.

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Fig. 1. Reaction scheme for the preparation of monoliths and their functionalization via “thiol-ene” click reaction.

Fig. 2.

Reversed-phase separation of proteins using monolith M-1 (a) and M-6 (b). Column: 121 mm $\times$ 100 μ m i.d. Mobile phase: A - 0.1% aqueous trifluoroacetic acid, B - 0.1% trifluoroacetic acid in acetonitrile, gradient from 20 to 60% B in A in 10 min, flow rate 1.0 μ L/min (a); gradient from 20 to 70% B in A in 15 min, flow rate 0.5 μ L/min (b), UV detection at 210 nm. Peaks: impurity (1), ribonuclease A (2), cytochrome C (3), myoglobin (4).

Fig. 3. Scanning electron micrographs of the internal structures of monoliths M-0 (a), M-1 (b), M-6 (c), and M-11(d).

Fig. 4. Reversed-phase separation of uracil and alkylbenzenes using generic poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (left) and lauryl methacrylate clicked (right) monolithic columns. Columns: generic 167 mm $\times$ 100 μ m i.d. (left), M-6 150 mm $\times$ 100 μ m i.d. (right); mobile phase: 40% water, 58% acetonitrile, 2% tetrahydrofuran, flow rate 0.5 μ L/min, UV detection at 254 nm. Peaks: uracil (1), benzene (2), toluene (3), ethylbenzene (4), propylbenzene (5), butylbenzene (6), amylbenzene (7).

Fig. 5.

Separation of peptides (left) and nucleotides (right) in HILIC mode using monolith M-11 with clicked [2-(methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl) ammonium betaine. Column: 109 mm $\times$ 100 μ m i.d. Mobile phase: left - A - 10 mmol/L triethylammonium phosphate buffer (pH 2.8), B - 5:95 vol.% 10 mmol/L triethylammonium phosphate buffer (pH 2.8) in acetonitrile, gradient from 100 to 60% B in A in 10 min, flow rate 1.0 μ L/min, UV detection at 214 nm; (right) 10:90 vol.% 20 mmol/L ammonium formate aqueous buffer (pH 3.2) in acetonitrile, flow rate 0.5 μ L/min, UV detection at 254 nm. Peaks: Impurities (1), Phe-Gly-Phe-Gly (2), Val-Try-Val (3), Gly-Leu (4), Gly-Try (5), Lys-Val (6), Gly-Gly-Gly (7), uracil (8), adenosine (9), cytidine (10), guanosine (11).

Table 1

Thiol-ene click reactions used for functionalization of porous polymer monoliths and their chromatographic properties.

Monolith	M ^a	Initiation ^b	c	t ^d	ΔP^e	k ^f
M-0 ^g	none	-	-	-	44.9	0.6
M-1	LMA	UV/DMPA	0.05	0.5	50.6	9.3
M-2	none	UV/DMPA	0.05	0.5	45.9	-
M-3	LMA	UV/BP	0.05	0.5	53.5	8.8
M-4	none	UV/BP	0.05	0.5	46.4	-
M-5	LMA	Thermal/AIBN	0.1	4	153.5	11.9
M-6	LMA	Thermal/AIBN	0.05	4	120.0	10.7
M-7	LMA	Thermal/AIBN	0.1	2	129.7	11.1
M-8	LMA	Thermal/AIBN	0.05	2	108.6	10.2
M-9	LMA	Thermal/AIBN	0.03	2	102.3	9.3
M-10	none	Thermal/AIBN	0.05	4	50.3	-
M-11	SPE	Thermal/AIBN	0.05	4	56.7	-
M-12	SPE	UV/DMPA	0.05	0.5	53.6	-

^aMonomers LMA - lauryl methacrylate, SPE - [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium betaine.

^bUV initiation at room temperature, thermal initiation at 80 °C. DMPA - 2,2-dimethyl-2-phenylacetophenone, BP - benzophenone, AIBN - 2,2'-azobis(2-methyl-propionitrile).

^cConcentration of initiator, mmol/L.

^dThiol-ene reaction time, h.

^eNormalized back pressure in the column MPa/m. Mobile phase acetonitrile-water 60:40 vol%; flow rate 0.5 μ L/min.

^fRetention factor for amylbenzene.

^gGeneric poly(glycidyl methacrylate-co-ethylene dimethacrylate) monolith.