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Preparation of molecularly imprinted polymers using nitroxide-mediated living radical polymerization

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Abstract

The use of molecularly imprinted polymers (MIPs) in chemical and bioanalytical applications has been gaining in interest in recent years. Compared to their biological receptor counterparts, MIPs are easy to prepare, have long shelf stability and can be used under a variety of harsh conditions. The majority of MIPs currently used are produced by traditional free radical polymerization. One drawback with the use of standard free radical initiators is that little control can be exerted over the chemical processes that form the final imprinted cavities. In this study we set out to investigate the application of controlled (living) free radical polymerization to the preparation of MIPs. This was exemplified by the synthesis of cholesterol-imprinted bulk polymers by nitroxide-mediated polymerization (NMP). A sacrificial covalent bond was employed to maintain imprinting fidelity at elevated temperature. Selective uptake of cholesterol from solutions in hexane was studied with imprinted polymers prepared under different conditions. The imprinted hydrolyzed MIP prepared by NMP displayed higher selective cholesterol binding than that prepared by a traditional radical polymerization.

Keywords: Molecular imprinting; Living radical polymerization; Nitroxide-mediated polymerization; Cholesterol

1. Introduction

Molecular imprinting is a powerful method for the preparation of synthetic receptors for a given target guest molecule. This synthetic approach typically involves polymerization of functional and cross-linking monomers in the presence of a molecular template, which controls the distribution of functional groups in the resulting three-dimensional polymer network. After polymerization and template removal, specific binding sites are left in the polymer material, which can be used to afford effective separation, chemical sensing or selective catalysis. The widespread use of traditional free radical polymerization methods for the preparation of molecularly imprinted polymers (MIPs) can be attributed to a tolerance for a wide range of functional groups and template structures. In essence, the free radicals generated during the addition polymerization do not interfere with the intermolecular interactions critical for the non-covalent imprinting system. Despite efforts to optimise the selection of functional monomers and improvements in the physical form and morphology of MIPs, binding of the target is often associated with a relatively low affinity, broad site heterogeneity and slow kinetics. The poor performance of MIPs can perhaps be partly explained at a molecular level: the polymerization chemistry itself, which largely influences the local structure of the cross-linked polymer network has yet to be thoroughly investigated and is currently poorly understood. Futhermore traditional free radical polymerization processes are difficult to control with regard to chain propagation and termination. This situation is in stark contrast to the synthesis of small organic receptors by a series of stepwise reactions, where precisely controlled conditions are used at each stage to furnish the desired intermediates. By gaining better control over the polymerization reaction therefore, it is

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expected that improved molecular recognition with MIPs can be achieved.

Recently, Zimmerman and co-workers used metal-catalyzed ring-closing metathesis polymerization to prepare monomolecularly imprinted dendrimers, in which a porphyrin binding site was formed by cross-linking of the peripheral vinyl groups (Zimmerman et al., 2002). Steinke and co-workers used ring-opening metathesis polymerization to prepare bulk polymers that displayed chiral-selective binding for L- and D-menthol (Patel et al., 2003). The use of the special catalysts in these studies required that a covalent imprinting – non-covalent binding strategy be adopted.

Research on living radical polymerization systems has resulted in significant advancement in the methodology during recent years. The problematic chain termination encountered in traditional addition polymerization can be minimized when using living radical initiators, resulting in a more constant rate for polymer chain growth, and a narrow molecular weight distribution for linear (non cross-linked) polymers. Living radical polymerization has so far been mainly used to synthesize non-cross-linked polymers with different terminal functional groups. The application of living radical polymerization to cross-linked systems has been little studied. Among the most feasible living radical polymerization methods, only atom-transfer radical polymerization (ATRP) (Matyjaszewski and Xia, 2001) has been used to prepare MIPs in a non-covalent imprinting system (Wei et al., 2005). The other living radical polymerization methods, i.e. reversible association fragmentation polymerization (RAFT) (Chiefari et al., 1998) and nitroxide-mediated polymerization (NMP) (Benoit et al., 1999) have not so far been used for MIP preparation.

The present work was aimed at studying nitroxide-mediated polymerization for the preparation of molecularly imprinted polymers. We were particularly interested in NMP because it does not require any additional catalyst or chain transfer reagent: a single NMP initiator is sufficient to achieve reaction control. In addition, NMP can also simplify surface modification for MIPs to bring about better compatibility with different solvent systems. Furthermore, NMP has the potential to be used in both covalent and non-covalent imprinting systems, given that new low temperature NMP initiators are being developed (Hintermann et al., 2002). In the present study we used a nitroxide reagent that requires a relatively high activation temperature (125 °C). To maintain stable functional monomer – template complexation under these conditions, we selected to adopt the sacrificial spacer method in which the cholesterol template remains covalently bound to a monomer during its copolymerization with cross-linker (Whitcombe et al., 1995). After polymerization and template removal by hydrolytic cleavage, the resulting MIP contained specific binding sites that could take up cholesterol via non-covalent hydrogen bond interaction in a non-polar solvent. The binding performance of the MIP prepared by NMP was compared to that prepared with a traditional radical polymerization initiator.

2. Experimental

2.1. Materials

Divinylbenzene (DVB, technical, mixture of isomers, 80%), 4-tert-butylstyrene (TBS, 93%), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 99%), 2,2′-azobisisobutyronitrile (AIBN, 98%), benzoyl peroxide (BPO, 75%), 4-acetoxystyrene (96%), cholesteryl chloroformate (98%) and m-xylene (anhydrous, 99+%) were purchased from Aldrich. TBS was purified by vacuum distillation. AIBN and BPO were purified by recrystallization from methanol. Prior to use, DVB was passed through an aluminium oxide column to remove the stabilizer, 4-tert-butylcatechol. [1α,2α-^{3}H(N)]cholesterol (specific activity 41.3 Ci mmol^{-1}) was supplied by Sigma. Scintillation liquid, Ecoscint A, was from National Diagnostics (Atlanta, GA, USA). The NMP initiator, 3-(4-tert-butylphenyl)-1,1-dimethyl-3-(2,2,6,6-tetramethylpiperidinooxy)propyl cyanide (**1**), was synthesized using a literature method (Abrol et al., 1997). Cholesteryl (4-vinyl)phenyl carbonate was synthesized following the published protocol (Whitcombe et al., 1995). Other solvents and reagents were of analytical grade unless otherwise stated. Elemental analysis for oxygen content was performed by MikroKemi AB, Uppsala, Sweden.

2.2. Polymer preparation

Cholesterol-imprinted polymers (MIP) and non-imprinted polymers (NIP) were prepared using either the NMP reagent **1** or BPO as initiator (Table 1). The monomers and initiator were dissolved in 0.88 mL of m-xylene, the solution was purged

Table 1
Preparation and characterization of molecularly imprinted polymers

Polymer	Initiator	Monomer (mmol)		Hydrolysis treatment	BET surface area ($m^2\ g^{-1}$)	O content (%)	High affinity sites		Low affinity sites	
		2	DVB				K_D (μM)	B_{max} (nmol g^{-1})	K_D (mM)	B_{max} (μmol g^{-1})
MIP(NMP)	**1**	0.29	5.51	No	470	1.1				
MIP(NMP)-H	**1**	0.29	5.51	Yes	621	0.4	4.5 ± 1.2[a]	61 ± 15	31 ± 5.4	33 ± 5.6
NIP(NMP)-H	**1**	0	5.81	Yes	705	n.d.[b]				
MIP(BPO)	BPO	0.29	5.51	No	571	1.2				
MIP(BPO)-H	BPO	0.29	5.51	Yes	730	0.8	8.1 ± 2.5	73 ± 22	12 ± 2.6	84 ± 18
NIP(BPO)-H	BPO	0	5.81	Yes	789	n.d.				

[a] Standard error of the linear regression.
[b] n.d.: not determined.