Biomaterials 31 (2010) 4417–4425

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Decontamination of chemical and biological warfare agents with a single multi-functional material

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ARTICLE INFO

Article history:
Received 19 January 2010
Accepted 2 February 2010
Available online 2 March 2010

Keywords:
Bioactive polymer
Antimicrobial
Chemical warfare
Biological warfare
Multifunctional materials
Enzymes

ABSTRACT

We report the synthesis of new polymers based on a dimethylacrylamide-methacrylate (DMAA-MA) co-polymer backbone that support both chemical and biological agent decontamination. Polyurethanes containing the redox enzymes glucose oxidase and horseradish peroxidase can convert halide ions into active halogens and exert striking bactericidal activity against gram positive and gram negative bacteria. New materials combining those biopolymers with a family of *N*-alkyl 4-pyridinium aldoxime (4-PAM) halide-acrylate co-polymers offer both nucleophilic activity for the detoxification of organophosphorus nerve agents and internal sources of halide ions for generation of biocidal activity. Generation of free bromine and iodine was observed in the combined material resulting in bactericidal activity of the enzymatically formed free halogens that caused complete kill of *E. coli* (>6 log units reduction) within 1 h at 37 °C. Detoxification of diisopropylfluorophosphate (DFP) by the polyDMAA MA-4-PAM iodide component was dose-dependent reaching 85% within 30 min. A subset of 4-PAM-halide co-polymers was designed to serve as a controlled release reservoir for *N*-hydroxyethyl 4-PAM (HE 4-PAM) molecules that reactivate nerve agent-inhibited acetylcholinesterase (AChE). Release rates for HE 4-PAM were consistent with hydrolysis of the HE 4-PAM from the polymer backbone. The HE 4-PAM that was released from the polymer reactivated DFP-inhibited AChE at a similar rate to the oxime antidote 4-PAM.

1. Introduction

Weapons of mass destruction pose almost unimaginable threats to our society. In response to these threats, scientists have long searched for environmentally benign approaches to decontamination of both biological and chemical agents. Since terrorists are unlikely to announce what type of agent has been deployed, the ideal approach is the development of broad spectrum decontaminants that are simple to use, active against both chemical and biological agents, and do not destroy the environment into which they are deployed. This has proven to be a vexing problem. For almost two decades we have been developing materials that can detect and decontaminate chemical and biological agents [1–6], but we have recently sought to bring together multiple approaches into one material. Such a material would rapidly destroy biological and nerve agents upon hydration. A schematic of how such a multi-functional biomaterial could generate biocidal halogens while releasing nerve agent detoxifying agents that could be used for decontamination or therapy is shown in Scheme 1. The minimum ingredients necessary to activate broad spectrum chem–bio defense are the multi-functional polymer–enzyme biomaterial [7], glucose and water. The ready availability of glucose and water in blood and bodily fluids would allow the biomaterial, as described herein, to be used internally or as a wound dressing. In the case of a wound dressing, the material would have the capacity to kill bacteria, viruses and even spores. In addition, the multi-functional material could also release oximes in a controlled way to combat the effects of cholinesterase inhibitors.

The ability of halogens to kill a wide variety of microorganisms including antibiotic-resistant bacteria, viruses, and fungi has been known for centuries [8,9]. For instance, multiple isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), a major nosocomial pathogen, were susceptible to povidone iodine within 30 min at a minimal biocidal concentration of 512 ppm [10]. Polyquaternary ammonium compounds spear-headed by positively charged quaternary ammonium units also exhibit remarkably broad bactericidal activity both in solution and when delivered

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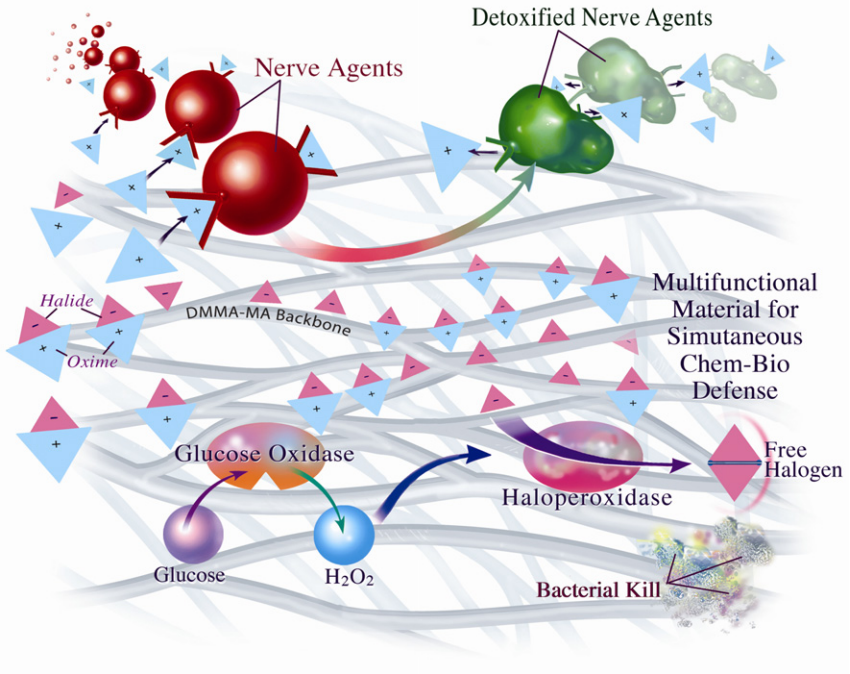
0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.02.004



Scheme 1. Schematic Depiction of a multi-functional polymer that can detoxify chemical and biological warfare agents.

from a surface [5,11]. Interestingly, quaternary pyridinium aldoximes (e.g. 2-PAM, 4-PAM, and toxogonin) are efficient reactivators of cholinesterases (ChE) inhibited by organophosphate (OP) toxins and are currently used as antidotes against OP pesticides and nerve agent poisoning [12–14]. Further, the direct interaction of pyridinium aldoximes with toxic OPs results in slow detoxification of the OP agent [15]. This slow detoxification activity was enhanced in polymers carrying nano-magnetoparticles complexed with oxime groups which displayed catalytic degradation of the nerve agent analog diisopropylfluorophosphate (DFP) [16,17]. The addition of positively charged groups was shown to further enhance the reaction rates of pyridinium oximes against toxic OPs [18] through a "charge effect" that likely increased the nucleophilic activity of the oxime group and resulted in reaction rates higher than calculated from Bronsted's law [19]. It follows that PAM-halide polymers could provide multiple, positively charged pyridinium groups thereby enhancing nucleophilic activity toward OPs as in the upper path of Scheme 1. Further, the negatively charged halide counter ions (I^- and Br^-) of the pyridinium aldoxime moiety could serve as the substrate for in-situ redox enzyme-catalyzed generation of biocidal halogens [6,9] as depicted in the lower path of Scheme 1.

We have previously shown that when glucose oxidase (GOX) and horseradish peroxidase (HRP) were electrospun into a non-reactive polyurethane fiber mesh, the generation of free iodine by the tandem redox reactions in the presence of sodium iodide and glucose killed gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria [6]. Modifying that matrix to include a polymer containing multiple positively charged aromatic nucleophiles (such as 4-PAM) with halides as counter ions could provide both a substrate for enzymatically-generated free halogen and a detoxification activity toward nerve agents. In this combined polymer matrix both positively charged nucleophiles and their negatively charged halide counter ions would serve as complementary components in a multi-functional material for decontamination of microorganisms and toxic chemicals. Further, the nerve agent detoxification component of the composite could also be a delivery vehicle for PAM-based therapeutics by conjugating the 4-PAM groups to the polymer backbone via hydrolytically metastable ester bonds. Hydrolysis of the ester bond would release 4-PAM derivatives which could then reactivate OP-inhibited AChE. We describe herein how such materials are true broad spectrum decontaminants both on surfaces and in liquid environments.

2. Materials and methods

2.1. Materials

Horseradish peroxidase (HRP) (1500 U/mg) and *Aspergillus niger* glucose oxidase (GOX) (100 U/mg), butyrylcholinesterase (BChE) (horse serum, 1690 U/mg), acetylcholinesterase AChE (electric eel 250 U/mg), iodine, bromine, sodium iodide, sodium bromide, partially saponified (87%) polyvinyl alcohol (PVA), hexafluoroisopropanol (HFIP) and glucose were purchased from Sigma St Louis MO. Medical grade polyurethane ChronoFlex AR is a product of AdvanSource Biomaterials Corp, Wilmington, MA.

γ-Aminobutyric acid, *p*-toluenesulfonic acid monohydrate (TosOH), methacryloyl chloride, 2-bromoethanol, *N,N,N*-triethylamine (TEA), *N,N*-dimethylacrylamide (DMAA), 2,2′-Azobis(2-methylpropionitrile) (AIBN), 4-pyridine aldoxime (4-PAM), 3-iodopropanol, 4-hydroxybenzophenone, pyridine, *N,N*-dimethylethylamine (DMEA), 3-bromopropanol, toluene, diethyl ether, 2-propanol, ethanol, dichloromethane (CH_2Cl_2) and acetonitrile were purchased from Sigma–Aldrich Chemical Co (St Louis MO).

2.2. Synthesis of multi-functional decontamination polymers

Polymers were prepared from a dimethylacrylamide (DMAA) – methacrylate (MA) co-polymer backbone that contained repeating quaternary 4-pyridinium aldoxime (4-PAM) covalently attached via halo-propionyl side chains. Detailed synthetic data are provided in supplementary materials.

Scheme 2 describes the strategy for synthesis of DMAA MA-3-Propionyl-ethyl 4-PAM bromide (polymer **2**). The precursor polymer DMAA-MA-3-propionyl ethyl bromide **1** was synthesized by radical polymerization from the corresponding monomers. Following the co-polymerization of DMAA with MA-propionyl bromide ester monomers, the bromo-ethyl-propionyl side chains of polymer **1** were reacted with 4-pyridine aldoxime to form the quaternary 4-PAM polymer **2**. The quaternary polymer product **2** contains *N*-hydroxyethyl 4-pyridinium aldoxime units (**7**, Scheme 2) tethered to the polymer backbone through ester bonds. The ester bonds connecting **7**, (*N*-HE-4-PAM, Scheme 2) to the polymer backbone are prone to spontaneous or enzyme-induced hydrolysis rendering polymer 2 a macromolecular carrier for sustained drug delivery (see release of **7** in Scheme 2).

Scheme 3 describes the synthetic pathway to four co-polymer structures. First, a non-quaternary co-polymer backbone containing propyl iodide side chains and benzophenone DMAA MA-propyl iodide – MA-propyl 4-pyridinium aldoxime MA-