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Plastic antibody for the recognition of chemical warfare agent sulphur mustard

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Abstract

Molecularly imprinted polymers (MIPs) known as plastic antibodies (PAs) represent a new class of materials possessing high selectivity and affinity for the target molecule. Since their discovery, PAs have attracted considerable interest from bio- and chemical laboratories to pharmaceutical institutes. PAs are becoming an important class of synthetic materials mimicking molecular recognition by natural receptors. In addition, they have been utilized as catalysts, sorbents for solid-phase extraction, stationary phase for liquid chromatography and mimics of enzymes. In this paper, first time we report the preparation and characterization of a PA for the recognition of blistering chemical warfare agent sulphur mustard (SM). The SM imprinted PA exhibited more surface area when compared to the control non-imprinted polymer (NIP). In addition, SEM image showed an ordered nano-pattern for the PA of SM that is entirely different from the image of NIP. The imprinting also enhanced SM rebinding ability to the PA when compared to the NIP with an imprinting efficiency (α) of 1.3.

Keywords: Molecular imprinting of polymer; Plastic antibodies; Chemical warfare agent; Sulphur mustard (yprite); Nano-pattern

1. Introduction

The idea of using a target molecule, as a template to direct the assembly of its own recognition site was first suggested by Pauling (1940) to explain the workings of the immune system and this formed basis for the preparation of plastic antibodies (PAs) by molecular imprinting technique. Molecular imprinting is a means of introducing sites of specific molecular arrangement into an otherwise uniform polymeric matrix. This is achieved by formation of a pre-polymerization complex between complementary monomers and the template molecule. Subsequent polymerization in the presence of a crosslinker, in a porogenic environment, results in the production of a microporous or nanoporous polymer capable of specific molecular recognition (Syu et al., 2004; Piletsky et al., 2001).

Commonly, three methods are adopted for the preparation of PAs and among these, one is covalent method which was pioneered by Wulff and Sarhan (1972), however, another one is non-covalent method that was introduced by Arshady and Mosbach (1981), from the early 1980s and up to now it remains the most popular method of imprinting due to its advantages than covalent method. The popularity of this approach is a reflection of its versatility. Indeed, a range of non-covalent interactions such as hydrogen bonding, dipolar association, ion pairing, hydrophobic interactions, etc., can be exploited in the formation of the initial template monomer complex using simple monomers (Whitcombe and Vulfson, 2001). Finally, the third method is based on the sacrificial spacer approach that was first introduced by Whitcombe et al. (1995).

PAs are having many advantages than natural antibodies in terms of preparation, storage, reusability and harsh environment utility (Haupt and Mosbach, 2000). An extensive literature survey reveals numerous applications of PAs in many areas. Recent reviews summarizes about current status of molecularly imprinted polymers (MIPs) as alternatives to antibodies in sorbent assays (Lavignac et al., 2004), MIPs and their use in biomimetic sensors (Haupt and Mosbach, 2000), MIPs as new molecular recognition materials for selective solid-phase extraction of organic compounds (Esteban, 2001), MIPs for drug delivery (Lorenzo and Concheiro, 2004), separation and

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screening of compounds of biological origin using MIPs (Xu et al., 2004), electrochemical sensors based on MIPs (Lopez et al., 2004), MIPs for biosensor applications (Yano and Karube, 1999) and MIPs potential and challenges in analytical chemistry (Mahony et al., 2005). In addition, other papers also bring out the applications of PAs in many areas including a custom synthesized PA for biotechnological application in special relevance to selective sensing of tylosin (Piletsky et al., 2004) and separation of individual antiviral nucleotide prodrugs from synthetic mixtures using cross-reactivity of a molecularly imprinted stationary phase (Allender et al., 2001).

Our establishment is working on defences against chemical and biological warfare agents; hence, we made efforts to make PAs for chemical and biological warfare agents in order to achieve their specific recognition. Literatures are minimum in this and related field based on molecular imprinting, however, available literatures are presented here. Determination of degradation products of nerve agents in human serum by solid phase extraction using MIP (Hui and Qin, 2001) and MIPs containing imidazoles and bivalent metal complexes for the detection and degradation of organophosphotriester pesticides (Yamazaki et al., 2001) were reported. Influence of quaternary amine organosilane structure on the formation and adsorption properties of surface-imprinted silicates for the soman hydrolysis product pinacolyl ethylphosphonate (Markowitz et al., 2001), potentiometric sensing of chemical warfare agents (Zhou et al., 2004), biomimetic catalysis of an organophosphate by molecularly surface imprinted polymers (Say et al., 2005) and non-covalent imprinting of phosphorous esters (Hall et al., 2005) were also studied. In addition, molecular imprinting of biological warfare agent Ricin (Lulka et al., 2000) and its A and B chains to organic silanes and MIPs for chemical warfare agents such as soman, sarin, VX and EA2192 detection in multiple water matrices were also achieved (Jenkins and Bae, 2005). Moreover, PAs were also available for the hydrolysis products of chemical warfare agents soman and sarin (Jenkins et al., 1999), pesticide and insecticides (Jenkins et al., 2001) and biological warfare agent microcystin-LR (Chianella et al., 2003). SM is a blistering agent, which is having the capability to alkylate body cells on short-term exposure and was used in the Iran–Iraq war. Moreover, its production, stockpiling and use are prohibited under chemical weapons convention (CWC, 1997) and monitored by an international organization known as organization for the prohibition of chemical weapons (OPCW), located at The Hague, The Netherlands. Thus, the recognition of SM is very essential in many aspects. To our knowledge, so far no report is available in the literature for the preparation and characterization of PA for the blistering chemical warfare agent SM based on molecular imprinting technique, hence, the present study was undertaken.

2. Materials and methods

2.1. Chemicals

Methacrylic acid (Aldrich), ethylene glycol dimethacrylate (Fluka), 2,2′-azobis isobutyronitirle (Otto Kemi), methanol (Merck) and carbon tetrachloride (SD fine) were analytical grade reagents and further appropriate purification was performed to remove the stabilizers from the monomers before use. Sulphur mustard was synthesized in our establishment and was found to be above 99% pure by gas chromatographic analysis. Utmost care was taken during the synthesis of SM in the declared facility of the establishment (Inspected by the Inspectors of the Organization for the Prohibition of Chemical Weapons, The Hague, The Netherlands). The experiments in this study with SM are performed in a fume hood with appropriate protection measures.

2.2. Instruments

AP2C hand held detector (Proengin, France), Chemito GC-1000 (India) with Pulsed Flame Photometric Detector (O-I Analytical, USA), Chemito GC 8610-FID (India), ESEM-EDX (Quanta 400-ESEM with EDAX-FEI, The Netherlands) and surface area analyzer (Autosorb 1C-AS1C-RGA2, Quanta Chrome, USA) were used.

2.3. Preparation of plastic antibody

PA was prepared by molecular imprinting technology. Molecular imprinting technology consisted of four steps, which are indicated as Scheme 1. In the first step, pre complexation was performed between the monomer methacrylic acid (25.0 mmol) and the target blistering chemical warfare agent SM (5.0 mmol) for 10 min in an inert atmosphere. In the second step, ethylene glycol dimethacrylate (100 mmol) was added as cross linker and subsequently in the third step, radical initiating agent 2,2′-azobis isobutyronitirle (2.5 mmol) which acts as polymerization catalyst was introduced in to the mixture. All the reactions and additions were performed in an inert atmosphere and finally the vial was kept in an ultrasonicator for 90 min and then placed in a vacuum oven at 70 °C for overnight in order to get the polymer. In the fourth step, removal of target molecule SM from the polymer was performed with methanol. The methanol treatment was performed repeatedly until not getting any signal for SM in AP2C hand held detector and GC-PFPD system. Methanol treated with the polymer was first subjected to AP2C detection, which is a hand held point detector for chemical warfare agents. After every extraction, the methanol and polymer-containing vial both were shown to AP2C for not showing any signal for the presence of SM. Again the polymer was treated with methanol for SM extraction and then 1 μl of the extract was injected to the GC-PFPD system that is having more sensitivity than AP2C. After every extraction, the extract was injected to the GC-PFPD system until not getting any signal for the SM in the chromatogram. Later, SM imprinted PA was kept in a vacuum oven for 1 h to remove the solvent methanol that is used for the removal of SM. Then the SM imprinted PA was crushed in a mortar and pestle to get fine powder PA and then preserved in a vial to use for further studies. A control non-imprinted polymer (NIP) was also prepared in the similar fashion as PA without the target molecule SM in order to see the effect of imprinting on SM rebinding ability of PA and to compare the results.