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Dual-functional OPH-immobilized polyamide nanofibrous membrane for effective organophosphorus toxic agents protection

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ABSTRACT

In both civilian and military domains, the increasing use of highly toxic organophosphates (OPs) has created an urgent demand for developing an effective method that could protect the human body from OP poisoning. In this study, a novel dual-functional protecting material with both OP filtration and degradation functions was designed and fabricated. The filtration function was endowed by the electrospinning polyamide 66 (Nylon 66, PA-66) nanofibrous membrane, while the OP degradation function was based on the immobilization of organophosphorus hydrolase (OPH) on membrane through glutaraldehyde (GA) crosslinking. A systematic study of the relationship between the membrane structure, filtration ability and OP degradation activity revealed that PA-66 nanofibrous enzyme with 20 μm thickness exhibited perfect uniform morphology, and the particle filtration efficiency was over 99%. The nanofibrous enzyme exhibited a specific activity of 41 U/g; the enzyme activity recovery was 53.3%, and the methyl parathion (MP) degradation efficiency was 40%. Moreover, the nanofibrous enzyme expressed excellent organic solvent stability; approximately 70% of its initial activity was retained after incubation in xylene solvent for 24 h at 25 °C. The residual activity was 40% after being stored for 30 d at 25 °C. After 10 repeated uses, the residual activity of the nanofibrous membrane was 37%. This study demonstrated the application potential of dual-functional PA-66 nanofibrous enzyme in biochemical protection, aimed at various military and civilian applications.

1. Introduction

Protection of the human body from toxic agents existing under certain circumstances was universally considered an issue of crucial importance, especially regarding protection from highly toxic organophosphates [OPs, (organophosphorus compounds)] in the air. The high toxicity and lethality of OPs were owed to its ability to cause neuromuscular paralysis throughout the body, ultimately leading to death by asphyxiation [1]. This extremely toxic property of OPs promoted its worldwide use in manufacturing different kinds of neurotoxin warfare agents and organophosphorus pesticides [2], which also indicated that OP usage required prior protection, not only in the agricultural field but also modern warfare.

In order to protect the human body from OP exposure, a physical protective mask and clothing equipped with an activated carbon layer were first adopted [3]. Although activated carbon's highest adsorption capacity for OPs reached 878 mg/g [4], this traditional carbon-based protective material still bore the limitation of deactivation once the adsorption equilibration was reached. Thus, based on the above physical protective method, a chemical catalyst was added to the activated carbon protective materials in order to help partly hydrolyze the OPs. Ramaseshan et al. synthesized a chemical catalyst using β-cyclodextrin and *o*-iodosobenzoic acid [5]. The protective capability of this synthesized catalyst on OPs was found to be 11.5 times higher than the protective capability of pure activated carbon. However, the chemical catalysis method could not completely degrade OPs into a nontoxic product; the toxic intermediate products inevitably caused secondary pollution to the human body and its surroundings.

Detoxification of OPs through the enzymatic degradation method using organophosphorus hydrolase (OPH) has recently been of particular interest [6]. OPH could hydrolyze OPs with broad substrate specificity and high efficiency [7–9]. Through cleaving P—S, P—O, P—CN, and P—F bonds [10,11], OPs were completely detoxified into nontoxic products. Although the high degradation ability of OPH on OPs has been demonstrated [6], the usage of

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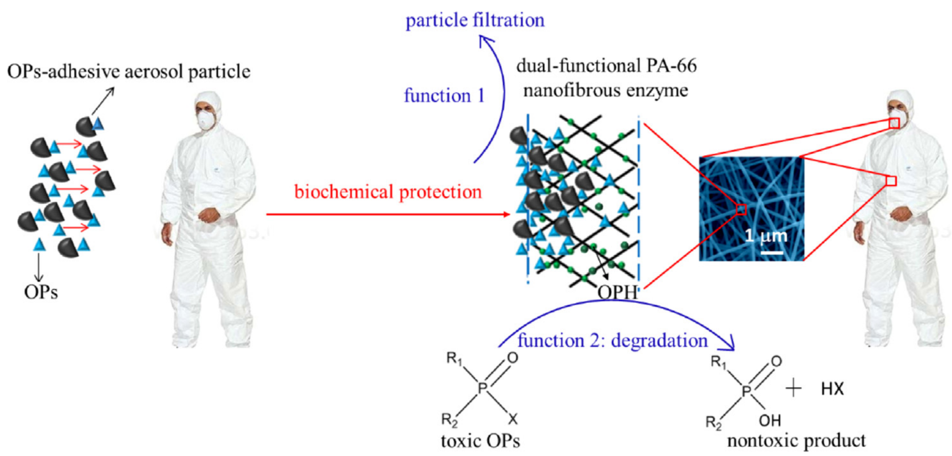

Fig. 1. Schematic diagram of biochemical protection mechanism of dual-functional PA-66 nanofibrous enzyme.

native OPH was still confined to spraying OPH solution on OPs polluted locations. OPH still could not be used in real-time to protect the human body from OP poisoning due to the lack of long-term operational stability and difficulty in recovery [12–15].

Enzyme immobilization could provide an effective method to stabilize OPH activity. The OPH immobilized on silicone polymers retained more than 90% of its activity after stored for 180 d, while native OPH only exhibited a half-life of 2.7 d under the same storage conditions [16]. However, the existing immobilization carrier also suffered severely from low OPH loading amount and activity recovery. Li et al. immobilized OPH onto insoluble bovine collagen fibers; the residual activity of immobilized OPH reached 98% after being stored 240 d, but the activity recovery of OPH after immobilization was only 35% [17]. Consequently, it was still necessary to develop an immobilization carrier that could simultaneously bring about enhanced activity and stability for OPH.

Furthermore, OPs released from biochemical weapon explosions or pesticide spraying tended to combine with moisture and dust in the air, transforming into OPs-adhesive aerosol particles [18]. From this perspective, protecting the human body from OP injury should also bring aerosol particles into consideration, but this matter has been ignored by most of the existing researchers. Suppose that OPH was immobilized on a carrier with a particle filtration function; OPs-adhesive aerosol particles would be effectively captured by carrier, thus promoting the accessibility of OPH with OPs substrates, meaning that the total OPs prevention capacity would also be improved. Therefore, novel functional carrier materials with high OPH immobilization efficiency and particle filtration capacity were an urgent necessity for OPs biochemical protective applications.

One distinctive feature of the nanofibrous membrane was the nano-scale fiber diameter, which varied depending on electrospinning conditions, endowing nanofibers with controllable porosity and a super-high surface area. Therefore, nanofibers possessed unique superiority in air permeability, particle filtration and adsorption, automatically becoming the appropriate choice for preparing protective masks and clothing. Regarding nanofibers with a pore size of around 100 nm, the filtration efficiency for particles with a 200 nm diameter reached over 95% [19,20]. On the above basis, other properties of nanofibers, such as more easily modified surface functionalities and a membrane-like appearance were suitable for enzyme immobilization and reusability [21–27]. Lipase was immobilized on polyacrylonitrile nanofibers with a high enzyme loading of 21.2 mg/g-fiber; enzyme activity recovery was 81%, and over 95% of the initial enzyme activity was retained after being stored for 20 d [28]. It could be seen that in the fields of filtration and enzyme immobilization, electrospun nanofibrous membranes all exhibited unusual superiority. Unfortunately, to date there have not been reports regarding the immobilization of OPH onto electrospun nanofibers, not to mention the application of nanofibrous enzymes in biochemical protection.

The above research status inspired us to design a dual-functional nanofibrous enzyme to be used for biochemical protection in various military and civilian applications. In order to comprehensively defend the human body against toxic OPs molecules and OPs-adhesive aerosol particles, nanofibrous enzyme membrane with "dual-function" including particle filtration function and OPs detoxification function were constructed. The dual-function of membrane was further illustrated in Fig. 1. When OPs molecules and OPs-adhesive aerosol particles attempted to diffuse across the polyamide 66 (PA-66) nanofibrous enzyme membrane, on the one hand, the membrane with suitable pore diameter and high surface area performed the first function of particle filtration by preventing the penetration of OPs-adhesive aerosol particles. In detail, particles would be directly excluded at the outer surface of membrane or be retained finally inside the membrane near the surface. On the other hand, OPH was immobilized on the PA-66 nanofibrous membrane through gluaraldehyde (GA) crosslinking, and the immobilized OPH would play the second function of degradation. Toxic OPs molecules and OPs adhered on aerosol particles would all be enzymatically detoxified into nontoxic products in a moist atmosphere condition. The collaboration of the above filtration and degradation dual-function was expected to fundamentally strengthen the defense capability of nanofibrous enzyme to OPs. In the present study, Methyl parathion (MP), a common high toxic OP generally used in military warfare agents and organophosphorus pesticides, was studied as the model of the OPs substrate. The relationship of the membrane structure and the dual-functionality of the nanofibrous membrane with OPH loading were systematically investigated. The kinetics and stability of nanofibrous enzyme were also detected. This research would lay a theoretical foundation and feasible framework for the further development of nanofibrous enzyme used in practical conditions.

2. Experiment

2.1. Materials

Polyamide 66 (Nylon 66, PA-66, density 1.37 g/cm^3) was obtained from BASF SE (Germany). Organophosphorus hydrolase (OPH, EC 3.1.8.1) was purchased from Schengenbiya Bioengineering Technology Co., Ltd. (Biva enzyme, China). Coomassie Brilliant Blue (G-250) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich Co., LLC. (Saint Louis, MO, USA). Methyl parathion