The generation of tri-block copolymers capable of detecting and detoxifying organophosphates

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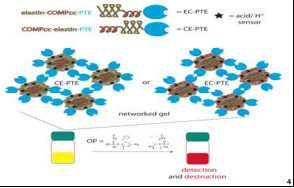
Abstract

The use of organophosphates (OP) as a chemical warfare agent poses a credible threat. The development of materials capable of detecting and detoxifying these agents is an effective manner to counter these threats. A number of enzymes exists which are capable of degrading a wide range of OP compounds however, to maximize the effectiveness of these enzymes for biocatalytic sensing and decontamination they need to be immobilized. Employing the biophysical properties of three different proteins we propose the formation of a novel tri-block copolymer capable of detecting and detoxifying OPs. The tri-block is in A.B.C or B.A.C configuration where the A block is an elastin like-peptide capable of undergoing a reverse phase transition, the B block is COMP a small protein capable of housing small molecules and the C block is phosphotriesterase (PTE) an enzyme found in soil bacterium which has been shown to have high catalytic towards a broad range of OPs.

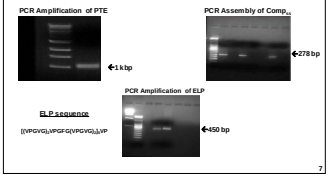
1

Assumed formation of PTE hydrogel



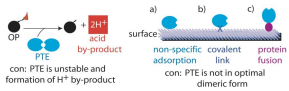
4

PCR construction of biosynthetic parts



7

Current problems facing PTE biosensors



con: PTE is unstable and formation of H^+ by-product

con: PTE is not in optimal dimeric form

- *Organophosphates (OPs) are neurotoxic agents used as chemical warfare agents.*
- *Phosphotriesterase (PTE) is an enzyme capable of detoxifying OPs. To stabilize and improve the efficacy of PTE, many have attempted to immobilize using non-specific, covalent and fusion based methods. However, the dimeric form of PTE is not necessarily maintained when immobilized. Also, 2 H^+s are liberated upon hydrolysis of OP, deactivating PTE.*

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Assessment of hydrogel effectiveness

	stability			
	activity	pH	T	recyclability
PTE*	$6.8 \times 10^6\ M^{-1}s^{-1}$	~ 8.5	≤ 35°C	●
EC-PTE	●	●	●	●
CE-PTE	●	●	●	●

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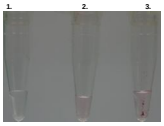
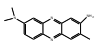
Binding of pH small molecule indicator to EC construct



1. Purified EC at 4°C
2. EC + Neutral red at 4°C pH 6.5
3. EC + Neutral red at 55°C pH 6.5



Neutral red is a small molecule pH indicator which yields a yellow color under basic conditions and turns red as the solution becomes more acidic

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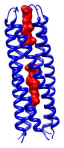
Biophysical properties of A &B blocks



Block A

- Elastin like-peptide (ELP)
- Solube in water <25°C but undergo a reverse phase transition > 25°C
- Sequence can be tailored to adjust reverse phase transition

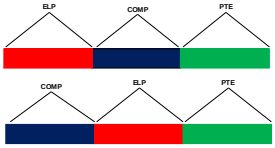
Block B

- Coiled coil region of Cartilage Oglomeric Protein (COMP)
- Pentameric protein domain with a hydrophobic pore capable of housing small hydrophobic molecules
- Has been shown to undergo reversible structural transition

Li, B., *Mol Biol* 2001, 305: 581-92.
VOstvik, S., EMBO J 2002, 21: 5960

3

Development of block polymers using recombinant DNA Technology



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Future Direction

- ♥ Generate C/E/PTE & E/C/PTE constructs
- ♥ Express and purify fusion proteins
- ♥ Develop assay for PTE hydrogel formation
- ♥ Assess the functionality of PTE hydrogels

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Acknowledgements

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