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Justin R. Whitfield^a; Alessandra Morelli^a; John C. Warner^a

^a Center for Green Chemistry, University of Massachusetts Lowell, Lowell, MA, USA

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Enzymatic Reversal of Polymeric Thymine Photocrosslinking with *E. coli* DNA Photolyase

JUSTIN R. WHITFIELD, ALESSANDRA MORELLI,
AND JOHN C. WARNER

Center for Green Chemistry, University of Massachusetts Lowell, Lowell,
MA, USA

Polymers containing pendant thymine groups undergo a $2\pi + 2\pi$ cycloaddition cross-linking reaction. The enzyme DNA photolyase has been demonstrated to reverse this photocrosslinking in thymine containing styrene derivatives.

Keywords biotransformations, green chemistry, photocrosslinking, polymers

Introduction

For a variety of environmental and economic reasons, the search for recyclable polymeric materials has been going on for quite some time (1). Indeed, the principles of Green Chemistry specifically address the need to consider the renewability of materials as well as their degradative fate (2). Unfortunately, the problem is almost paradoxical. On the one hand, we need strong, robust materials that can withstand the rigors of commercial use, while on the other hand, we do not want these materials to persist in the environment after their use. Several years ago, water soluble polystyrene polymers containing pendant thymine groups were reported to undergo photodimerization via a $2\pi + 2\pi$ cycloaddition reaction (3).

This process has been well characterized in DNA (4), and is considered one of the fundamental mechanisms leading to the development of certain skin cancers (5). Highly efficient photoresist materials have been developed and investigated based on these water soluble bioinspired synthetic polymers (6).

Humans have certain enzymatic protection schemes that serve to protect us from mild UV irradiation (7). There are enzymes present in our cells that recognize regions of DNA that have led the photo $2\pi + 2\pi$ reaction to occur. These excisionases “snip out” the photodamaged region and replace it with fresh material. Some organisms are more conservative in their nucleic acids and use a specific enzyme DNA photolyase to directly reverse the photodimerization reaction so as to return to the unaltered form (8). Having a polymeric crosslinking mechanism in hand that is based on the same thymine

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Address correspondence to John C. Warner, Center for Green Chemistry, University of Massachusetts Lowell, 1 University Avenue, Suite 4, Lowell, MA 01854, USA. E-mail: john_warner@uml.edu

cycloaddition reaction, we decided to explore if the enzyme *E. coli* DNA photolyase could, in fact, be used to reverse our $2\pi + 2\pi$ crosslinking mechanism.

Results and Discussion

As a model system to investigate the feasibility of this enzymatic reversal, we decided to use a simple pigment-toned photoresist process (Figure 1) (9). In this system, a thin film of a water soluble thymine containing polymer is coated on a polyethylene terephthalate substrate. The polymer that we chose to use was a 1 : 4 copolymer of vinylbenzylthymine and vinylbenzyltriethylammonium chloride because we have had a great deal of experience using this particular system.

The polymeric film was then irradiated with short wave ultraviolet light so as to generate an area of immobilized photocrosslinked polymer. The irradiated film was then washed thoroughly with water to remove any of the unreacted polymer. In order to efficiently visualize the immobilized polymer, we took advantage of the cationic nature of the solubilizing monomer, and toned the film in a bath of the anionic dye FD&C Red #40. The electrostatic interactions allow for clear visualization of the regions of the polyethylene terephthalate film that has immobilized the remaining polymer (Fig. 2).

To evaluate the effectiveness of the DNA photolyase to reverse the crosslinking of the immobilized polymer, regions that were pre-irradiated and washed were subjected to a buffered solution of the enzyme. In order to investigate the reusability of the enzyme system, subsequent applications demonstrated continued efficacy. A control with the buffer solution without the enzyme was also prepared. The regions of pre-irradiated polymer that had been subjected to the photolyase were washed thoroughly and "toned" with the anionic dye. The resulting color densities of dye in these experiments are summarized below (Fig. 3).

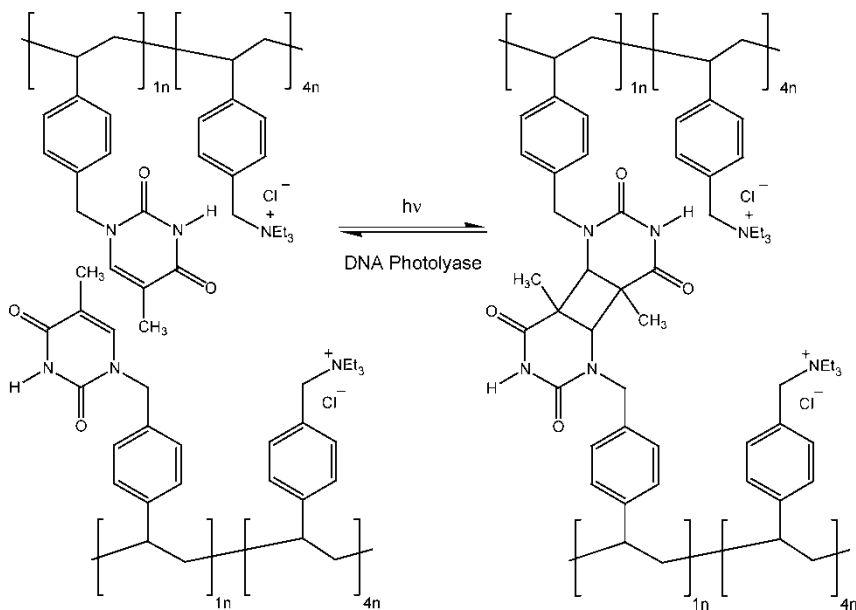


Figure 1. $2\pi + 2\pi$ cycloaddition and enzymatic reversal in polystyrene polymer system.

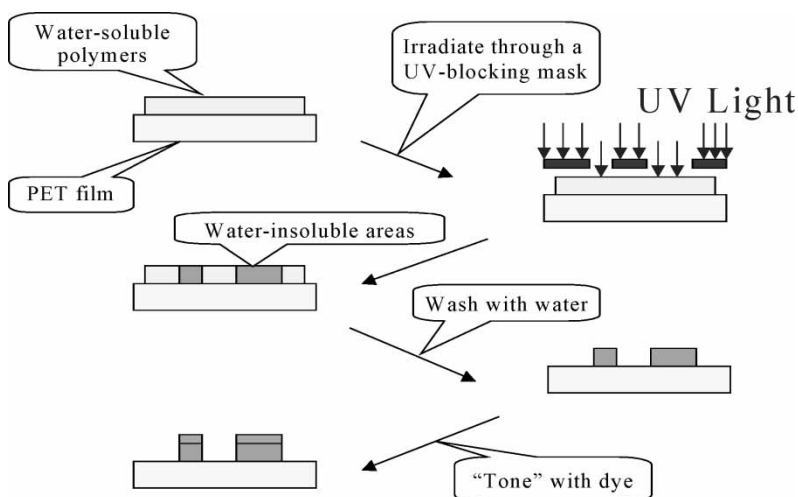


Figure 2. Photoresist-toning procedure.

It can be seen that the buffered solution without any enzyme [control] has a negligible effect on the immobilized polymer. The application of the enzyme solution removed almost all of the immobilized polymer, reducing the color density to 6%. The reuse of this solution a second and then a third time demonstrated that the enzyme remained effective at 7% and 12%, respectively, however it showed some sign of loss of activity.

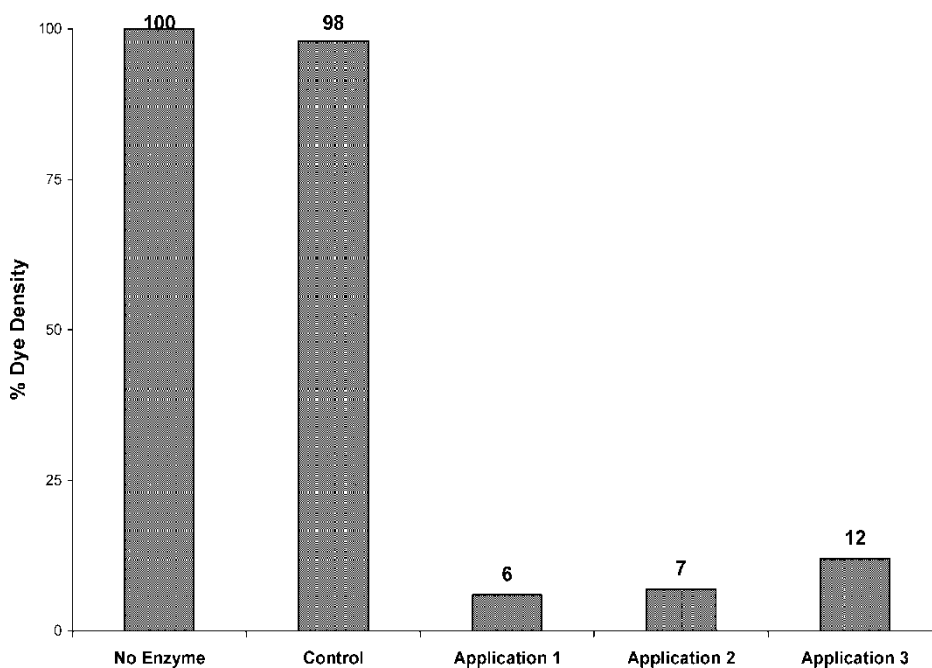


Figure 3. Effect of DNA Photolyase on "toned" dye density of crosslinked films.

In order to verify that the mechanism of action of the enzyme involved removal of the immobilized polymer, scanning electron microscopy was used. Micrographs of the system show the immobilized polymer absent in the regions where the photolyase was applied (Fig. 4).

Clearly, DNA photolyase is an efficient reversal agent for thymine $2\pi + 2\pi$ photo-dimerization in these polymeric systems. Future work is underway to elucidate the exact mechanism of this reversal and to explore the practical applications available.

Experimental

Materials

Vinylbenzylthymine : vinylbenzyltriethylammonium chloride 1:4 copolymer was prepared as described previously (7). *E. coli* DNA Photolyase and RECTM Buffer 14 was purchased from Trevigen[®].

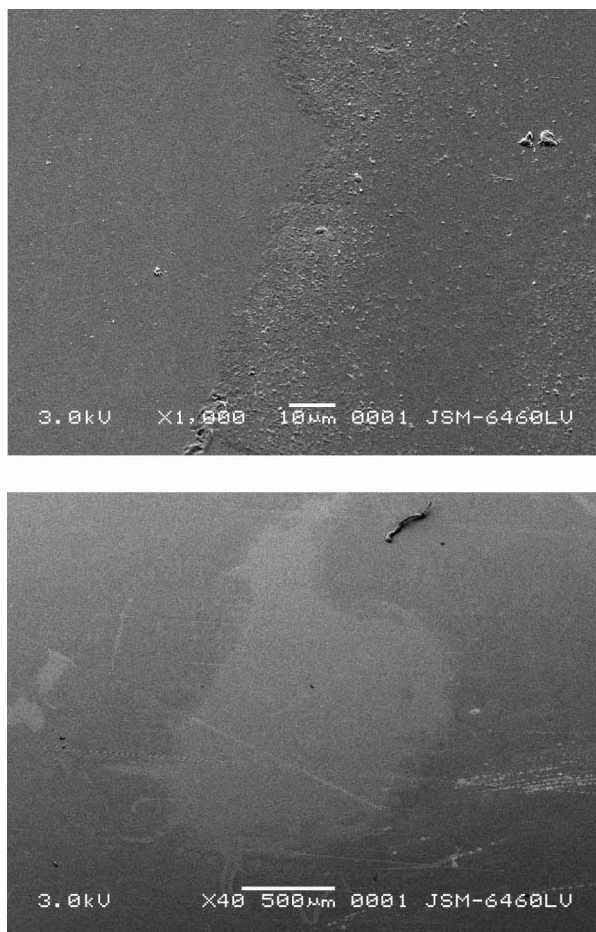


Figure 4. SEM of photoresist film showing region of removal at (a) 1000X and (b) 40X, the particle at top center is a contaminant.

Copolymer Coating

A 10% by weight solution of 1 : 4 vinylbenzylthymine : vinylbenzyl triethylammonium chloride was cast to a film with a #3 Meier type [R.D. Specialties, Inc] coating rod to a polyethylterephthalate sheet [X4C1 hydrophilic subcoating] to deposit approximately 6.8 cm wet film thickness. This film was then allowed to air dry.

Photoirradiation and Imaging

Using an Entela[®] Model UVLS-28 UV lamp, the film was irradiated for 30 sec with the 254 nm light setting at a distance of 1.5 cm from the film surface. The film was then run under tap water and gently wiped to remove uncrosslinked VBT/TEQ polymer.

Enzymatic Processing

The enzyme and 10X REC[™] Buffer 14 vials were removed prior to being used from storage at -80°C . Once they had reached room temperature, the vials were spun briefly to concentrate samples to the bottom of the vials. A mixture of 10 μl of *E. coli* DNA Photolyase as received from Trevigen[®] (100 g/40 μl) and 20 μl 1X REC[™] Buffer 14 (from 900 μl of pH 7.0 KOH and 100 μl of 10X REC Buffer 14) was spotted onto the film with a P 100 VWRBrand[®] autopipette. The spot of enzyme solution was agitated intermittently with the autopipette tip to ensure proper mixing. After 8 $\frac{1}{2}$ min, the spot was reacquired with the autopipette and moved to a fresh location on the film. The spot was agitated as previously. After an additional 3 $\frac{1}{2}$ min, the spot was reacquired and moved again. The spot was agitated as previously. The film was washed as previously under tap water to remove the enzyme solution as well as uncrosslinked polymer. To visualize the results of this experiment the film was placed polymer coating side down into a dilute solution of FD&C Red #40 in water for approximately one minute. Dye densities were measured via inverse pixel quantification from scanned images of the films.

Acknowledgment

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Supporting Information Available

Full experimental procedure is available via email from john_warner@uml.edu. For additional information please visit www.greenchemistry.uml.edu.

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