Chemo-enzymatic cascade reactions of flavin-dependent monooxygenase and firefly luciferase for detection of halogenated and nitro phenols

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Abstract

Chemo-enzymatic cascade reactions of flavin-dependent monooxygenase and firefly luciferase for detection of halogenated and nitro phenols
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Halogenated and nitro phenols are used widely as agro- and industrial chemicals. Their accumulations in environment are seriously concerned because they are highly toxic. Their usage and environmental contamination are regulated by United State Environmental Protection Agency (US-EPA). Our group has studied enzymatic reactions of flavin-dependent monooxygenase (HadA) which is able of catalyzing dehalogenation and denitration of 4-halogenated and 4-nitro phenols to generate *p*-benzoquinone. As *p*-benzoquinone from the HadA reactions can react with D-cysteine to generate D-luciferinwhich is a substrate for firefly luciferase, we have developed a novel chemo-enzymatic method for detection of p-halogenated and p-nitro-phenols. Ratios of *p*-benzoquinone and D-cysteine were varied and monitored absorption (330 nm) and fluorescence (*Em* 530 nm) spectrum changes to obtain the best condition for D-luciferin synthesis. We obtained the best ratio of D-luciferin synthesis as 1:4 of *p*-benzoquinone:D-cysteine. We coupled chemo-enzymatic detection to detect

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the conversion of p-nitrophenol to D-luciferin using LC-ESI-qTOF techniques. The results showed that not only D- luciferin could be formed but a6-benzothiazole-2-carbaldehyde was also detected. However, this novel chemo-enzymatic reactions can be coupled with the reaction of firefly luciferase to generate light, indicating that this method can be used to detect halogenated and nitro-phenols.

Keywords: Chemo, enzymatic cascade, Flavin, dependent monooxygenase, Firefly luciferase, D, luciferin, Halogenated phenols, Nitro phenols