Improvement of glycine oxidase by DNA shuffling, and site-saturation mutagenesis of F247 residue (Abstract)

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ABSTRACT

Glyphosate is a broad spectrum herbicide widely used throughout the world, and it could be degraded by glycine oxidase (GO) through cleavage of C-N bond. For a better understanding of the structure-function relationship and improving activity of B3S1 (GO from *Bacillus cereus*) (Zhan et al, 2013), DNA shuffling was performed. A mutant B4S7 (K_m, V_{max}, k_{cat} and k_{cat}/K_m on glyphosate were 0.1 mM, 0.002401 mM min^{-1},3.62 min^{-1} and 36.2 mM^{-1} min^{-1}, respectively. The four parameters on glycine were 50.34 mM, 0.02098 mM min^{-1}, 2.18 min^{-1} and 0.04 mM^{-1} min^{-1}, respectively) was obtained from 10,000 clones, which presented a 3.9-fold increase of specificity constant (the k_{cat}/K_m ratio between glyphosate and glycine) compared with B3S1. Especially, the K_m value of B4S7 to glyphosate was much less than those reported GO. Structure modeling and molecular docking indicated that the novel mutation point F247S was close to the active site of the enzyme. To identify the role of the site, the remaining 19 amino acids were introduced into the site by site-saturation mutagenesis. The result showed that compared with B3S1, the specificity constant of mutant F247S and F247R increased 0.64-fold and 1.04-fold, separately. While to F247E, it decreased 2.01-fold. Therefore, the site 247 plays a crucial role in regulating substrate specificity. This study provides new information on the structure-function relationship of glycine oxidase and the development of glyphosate tolerance crops.

Keywords: Glycine oxidase by, DNA shuffling, site-saturation mutagenesis, F247 residue