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Molecular Dynamics Approach in the Comparison of Wild-Type and Mutants of D-hydantoinase

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Pregabalin (PGB), has already shown great potential in clinical applications as an analgesic, anticonvulsant, and anxiolytic drug [1]. A suitable reaction for the production of PGB is the enzymatic desymmetrization of 3-isobutyl glutarimide (IBI) to *R*-3-isobutyl glutaric acid monoamide (*R*-IBM), which can be converted to *S*-PGB by the Hofmann rearrangement. PGB is a chiral compound and *S*-PGB exhibited a much greater level of anticonvulsant activity than *R*-PGB [2]. Therefore, *R*-selective enzymes are being researched for the asymmetric hydrolysis of IBI to yield *R*-IBM. An enzyme that can be used is D-hydantoinase, which belongs to the family of cyclic amidohydrolases and hydrolyzes bulky cyclic imides into their corresponding half-amides [3]. Molecular docking indicated that three residues, Met-63, Leu-65, and Cys-317, may affect the substrate binding [4]. In this work, we did molecular dynamics (MD) simulations on both wild-type and mutant enzymes (cf. Table 1) to determine the effects of the mutations on the local structure of the active site and the overall structure of the enzyme, as well as whether there is a relationship between these changes and *ee* and k_{cat}. Based on the RMSD, RMSF, and Rg analysis of MD simulations results, M63AL65AC317T was determined to be the best mutant of this enzyme. This result is in good agreement with the experimental results, which indicate that this mutation has 96.52% ee_p [5].

Table 1. The ee_p value and the average Rg, RMSF, and RMSD values (in Å) over the 200 ns production simulations from the MD simulations.

	ee_p (%)	Rg	RMSF	RMSD
WT	38.77 ± 0.95	21.80	14.55	1.53
M63A	89.83 ± 0.10	21.72	13.33	1.46
M63AL65A	93.99 ± 0.24	21.81	14.90	1.56
M63AL65H	95.66 ± 0.16	21.75	15.12	1.51
M63AL65AC317T	96.52 ± 0.27	21.77	12.54	1.37
M63AL65HC317T	97.00 ± 0.54	21.80	19.15	1.72

Keywords: Pregabalin, D-hydantoinase, Molecular docking, MD simulations

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