Proc. KMB Annual Meeting
June 24-26, 2003, Muju, Korea



# Structure-Based Design of Substrate Specificity of D-Hydantoinase

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D-Hydantoinases are versatile industrial enzymes for the synthesis of optically pure d-amino acids which are extensively used in pharmaceutical fields. These enzymes are found in various microorganisms and have diverse substrate specificities. Of them, the d-hydantoinase from *Bacillus stearothermophilus* SD1 has many advantages, such as strict enantioselectivity, easy over-expression, and thermostability. But its activity is relatively low for aromatic hydantoin derivatives whose products are used as building blocks for important pharmaceuticals, such as semisynthetic antibiotics. On the other side, the recently cloned phenylhydantoinase from *Escherichia coli* has distinct activity for the aromatic hydantoin derivatives. Based on the recently reported three dimensional structure of d-hydantoinase, we compared the active sites of the two enzymes and designed new d-hydantoinase with desirable substrate specificity. The designed mutant enzymes show a remarkable change in substrate specificity. Particularly, the mutation of F159 into smaller amino acids, such as alanine and serine, resulted in the dramatic change in the substrate specificity. The strategy of tailing the substrate specificity and the characteristics of the designed enzyme will be discussed in detail.

## References

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