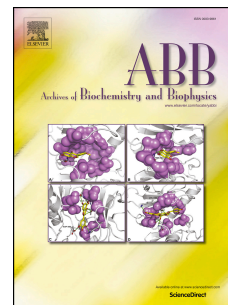


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Increase of *Bacillus badius* Phenylalanine Dehydrogenase specificity towards Phenylalanine Substrate by Site-directed Mutagenesis

Farzad Yousefi^{1,2}, Farangis Ataei¹, Seyed Shahriar Arab³, Saman Hosseinkhani^{1*}

¹ Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

² Afghanistan Specialists in Medicine Association (ASMA)

³ Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

*Address correspondence to: Saman Hosseinkhani, Tel: (98)-21-82884407; Fax: (98)-21-82884484;

E-mail: (saman_h@modares.ac.ir)

Abstract

Phenylalanine dehydrogenase (PheDH) is a key enzyme in medical diagnostic for determining the amount of phenylalanine to detect phenylketonuria (PKU) disease. However, determination of phenylalanine can be usually disturbed in presence of tyrosine in blood samples. Position N145 of *B.sphaericus* PheDH, has been previously showed a crucial role in substrate binding, which corresponded by position V144 in *B. badius* PheDH. In this study, the PheDH of *B. badius* due to reasonable activity was cloned and subjected to site-directed mutagenesis at mentioned position, followed by kinetic and structural studies to find more exclusive mutants. The results showed that the V¹⁴⁴L mutant considerably increases specificity toward phenylalanine and decreases toward L-tyrosine, while in V¹⁴⁴N mutant, the specificity reduces toward phenylalanine and increases toward tyrosine. Moreover, concerning the mutated V¹⁴⁴D, significantly reduced k_{cat} and also decreased k_m value for phenylalanine relative to that of wild type. The Phe/Tyr specificity constant in V¹⁴⁴L increased more than 4-fold compared to wild type, makes it to be a suitable candidate for more specific identification of PKU. Finally, docking and molecular dynamic simulation on wild type and mutants clarified the structural basis behind more specificity of V¹⁴⁴L mutant for phenylalanine substrate.

Keywords: Phenylalanine dehydrogenase , Mutation, specificity constant.

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