Research Article

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Implications of Stisa2 catalytic residue restoration through site directed mutagenesis

Bölgeye yönlendirilmiş mutajenez ile Stisa2 katalitik amino asit restorasyonunun olası sonuçları

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Abstract

Objective: Restoration of catalytic activity of Isa2 from *Solanum tuberosum* (Stisa2) through restoration of conserved catalytic residues by site directed mutagenesis.
Methods: The six conserved amino acid residues absent in the Stisa2 gene were restored by mutation using the overlap extension PCR and the asymmetrical overlap extension PCR methods. Next, mutant Stisa2 with restored catalytic residues was expressed in *E. coli* Rosetta 2 under optimized conditions. Evaluation of debranching activity on starch, amylopectin and β-limit dextrin was carried out by measuring the amount of glucose equivalents released using the bicinchoninic acid assay.
Results: Both qualitative and quantitative analysis showed that the restoration of the conserved residues in the catalytic site did not restore starch debranching activity. Molecular modeling showed greater than expected distances between the catalytic triad in mutant Stisa2. These additional distances are likely to prevent hydrogen bonding which stabilizes the reaction intermediate, and are critical for catalytic activity.
Conclusions: These results suggest that during evolution, mutations in other highly conserved regions have caused significant changes to the structure and function of the catalytic network. Catalytically inactive Isa2, which is conserved in starch-producing plants, has evolved important non-catalytic roles such as in substrate binding and in regulating isoamylase activity.

Keywords: Isoamylase; Site directed mutagenesis; Catalytic site; Starch debranching; Isa2.

Özet

Amaç: Bölgeye yönlendirilmiş mutajenez ile *Solanum tuberosum* Isa2 (Stisa2) katalitik aktivitesinin, korunmuş katalitik amino asit restorasyonu ile geri kazanılması.
Metod: Stisa2 geninde bulunmayan 6 korunmuş amino asit, örtüşen uzatmalı PZR ve asimetrik örtüşen uzatmalı PZR yöntemleri ile gene eklendi. Mutasyana uğratılarak katalitik bölgesi restore edilen Stisa2 geni optimize edilmiş şartlarda *E. coli* Rosetta 2 de ifade edildi. Bicinchoninik asit assayi kullanılarak; Stisa2 gen ürününün nişasta, amylopektin veβ-limit dextrin üzerindeki budayıcı aktivitesi glikoz türevlerinin salınım oranı ölçülerek değerlendirildi.
Bulgular: Yapılan nicel ve/ve ya nitel çalışmalar katalitik bölgedeki korunmuş amino asitlerin restorasyonunun nişasta budayıcı aktivitesini restore etmeye yetmediğini gösterdi. Mutant Stisa2 ile yapılan moleküler modelleme çalışmaları katalitik triad arasındaki uzaklıkların beklenenden çok daha uzun olduğunu gösterdi. Bu uzaklıklar muhtemelen ara ürün sabitlenmesinde ve dolayısı ile katalitik aktivitede önemli olan hidrojen bağının gerçekleşmesini önlemektedir.

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