Cloning of three bifunctional and one trifunctional enzyme chimerae.

Arruda, L. M.[1]; Ward, R. J.[2]

[1]***Biochemistry and Immunology Department, FMRP-USP, Brazil***

[2]***Chemistry Department, FFCLRP-USP, Brazil.***

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Rational design of hybrid enzymes is a proven strategy of fusion constructions preserving secondary and tertiary structures in protein chimerae. Four molecular models were created by rational design consisting of catalytic domains derived from parental enzymes that degrade lignocellulosic material: arabinofuranosidase (Ara) and xylanase (Xyl) from <i>*Bacillus subitilis<i/>,* and feruloil esterase (Fae) from <i>*Clostridium thermocellum<i/>,* in which all, two by two combinations, generated three bifunctional chimeric enzymes (<i>*AraFae<i/>,<i>AraXy<i/>l and <i>FaeXyl<i/>)* and one trifunctional (<i>*AraFaeXyl<i/>).* The *in silico* fusion of the parental enzymes were inserted a given enzyme in to a surface loop of the other enzyme using as a starting point the respective crystal structure deposited in the PDB data bank (3C7G for Arabinofuranosidase; 1GKK for Feruloil Esterase and 1XXN for Xylanase). The fusions were constructed using overlap PCR in several sequential reactions, in which each reaction amplifies a fragment with overlap regions at both extremities. The fragments with overlap regions generated in separated PCR reactions are annealed together in a single PCR reaction that generated the fusion products. The primers at the 5' and 3' ends of the fusion products include restriction enzyme sites to facilitate the cloning and subcloning procedures. All four chimeric enzymes were successfully constructed and cloned in the vector pT7T3-18U, and the expected constructs were confirmed by DNA sequencing. In conclusions, rational design is a innovative methodology for creating protein fusions that has been used to construct bifunctional and trifunctional chimerae. These constructions are currently been expressed and present potential for application in bioethanol industries and cellulosic bleaching industries.

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