COVALENT IMMOBILIZATION OF THE TOBACCO ETCH VIRUS (TEV) PROTEASE TO BE USED IN THE CLEAVAGE OF THE HISTIDINE-TAG OF RECOMBINANT PROTEINS FROM *T. CRUZI*

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TEV is the most frequently used protease to cleave recombinant protein tags. It is specific for the sequence E-N-L-Y-F-Q*G and is catalytically active under a broad range of conditions. Although the His-tags facilitate purification of recombinant proteins by affinity chromatography, their presence may disturb protein activity, interactions, and the formation well diffracting crystals. TEV is commercially available in soluble form, but its use in soluble form increases the steps and the cost of the purification process. The objective of this work is to obtain TEV in an insoluble form to replace for the soluble one, by covalent immobilization in different matrices. The yield of purified protein from 1liter of culture was 20mg for TEV and 5mg for an 19kDa TEV-substrate (Tyrosine phosphatase). The soluble form of TEV was active, but after immobilization by the amino groups of Lys (8 exposed), no activity was detected, suggesting the inactivation of TEV under this multi-point immobilization procedure. Protease activity however, was detected after one/bi-point immobilization by reaction of the SH groups of their Cys (2 exposed) with the thiolsulfinate groups of the matrix. We are analyzing the uni/bi-point immobilization procedure to other kind of activated matrices, to select the best derivative of immobilized TEV-protease.

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