A fluorogenic substrate as quantitative in vivo reporter to determine protein expression and folding of tobacco etch virus protease in Escherichia coli.

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Abstract

Quantitative and folding reporters are adequate tools to optimize recombinant protein expression in various host organisms, including Escherichia coli. To determine the yield of soluble active protease from the tobacco etch virus (TEV), we developed a single-molecule assay based on the fluorogenic substrate ANA-QS-MCA. This substrate consists of a 10 amino acid peptide (ENLYFQSGTK) containing the proteolytic cleavage sequence of the TEV protease. The peptide works as a linker N-terminally tagged with a fluorescent donor group (7-Methoxycoumarin-4-yl)acetyl (MCA) and C-terminally tagged with the acceptor group 5-Amino-2-nitrobenzoic acid (ANA). Fluorescence can be observed after specific cleavage of the substrate at the Gln-Ser bond by active TEV protease. Purified His-tagged TEV protease was used for in vitro analysis. Through determination of proteolytic activity in living E. coli cells and through application of Confocal Laser-Scanning-Microscopy we demonstrate that the peptide is well suited to in vivo expression analysis. This provides an effective tool to monitor the

accumulation of active recombinant TEV protease in crude extracts and intact cells.

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