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Use of the bacterial two-hybrid system for the development of quasi-homodimeric papain-like cysteine proteases

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PURPOSE OF THE ABSTRACT
Oligomeric proteins account for 30-50 % of all proteins in nature [1]. The abundance of oligomers is explained by their functional and structural advantages over monomeric variants. These include increased stability, resistance to denaturation and degradation, additional ability to regulate enzyme activity, and cooperativity [2]. Oligomeric proteins are formed in nature in several ways. The most common is the interaction of polypeptide chains via complementary surfaces with non-covalent interactions, but it is also possible to form oligomers via the domain swapping mechanism, formation of common metal ions and disulfide bonds.

The subject of our research is the family of papain-like cysteine proteases, which are mostly monomeric. Our goal is to produce these enzymes in the form of stable dimers. Due to the advantages of the oligomeric state of enzymes compared to the monomeric state, we expect that the produced recombinant dimeric enzymes will have better regulatory mechanisms and kinetic properties compared to natural enzymes, making them interesting for biotechnological applications. Our main target is the prokaryotic cysteine protease xylellain from the bacterium Xylella Fastidiosa, which is a suitable model protein due to its ease of production in the bacterial expression system. In addition, we aim to produce dimeric forms of ficin, stem bromelain, and fruit bromelain, three representatives of the plant papain-like proteases that are indispensable in biotechnological processes in the production of meat, dairy products, and juices.

Our approach is based on the production of dimeric forms of the target by optimizing the existing surfaces of the protein. We have identified the back and bottom surfaces of the protein with respect to the standard orientation as suitable surfaces for engineering oligomerization, as these surfaces are planar and large enough to allow the formation of stable interfaces. We have prepared several site-directed saturated libraries to be studied using a commercially available bacterial two-hybrid system originally developed for the discovery of interaction partners in-vivo [3]. The two-hybrid system is based on co-transformation of bacteria with plasmids pUT18 and pKT25, which express the fusion of the target protein with one of the two fragments of adenylate cyclase. Screening is performed on plates using the blue-white screening protocol. Because the system uses two differently mutated forms of the target protein that otherwise contain the mutation at the same position, we have termed the resulting dimers quasi-homodimers.

So far, we have identified 15 pairs of potential quasi-homodimers of xylellain using the bacterial two-hybrid system. The identified mutants had predominantly hydrophobic residues on the interaction surface, but cysteine, arginine, glutamic acid, threonine, and histidine also occurred. Mutants expected to contribute to quasi-homodimerization were mutated in-silico, and the potential structures of the symmetric dimers were calculated using the program M-ZDOCK. Experimental verification using purified recombinant proteins is underway.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Protein engineering | Xylellain | Oligomerization

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