Synopsis of Original Research Paper

Properties, evolution, and cosmetological aspects of trappins that have recently been discovered as a new protein family and shown to have anchoring sequences

Shigehisa Hirose

Department of Biological Sciences, Tokyo Institute of Technology

Trappins are a family of unique proteins that have recently been identified and shown to have an anchoring sequence at their N-termini through which they become covalently trapped at the site of action. A typical example of the trappin family members is the elastase inhibitor elafin which is composed of two domains: the N-terminal transglutaminase substrate domain or "cementoin" domain that serves as an anchoring sequence and the C-terminal inhibitor domain having a compact structure stabilized by four disulfide bonds and therefore called "four disulfide core" or "WAP motif". In this study, as summarized below, we characterized the cementoin moiety which has an important potential application in developing intelligent cosmetic materials and further carried out a series of basic research on trappins such as molecular evolution of trappins.

Two types of derivatives of the cementoin-like anchoring sequence of trappin-1 were produced and characterized. The cementoin-like sequence (cem) was first elongated by tandemly linking its cDNA and expressing it in *E.* coli. Using the genetically engineered cem-cem, the anchoring sequence rich in Gln, Lys, and Pro was shown to have a random coil structure and to serve as a good substrate for transglutaminase, a characteristic very useful for developing the biotechnology of protein cross-linking or protein gluing. As a second construct, we prepared a fusion protein of cem and green fluorescent protein (GFP). The useful properties of the component proteins are maintained in the fusion protein, namely we succeeded to prepare a fluorescent cementoin moiety that will be of special interest and importance in protein engineering.

Previous studies showed that trappin genes, especially of the pig, have undergone rapid evolution, producing trappins with a broad spectrum of action. To understand the evolution of such a rapidly evolving multigene family, we characterized the trappin genes and found that the intron sequences are homogenized by gene conversion. Similar mechanisms may also operate in the other genes whose intron sequences are conserved much highly than the exon sequences.

In summary, 1) using the newly discovered adhesive protein "cementoin" and its family members, we developed a powerful method for protein cross-linking. 2) The secondary structure of cementoin was determined using recombinant cementoin. 3) Fluorescent cementoin was prepared by fusing it with green fluorescent protein. 4) Evolution of cementoin family genes was clarified.