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Characterization of Domain 3 and Its α-Helical Region of the Hemolytic Lectin CEL-III Expressed as Glutathione S-Transferase-Fusion Proteins

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Abstract

CEL-III is a hemolytic lectin containing two carbohydrate-binding domains (domains 1 and 2) and a β-sheet-rich domain (domain 3). In domain 3, there is a hydrophobic region containing two α-helices (H8 and H9) and a loop between them, in which alternate hydrophobic residues, especially Val residues, are present. Synthetic peptides corresponding to the loop and second α-helix (H9) showed the strongest antibacterial activity. The recombinant glutathione S-transferase (GST)-fusion proteins containing domain 3 or the α-helical region peptide formed self-oligomers, whereas mutations in the alternate Val residues in the α-helical region lead to decreased oligomerization ability of the fusion proteins. These results suggest that the α-helical region, particularly its alternate Val residues are important for its oligomerization.

Introduction

The body fluid of marine invertebrate *Cucumaria echinat*a (sea cucumber) contains a Ca^{2+}-dependent hemolytic lectin CEL-III (1). This lectin forms oligomers consisting of 6-7 monomers in target cell membranes, leading to the formation of ion permeable pores (2). CEL-III consists of two carbohydrate-binding domains (domains 1 and 2) and one oligomerization domain (domain 3) (Fig. 1) (3). In domain 3, there is a hydrophobic region involving two amphiphilic α-helices, which was suggested to play an important role in oligomerization and following insertion into cell membranes. In this study, we synthesized this α-helical region as well as a whole domain 3 as fusion proteins with glutathione S-transferase (GST), and examined

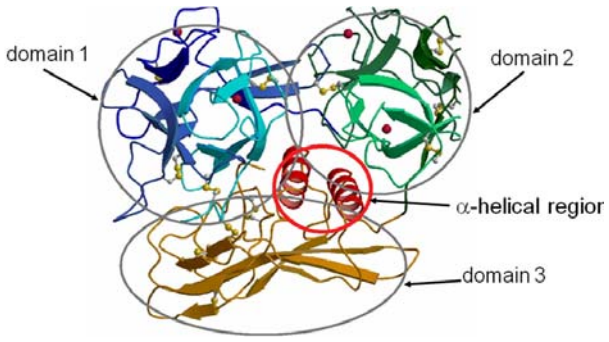

Fig. 1. Tertiary structure of CEL-III.

their properties.

Results and Discussions

Domain 3 or the α-helical region as GST-fusion proteins (GST-domain3 or GST-helix, respectively) were expressed in *E. coli* cells. The expressed proteins showed a strong tendency to form oligomer, which may be closely related to the ability of domain 3 to form membrane pores. To identify the amino acid residues responsible for such an oligomerizing ability, Val residues located in the α-helical region were replaced by Ala residues (V → A-GST-helix), because of their characteristic repetitive arrangement. Consequently, expressed V→A-GST-helix mutants lost the oligomerizing ability (Fig. 2).

These results strongly suggest that the repetitive Val residues in the α-helical region are closely associated to interaction between domain 3 to form CEL-III oligomers. It seems possible that a-helical region in domain 3 forms two-stranded antiparallel β-sheet, leading to β-barrel structure in the membrane by strong interactions between characteristic repetitive Val residues.

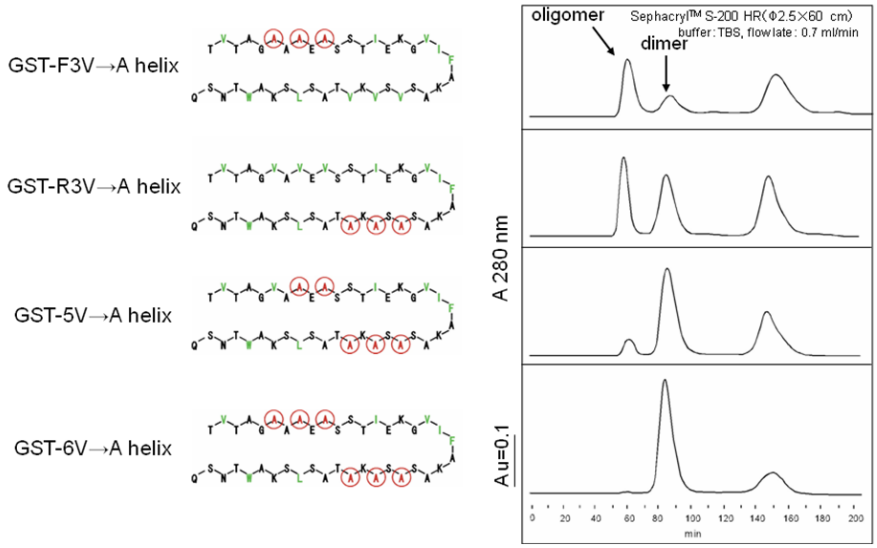

Fig. 2. Gel filtration of V→A-GST-helix mutants.

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