Peptide Induce Membrane Fusion: Peptide Structure Required for the Fusion

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Amphiphilic α-helical peptides may induce biomembrane fusion. Measurements of fusion activity of about 80 peptides having modified amino acid sequences of influenza hemagglutinin HA-2 subunit N-terminal domain revealed that, in addition to amphiphilic properties, intermittent distribution of bulky hydrophobic residues is crucial for peptides to be active in triggering membrane fusion.

Keywords: Synthetic peptides/ Membrane fusion/ α-Helix/ Amphiphilic peptide/ β-Structure

The subdivision of Biopolymer Structure has two activities: Physicochemical studies of synthetic peptides as a model of protein structure in the aspects of stability of secondary or super secondary structures and function, and elucidation of protein structures by X-ray crystallography. This year, we will focus on the recent results in the former activity, mainly a structure formation of small peptides in biomembranes and a peptide function to induce lipid membrane fusion.

Phospholipid bilayers consist of a basic structure in living organisms. They form not only a cell wall to segregate a living system from the environment, also intracellular vesicles called organelles such as nucleus, mitochondrion, Golgi apparatus, endosome, etc., each of which takes a specific action in a living cell. As a cell is encapsulated by cell wall, incorporation or secretion of substances (except small molecules) into or from a cell requires a specific mechanism to pass through the membrane. A Golgi system and endosome are responsible for these processes. For example, infection of enveloped viruses, a release of viral genomes in cytoplasm, takes place either by direct fusion of a viral envelope with cell membrane or by fusion of viral membrane with endosomal one after incorporation of viral particles in an endosome (endocytosis). The influenza virus infects a living cell by an endocytic pathway. The viral envelope fuses to an endosome membrane when pH inside the organelle was lowered below 5.5 in the process of endocytosis. A specific protein, hemagglutinin, was identified to be responsible to trigger the fusion at acidic pH, while it is inactive at neutral. Hemagglutinin is a multifunctional protein embedded in a viral envelope and its subunit HA-2 has a stretch of hydrophobic amino acids as an N-terminal segment, which had been called putative fusion peptide. We found that a synthetic 20-residue peptide having the same amino acid sequence as that of influenza virus strain A/PR/8/34 (H-1) could induce lipid vesicle fusion with the similar dependency on pH [1]. Several aspects have been revealed: (1) the peptides that cause membrane fusion must interact with lipid membranes and have an amphiphilic nature by forming ordered secondary

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Scope of research

(1) Peptide secondary or supersecondary structures in aqueous or hydrophobic environments are studied to get a principle of protein architecture, employing various spectroscopic methods. (2) Protein X-ray crystallography is carrying out to reveal a tertiary structure of protein. Efforts are also paid on elucidation of structure-function relationships of enzymes.

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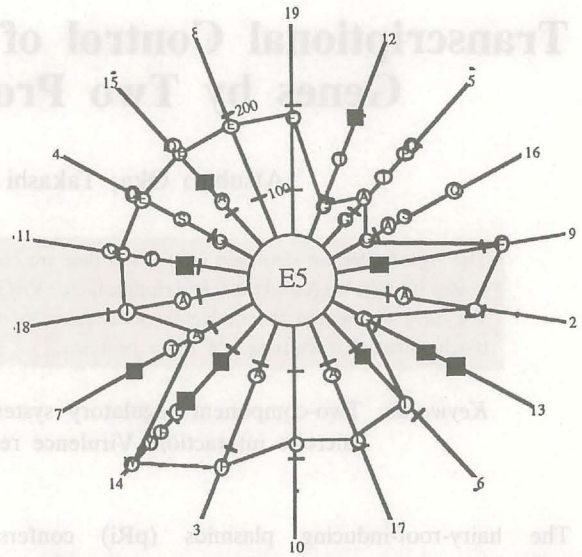
structures; (2) complimentarily structured peptides, or when a peptide was structured with the aid of other substances, also could trigger the fusion ; and (3) a fusion-active peptide takes α-helix in lipid bilayers, with the helix axis 30° from the membrane plane [2].

Not all of amphiphilic peptides are active fusogen of lipid membranes. It is quite apparent that specific amino acid sequences or compositions must be required. We have tried to reveal the necessary conditions that are required for peptides to be active in inducing membrane fusion from a study of amino acid sequence-modified peptides starting from the original influenza HA-2 peptede, which is shown below. Sequence modifications

HA-Peptide GLFGAIAGFIEGGWTGMIDG

were such as a one residue was substituted by other amino acid at a time or a group by a group and about 80 peptides were obtained in this way. In every situation, amphipathic nature of the peptides was conserved since that was one of the necessary conditions for peptide to interact with lipid bilayers. Membrane fusion activities of these peptides were collected, summarized, and evaluated to yield a structure-activity relationship [3]. Point (one residue) modifications usually reserved the activity, at some specific points such as N-terminal, however, the nature of the substituted residue critically affected the activity. For example, an introduction of a charged group at N-terminus abolished the activity while substitutions with neutral amino acids, either large or small, hydrophobic or hydrophilic, did not affect the activity. At the position 14, substitutions with large hydrophbic residues reserved the activity but small or charged did not. The place necessary for activity expression is not localized because those peptides having amino acid sequences doubled for the N- or C-terminal half of the original peptide were inactive. Furthermore, interchange of the N- and C-terminal halves gave a peptide which was still active. This suggests that a combination of residues more than half of the original peptide is critical for the activity. Our present explanation what properties of amino acid residues are correlated to the activity of a peptide is following. Peptides under consideration are considered to be helical in lipid bilayers. To fuse apposed membranes some perturbation must be caused by the peptide-lipid interactions. It is reasonable to assume such interactions are due to contacts of peptide surface and surrounding lipid molecules. We considered the surface area of amino acid residue as most important in the view of peptide-lipid interactions and, therefore, plotted calculated surface area of each residue side chain ($Å^2$) as a helical wheel representation (Figure below), where the outermost numbers showed residue numbers, marked circles for residues by which substitution afforded fusion-active peptides, and filled squares for residues to reduce the activity, respectively (the kite-like polygon represents



a residue surface area profile of a peptide called E-5 taken as a reference). We could notice a remarkable feature on the picture, namely a small residue was required at some points for a peptide to be fusion active, or in other words, constrictions in a surface area profile must be present somewhere. Our inactive peptides, such as those having N- or C-terminal half-doubled or having an optimized amino acid sequence for an amphipathic α-helix, show uniform or smooth (without constrictions) surface area profiles. Also we surveyed other fusion peptide sequences reported for various viruses and could recognize a similar feature as shown in above figure. As our acidic peptides become active at pH below 6, protonations of carboxylate groups are apparently related to the fusion activity. Presumably the protonation increases hydrophobicity of the peptides and makes the peptides deeper inserted inside lipid bilayers. Deeper insertion of peptides causes a larger amount of perturbation on the order of lipid bilayer structure, crossing a critical degree of disorder or perturbation will trigger fusion between closely apposed membranes.

As an application of fusion-active peptides to other field of science, Hirata's group have found the presence of a fusion-active complementary peptide pair significantly enhanced incorporation of recombinant DNA into cells by a lipofection method [4].

References

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