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The dynamics of melittin within the liposome using mass spectrometry and chemical modification

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The membrane-associated peptides have common physicochemical properties; they form amphipathic secondary structures, which can be accommodated into membranes. For example, melittin, the toxin of honey bee (*Apis mellifera*), adopts amphipatic alpha-helical conformation, thus melittin has been used as a suitable model peptide for monitoring lipid-protein interactions in membranes. However, the terminal topology of melittin into membranes is still a problem. In this study, we investigated the terminal topology and the dynamics of melittin within the liposomes using mass spectrometry combined with chemical modification.

First, melittin within the liposomes was acetylated with *N*-hydroxysulfosuccinimidyl acetate (NHSSAc) for 30 min at room temperature, with molar ratio of melittin to NHSSAc of 1 : 50. In the MALDI-TOF mass spectra of these melittin, peaks were detected at *m/z* 2846 and 2888. The peaks at *m/z* 2846 and 2888 were identified as melittin and the mono-acetylated melittin, respectively. According to MALDI-QIT-TOF MS/MS analysis of the peak at *m/z* 2888, the acetylated position was identified as N-terminal amine. Consequently, it was found that melittin within the liposomes adopted an N-terminal-outside topology (Figure 1).

Next, melittin within the liposomes was acetylated with NHSSAc for 2~180 min at room temperature. This acetylation rate of melittin within the liposomes followed first-order kinetics and the rate constant was 4.83×10^{-4} s^{-1}. It was suggested that this rate constant was too slow for the rate constant of the acetylation of melittin in aqueous solution. Similar experiments were carried out between 6 and 30 ˚ C. The rate constants were plotted in the form of the Arrhenius plot. The plot was linear with a slope corresponding to an activation energy of 74 kJ mol^{-1}. Activation free energies for dissociation of a phospholipid monomer or a hydrophobic transmembrane peptide from the vesicle were estimated to be about 70 kJ mol^{-1} [1, 2].

Thus, we concluded that this activation energy was due to the energy that the N-terminal region of melittin is released to the water phase from the hydrophobic core of the liposomes. This result suggested that melittin has a longitudinal motion within the liposomes like a float (Figure 1.).

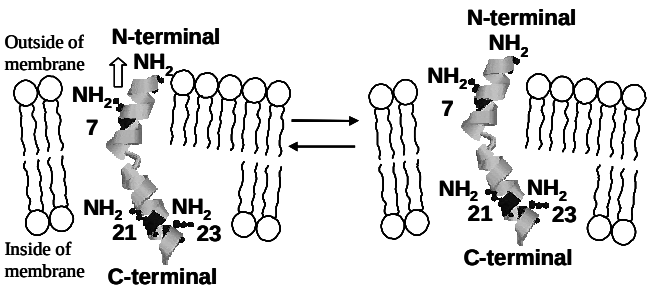

Figure 1. Speculated model of the dynamics of melittin within the liposomes

(References)

1)Y. Yano *et al*, *Biochemistry*, **41**, 12407 (2002).

2) J.W. Nichols, *Biochemistry*, **24**, 6390 (1985).