

PROBING ANTIMICROBIAL PEPTIDE-MEMBRANE INTERACTIONS USING CALORIMETRY AND DETERMINING VESICLE LEAKAGE RATES

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The eighteen amino acid ζ CRRLCYKQRCVITYCRGR) Gomesin, GM, a antimicrobial peptide isolated from hemocytes of the Brazilian spider *Acanthoscurria gomesiana*, has with two disulfide bridges (Cys²⁻¹⁵/Cys⁶⁻¹¹). CD studies evidence a β -hairpin structure (Fázio et al., Biopolymers, 84: 205-218, 2006). A linear analogue of GM was obtained (Cys \rightarrow Ser) ([Ser^{2,6,11,15}]-Gm). Here we investigate peptide-membrane interactions of GM and [Ser^{2,6,11,15}]-Gm, using leakage kinetics of 5,6-carboxyfluorescein (CF) entrapped in lecithin large unilamellar vesicles (LUV) and Isothermal Titration Calorimetry (ITC)). Both GM and [Ser^{2,6,11,15}]-Gm increased the rate of CF leakage from LUVs containing 13 mol% cardiolipin (CL). A 20-fold higher concentration of [Ser^{2,6,11,15}]-Gm was necessary to obtain the same extent of GM-induced leakage. Unexpectedly, the observed rate constant, k_{obs} , obtained at a peptide/lipid ratio of 0.3, was $1.2 \times 10^{-3} \text{ s}^{-1}$ for [Ser^{2,6,11,15}]-Gm and $5.5 \times 10^{-4} \text{ s}^{-1}$ for GM, i.e., k_{obs} was 2-fold higher for [Ser^{2,6,11,15}]-Gm than for GM. For LUVs without CL, the maximum CF leakage induced by GM was independent of peptide concentration while [Ser^{2,6,11,15}]-Gm did not produce complete CF leakage. Calorimetric results showed that the interaction of GM with negatively charged membranes is more exothermic (-14 kcal/mol with CL and -2 kcal/mol no CL).

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