Molecular basis of the latarcin Ltc1 antibacterial activity as studied with optical spectroscopy and microscopy methods

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Latarcin Ltc1 produced by spider *Lachesana tarabaevi* venom gland [1] is highly toxic for bacteria *E.coli*, *P.fluorescens*, *R.equi*, *B.subtilis*, *M.luteus*, and its bactericidal kinetics is fast (time course of killing is less than 30 min). To clarify the structure-function basis of antibacterial activity, we subjected the synthetic Ltc1 to spectroscopic and microscopic study *in vivo* and in model systems, e.g. phospholipid liposomes.

Characteristic changes in tryptophan fluorescence confirm that the peptide associates with dioleoylphosphatidylcholine (DOPC) liposomes, and the binding increases drastically when liposomes contain negatively charged lipids. According to circular dichroism data, Ltc1 adopts an α-helical structure in membrane-mimetic environment, *viz* sodium dodecyl sulfate micelles and DOPC liposomes. Ltc1-induced calcein leakage from DOPC liposomes proves that the peptide is a membrane-acting agent. Förster resonance energy transfer (FRET) occurs from nitrobenzofurazan- to sulforhodamine- conjugated Ltc1 in the sodium dodecyl sulfate micelles as well as in DOPC liposomes revealing oligomerisation of the peptide.

Fluorescent membrane probes were applied to study how Ltc1 affected bacteria. Changes in fluorescence emission of voltage-sensing dye 3,3`-dipropylthiadicarbocyanine show that Ltc1 disrupts membrane potential in Gram-positive bacteria *S.aureus*. These data are in agreement with the assumed membrane-lytic mechanism of Ltc1 action. Enhancement of 1-N-phenylnaphtylamine uptake shows that Ltc1 increases the permeability of outer membrane of Gram-negative bacteria.

Ltc1 binds effectively to Gram-negative bacteria as revealed with the laser scanning confocal microscopy. FRET between nitrobenzofurazan- and sulforhodamine-labelled Ltc1 allows one to suppose that the bound molecules are closely spaced on the bacterium surface at the growth-inhibitory concentration, and Ltc1 may disturb membrane integrity through so-called carpet mechanism. Definitely, a negative charge of bacterial membrane plays an essential role in this process.

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[1] *S.A. Kozlov, A.A. Vassilevski, A.V. Feofanov, A.Y. Surovoy, D.V. Karpunin, E.V. Grishin*. Latarcins, antimicrobial and cytolytic peptides from the venom of the spider *Lachesana tarabaevi* (Zodariidae) that exemplify biomolecular diversity. // *J. Biol. Chem.* **281**, 30 (2006). Pp. 20983–20992.