

COMBINED ANTIBACTERIAL ACTION OF SALIVARY CATIONIC PROLINE-RICH PEPTIDES AND ANTIMICROBIAL PEPTIDES

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СОВМЕШНОЕ АНТИБАКТЕРИАЛЬНОЕ ДЕЙСТВИЕ КАТИОННЫХ ПРОЛИН-БОГАТЫХ ПЕПТИДОВ СЛЮНЫ И АНТИМИКРОБНЫХ ПЕПТИДОВ

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Saliva is an important biological fluid that reflects human's health. Its main function is protection of the oral cavity from pathogens. Antimicrobial peptides (AMPs) of the innate immunity may play an important role in anti-infectious defense of the oral cavity, but their relative amount in saliva is low. It's major component is Proline-rich peptides (PRPs), whose impact in antimicrobial protection remains poorly understood. We suggest that salivary PRPs may reveal their defensive functions upon interaction with other molecules, in particular with AMPs. The aim of this work is an investigation of the combined antibacterial action of salivary PRPs (fragments of Basic salivary proline-rich protein 1: P-H (37-51), IB6 (98-116), p1932) with antimicrobial peptides (histatin 5 and cathelicidin LL-37 and beta-defensin hBD3). Listed PRPs have been obtained by chemical solid-phase synthesis. The method of broth microdilutions was used to compare minimal inhibitory concentrations (MICs) of individual fractions of AMPs and their MICs in the presence of salivary peptides. It was found that in the presence of peptides IB6 (98-116) or P-H (37-51) the activity of defensin hBD3 was increased (reduction of MICs by 2 times) against *Staphylococcus aureus* SG511. In the presence of IB6 (98-116) or p1932 the activity of this defensin against *E. coli* ML35p was also improved (MICs of hBD3 was lowered by 2 times). For other combinations of the peptides, this effect was not observed. The obtained data confirm the assumption that the combined action of varied salivary peptides, including cationic Proline-rich peptides, plays an important role in anti-infectious protection of the oral cavity.

Keywords: saliva; proline-rich peptides; antimicrobial peptides; innate immunity.

Слюна является важной биологической жидкостью, состав которой отражает состояние здоровья человека. Ее главная функция состоит в защите ротовой полости от болезнетворных микроорганизмов. В обеспечении противoinфекционных свойств слюны немаловажную роль могут играть антимикробные пептиды (АМП) врожденного иммунитета, хотя относительное содержание их в слюне невелико. Ее мажорным компонентом являются пролин-богатые пептиды, функции которых в обеспечении антимикробной защиты остаются малоизученными. Можно предположить, что они реализуются при совместном действии с другими молекулами, в частности с АМП. Целью работы явилось изучение совместного антибактериального действия катионных пролин-богатых пептидов (ПБП) слюны (фрагментов катионного белка слюны 1: Р-Н (37-51), IB6 (98-116), p1932) с антимикробными пептидами (гистатин 5, кателицидин LL-37, бета-дефенсин hBD3). Пептиды были получены с помощью твердофазного химического синтеза. Методом серийных разведений в жидкой питательной среде проведено сравнение минимальных ингибирующих концентраций (МИК) индивидуальных фракций АМП и их МИК в присутствии пептидов слюны. Установлено, что в присутствии пептидов IB6 (98-116) и Р-Н (37-51) повышается активность дефенсина hBD3 (МИК снижается в 2 раза) в отношении *Staphylococcus aureus* SG511. В присутствии пептидов IB6 (98-116) и p1932 повышалась активность дефенсина в отношении *E. coli* ML35p (МИК hBD3 снижалась в 2 раза). Для остальных пептидов подобный эффект не наблюдался. Полученные данные подтверждают предположение, что сочетанное действие факторов пептидной природы, содержащихся в слюне человека, в том числе катионных пролин-богатых пептидов, играет важную роль в обеспечении противoinфекционной защиты ротовой полости.

Ключевые слова: слюна; пролин-богатые пептиды; антимикробные пептиды; врожденный иммунитет.

Introduction. Nowadays an interest to saliva as a promising diagnostics tool is rising. The content of this biological fluent perfectly reflects hu-

man's health. The main function of saliva is keeping integrity of an upper region of digestive tract mucus and protection from pathogens [1]. Among

salivary components there are varied antimicrobial peptides (AMPs): alpha- and beta-defensins, cathelicidins, histatins, but their concentration is relatively low [2]. The major protein fraction of saliva includes Proline-Rich proteins and their proteolytic fragments — Proline-Rich Peptides (PRPs). The biological role of these substances is still poorly understood [3]. We suggested that PRPs may contribute to antibacterial defense working together with AMPs. The aim of the present study was an investigation of a combined action of salivary PRPs (P-H (37-51), IB6 (98-116), p1932) and AMPs (histatin 5, cathelicidin LL-37, beta-defensin hBD3).

Material and methods. The listed PRPs have been produced by a solid phase synthesis using Fmoc/tBu-strategy on a 2-chlorotrityl chloride resin [4]. The peptide sequence was built up in the automatic peptide synthesizer Symphony X (Protein Technologies, Inc.), USA, and also by use of manual and automatic peptide synthesis on the equipment of Institute of Chemistry of Molecular Recognition (Milan, Italy). Synthesized peptides have been purified by reversed-phase high-performance liquid chromatography (RF-HPLC) at the Gold System chromatograph (Beckman, USA). Their purity confirmed by means of analytical RF-HPLC and mass spectrometric analysis (MALDI-TOF MS) was not less than 95%. Chemically synthesized peptides histatin 5 and cathelicidin LL-37 were purchased from Anaspec Inc., USA.

Antimicrobial activity of peptides was determined by broth microdilution assay [5] against gram-negative bacteria *Escherichia coli* ML35p and gram-positive bacteria *Staphylococcus aureus* SG511. To determine the minimum inhibitory concentrations of the peptides we performed their serial twofold dilutions in sterile Terasaki microchambers in 0.01 M sodium-phosphate buffer containing 0.1% bovine serum albumin (5 µl/well). Suspension of microorganisms in 2.1% Mueller-Hinton broth M391 (HiMedia, India) was added to wells of Terasaki chambers (5 µl/well). Microchambers were incubated for 20 h at 37 °C. The

results are presented as medians obtained from 3–5 independent experiments (each sample was done in triplicates).

Results and discussion. Antimicrobial activity of individual fractions of PRPs (P-H (37-51), IB6 (98-116), p1932) against *Escherichia coli* ML35p and *Staphylococcus aureus* SG 511 was insignificant in the range of concentrations of the tested peptides from 1 to 1000 µM. Because of the lack of MIC values for all these salivary peptides it was not possible to use a standard method of “Checkerboard titration” for characterization of their combined antimicrobial action with AMPs. We studied effects of salivary PRPs, taken in single concentration — 64 µM, on the antimicrobial activity of AMPs (histatin 5, cathelicidin LL-37, beta-defensin hBD3) applied in serial (twofold) dilutions. Comparative analysis of MICs of AMPs alone or in the presence of salivary PRPs was carried out. It was shown that the antibacterial activity of defensin hBD3 towards *S. aureus* SG511 is enhancing in the presence of IB6 (98-116) or P-H (37-51): MIC of hBD3 alone was 1 µM, MIC of hBD3 with the IB6 (98-116) or P-H (37-51) — 0.5 µM. The peptide p1932 did not affect the activity of defensin against this strain of staphylococci. The antimicrobial activity of hBD3 towards *E. coli* ML35p raised in the presence of salivary PRPs IB6 (98-116) or p1932 (MIC of the defensin was 30 µM, MIC of hBD3 with IB6 (98-116) or p1932 — 15 µM). In the case of other PRPs this effect was not observed. The antimicrobial activity of AMPs histatin 5 and LL-37 was not changed in the presence of 64 µM of salivary peptides IB6 (98-116), p1932 or P-H (37-51).

Conclusion. The obtained data confirm the suggestion on the biological importance of the combined action of human salivary peptides of varied structures, in particular cationic proline-rich peptides and antimicrobial peptides, in implementation of anti-infectious defense of the oral cavity.

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