Functional role of individual parts of *B. cereus* hemolysin II

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Hemolysin II of *Bacillus cereus* sensu lato is synthesized in a bacterial cell in the form of a water-soluble secreted monomer and penetrates into eukaryotic membranes. The HlyII protein has a C-terminal extension (HlyIICTD), includes 94 amino acid residues [1]. Using monoclonal antibodies (MAbs) against recombinant HlyIICTD [2] reveals epitopes on the surface of HlyIICTD that are located in such a way that these MAbs in the immune complexes do not allow HlyIICTD to interact with red blood cells. The interaction of HlyIICTD with red blood cells was observed only when using certain antibodies, the epitopes of which on the domain molecule did not affect its binding to cells. This fact indicates that the HlyIICTD interaction is oriented relative to the surface of the red blood cell. Hemolysin II attacks erythrocytes of various origins with varying effectiveness. We found that at identical conditions the most sensitive were rabbit (55 HU) and human (14 HU) erythrocytes whereas bovine (8 HU) and mouse erythrocytes (1 HU) were the least sensitive [3]. At the same time, the affinity coefficients in the interaction of HlyIICTD with these erythrocytes are almost identical. This may indicate that HlyIICTD is not involved in the recognition of the erythrocyte surface, which differs depending on their origin, and the differences associated with the recognition of red blood cells are determined by HlyII core. However, the affinity coefficients in the interaction of HlyIICTD with eukaryotic cells J774 mouse monocyte (macrophage) Jurkat immortalized line of human T lymphocyte cells are significantly different from those when interacting with erythrocytes, that is, HlyIICTD recognizes differences associated with the nature of eukaryotic cells. HlyIICTD in water solution is able to form oligomeric structures. In the presence of membrane HlyIICTD exits in oligomeric form while monomeric forms are almost completely absent. HlyIICTD trimerized in the presence of 4M urea, forming a possibly some structure that can be integrated into the artificial bilayer membrane with the formation of pores. The current-voltage characteristic of these channels was determined. Comparing the current characteristics of the membrane in the presence of HlyII (0.236 mg/ml) and HlyIICTD (1.99 mg/ml), it was shown that both proteins form pores in the membrane. But HlyIICTD, in contrast to the whole molecule HlyII, is less dependent on the sign of the applied potential, and the conductivity of the membrane at the same positive and negative voltages [4] has almost the same value. Such protein structures are characteristic of trimeric autotransporter proteins [5]. In this case, the secreted full-sized monomeric form of hemolysin II acts as a passenger, and HlyIICTD acts as an element involved in adhesion to membrane and secretion from bacterial cells. The materials presented in this paper demonstrate that hemolysin II may belong to trimeric autotransporter proteins – the first case of the description of this family of molecules among Gram positive microorganisms.

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References

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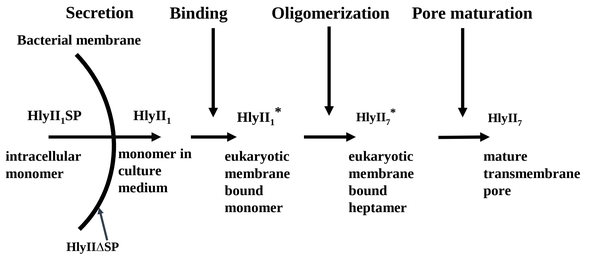
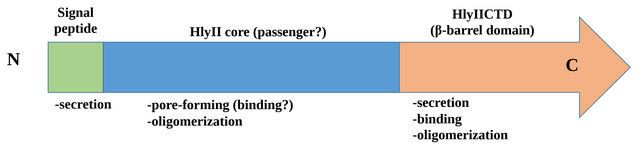
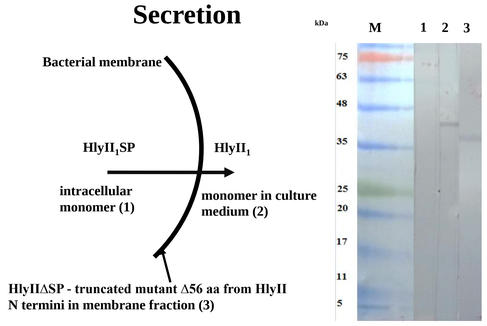
General mechanism of pore formation by hemolysin II from *Bacillus cereus*



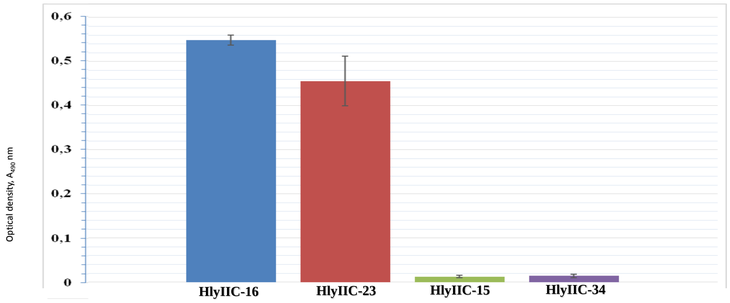
Presumed topographic scheme of HlyII *Bacillus cereus*



Secretion



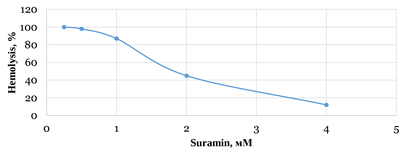
Binding



The effectiveness of the interaction of HlyIICTD with erythrocytes as part of immune complexes with biotinylated monoclonal antibodies as described in [2]

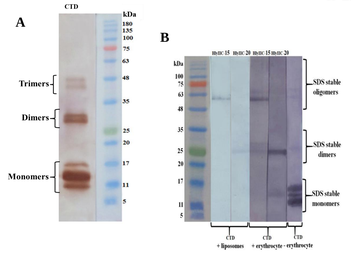
Used cells	HlyIICTD affinity constants
Rrbc (rabbit erythrocytes)	**$5{,}63 \times 10^6$ M^{-1}**
Mrbc (mouse erythrocytes)	**$5{,}125 \times 10^6$ M^{-1}**
Hrbc (human erythrocytes)	**$5{,}27 \times 10^6$ M^{-1}**
J774 (mouse monocyte; macrophage)	**$6{,}77 \times 10^6 M^{-1}$**
Jurkat (immortalized line of human T lymphocyte cells)	**$19{,}4 \times 10^6$ M^{-1}**

Determination of Kaff in Scatchard coordinates



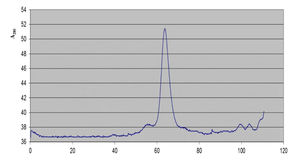
Determination of the effective concentration of suramin for hemolytic activity HlyII
Treatment of rabbit erythrocytes with receptor antagonists (suramin) led to a decrease in the hemolytic activity of hemolysin II

Oligomerization of HlyIICTD



Oligomerization of HlyIICTD in solution (A) in the presence of liposomes and erythrocytes (B) [6, 7]. Oligomerization and inhibition of oligomerization HlyII in the presence of erythrocytes by a monoclonal antibody HlyIIC-20 (C) [7].

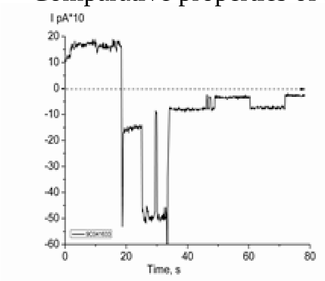
Trimerization of HlyIICTD



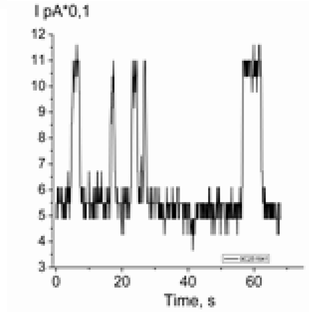
Gel filtration of HlyIICTD on HiLoad 16/60 Seperdex 75 column in the presence of 4M urea

Pore maturation

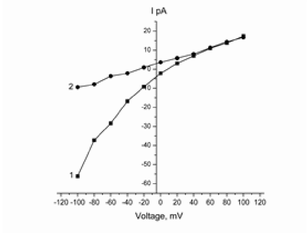
Comparative properties of the channel formed by HlyII and HlyIICTD



Fluctuations of the current on the membrane when applied from one side (out) 1 μg/ml HlyII; KCl solution 1M; Membrane voltage + - 100mV



Fluctuations of the current on the membrane when applied from one side (out) 63 μg/ml HlyIICTD; 1M KCl solution; Membrane voltage + 100mV



Current-voltage characteristics obtained in the presence of HlyII and HlyIICTD introduced on one side of the membrane (out) at a concentration of 1 μg/ml and 500 μg/ml, respectively. 1 - HlyII, 2 – HlyIICTD.
Bilayer lipid membranes (BLM) were formed according to [4] using a solution containing 20 g/l of soybean lecithin

CONCLUSION AND OPEN QUESTIONS

Studying a functional role of individual parts of *B. cereus* HlyII it was assumed that HlyIICTD can form oligomers and during trimerization is able to form a β-barrel structure, since pore-forming proteins can spontaneously form oligomers which is a feature of many trimeric autotransporter adhesins with a β-barrel structure in C-terminal part. The possibility to form of a trimeric oligomer channel in bilayer membranes by the C-terminal domain of HlyII and the manifestation of its adhesive properties suggest that HlyII can be presumably attributed to trimeric autotransporter proteins. In this case, HlyII itself stands out as a passenger of the trimeric autotransporter adhesin. The topographic scheme of HlyII (as shown in presumed topographic scheme of HlyII *Bacillus cereus)* is similar to that described for trimeric autotransporter adhesins, and the protein itself can be assigned to this family.