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INTERACTION OF BACTERIOCINS WITH THE RECEPTORS OF SENSITIVE BACTERIAL CELLS

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ABSTRACT

Bacteriocins are proteins and long chain peptides secreted by many species of bacteria that kill bacterial recipient cells in the struggle for vital resources. Their mechanism of action is different from action of traditional antibiotics. A number of studies have shown that they have advantages over traditional antibiotics. The reasons for their effective action on recipient bacterial cells are discussed.

Key words: Bacteriocin, Enterolysin, Catalytic act, Enzymatic bacteriocins, Receptor substrate.

INTRODUCTION

Bacteriocins are a large group of antimicrobial protein substances (bacteriolytic enzymes, non-enzyme proteins, peptides) secreted by a wide range of bacteria (1). Some of them are synthesized on ribosomes, while others are produced as precursors and biologically active bacteriocins are formed post-translational. According to some data, bacteriocins are non-reactogenic and low-toxic in contrast to conventional antibiotics. Their action on sensitive cells is much stronger (sometimes hundreds of times) than that of traditional antibiotics (2). In addition, bacteriolytic enzymes can synergistically enhance their action in the presence of antimicrobial peptides and proteins (nisin, lactoferrin, etc.) (3).

There is no still generally accepted classification of bacteriocins. They are usually divided into three classes:

1) Peptide bacteriocins with a molecular mass of 3-5 kDa containing lanthionine and some other unusual amino acids;

2) Peptide bacteriocins with a molecular mass of 5-9 kDa containing no unusual amino acids;

3) Non-enzyme proteins and bacteriolytic enzymes, among which the most known are enterolysin, lysozyme and lysostafin (4).

This work is a hypothesis about the action of three classes of bacteriocins on sensitive bacterial cells and gives a possible answer to the following questions:

How and why do bacteriocins act upon sensitive cells? How and why do some bacteriocins act on cells of only one or closely related species, whereas others act on cells of a wide range of species, among which there may be both Gram-positive and Gram-negative microorganisms (5). Why do bacteriocins kill sensitive cells much more effectively than traditional antibiotics do; how and why does the death of sensitive cells occur under the action of bacteriocin; what are the mechanisms of action of bacteriocins.

In the case of enzymatic bacteriocins, the reason for the effectiveness is clear. The binding of a bacteriocin to the receptor substrate plays a crucial role ("The better binding gives the better catalysis!"). Then a catalytic act takes place and the enzyme leaves the reaction area. The cycle repeats many times.

Peptide bacteriocins function in the membrane bilayer of sensitive cells. Since most of them are cationic peptides, binding may occur at the place of the largest negative charge of the bilayer. Since the overwhelming majority of these bacteriocins are cationic peptides, binding can occur at the site of the greatest negative charge of the bilayer.

Such places are lipid phosphate "heads". However, this interaction is reversible due to the high dielectric constant of water. Therefore, almost irreversible binding may be only with other components of the membrane bilayer, proteins. Since this intermolecular interaction is not only strong, but also specific, there must be a site ("active centre") on the bacteriocin for binding to the receptor in the bilayer. Because of the specificity, the "active centre" binding to the receptor has to be present in a non-enzyme protein bacteriocin as well. There also should be a specific site on the receptor of the sensitive cell, which binds to the "active center" of the bacteriocin. The binding itself in peptide and protein non-enzyme bacteriocins, as well as in a bacteriolytic enzyme, must be complementary. The binding is mainly hydrophobic because of weak ionic bonds. Charge-transfer complexes in protein substances are also weak, and do not give a strong additional band like classical complexes (6).

Hydrogen bonds cannot be decisive because of competitive water hydrogen bonds. Thus, the specific binding plays a key role in the interaction with receptors in protein bacteriocins, as well as in peptide ones.

The important role of hydrophobicity and complementarity in the binding of bacteriocins is shown (7). In this research a bacteriocin carrying three tryptophan residues in its composition was used. Tri-33 turned out to be essential to manifestation of the activity. When replaced with hydrophobic residues of leucine or phenylalanine, the bacteriocin activity remained practically unchanged. Its substitution for more hydrophilic tyrosine or arginine residue resulted in 10-20-fold and 500-1000-fold decrease in the activity, respectively. The replacement of Try-18 and Try-41 residues with any hydrophilic or hydrophobic amino acid resulted in complete loss of the activity. They are likely a part of the "active centre" of the bacteriocin, and their substitution leads to the loss of complementarity and, as a result, to a loss of activity.

Class I biologically active peptide bacteriocins with molecular mass of up to 5 kDa unlike Class II bacteriocins carrying unusual amino acids in their composition, as well as high- molecular mass protein ones, cannot be synthesized on ribosomes and are posttranslational produced. Apparently, the presence of the unusual amino acids is not accidental. The "active centre" is much easier to be formed by a peptide with a larger molecular mass (up to 9 kDa). Besides, Class II bacteriocins are much more numerous than Class I bacteriocins. The number of conventional acid residues in Class I bacteriocins is limited, and for strong specific binding to the receptor protein in sensitive cells unusual acids probably appeared. Since, stoichiometrically, upon strong binding, one "active centre" of peptide and non-enzymatic protein bacteriocin has one binding site for the receptor protein of a sensitive cell, such bacteriocins are much more effective than traditional antibiotics.

Amino acid substitutes at the binding site of the receptor protein of a sensitive cell to the bacteriocin "active centre" may lead to

decreased sensitivity (binding constants) and even to its loss. If specific binding site in the receptor protein of cells belonging to the same species, it is "unique". The appropriate bacteriocin will bind target cells of this species only. If the same or similar in primary structure binding site in cells of other species (or species), all they will interact with this bacteriocin. Both Gram-positive and Gram-negative microorganisms may be among them. In the research (8). a recombinant bacteriocin (avicin) naturally produced by *Enterococcus avium* was used (7).

Avicin strongly binds to *Listeria monocytogenes* cells and (weaker) to *Streptococcus faecalis*. However, it turned out that avicin interacts with Escherichia coli ATCC 25932 cells with the same binding constant as with *S. faecalis* cells.

The bacteriocin formed weak bonds with *E. coli* C 600 and almost did not act on cells of the other three *E. coli* strains. This suggests that the binding site of the receptor protein in *E. coli* ATCC 25932 cells is the same (or similar) as in *S. faecalis* cells. In other *E. coli* strains the binding is weakening and almost disappears with increasing the number of substitutes in the amino acid sequence of the receptor protein.

The death of sensitive bacterial cells occurs in different ways depending on the receptor protein of the cell. Thus, as a result of binding, the conformation of the receptor protein can change with the formation of pores, which are retained during the lateral movement of the components of the membrane bilayer. After the formation of pores, the permeability of the membrane is disrupted, which leads to cell death. Cell death also takes place if the receptor is a protein responsible for some cell vital functions, e.g. regulatory, transport (8**).** and enzymatic ones (9). Thus, under the action of avicin on *L. monocytogenes* cell, such a protein was mannose transferase. To kill the population of these sensitive cells, it was enough to use avicin at concentration of 3.3 nM (9). Thus, under the action of protein and peptide bacteriocins on the target microbial cell, a strong specific binding determined by complementarity and hydrophobicity plays a decisive role. After binding enzyme bacteriocins a catalytic reaction takes place, and after binding peptide and protein non–enzyme bacteriocins an effector reaction does. In both cases, the process ends with death of sensitive cells.

CONCLUSION

Over 50 years of studying bacteriocins many mechanisms of their action have been proposed. However, they usually relate to either one or several bacteriocins. In this paper, a possible mechanisms of action for three classes of bacteriocins is proposed. Mechanisms of action- description of the process with successive stages (10). In this case the process is the action of bacteriocins on sensitive cells. So, enzymatic bacteriocins act according to a three-stage mechanism (binding, catalytic act, exit from the reaction zone), and peptide and protein non-enzymatic ones, according to a two-stage mechanism (binding and effector reaction).

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None declared.

AUTHORS CONTRIBUTION

All authors have contributed to the conduct of this work. All authors also declare that they have read and approved the final version of the manuscript.

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