CHEMICAL-BIOLOGICAL MECHANISMS AND DIRECTIONS OF DEVELOPMENT OF ANTIMICROBIC PHOTODYNAMIC THERAPY IN DENTISTRY

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- Antimicrobial photodynamic therapy - a treatment method based on the use of the energy of photochemical reactions to destroy cells and factors of pathogenic microorganisms

- The clinical procedure for antimicrobial photodynamic therapy consists of two stages: applying a photosensitizer in the form of a solution, gel or ointment to the surface of infected biological tissue and subsequent irradiation of the treated surface with light radiation with a wavelength corresponding to the peak absorption of the photoagent. An excited photoagent initiates the formation of free radicals that have a destructive effect on the structural elements of the cell

- The main elements of the implementation of the photodynamic reaction are light, photosensitizer and molecular oxygen

- A photosensitizer is a compound whose molecule, due to the peculiarities of its chemical structure, is able to absorb the energy of electromagnetic radiation and, in an excited state, trigger a cascade of free radical reactions. This is done either by first detaching the electron from the substrate molecule or by transferring energy to oxygen dissolved in the biological fluid with the formation of its singlet form

Excitation of the photosensitizer molecule with the formation of its triplet and excited singlet forms:

$$Sens + hv \longrightarrow {}^3Sens^*$$

$$^3Sens^* + hv \longrightarrow {}^1Sens^*$$

<u>Type I reaction:</u>

Electron detachment from the substrate molecule with the formation of a cationic radical of the substrate:

$$^3Sens^* + X \longrightarrow {}^-Sens\cdot + {}^+X\cdot$$

Electron transfer to molecular oxygen with the formation of a superoxide-anion radical:

$$^-Sens\cdot + O_2 \longrightarrow Sens + {}^-O_2\cdot$$

The result of the type I photodynamic reaction is the formation of two radicals: the cationic radical of the substrate $^+X\cdot$ and the superoxide anion radical $^-O_2\cdot$, which are the initiators of the subsequent chain reaction

Type II reaction:

in type II reactions, the excited triplet and short-lived singlet forms of the photosensitizer interact with molecular oxygen, in which the latter acts as an energy acceptor and transforms into a singlet form

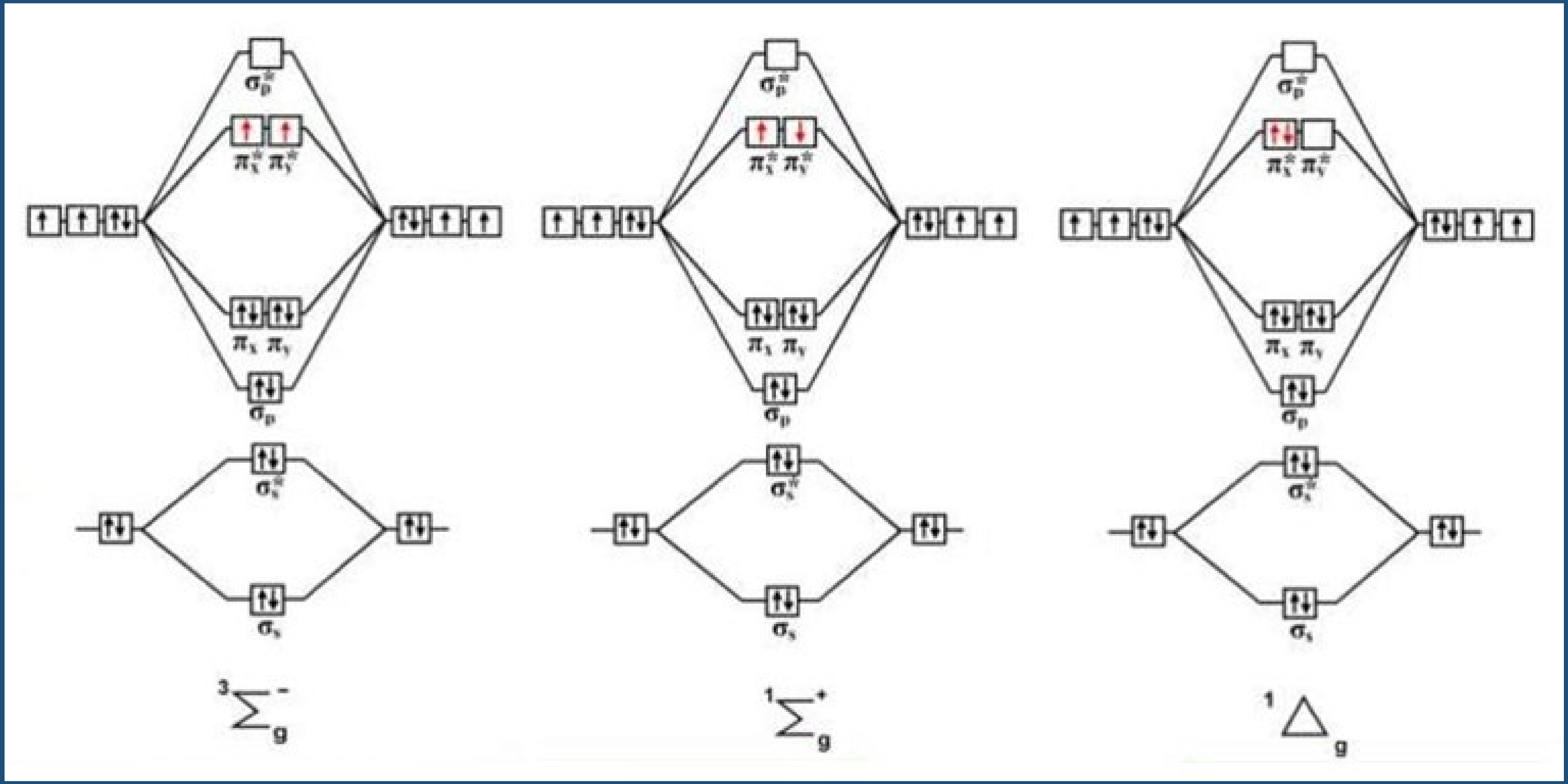
$$^{1}Sens^{*} + {}^{3}O_2 \rightarrow {}^{3}Sens^{*} + {}^{1}O_2$$

$$^{3}Sens^{*} + {}^{3}O_2 \rightarrow Sens + {}^{1}O_2$$

In quantum mechanics, the system of energy sublevels into which the energy shell of an atom or molecule splits as a result of spin-orbit interaction is called the fine structure. Spin multiplicity is a parameter that describes the quantitative characteristics of the fine structure and is calculated by the formula $M = 2S+1$, where S is the spin quantum number, defined as the total spin of all electrons in the system. The numerical value of the spin can be +½ and -½, which means that, for atoms or molecules with an even number of atoms and antiparallelism of spin moments, the spin quantum number will be 0, and the spin multiplicity 1. This state is characterized by the non-splitting of the particle's energy shell and is called singlet

Due to the mutual repulsion of electrons with antiparallel spins, the singlet state is the highest in energy. If the spin moments of two electrons, as, for example, in an oxygen molecule, are parallel, the spin quantum number will be $½ + ½ = 1$, and the spin multiplicity will be $2 \times 1 + 1 = 3$. In this state, the energy shell of the molecule is split into 3 energy sublevels, and the state itself is called a triplet. This condition is stable and is the main one for oxygen. When energy is transferred to an oxygen molecule from outside, for example, from an excited photosensitizer molecule, or due to direct irradiation of the substrate, the mutual orientation of the spins of unpaired electrons of the outer electron shell to antiparallel can occur, and the corresponding transformation of the triplet energy state to a state with a higher energy is a singlet

OXYGEN MOLECULE

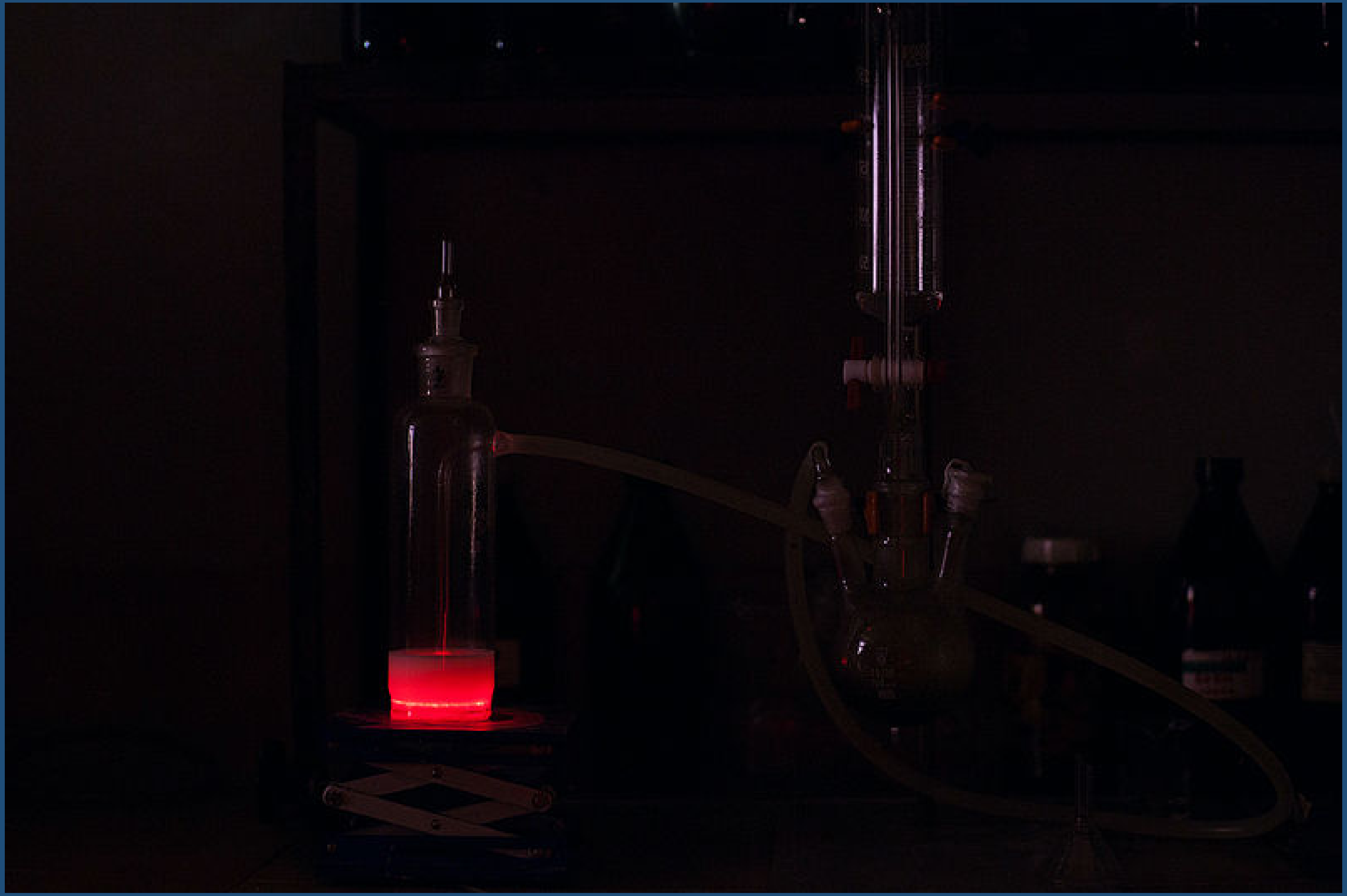


triplet state (main) | first singlet state | second singlet state

Depending on the location of unpaired electrons on electronic orbitals, the first and second singlet states of oxygen are distinguished, the characteristics of which are somewhat different. So, for example, the lifetime of an oxygen molecule in the gas phase in the first singlet state is about 7 seconds, and in the second 72 minutes. In the liquid phase, which we deal with during photodynamic therapy, the lifetime of a singlet oxygen molecule is reduced to 10^{-9} - 10^{-8} seconds

An example of a qualitative reaction of the formation of singlet oxygen is the interaction of chlorine gas with sodium hydroxide and hydrogen peroxide in solution, as a result of which singlet oxygen is released through the stages of the formation of hypochlorite and chlorine peroxide ions. Upon its transition to a stable triplet form, light emission is detected

QUALITATIVE REACTION OF SINGLET OXYGEN FORMATION



Particles formed as a result of reactions of type I and II interact with biological macromolecules, oxidizing them

Singlet oxygen $^1O_2$, formed as a result of type II reactions, is an extremely active agent, but its lifetime and diffusion length are extremely short: 10^{-8}-10^{-9} seconds and 10-20 nm, respectively. Therefore, for its interaction with the target molecule, direct contact of the photosensitizer with the substrate is required, as well as its ability to form this contact (affinity)

To make this contact, it is necessary to transport the photosensitizer molecule to the microbial cell and its capture

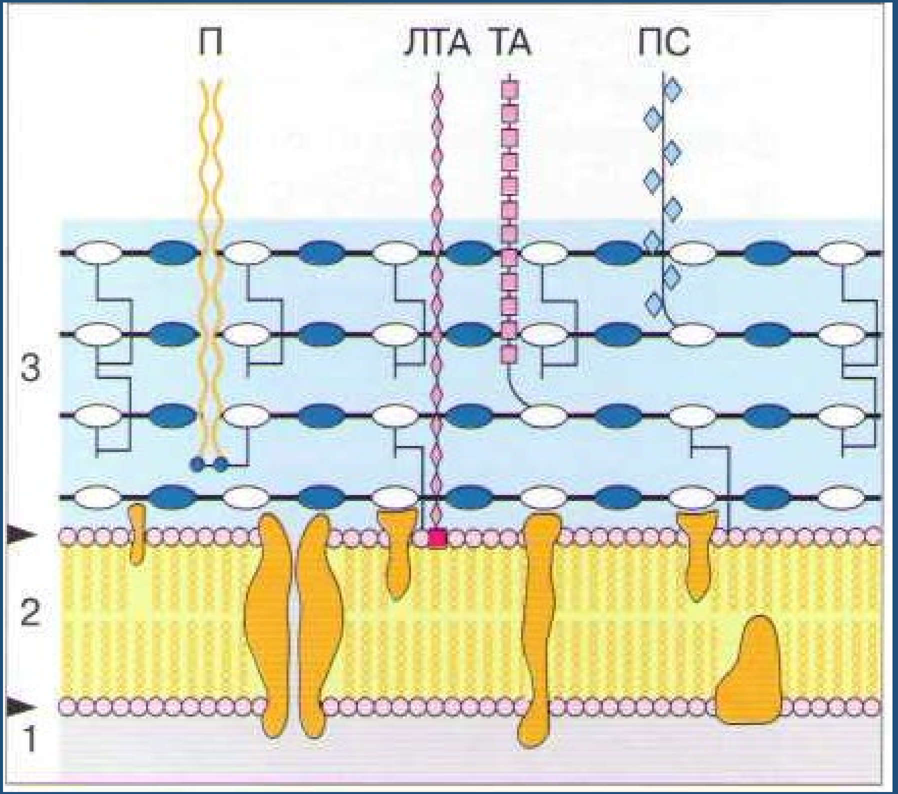
At the transportation stage, in particular during the application of a photosensitizer in dentistry, difficulties may arise due to the inability to ensure absolute dryness of the mucous membrane and instability of the pH of the medium

In the future, the effectiveness of the interaction of the photosensitizer molecule with microbial cells may vary significantly. So, if for grams-positive microorganisms this process is relatively easy, then for gram-negative, with the use of certain groups of photosensitizers, it may not be feasible

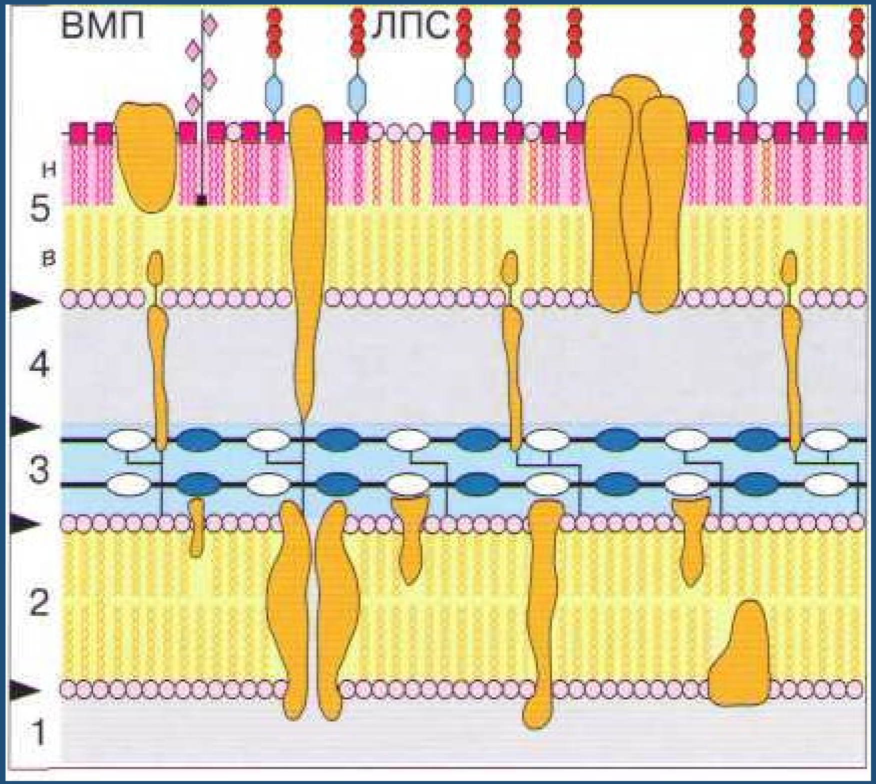
The use of the first photosensitizers was ineffective against gram-negative microorganisms. The presence in the structure of the cell wall of the outer membrane, as well as a number of other morphological and physiological features, made it impossible for sorption and intracellular penetration of photoactive agents

The first attempt to overcome the microbial resistance of gram-negative microorganisms was the use of vectors - chemicals or biological agents that change the permeability of the cell wall of a microorganism due to enzymatic degradation. In the role of such agents were used calcium chloride, EDTA, polymyxin B.

Differences in the structure of the cell wall of gram-positive and gram-negative microorganisms



gram-positive



gram-negative

П – cell wall proteins
ТА – teichoic acid
ЛТА – lipoteichoic acid
ПС –specific polysaccharides
ВМП – external membrane proteins
ЛПС - lipopolysaccharides

In the course of further work, a key factor in the interaction of the photosensitizer and gram-negative microorganisms was determined. This is the positive charge of its molecule. It was shown that it is the cationic form of the photochemical agent that contains fractions that bind strongly to the cell wall of microbial cells and are not removed by repeated washes

In addition, it was shown that different mechanisms work during the inactivation of gram-positive and gram-negative microbial cells

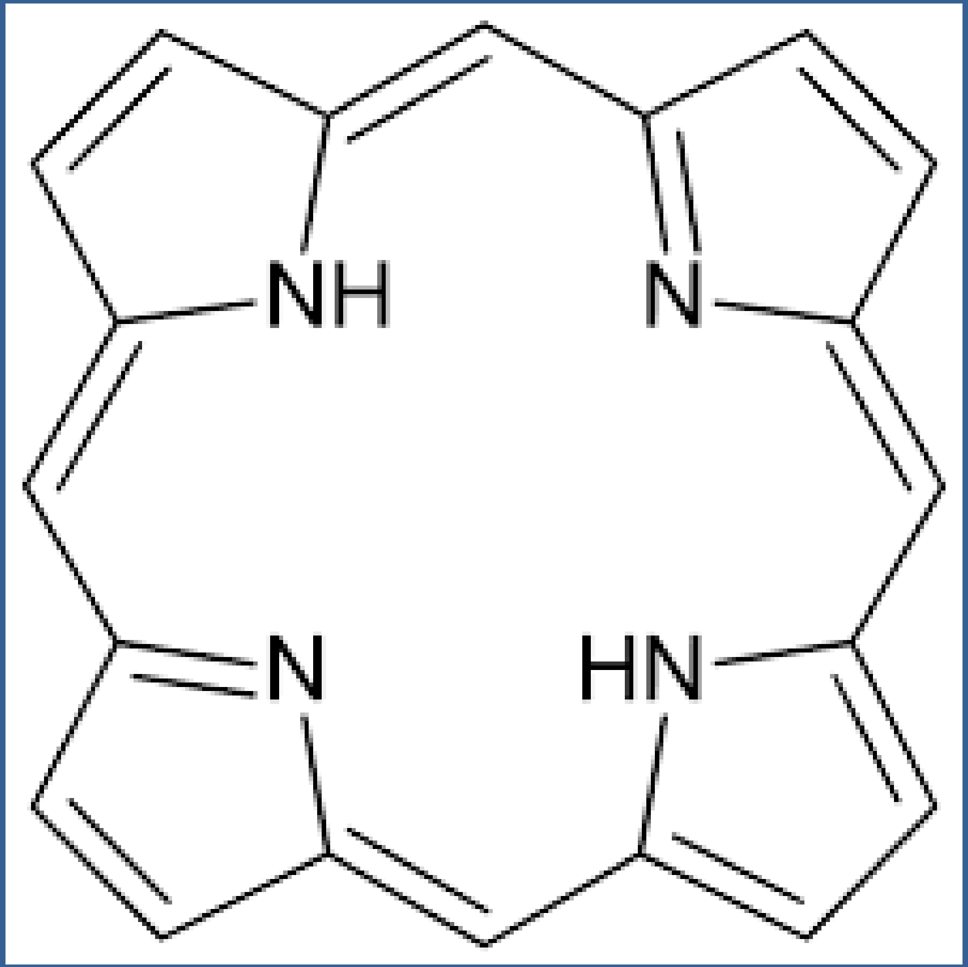
The photodynamic destruction of gram-positive microorganisms proceeds only in the second type of reaction with the formation of singlet oxygen, while lethal photosensitization of gram-negative sequentially includes both mechanisms. First of all, a type I reaction is triggered, which leads to a change in the permeability of the cell wall, after which a type II reaction, causing cytolysis, is attached

Today, there are more than 500 chemical compounds belonging to different classes and possessing photodynamic activity

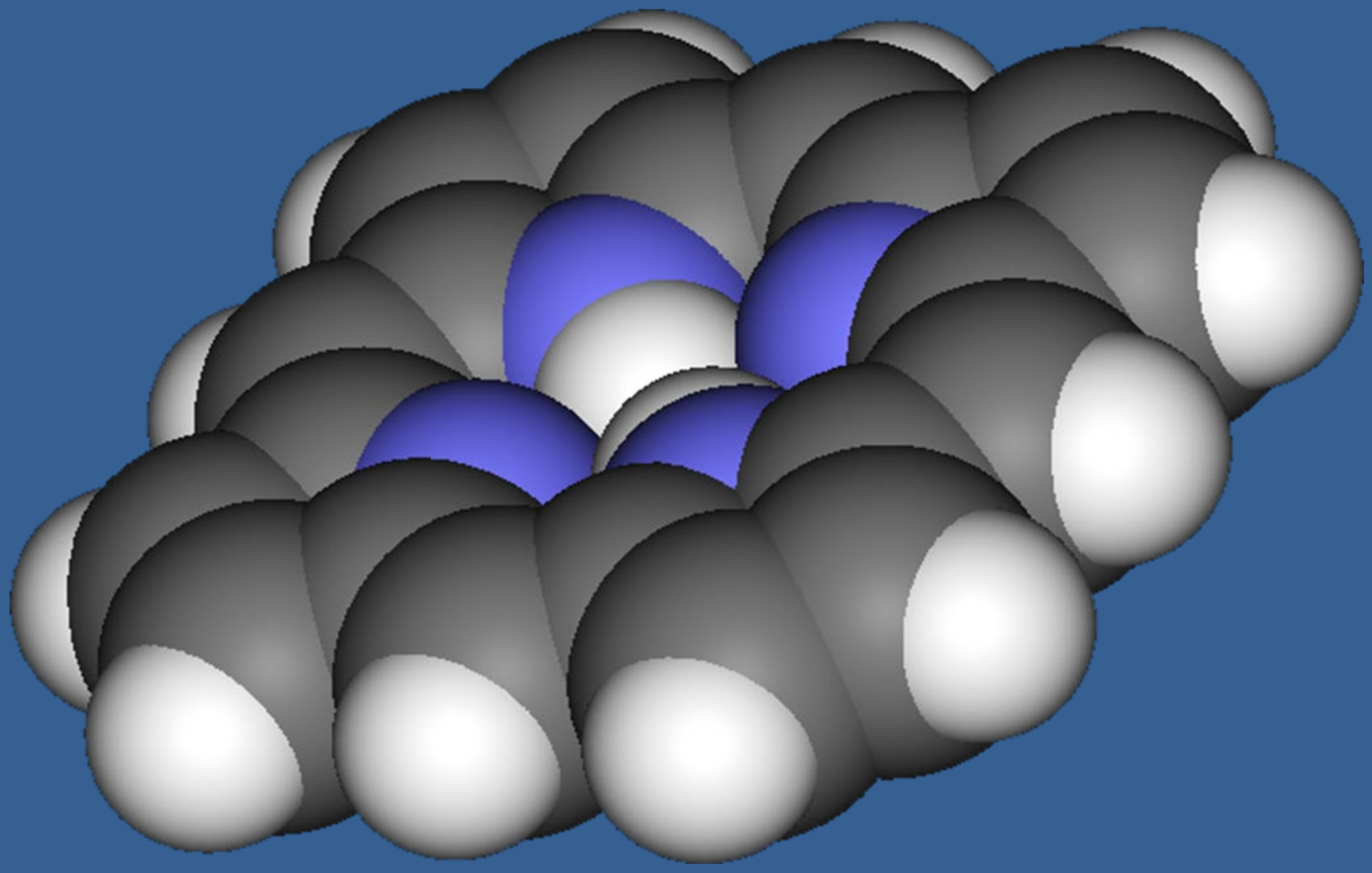
As one of the first and key it is necessary to mention porphyrin, whose molecule, due to the peculiarities of the chemical structure, has a number of unique qualities

Being a tetrapyrrole macrocycle in which four nitrogen-containing nuclei are connected to each other by four methine bridges, the porphyrin molecule has an extended conjugated electron system, which underlies an extremely high (about 105) molar light absorption coefficient. In addition, due to electron pairs of nitrogen atoms, porphyrin is able to act as a powerful chelating ligand

Porphyrin molecule



3D-model of the porphyrin molecule

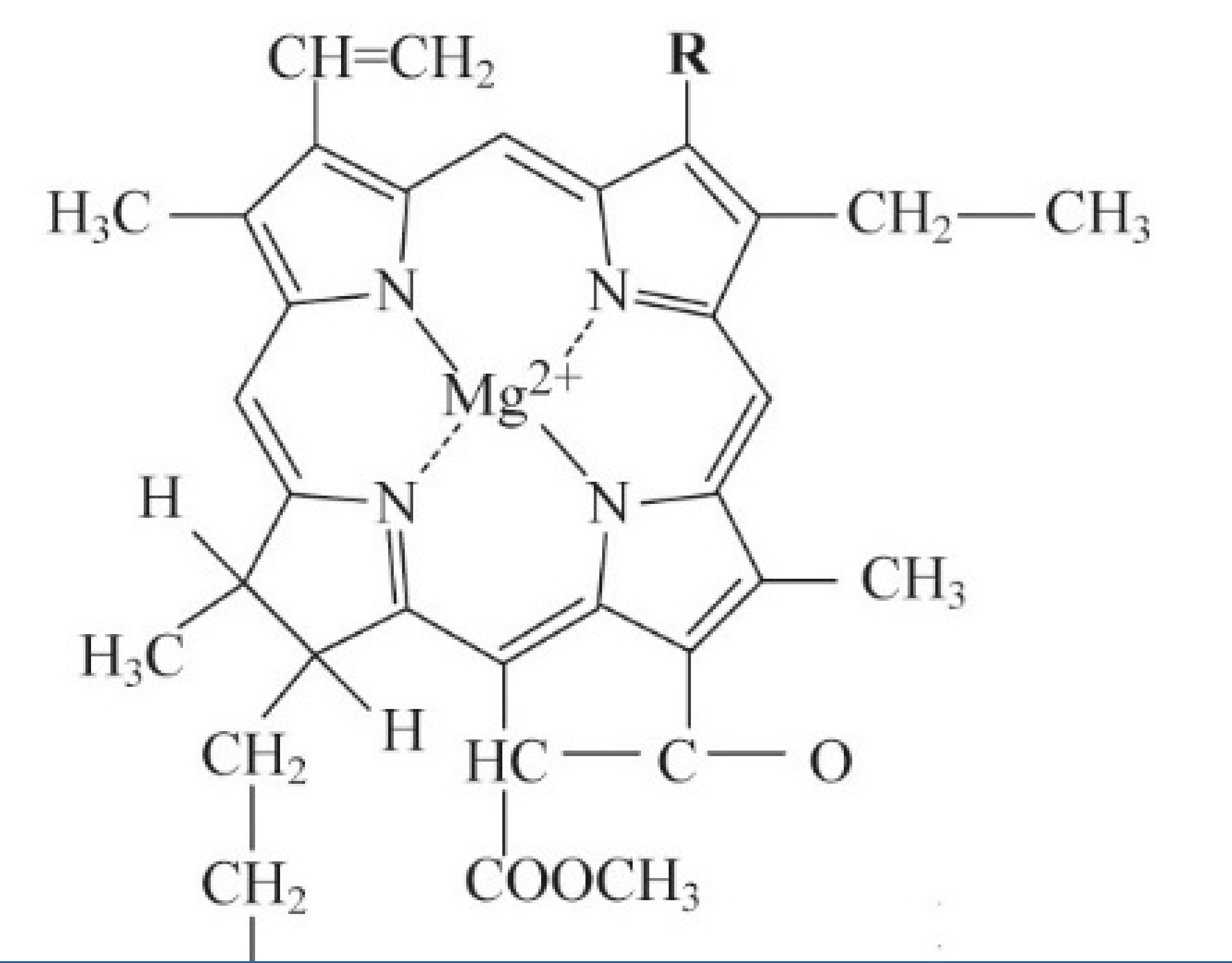


An example of a porphyrin complex compound is chlorophyll. Chlorophyll is a natural photosensitive compound that provides oxygenic photosynthesis reactions that occur in the chloroplasts of green plants

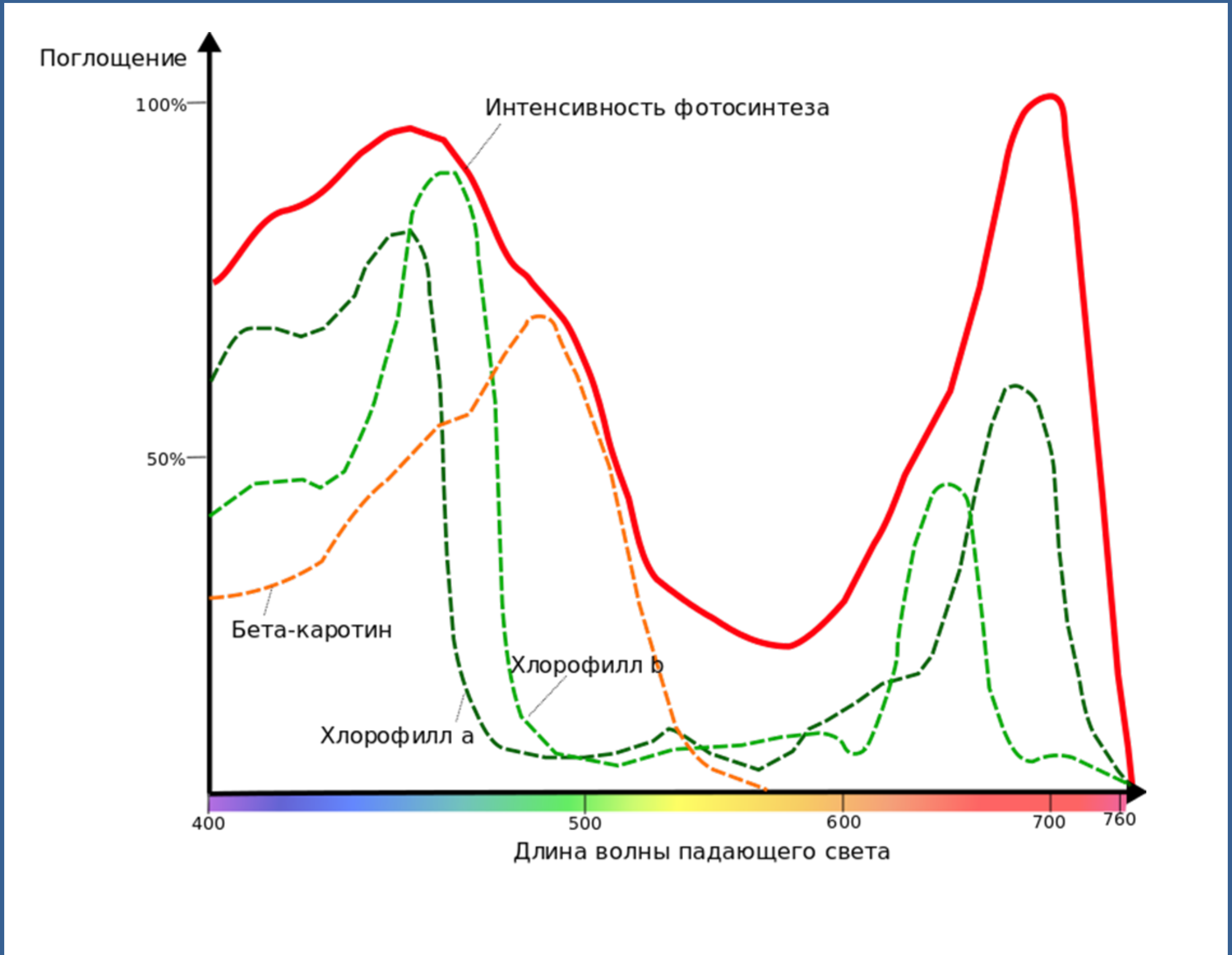
Moreover, the photoacceptor system of the chlorophyll molecule includes two subsystems that determine two levels of excitation and, accordingly, two maxima in the spectrum of photosynthesis:

- conjugated double bond electron system
- system of unpaired electrons of nitrogen atoms and magnesium ion

The core of the chlorophyll molecule



The spectrum of photosynthesis



To date, 3 generations of photosensitizers are distinguished:

I generation: hematoporphyrin derivatives, ineffective against gram-negative microorganisms

The following main groups of compounds belong to the II generation:

1) chlorophyll A derivatives
2) derivatives of bacteriochlorophyll A
3) tetranitroporphyrins
4) derivatives of δ-aminolevulinic acid (5-aminolevulinic acid)
5) porphyrin analogs

The third generation of photosensitizers is also distinguished

The molecule of the compounds of this group includes a radioactive radical, which determines the selective binding of the agent to certain proteins and minimizes the accumulation of the photosensitizer in healthy cells

The clinical use of the method of antimicrobial photodynamic therapy in dentistry has shown its high efficiency in the treatment of infectious and inflammatory diseases of various localization

At the same time, a number of drawbacks of the method were identified due to the qualities of the photosensitizers used:

1. complexity and high cost of production
2. low photodynamic activity
3. the increase in toxic effect with increasing concentration
4. the mismatch between the selectivity of accumulation and destruction
5. sensitivity to a specific wavelength
6. the dependence of work efficiency in application from fluctuations in the pH of the medium, the amount of blood, saliva, gingival fluid

PROSPECTS FOR THE DEVELOPMENT OF ANTIMICROBIAL PHOTODYNAMIC THERAPY

Today, there are 3 main areas of development of the method:

1. The use of endogenous compounds localized in a microbial cell as photosensitizers

The possibility of triggering a photodynamic reaction due to the excitation of endogenous photosensitizers of the microbial cell itself has been proved. The latter include endogenous porphyrins and endogenous flavins, the maximum absorption of which occurs at 405 and 445 nm, respectively. Using the methods of spectrophotometry and spectrofluorophotometry, one can determine the level of saturation of microbial cells with these substances. A necessary condition for this type of interaction to occur, in addition to the required wavelength of the initiating radiation, is a sufficient level of its power density. Activation of endogenous photosensitizers eliminates the use of exogenous compounds

2. Direct generation of singlet oxygen

The intracellular environment of any cell contains dissolved oxygen in triplet form. The spectral absorption maximum of its molecules corresponds to wavelengths of 1273 nm, 1073 nm, 765 nm and 690 nm. With direct irradiation of microbial cells with radiation with the appropriate wavelength, molecular oxygen can transition from the main triplet to the singlet state.

The main condition for the implementation of such an energy transformation is a high level of power density of the used radiation (more than 300 mW/cm^2). In a biological system, the application of these radiation parameters leads to the appearance of a concomitant thermal effect. Unacceptable tissue heating can be avoided if energy is used not in the form of a continuous stream, but in the form of discrete, successive pulses. In such conditions, it is possible to transfer the necessary excitation to the biosubstrate in a very short time, insufficient for the development of side effects

3. Search and creation of photosensitizers devoid of the above disadvantages

This can be the use of known substances with photochemical activity, but not previously used as photosensitizers in dentistry, or the extraction and synthesis of fundamentally new compounds

Our research includes work in all three areas. Namely, the study of the effectiveness of antimicrobial photodynamic therapy for the treatment of infectious and inflammatory diseases of various localization in dentistry using endogenous photosensitizers, direct generation of singlet oxygen, as well as new chemical compounds with photodynamic activity

THANK YOU FOR ATTENTION!