BACTERIOCHLORIN $e_6$ AS A SENSITIZER FOR PHOTODYNAMIC THERAPY

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A simple scheme of modification of the native pigment bacteriochlorophyll $a$, extracted from the biomass of phototropic bacteria, for the purpose of obtaining a water-soluble derivative — bacteriochlorin $e_6$ — which is similar in molecular structure to the effective sensitizer chlorin $e_6$ and, in addition, has an intense longwave absorption band in the near-infrared region of the spectrum where biological tissues weakly absorb light — has been developed. The ease of production of bacteriochlorin $e_6$ in combination with its physicochemical and spectral properties allow it to be considered as a promising sensitizer for photodynamic therapy.

Keywords: photodynamic therapy, photosensitizer, bacteriochlorophyll $a$, bacteriochlorin $e_6$.

Introduction. Bacteriochlorophyll compounds are actively being studied in many scientific centers. Even though the majority of such investigations are devoted to the role of these pigments in the photosynthesis processes, increasing interest is being shown in the medical application of bacteriochlorophyll and its derivatives [1]. The features of the molecular structure of the above-mentioned compounds, determining their spectral characteristics (existence of a longwave absorption band ($\lambda_{\text{max}} > 700 \text{ nm}$) with a large extinction ratio ($\varepsilon > 4.0 \cdot 10^4 \text{ M}^{-1} \text{cm}^{-1}$) in their spectrum and high efficiency of photosensitized formation of singlet molecular oxygen), allow the bacteriochlorophyll pigments to be considered as potential sensitizers for photodynamic therapy [1, 2]. At the same time, it is known [1] that native bacteriochlorophyll $a$ is poorly dissolved in water, which hampers the transport of the photosensitizer to tumor tissues and its subsequent removal from the organism. On the other hand, the bacteriochlorophyll molecule has a low oxidation potential because of the small energy gap between its ground $S_0$ and lower excited $S_1$ states, which leads to a rapid degradation of this compound, especially in the presence of molecular oxygen [1–4]. Considerable recent attention has been focused on the search for new bacteriochlorophyll derivatives, possessing all the positive properties of the initial compound and free of its drawbacks. The properties of a large variety of bacteriochlorophyll $a$ derivatives: bacteriophaeophorbide $a$ [5, 6], bacteriochlorin $a$ [7, 8], bacteriopurpurin $a$ [9, 10], and their conjugates with amino acids [1, 2, 11, 12], have been investigated. Moreover, methods of obtaining and investigating synthetic bacteriochlorins are being developed [1, 13]. The photodynamic activity of bacteriochlorophyll $a$ derivatives in vitro and in vivo has been investigated in [7, 8, 14]. It has been shown that in these compounds there can occur photodynamic effects that lead to the formation of radical products and singlet molecular oxygen. The solubility of bacteriochlorophyll $a$ derivatives and, accordingly, their transport to the tumor tissues can be enhanced with the use of phosphatidylcholine liposomes, which makes it possible to significantly increase the photodynamic effect [15].

The aim of the present work is development of a simple scheme of obtaining, from a bacterial biomass, a bacteriochlorophyll derivative — bacteriochlorin $e_6$ — with a structure similar to the structure of the well-known photodynamic sensitizer chlorin $e_6$.

Methods of Obtaining Bacteriochlorin $e_6$. Bacteriochlorin $e_6$ can be obtained in the form of trimethyl ether [16]. For this purpose, a solution of bacteriochlorophyll $a$ extracted from Rhodospirillum rubrum bacteria is subjected to reetherification (HCl/CH$_3$OH) followed by the formation of an intermediate product — methylbacteriophaeophorbide — in which the cyclopentanone ring is cleaved under the action of a weak alkali in the presence of methanol and the

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finite product is formed. Another known method of obtaining bacteriochlorin e6 involves saponification and acid hydrolysis, with the use of sodium acetate, of bacteriochlorophyll a extracted from Rhodospirillum rubrum bacteria [17] with the resulting formation of an intermediate product — bacteriochlorophyllipte.

Figure 1 shows the scheme of obtaining bacteriochlorin e6, developed by us [18]. At the first stage, bacteriochlorophyll a is extracted from the biomass of Rhodospirillum rubrum or Rhodobacter sphaeroides bacteria with the use of a mixture of solvents (acetone and methyl alcohol) and the extract obtained is subjected to chromatography with the use of saccharose. Then bacteriochlorophyll a is boiled in a saturated alcoholic solution of an alkali (KOH or NaOH) for a short time until the potassium or sodium salt of the bacteriochlorin e6 magnesium complex precipitates. The product obtained was purified by ether-acid fractionation, in the process of which the magnesium ion was removed from the center of the porphyrin macrocycle and the tripotassium or trisodium salt of bacteriochlorin e6 was formed. Molecules of the tripotassium or trisodium salt of bacteriochlorin e6 in a phosphate-salt buffer dissociate into sodium or potassium cations and bacteriochlorin e6 anions, due to which bacteriochlorin e6 becomes soluble in aqueous media.

The absorption spectra of the pigments were recorded on a Beckman-5270 spectrophotometer (USA). The solvents were purified by standard methods and were additionally rectified.

Results and Discussion. In the works of G. P. Gurinovich and his disciples, it is shown (see, e.g., [19, 20]) that chlorin e6, representing a derivative of the native pigment chlorophyll a, can be used as an effective photosensitizer in photodynamic therapy of tumors. Chlorin e6, due to the presence of three carboxyl groups in its molecule, is hydrophilic and, therefore, can be effectively transported and accumulated in the tumor tissues, which, in combination with its ability to effectively absorb a longwave light and, as a result, sensitize singlet molecular oxygen, enables it to be photodynamically active [1]. Clinical tests of some photosensitizers based on chlorin e6 [Fotolon ("Belmedpreparaty," Minsk, Belarus), Chlorin ("Dialek," Minsk, Belarus), and MACE (Nippon Petrochemicals, Tokyo, Japan), have given satisfactory results [1, 21–23]. At present, such photosensitizers are being tested in China [24].

It is thought that each of the sensitizers available, in a market of medicines, should possess concrete properties that would allow it to be used for treatment of a definite type of tumors. The potential of bacteriochlorin e6 as a photodynamic sensitizer is anticipated from its structural features that are similar to those of the known sensitizer chlorin e6 and allow it to provide an optimum hydrophobic-hydrophilic balance and, consequently, to be effectively transported to the tumor tissues and then easily removed from the organism. At the same time, sensitizers based on bacteriochlorophyll a derivatives (as compared to the sensitizers based on chlorophyll a derivatives) have a more intense longwave absorption band (denoted, as a rule, by $Q_y$), which is shifted to the region of higher transparency of biotissues.

The structure of the side substituents in the bacteriochlorin e6 molecule is similar to that of the chlorin e6 molecule. Additional hydrogenation of pyrrolophenine ring II does not significantly change the solubility of the tetrpyrrole pigments in aqueous media [1] but influences the $\pi$-system of the macrocycle [25], with the result that the maximum of the absorption band of the first electron transition in bacteriochlorin tetrahydroderivatives shifts to the longer-wave region of the spectrum as compared to that of chlorin dehydroderivatives. If the maximum of the $Q_y$ absorption band of chlorin e6 (in different solvents) lies in the range 660–670 nm, the maximum of this band of bacterio-