MECHANISM OF COMPLEXING OF CHLORIN e₆ AND TETRA(p-CARBOXYPHENYL)PORPHYRIN WITH MALATE DEHYDROGENASE


The mechanism of complexing of the tetrapyrrole-nature photodynamic sensitizers chlorin e₆ and tetra(p-carboxyphenyl)porphyrin (TCPP) with the Krebs-cycle enzyme malate dehydrogenase (MDG) has been investigated by spectral-luminescence methods. It is shown that each subunit entering into the composition of the MDG dimer molecule forms an equilibrium complex with one dye molecule. However, if the sites of bonding of TCPP on a protein molecule are independent, the MDG–chlorin e₆ complex has a negative cooperativity since after the dye is incorporated into the first subunit of the macromolecule, its penetration to the second subunit becomes difficult. This is explained by the fact that conformation transformations, arising as a result of the incorporation of chlorin into one of the MDG subunits, are transferred to the site of its bonding on other MDG subunits through the intersubunit contacts of the enzyme. It has been established that photosensitizers compete with the hydrophobic fluorescent probe 8-aniline-1-naphthalenesulfonate (ANS) (whose position in the MDG macromolecule was described in detail in the literature) for the sites of bonding on the protein molecule, which allows the conclusion that TCPP and chlorin e₆ are localized in the catalytic domain of MDG. In this case, the sites of bonding of these dyes and the sites of bonding of nicotinamide-adenine dinucleotide, occupying the MDG domain that bonds coenzymes, do not interact with each other.

Keywords: malate dehydrogenase, chlorin e₆, tetra(p-carboxyphenyl)porphyrin, photosensitizer, enzyme, complex, cooperative bonding, apoptosis, fluorescence, polarization.

Introduction. As is known [1, 2], irradiation of animal or human cells in the presence of an exogenous dye-sensitizer and molecular oxygen dissolved in the medium damages the vital organelles of these cells, which can lead to their death. Such photochemical destruction of tumor cells with the use of tetrapyrrole dyes, characterized by an increased ability of accumulating in tumor tissues, forms the basis for the method of photodynamic therapy of oncological diseases [3, 4]. It is commonly supposed [2, 5–7] that cells exposed to photodynamic action can die as a result of their necrosis or apoptosis. The more acceptable mechanism for an organism is apoptosis, in the process of which certain cells are destroyed under an external action; therefore, apoptosis is considered as one of the most important results of a treatment [8]. The mechanism of death of cells — apoptosis or necrosis — depends on the type and concentration of the dye used, the sites of localization of the dye in the most important cellular structures, and the intensity of the acting radiation [5–7]. The apoptosis process is initiated under photodynamic action when the intracellular level of adenosine triphosphate decreases to a certain level [7–12]. If a photodynamic action causes a sharp decrease in the adenosine triphosphate level and, as a result, an almost complete disruption of the energy exchange in a cell, the apoptosis can be quickly changed to the less desirable necrosis [12]. The mechanism of change from apoptosis to necrosis under photodynamic action is not clearly understood because the primary molecular targets through which light exerts destructive action upon cells in the presence of a dye-sensitizer are a question.

It is known [13] that the amount of adenosine triphosphate, which is necessary for the vital activity of cells, is produced as a result of a number of Krebs-cycle and glycolysis-system biochemical reactions occurring only in the

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presence of protein-nature catalyzers — enzymes. It may be suggested that the photodestruction of enzymes under photodynamic action is responsible for the decrease in the adenosine triphosphate level and, consequently, for the death of tumor cells due to necrosis or apoptosis. Since in a heterogeneous biological medium, all things being equal, the macromolecules near which a sensitizer is localized, i.e., which form a complex with it, will be damaged first of all, of particular interest is the study of the complexing of photodynamic dyes with the molecules of enzymes catalyzing biochemical energy-exchange reactions in a cell.

The aim of the present work is to investigate the mechanism of complexing of the Krebs-cycle enzyme malate dehydrogenase (MDG) with the tetrapyrrole-nature photodynamic sensitizers chlorin $e_6$ and tetra($p$-carboxyphenyl)porphyrin (TCPP). Spectroscopic verifications of the formation of complexes of these sensitizers with MDG molecules have been obtained earlier [14, 15].

Materials and Methods. We used the cytoplasmic (supernatant) MDG (L-malate: NAD-oxidoreductase, catalogue of enzymes 1.1.1.37) from the pig heart (Sigma, USA). TCPP and chlorin $e_6$ were synthesized at the Institute of Molecular and Atomic Physics of the National Academy of Sciences of Belarus. The localization of the sites of bonding of the dyes studied on the MDG macromolecule was determined by the localization of a specific ligand — the coenzyme reduced nicotinamide adenine dinucleotide (NADH) of the Reanal Company (Hungary) — and the fluorescent probe 8-aniline-1-naphthalene sulfonate (ANS) (Sigma, USA). The solutions were prepared based on a the 0.01-M phosphate buffer, pH 7.3.

The formation of complexes of enzyme molecules with a sensitizer was investigated using differential spectrophotometry (M40 UV VIS Specord, Carl Zeiss, Jena, Germany) and spectral-luminescence and polarization methods (SFL 1211A, "Solar," Belarus). To exclude the errors in determining the optical density, which can arise due to the scattering of radiation in the enzyme solution, we carried out spectrophotometric measurements by the so-called method of four cuvettes (two cuvettes were placed in each channel of the spectrophotometer) [16].

We used this method instead of the method of two cuvettes because, in the case where one cuvette with a dye is placed in each channel of the spectrophotometer, the change in the optical density of the dye at the wavelength of its absorption band arising when a fairly large amount of an enzyme is introduced into it can be due to both the change in the absorption spectrum of the dye as a result of its complexing with the enzyme and to the light scattering (on enzyme macromolecules), whose intensity depends on the light wavelength and the concentration of the enzyme macromolecules. In the method of four cuvettes, an enzyme is introduced into one of the cuvettes of the measuring and comparison channels of the spectrometer, namely into the cuvette with a dye in the measuring channel and into the cuvette with a buffer (free of the pigment) in the comparison channel. Therefore, in this case the change in the absorption spectrum of the dye caused by the introduction of the protein into it can be only due to the change in the spectral characteristics of the pigment as a result of its complexing with the enzyme.

The parameters of the TCPP–MDG and chlorin–MDG complexes (association constant $K_{as}$, number of sites of bonding $n$ of chlorin on the dimer enzyme molecule, and Hill cooperativity coefficient $h$) were determined by the