PHOTOINDUCED SINGLET OXYGEN FORMATION IN AQUEOUS SOLUTIONS OF COVALENT PORPHYRIN–ANTIBODY CONJUGATES

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The photodynamic therapy (PDT) of cancer is based on the ability of certain sensitizers, more especially porphyrins, when introduced into the body, of accumulating selectively in tumor cells and inducing their photodynamic destruction [4]. This effect is most probably due to the formation of reactive oxygen molecules in the singlet $^1\Delta_g$ state ($^1O_2$), photoinduced by porphyrins. Drawbacks of the PDT method include the relatively low selectivity of porphyrin accumulation in tumor tissue compared with normal tissue (usually not more than ten times greater), and binding of prophyrins by serum proteins. Therapeutic doses of porphyrins are therefore relatively high and this leads to unfavorable effects (toxicoses, damage to normal tissue, prolonged rehabilitation) [5, 7].

These disadvantages can be overcome by using photoimmunotoxins, which were first suggested in [10], i.e., covalent conjugates of porphyrins with antibodies to specific tumor markers, which combine photodynamic action and oriented transport to tumor tissue [6, 10]. To obtain an equal effect the concentration of porphyrin conjugated with proteins can in this way be substantially reduced (by 50-100 times) compared with the free porphyrin level [9]. This effect is evidently associated with the more effective accumulation of antibodies specific for tumor markers in cancer cells.

The aim of this investigation was, by using a direct luminescent method of recording $^1O_2$ developed previously [7], to study the ability of photoimmunotoxins to sensitize $^1O_2$ formation and to compare their activity in this process with that of free porphyrins in aqueous solutions.

EXPERIMENTAL METHOD

Luminescence of $^1O_2$ was excited by nanosecond pulses from an LGI-21 nitrogen laser (337.1 nm) and recorded in the 1270 nm region by means of a cooled FÉU-83 photomultiplier through a high-transmission MS-80 monochromator (Central Design Office, Academy of Medical Sciences of the USSR). Signals from the FÉU-83 were stored in an NTA-1024 multichannel analyzer. The results were processed by EMG-666 microcomputer (Hungary) [2, 8].

Human γ-globulin (hIgG) of 99% purity ("Serva," West Germany), mouse monoclonal antibodies to carcinoembryonic antigen of glass IgG_{2a} ($mIgG_{2a}$), and mouse monoclonal antibodies to insulin: clone C7, class IgG_1 ($Migg_1$) and clone B9, class IgM (mIgM) provided by T. V. Cherednikova (A. N. Bakh Institute of Biochemistry, Academy of Sciences of the USSR, Moscow), were used. The monoclonal antibodies were isolated from ascites fluid by standard methods [1]. The purity of the antibody preparations, according to the results of ion-exchange HPLC, was not less than 96%.

2,4-Di(α-methoxyethyl)deuteroporphyrin IX (DMDP), cocoproporphyrin I (CP), cyclopentenecoproporphyrin I (CCP), and tetra(*p*-sulfophenyl)porphyrin (TSPP) were synthesized at the Institute of Biophysics, Ministry of Health of the USSR, Moscow (Fig. 1). Synthesis and isolation of covalent conjugates of porphyrins with antibodies were carried out by methods similar to those described in [4, 10]. Samples of the labeled proteins were not contaminated with noncovalently bound porphyrin.

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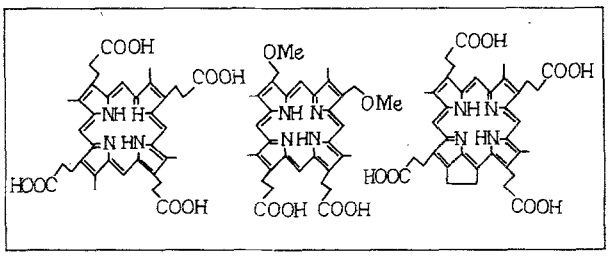

Fig. 1. Structural formulas of porphyrins. 1) CP, 2) DMDP, 3) CCP.

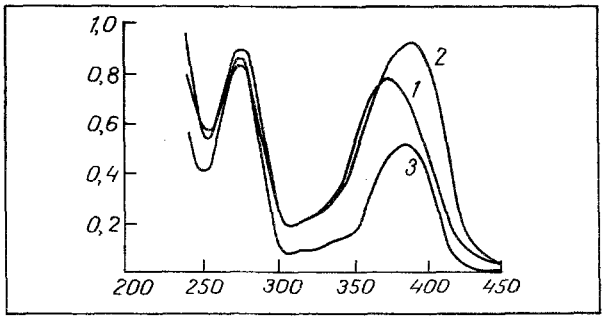

Fig. 2. Absorption spectra of solutions of covalent conjugates of monoclonal antibodies with porphyrins. 1) mIgG-CP (1:1.7, 3.8 μM), 2) mIgG-DMDP (1:1.9, 4 μM), 3) mIgG-CCP (1:1.2, 3.9 μM). Abscissa, wavelength (in nm); ordinate, optical density (optical density units).

The luminescence measurements were undertaken in 10 mM phosphate buffer, made up in deuterated water (D_2O), obtained from the "Izotop" Leningrad Opticomechanical Combine, containing not less than 99.9% D_2O (pD 7.4-7.8), and containing 0.15 M NaCl.

EXPERIMENTAL RESULTS

Absorption spectra of solutions of conjugates of porphyrins with monoclonal antibodies are shown in Fig. 2 and the concentrations and composition of the conjugates were determined from them by the method in [4]. The Cope absorption band for conjugates of porphyrins with antibodies was somewhat widened and its maximum shifted into the short-wave region compared with alcoholic solutions, containing monomeric porphyrin molecules. Aqueous solutions of porphyrins containing a mixture of monomers and associates have similar absorption spectra [2, 6].

After laser irradiation of air-saturated salt solutions of porphyrins and their conjugates, made up in D_2O, photoinduced luminescence of $^1O_2$ was observed with a maximum at 1270 nm and with a life (τ_Δ) of 61 sec for solutions of the free pigments and 58 μsec for solutions of conjugates with antibodies was observed. The very small decrease in τ_Δ in solutions of the conjugates was evidently due to quenching of the $^1O_2$ luminescence by protein molecules.

Values of absolute quantum yields of $^1O_2$ photogeneration (γ_Δ), determined by comparing the intensity of luminescence of $^1O_2$ of solutions of the test compound with a solution of TSPP by the method described previously [3, 7], are given in Table 1. It was shown that the porphyrins tested can effectively induce $^1O_2$ formation both in the free state and in the composition of covalent conjugates with antibodies, although in the latter case the efficiency of $^1O_2$ photogeneration as a rule is lower and is close to γ_Δ of solutions of porphyrins containing a high proportion of associates [3].

It follows from the results that the efficiency of $^1O_2$ generation by photoimmunotoxins is only weakly dependent on the nature of the protein and is independent both of the class of antibodies (IgG or IgM with mol.wt. of 150 and 950 kilodaltons, respectively) and of the source of their isolation (mouse or human globulins).

TABLE 1. Quantum Yields of Photogeneration of $^1O_2$ (γ_Δ; ± 10%) by Porphyrins and Their Conjugates with Monoclonal Antibodies in Aqueous Solutions

Object	γ_Δ^*
DMDP	0,53
$mIgG_1$-DMDP	0,19
CCP	0,54
$mIgG_1$-CCP	0,29
CP	0,18
$mIgG_1$-CP	0,25
$mIgG_{2a}$-CP	0,24
mIgM-CP	0,21
hIgG-CP	0,20

*Tetra(*p*-sulfophenyl)porphyrin, for which the absolute quantum yield under the conditions of measurement is 0.70, was used as the standard [3, 8].

It can thus be concluded from these experiments that conjugates of porphyrins with antibodies can be used as efficient sensitizers of $^1O_2$ formation and, consequently, they can initiate photodynamic cell damage. This, in turn, points to the good prospects for the use of covalent conjugates of porphyrins with antibodies specific for cancer cell antigens in order to make photodynamic cancer therapy more effective.

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