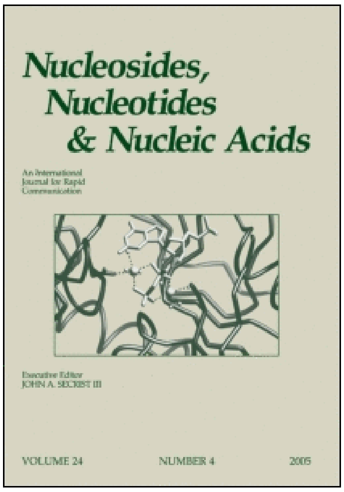

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PHOTOSENSITIZED AND CATALYTIC OXIDATION OF DNA BY METALLOPHTHALOCYANINE-OLIGONUCLEOTIDE CONJUGATES

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ABSTRACT

The synthesis of new metallophthalocyanine-oligonucleotide conjugates is reported. These conjugates can cause sequence-specific photosensitized or catalytic oxidation of DNA by molecular oxygen.

Phthalocyanines constitute an interesting family of compounds producing active oxygen species under photoirradiation or in dark conditions. Al(III)-, Zn(II)- and Si(IV)-phthalocyanines are used now for photodynamic (PDT) therapy of malignant tumors [1]. Phthalocyanines of Fe(II) and Co(II) are considered as a promising anticancer drugs in "dark" therapy [2, 3], because they can act as a very effective catalysts of oxidation with molecular oxygen. Therefore, such properties of metallophthalocyanines makes them very attractive as a reactive groups to be linked to antisense oligonucleotides for specific DNA modification *in vitro* and *in vivo*.

In this paper we describe the synthesis of the oligonucleotide conjugates with metallophthalocyanines **1**–**3**. The synthesis of conjugates **1**–**3** was performed as shown in Scheme 1. Oligodeoxyribonucleotide TCTTCCCA containing a -O-$(CH_2)_3NH_2$ linker at the 5′-end was synthesized by standard phosphoramidite

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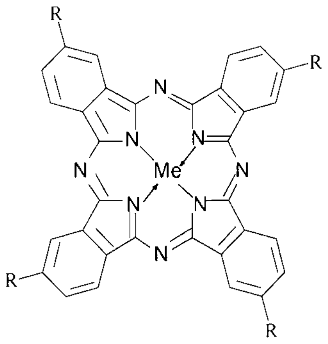
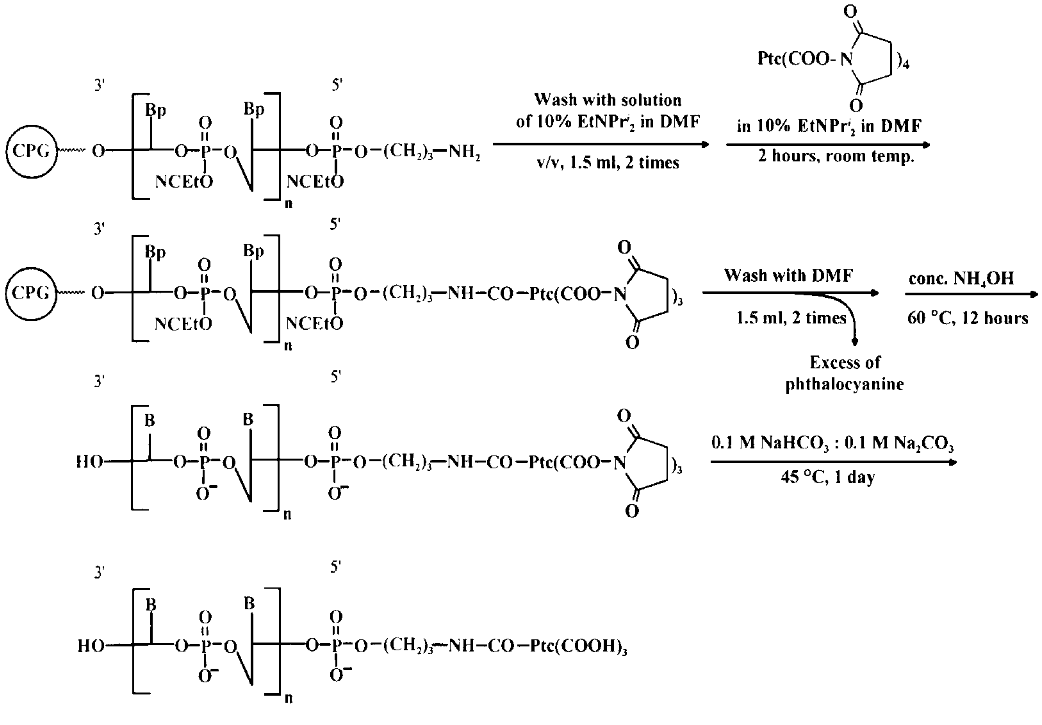


N	Me	R
1	Co(II)	COOH
2	Al(III)Cl	$SO_2NH(CH_2)_5COOH$
3	Zn(II)	SCH_2COOH

Scheme 1.

methodology [4]. Succinimide esters of phthalocyanines were coupled to the oligonucleotide while it was still attached to the control pore glass (CPG). The resulting conjugates were deprotected and cleaved from the CPG with concentrated aqueous ammonium hydroxide. Finally, the conjugates were purified by preparative reversed phase HPLC in acetonitrile/H_2O gradient. The yields of conjugates were 30–40%.



Ptc - phthalocyanine, $EtNPr^i_2$ - N-diisopropylethylamine, NCEt - β - cyanoethyl, Bp - protected base, B - base, DMF - dimethylformamide.

Figure 1.

The ability of the oligonucleotide-Co(II)Ptc conjugate to modify single-stranded DNA targets [^{32}P]-GGGAAGAGGGTCAG (14-mer) and [^{32}P]-TGAATG GGAAGAGGGTCAGGTT (22-mer) was assayed at 25°C in buffer: 0.16 M NaCl, 0.02 M NaH_2PO_4 (pH = 7.5), 1 mM Na_2EDTA, reaction time = 24 hours. It is known that Co(II)Ptc catalyses the formation of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}$OH), from molecular oxygen and reducing molecules. We used dithiothreitol, mercaptoethanol and ascorbic acid as reducing agents. The products of 14-mer and 22-mer modification were analyzed with 10% PAGE. The direct cleavage of the targets was not observed. The treatment of modified target with 1 M piperidine led to the cleavages at G^6 of 14-mer and at G^{11} of 22-mer when dithiothreitol or 2-mercaptoethanol were used as the reducing agents or if hydrogen peroxide was used as an oxidant instead of molecular oxygen and reducer. When ascorbic acid was used as the reducing agent the non-selective cleavage of the targets after piperidine treatment was observed. The maximal yields of the target modifications in the presence of $SHCH_2CH_2OH$ and H_2O_2 were 15% and 35%, respectively.

The modified targets were also treated with the repair enzyme 8-oxoguanine DNA glycosylase from *E.coli* (Fpg protein). This enzyme cleaves DNA chains at the positions of 8-oxoG and some another modified nucleosides. It was determined that the total yield of modification was about 60%. This indicates that 8-oxoG is the main product of modification in this systems, because this nucleosides does not lead to the cleavage of the chain under alkali treatment.

It was shown that the conjugates with Al(III) and Zn(II) modified the 22-mer on irradiation with light from a Hg-lamp (1000 W) at wavelengths longer than 340 nm or with light of a He/Ne laser at 633 nm. If the Hg-lamp was used as the light source (irradiation time was 3 hours) modification occurred at G^{20}, G^{19}, G^{15-13} and G^{11} residues, as was detected both with Fpg protein and piperidine treatment. The modification yields varied in the range 6–20%. The cleavage with Fpg protein led to lower yield than in the case of piperidine treatment.

Following irradiation with He/Ne laser light for 30 minutes the modification yields were 10% and 24% for conjugates with Al(III) and Zn(II), respectively, and were equal for both Fpg protein and piperidine treatment.

The data presented in this work demonstrates the dependence of the efficiency and the selectivity of DNA modification on the nature of the reactive group in the oligonucleotide conjugate. In the case of Co(II)Ptc-group the hydroxyl radicals may be considered as the main oxidative species. Due to very high reactivity ($E^0_{OH/OH^-} = 1.9$ V) this radical oxidizes the nucleoside residues of the target located close to phthalocyanine group of the conjugate in complementary complex. In contrast to Co(II)Ptc the photosensitizers Al(III)Ptc and Zn(II)Ptc produce the long-living singlet molecular oxygen $^1O_2$ ($^1\Delta_g$). This particle can migrate along the DNA chain and can cause the modification of nucleosides located far from the reactive group in the duplex. Thus, the efficiency and selectivity will be low. Further studies of such metallophthalocyanine-oligonucleotide conjugates are of interest in the development of sequence specific gene targeting reagents.

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