# Dimeric Fe–Co Phthalocyanine Complex as a Reagent for the Selective Damage of Nucleic Acids

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*The complexes of $Fe^{II}$ and $Co^{II}$ with phthalocyanines are extremely good catalysts for oxidation of organic compounds with molecular oxygen and hydrogen peroxide. Their solubility and reactivity are increased by conjugation with oligonucleotides and by formation of dimeric complexes between negatively and positively charged phthalocyanines. In our work such complexes were formed directly on single-stranded DNA through interaction between negatively charged $Co^{II}$ phthalocyanine in conjugate and positively charged $Fe^{II}$ phthalocyanine in solution. The resulting oppositely charged phthalocyanine complexes showed significant increase of catalytic activity compared with monomeric forms of phthalocyanines $Fe^{II}$. The site-directed modification of single- and double-stranded DNA by $H_2O_2$ in the presence of dimeric complexes of negatively charged $Co^{II}$ and positively charged $Fe^{II}$ phthalocyanines was detected. These complexes catalyzed the DNA oxidation with high efficacy and led to direct DNA strand cleavage.*

**Keywords:** $Fe^{II}$ and $Co^{II}$ phthalocyanines, nucleic acid, catalysis.

## Introduction

Many natural and synthetic compounds that could selectively modified various cell constituents including DNA are studied as potential anti-cancer drugs during past decades. Complexes of phthalocyanines (Pcs) with the ions of transition metals (Fe, Co, *etc*.) represent an interesting type of reactive group due to their ability to catalyze the formation of reactive oxygen species. Currently, phthalocyanine complexes of $Co^{II}$ and $Fe^{II}$ are being investigated as drugs for the catalytic therapy of cancer.[1]

The main problem of phthalocyanine usage in cancer therapy is their low solubility in water solutions. The solubility could be increased by Pcs conjugation with 6 or 8 charged substituents. But this method did not increase the specificity of resulting reagents.

On the other hand, using any affinity groups as substituents it is possible to increase the selectivity of action of the resulting conjugates. For example, the conjugation of Pcs with oligonucleotides[2] which are of interest as potential anti-cancer drugs themselves,[3] could has a positive synergetic effect in therapy of cancer.

In recent investigations, the sequence-directed oxidative cleavage of DNA with $O_2$ and $H_2O_2$ in the presence of conjugates of $Co^{II}$ and $Fe^{II}$ phthalocyanines attached to oligonucleotides were studied.[4,5] It was shown that single-stranded DNA is efficiently damaged in complexes with these conjugates. Moreover, the activity of Pcs could be increased by forming dimeric complexes between negatively and positively charged phthalocyanines.

The site-directed modification of single-stranded DNA by $O_2$ and $H_2O_2$ in the presence of heterogeneous dimeric complexes of negatively and positively charged $Fe^{II}$ and $Co^{II}$ phthalocyanines ($Fe^{II}Pc_{pos}\cdot Fe^{II}Pc_{neg}$ and $Co^{II}Pc_{pos}\cdot Co^{II}Pc_{neg}$, respectively) was investigated in our previous work.[6] These complexes were formed directly on single-stranded DNA through interaction between the negatively charged phthalocyanine in the oligonucleotide-conjugate and the positively charged phthalocyanine in the solution. The resulting phthalocyanine complexes showed a significant increase in catalytic activity compared to monomeric forms of phthalocyanines $Fe^{II}$ and $Co^{II}$[6] and led to direct DNA strand cleavage. It was determined that oxidation of DNA by molecular oxygen catalyzed by a complex of $Fe^{II}$-phthalocyanines proceeds with a higher rate than that catalyzed by $Co^{II}$-phthalocyanines; however, the latter led to a higher yield of target DNA modification.

In current work we decided to combine two phthalocyanines to form heterogeneous metal dimeric complex of negatively charged $Co^{II}$ and positively charged $Fe^{II}$ phthalocyanines ($Fe^{II}Pc_{pos}\cdot Co^{II}Pc_{neg}$). We study the modification of single-and double-stranded DNA by such complex.

## Experimental

*Chemicals and reagents.* Acrylamide, *N,N'*-methylene-bisacrylamide, urea, acetonitrile, DMF (Fluka, Switzerland), Tris and piperidine, were used. All solutions were prepared with double-distilled water using ultrapure reagents. Hydrogen peroxide (stabilized, more than 30%) was purchased from Fluka. T4 polynucleotide kinase was purchased from Sibenzyme (Russia). [$\gamma$-$^{32}$P]ATP (> 3000Ci/mmol) was purchased from Biosan (Russia). All experiments were carried out at 25 ºC in a buffer containing 0.16 M NaCl, 0.02 M sodium phosphate (p*H* 7.4), and 1 mM EDTA.

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*Phthalocyanines.* Cobalt(II) tetra-4-carboxyphthalocyanine CoPc was prepared and characterized as described previously.[7] Octakis-4,5-(*N*-β-aminoethyl-β'-*N,N*-diethylammonioethoxycarbonyl) phthalocyanine $CoPc_{pos}$ and $FePc_{pos}$ complexes were prepared with high yields by quaternization of the respective octakis-4,5-(β-chloroethoxycarbonyl)phthalocyanine complexes[7] with *N,N*-diethylethylenediamine by heating them in *N*-methylpyrrolidone in the presence of catalytic amounts of anhydrous sodium iodide.[6]

*Oligonucleotides and conjugates.* The oligonucleotides 5'd(AATGGGAATAAAAAAAAAAA)3' and 5'd(TATTCCCATT)3' (ODN) were synthesized and purified in the same way as reported previously.[6] Concentrations of the oligonucleotides were determined from their absorbance at 260 nm.[8] The 5'-termini of the oligonucleotides were $^{32}P$-labeled using a standard procedure with T4-polynucleotide kinase and [γ-$^{32}P$]ATP (> 3000 Ci/mmol).[9] The concentration of the labeled oligonucleotide solution did not exceed $2.5\cdot10^{-7}$ M. The oligonucleotide derivative CoPc-NH-$(CH_2)_6$-O-5'pd(TATTCCCATT)3' $Co^{II}Pc$-ODN was synthesized using a previously reported solid phase method[10] with a 30- 40% yield.

*Oxidation of the target oligonucleotides.* Oxidation of DNA target by hydrogen peroxide was carried out in DNA duplex (Figure 1). The concentrations of the target DNA and phthalocyanine-oligonucleotide derivatives were equimolar ($1.0\cdot10^{-6}$ M), the concentration of the $^{32}P$-labeled DNA-target was $1.0\cdot10^{-8}$ M. The concentrations of the hydrogen peroxide was $1.0\cdot10^{-3}$ M. The reaction was initiated by adding $H_2O_2$ or the reducing agents. Aliquots (10 μl) were taken from the reaction mixture at different times and immediately transferred into polypropylene tubes containing 200 μl of 2% $LiClO_4$ in acetone. The precipitate was pelleted by centrifugation, washed twice with 80% ethanol and once with acetone, and dried under vacuum. The samples were then either treated or not with 1 M piperidine.[11] The products of the modification were separated by 20% PAGE in the presence of 7 M urea. After electrophoresis, the gel was exposed to CP-BU X-ray film (Agfa-Gevaert, Belgium) for 10-20 h at -10 ºC. Autoradiograms were quantitated using Gel-Pro Analyzer 3.0 software (Media Cybernetics, MD). The yield of modification was calculated as the ratio of the areas of the product peaks to the sum of the areas of the product and the initial oligonucleotide peaks. The error did not exceed 20%.

## Results and Discussion

Oxidation of DNA target by $O_2$ and $H_2O_2$ in the presence of complexes of oppositely charged phthalocyanines of $Co^{II}Pc_{neg}$ and $Fe^{II}Pc_{pos}$ was studied within the DNA duplex containing two parts (Figure 1). The first part is a double-stranded fragment (A1-A10) formed between the oligonucleotide part of the phthalocyanine-oligonucleotide derivative and the complementary sequence of the single-stranded target. The other part is a single-stranded fragment (A11-A20) of the target, not involved in duplex formation. This region of the target DNA is located in close distance to the phthalocyanine moiety in $Co^{II}Pc_{neg}$-ODN and, therefore can be highly modified. Free $Fe^{II}Pc_{pos}$ can interact in solution with both double- and single-stranded parts of complex and negatively charged Pc residues.

In case of free $Fe^{II}Pc_{pos}$ the nucleotide oxidation depended on the region of interaction between target and $Fe^{II}Pc_{pos}$. As can be seen from Figure 2A the nucleotides G4-A7 are the mostly modified region with 5-8% modification yield. As far as we did not observe any preferable oxidation of other nucleotides, we could make a conclusion that free $Fe^{II}Pc_{pos}$ interacts with DNA and is located at the 5'-end of DNA target. The total modification yield was about 25% in the presence of $H_2O_2$.

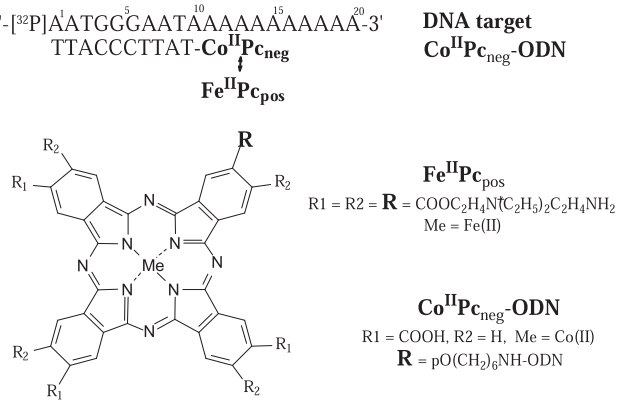


**Figure 1:** Structures of the DNA duplex and nucleotide sequences of the DNA target and $Co^{II}Pc_{neg}$-ODN; and the structures of the Pcs used in this work.

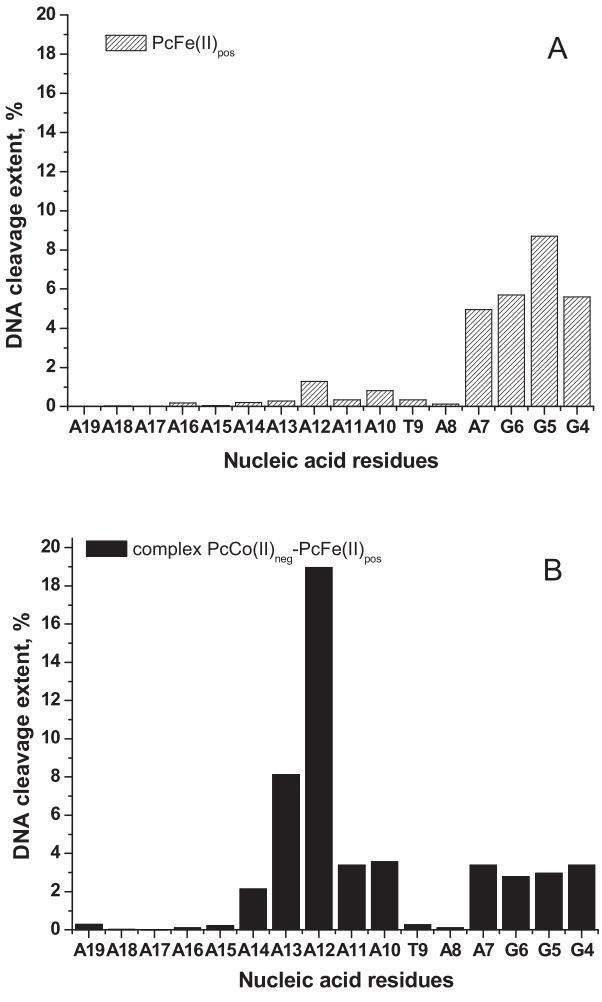


**Figure 2.** Distribution of base modifications in the target DNA by free $Fe^{II}Pc_{pos}$ (A) and by dimeric complex $Fe^{II}Pc_{pos}\cdot Co^{II}Pc_{neg}$-ODN (B). The modifications were revealed by treatment with 1 M piperidine.

The distribution of nucleotide modifications was more selective in the case of $Fe^{II}Pc_{pos}$ $Co^{II}Pc_{neg}$-ODN com-

plex. As for $Fe^{II}Pc_{pos}$ the G4-A7 region was oxidized with 3-4% modification yield, but A12 and A13 nucleotides were mainly modified with 19% and 8% yield, respectively (Figure 2B). Such selective oxidation corresponds to expected location of the reactive complex ($Fe^{II}Pc_{pos}$·$Co^{II}Pc_{neg}$) near A10-A15 region. The total modification yield was about 50% that in 2-fold higher than in the case of free $Fe^{II}Pc_{pos}$. The oxidation in the G4-A7 region in both cases seems to be due to reactivity and localization of the free $Fe^{II}Pc_{pos}$ within DNA duplex.

In comparison with the catalytic activities of the $Fe^{II}Pc_{pos}$·$Fe^{II}Pc_{neg}$ and $Co^{II}Pc_{pos}$·$Co^{II}Pc_{neg}$ complexes reported previously[6] the reactivity of the heterogeneous complex studied in this work was similar to the last one. Thus, the change of $Co^{II}$ into $Fe^{II}$ in $Pc_{pos}$ did not significantly influence the modification yield and DNA oxidation rate within first 24 hours, although the selectivity of action was increased.

## Conclusions

Overall, this study indicates that heterogeneous dimeric complex $Fe^{II}Pc_{pos}$·$Co^{II}Pc_{neg}$-ODN possesses the increased catalytic activity in the oxidative degradation of DNA with $H_2O_2$ in comparison with monomeric phthalocyanines.[4,12]

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