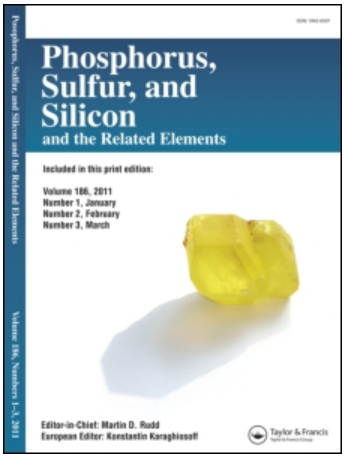

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OLIGONUCLEOTIDES SHACKLED WITH TETRAPHENYLPORPHYRIN

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Abstract Phosphorus(V)porphyrin derivative photocleaved a double stranded circular DNA, ϕx 174FRI, in the absence of oxygen. The potential of photoexcited phosphorus(V)porphyrin derivative has sufficient oxidation potential to oxidize nucleic acid bases. Photocleavage of DNA by a direct electron transfer mechanism was confirmed. Several definite-sequenced oligonucleotides shackled with tetraphenylporphyrin were synthesized. They hybridized with their complementary oligonucleotides, respectively. A sharply sequence targeted photocleavage of DNA through the direct electron transfer was suggested.

Key Words Oligonulceotide/ Tetraphenylporphyrin/ Artificial restrictive enzyme/ Oligonucleotide shackled with porphyrin

1. INTRODUCTION

Photofunctional oligonucleotide derivatives chemically modified by photoactive molecules are employed for non-radioactive probes, artificial photoenzymes, and tools for photochemical examining of gene expression. We have reported phosphorus(V)tetraphenylporphyrin (P(V)TPP) derivatives[1~7] are useful and artificial photonuclease in which photo-induced electron transfer was investigated.[8~12] In this paper, DNA photocleaving ability of P(V)TPP, syntheses of novel oligonuleotide derivatives shackled with tetraphenylporphyrin (TPP) at the phosphorus atom of an internucleotide phosphodiester without change of backbone of the nucleotide and their interactive properties with their complementary oligonucleotide are investigated, and the possibility of an artificial restrictive photonuclease is suggested.

2. P(V)TPP, OLIGONUCLEOTIDE SHACKLED WITH TPP AND THEIR DERIVATIVES

The followings are examples of P(V)porphyrins and oligonucleotide shackled with porphyrins. The oligonucleotides shackled with TPP were synthesized by DNA synthesizer using a key molecule which is shown in Fig.1.

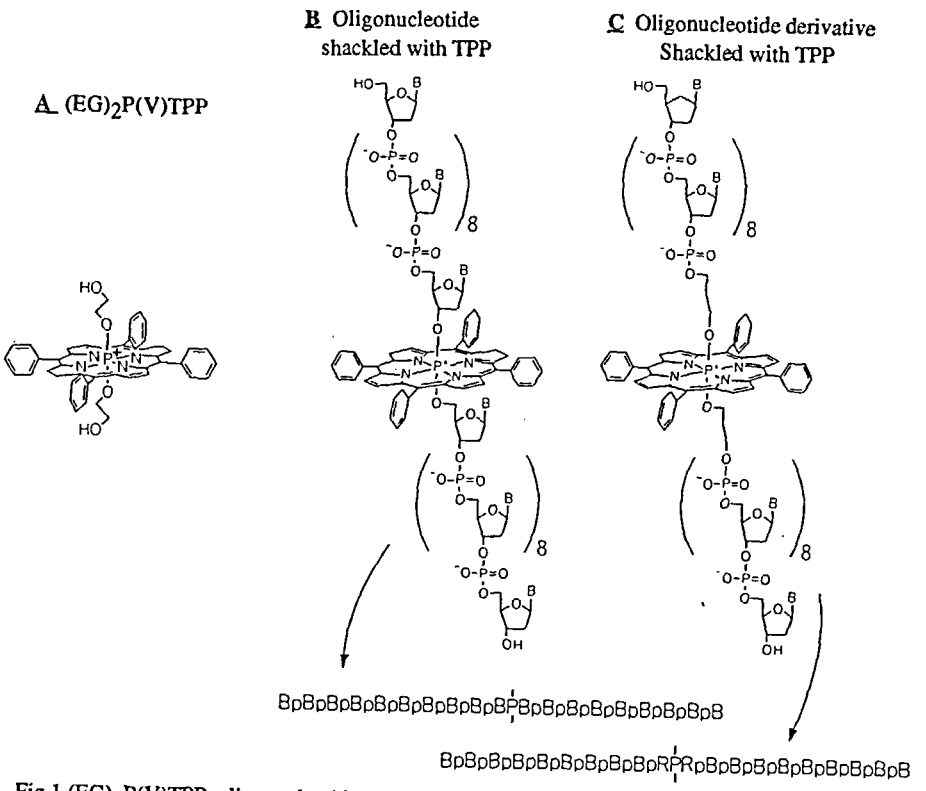

Fig.1 $(EG)_2P(V)TPP$, oligonucleotides shackled with TPP and their derivatives (examples)

3.PHOTOCLEAVAGES OF DOUBLE-STRANDED ΦX 174 DNA WITH P(V)TPP DERIVATIVE

The photograph in Fig.2 shows typical examples of the photoceavage of DNA by visible light irradiation of ϕx 174RFI DNA in the presence of the $(EG)_2P(V)TPP$ in aerated solution resulted in the appearance of open circular DNA (form II) and a small amount of linear DNA (form III). The photocleavage was enhanced by the increase of the concentration of the $(EG)_2P(V)TPP$ and irradiation time. No strand scission was observed in the absence of $(EG)_2P(V)TPP$ and in the dark . To detect the reactive oxygen species in the photoreaction, the effects of scavengers were examined. As shown in lanes 3-5, NaN_3 effectively inhibited the DNA cleavage. However, SOD and D-mannitol did not inhibit the DNA cleavage. These findings suggest that the main reactive species in the photocleavage in air is singlet oxygen ($^1O_2$), but not superoxide anion radical or hydroxy radical. Energy transfer from the triplet excited state of the $(EG)_2P(V)TPP$ to O_2 is considered to have resulted in the formation of $^1O_2$, which causes oxidative cleavage of the DNA. In fact, the time-resolved absorption spectroscopy indicated that the lifetime of the triplet excited state of the $(EG)_2P(V)TPP$ was shortened with O_2. Interestingly, the

single-strand scission of DNA was also observed even in the absence of $^1O_2$ (lane 6). This result suggests the photocleavage of DNA by the direct electron transfer between the $(EG)_2P(V)TPP$ and DNA. In general, decompositionof DNA by direct electron transfer is initiated from the oxidation of nuclei acid bases, mainly guanine in the absence of $^1O_2$. The reduction potentials of the $(EG)_2P(V)TPP$ in the singlet and the triplet excited states are estimated +1.83V and +1.44 (vs NHE), respectively. These values are considered to be high enough to oxidize all nucleic acid bases. Fig.3 shows mechanism of the photocleavage.

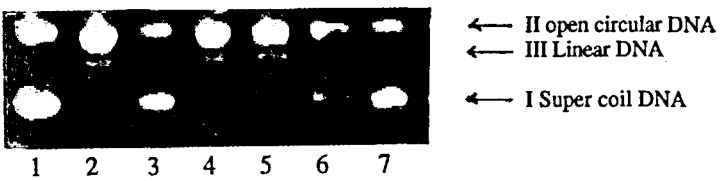

Fig.2. Agarose gel electrophoresis showing relaxation of ϕx 174RFI DNA (40mM) due to photocleavage by $(EG)_2P(V)TPP$ (20mM) in 20 mM Tris HCl buffer, pH 6.8 containing 10mM NaCl. Lane1, DNA only: Lane2, DNA+P(V)TPP in air: Lane3, Lane2+NaN3 (100mM). Lane4, Lane2+SOD (10ng/ml): Lane5, Lane2+D-mannitol (100mM): Lane6, DNA+P(V)TPP in argon saturated aqueous solution. All irradiation were performed for 1 hr. Control experiments, lane7 was performed without irradiation.

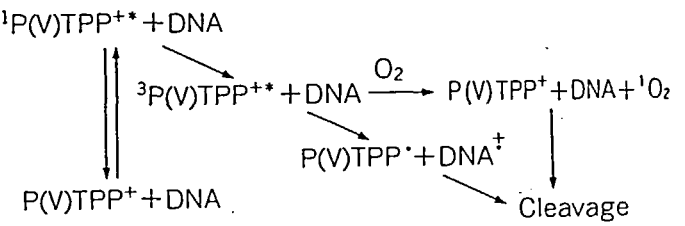

Fig.3. Mechanism of photocleavage of ϕx 174RFI DNA by $(EG)_2P(V)TPP$.

4. HYBRIDIZATION OF THE OLIGONUCLEOTIDE DERIVATIVES SHACKLED WITH TETRAPHENYLPORPHYRIN AND THEIR COMPLEMENTARY OLIGONUCLEOTIDE

The specific interactions, hybridizations, of the oligonucleotide derivatives with their complementary oligonucleotides were investigated by temperature-dependent UV spectra. Table 1 shows examples of melting points of hybrid. In table, Nos. 1 ~ 6 were for homooligonucleotides and Nos. 7 ~ 9 were for sequence-defined derivatives. Compared derative series (Nos. 2,3, and 4) with a native one (No.1), both porphyrin and abasic spacer decreases hybridization a little, however sharp meltings were observed. The sequence-defined derivatives seems to be more preferable for antisense pairing.

Fluorescence quenching were reduced in the molten temperature range, which suggests possibility of a direct electron transfer from a base to excited porphyrin in hybrid.
CD spectra of these hybrid were almost similar to those of complementary native oligonucleotide.

Table 1. Melting temperature of Hybrids

No.		mp/°C
1	TpTpTpTpTpTpTpTpTpTpTpTpTpTpTpTpTpT ApApApApApApApApApApApApApApApApApApA	48
2	TpTpTpTpTpTpTpTpTpTPTpTpTpTpTpTpTpTpT ApApApApApApApApApApApApApApApApApApA	33
3	TpTpTpTpTpTpTpTpTpRPRpTpTpTpTpTpTpTpT ApApApApApApApApApApApApApApApApApApA	32~33
4	TpTpTpTpTpTpTpTpTpRpRpTpTpTpTpTpTpTpT ApApApApApApApApApApApApApApApApApApA	32~33
5	TpTpTpTpTpTpTpTpTpRPRpTpTpTpTpTpTpTpT ···pApApApApApApApApApApApApApApApApApApAp···	33~34
6	TpTpTpTpTpTpTpTpTpRPRpTpTpTpTpTpTpTpT ApApApApApApApApApA	22
7	TpTpApTpApApApTpTpTpTpTpApApApTpApTpT ApApTpApTpTpTpApApApApApTpTpTpApTpApA	41
8	TpTpApTpApApApTpTpTPTpTpTpApApApTpApTpT ApApTpApTpTpTpApApApApApTpTpTpApTpApA	29
9	TpTpApTpApApApTpTpRPRpTpTpApApApTpApTpT ApApTpApTpTpTpApApApApApTpTpTpApTpApA	20~21

These results imply that hybrids of oligonucleotide shackled with TPP and its complementary oligonucleotide are almost similar tight shape as native ones, and a photoceavage of the targeted DNA sequence by photo-induced direct electron transfer is suggested.

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