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Palladium(II)-coproporphyrin I as a photoactivable group in sequence-specific modification of nucleic acids by oligonucleotide derivatives

O.S. Fedorova, A.P. Savitskii[+], K.G. Shoikhet, G.V. Ponomarev*

Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Lavrentiev Pr. 8, 630090, Novosibirsk, USSR, [+]Institute of Biochemistry, the USSR Academy of Sciences, Lenin Pr. 33, 117071, Moscow, USSR and **Institute of Biophysics of the USSR Ministry of Health, Zhivopisnaya Str. 46, 123182, Moscow, USSR*

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The 34-mer oligodeoxynucleotide was shown to be selectively modified at the G17 position upon photoirradiation in the presence of complementary 17-mer oligodeoxynucleotide bearing Pd(II)-coproporphyrin I covalently linked to the 5'-end phosphate group.

Sequence-specific modification; Pd(II)-coproporphyrin I; Photoirradiation

1. INTRODUCTION

Reactive derivatives of antisense oligonucleotides can be used for sequence-specific modification of nucleic acids (complementary addressed modification [1-3]). A number of reactive derivatives, containing alkylating [3,4], photoactivable [3-5], metal chelating groups [4,6-10] covalently attached to oligonucleotides have been synthesized to date. It is known that some metalloporphyrins can act as sensitizers for singlet oxygen formation and therefore can induce photodegradation of DNA [11], but, as far as we know, the oligonucleotide reagents of this type have not been synthesized yet. Here we present data indicating that the palladium (II)-coproporphyrin I derivative of heptadecadeoxyribonucleotide is capable of selective photomodification in solution of a 34-mer oligodeoxyribonucleotide containing complementary region. A probable mechanism of modification is discussed.

2. MATERIALS AND METHODS

The 17-mer d(pCATTAGTTCTGGGTGCC) (i) and 34-mer d(GGCACCCAGAACTAATGAATGAAGGTGGAGAGGT) (ii) were synthesized by the solid phase phosphoramidite procedure with Victoria 4M automated synthesizer, USSR [12]. The oligonucleotide sequences were confirmed by the Maxam-Gilbert procedure [13]. The 5'-end phosphate group was introduced in (ii) by means of T4-polynucleotide-kinase and [γ-^{32}P]ATP.

The attachment of palladium(II)-coproporphyrin I (fig.1) to the 5'-end phospate group of oligonucleotide (i) was carried out in accordance with the following scheme:

$$\mathrm{Por(COOH)_4} + \mathrm{NH_2CH_2CH_2NH_2} \xrightarrow[\mathrm{H_2O:DMF=2:1}]{\mathrm{CMC,\ pH\approx 6}} \longrightarrow \mathrm{(COOH)_3PorCONHCH_2CH_2NH_2}\ \ \textbf{(iii)} \qquad (1)$$

$$\textbf{(i)} + \mathrm{DMAP} \xrightarrow[\mathrm{DMF}]{\mathrm{Ph_3P,\ Py_2S_2}} \mathrm{DMAP^+} - \textbf{(i)}\ \ \textbf{(iv)} \qquad (2)$$

$$\textbf{(iii)} + \textbf{(iv)} \xrightarrow[\mathrm{DMSO/DMF}]{} \mathrm{(COOH)_3PorCONHCH_2CH_2NH\text{-}}\textbf{(i)}\ \ \textbf{(v)} \qquad (3)$$

At the first stage of synthesis $Por(COOH)_4$ was condensed with one equivalent of ethylenediamine in the presence of CMC. The formation of (iii) was monitored by means of cation exchange chromatography (Nucleosil SA, 5 μm, Machery-Nagel, FRG; elution: $LiClO_4$ gradient (0-1.2 M) in water/acetonitrile 1:1 (v/v) + 0.1% trifluoroacetic acid), product was isolated by reverse-phase chromatography (Silasorb C-8, 10 μm, Reanal, Hungary), molecular weight having been confirmed by mass-spectrometry. Bands at 1550-1650 cm^{-1} in the IR spectrum of the product, absent in that of $Por(COOH)_4$, confirm the CONH group formation. The reaction was stopped at 30% conversion degree to avoid formation of more substituted porphyrin amides.

The 5'-end phosphate group of oligonucleotide (i) activation yielding $DMAP^+$-derivative was carried out as in [14].

The reagent (v) was obtained in the reaction of 250 nmol of (iii) and 30 nmol og (iv) (1.5 days, room temperature) and isolated by reverse-phase chromatography (Nucleosil C-18, 5 μm, Machery-Nagel, FRG; elution: acetonitrile gradient (0-25%) in water, 0.1 M Tris-acetate pH = 7.2), yield being about 10%.

The absorption spectrum of (v) is a combination of the absorption spectra of the oligonucleotide moiety ($\epsilon^{260} \approx 1.5 \cdot 10^5\ M^{-1}\ cm^{-1}$) and the porphyrin moiety ($\epsilon^{395} \approx 1.1 \cdot 10^5\ M^{-1}cm^{-1}$).

The luminescence spectrum of (v) coincides with that of free palladium(II)-coproporhyrin I in solution containing: 0.05 M

Correspondence address: O.S. Fedorova, Institute of Bioorganic Chemistry, Siberian Division of the USSR Academy of Sciences, Lavrentiev Pr. 8, 630090, Novosibirsk, USSR

Abbreviations: Por $(COOH)_4$, palladium (II)-coproporphyrin I; CMC, *N*-cyclohexyl-*N'*-morpholinoethyl carbodiimide metho-*p*-toluenesulfonate; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DMAP, 4-dimethylaminopyridine

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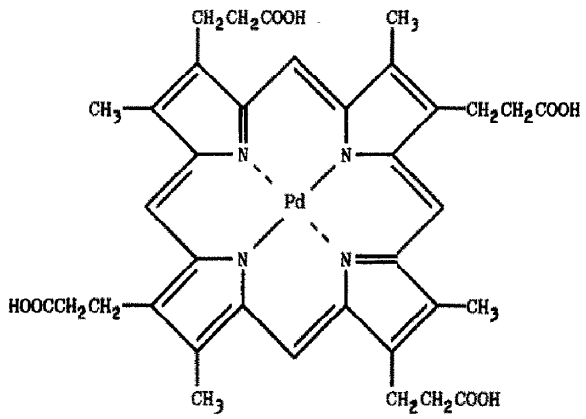

Fig.1. The structure of palladium(II)-coproporphyrin I [Por-$(COOH)_4$].

Na_2SO_3, 0.1% Triton X-100, 0.1 M KH_2PO_4, pH = 7.5, λ_{max} = 667 nm, λ_{ex} = 395 nm.

Samples were irradiated from the side in glass tubes with 2 mm inner diameter placed into quartz cells filled with water cooled to approximately 10°C. The light source was a high-pressure Hg lamp with filter to provide 330–410 nm radiation. Typically, a sample of 20–80 μl containing aerated buffer (0.2 M NaCl, 0.01 M KH_2PO_4, pH = 7.6), 5′-^{32}P-labelled oligonucleotide (ii) ($5 \cdot 10^{-7}$ M) and reagent (v) (about $2 \cdot 10^{-6}$ M) received approximately $2 \cdot 10^2$ W/m^2.

After irradiation, oligonucleotides were precipitated by addition of 2% $LiClO_4$ solution in acetone, treated or not with piperidine (1 M, 95°C, 30 min) and loaded onto denaturing 20% polyacrylamide gel (7 M urea, 0.05 M Tris-H_3BO_3, pH = 8.0, 1 mM EDTA). The gels were autoradiographed at −20°C with RM-V film with or without intensifying screens. Autoradiograms were quantified with UltroScan XL densitometer (LKB Bromma, Sweden).

3. RESULTS AND DISCUSSION

The photomodification of 5′-^{32}P-labelled oligonucleotide (ii) by reagent (v) in complementary complex

P-GGCA CCCA GAA CTAA TGAA TGAA
GGTGGA GA GGTCCGTGGGTCTTGATTACp-X

(X = -$NHCH_2CH_2NHCOPor(COOH)_3$

was investigated. The autoradiogram in fig.2 shows that crosslinking reaction takes place during irradiation. Besides, band 1 could contain products of 'latent' modification of target (ii). Therefore the products were isolated from bands 1–3 and treated with hot piperidine. The cross-linked products from bands 2 and 3 were partly decomposed to yield major bands corresponding to cleavage at G17 position and weaker bands at T16 position (fig.3). So was the product isolated from band 1, indicating that 'latent' piperidine-cleavable modification of (ii) did take place.

Data presented in fig.4 demonstrate that only weak non-specific modification of (ii) takes place in the presence of free palladium (II)-coproporphyrin I while the extent of specific photodegradation of (ii) caused by (v) was about 30%. The conversion curve was sigmoidal in shape and the reaction levelled off in 20–40 min of irradiation.

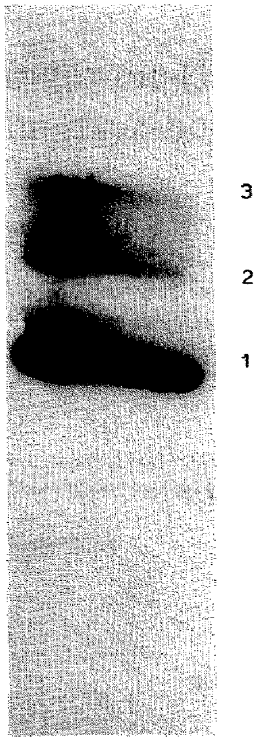

Fig.2. Autoradiogram of the gel corresponding to the reaction of reagent (v) and 5′-end ^{32}P-labelled target (ii), irradiation time 30 min. 2 and 3, crosslinked products, 1, oligonucleotide (ii) and products of its 'latent' modification.

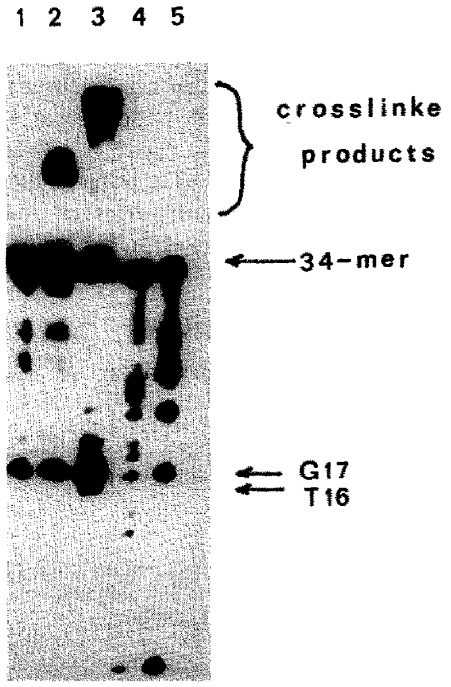

Fig.3. Piperidine treatment of products isolated from bands 1–3 (see fig.2), lanes 1–3 correspondingly; Maxam-Gilbert sequencing reactions: A + G, lane 4; G, lane 5.

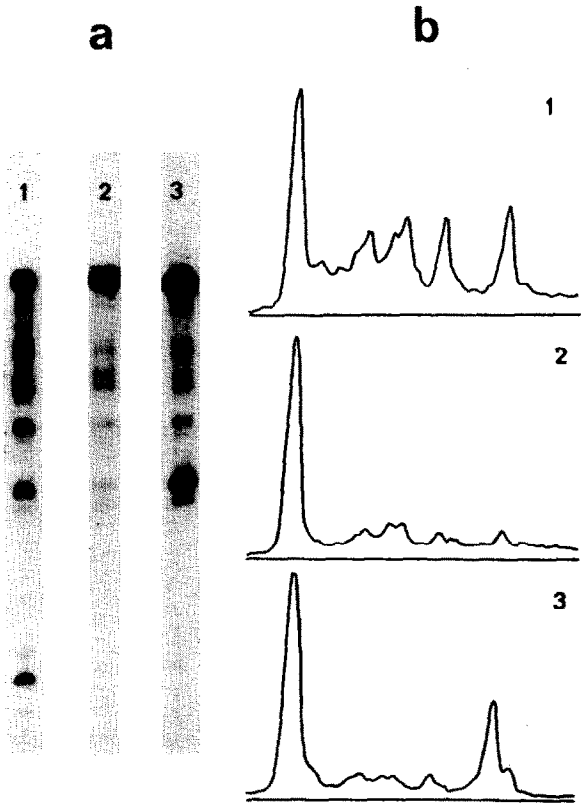

Fig.4. (a) Autoradiogram of the gel and (b) its densitogram. 1, G-sequencing cleavage; 2 and 3, correspond to reactions of $5 \cdot 10^{-7}$ M of target (ii) with free palladium(II)-coproporphyrin I ($2 \cdot 10^{-5}$ M, lane 2) or reagent (v) ($2 \cdot 10^{-6}$ M, lane 3); irradiation time, −60 min; products were treated with piperdine.

The reagent (v) was also destroyed under irradiation to yield mainly the product with electrophoretic mobility similar to that of 17-mer (i). Thus the self-degradation of reagent (v) prevents complete modification of target 34-mer.

Palladium(II)-coproporphyrin I can act as sensitizer for singlet oxygen generation [11,15]. The reactions of singlet oxygen $^1O_2$ with DNA are known to come almost entirely to guanine bases modification [16–18]. The participation of singlet oxygen in the reaction can be confirmed in the case of increase of reaction rate in D_2O, where the lifetime of $^1O_2$ is much higher than in H_2O [19], but this test is not suitable for investigation of reactions in complexes. Indeed, reaction in D_2O resulted in higher level of non-specific modification, not affecting that of specific modification. This points at the participation of $^1O_2$ at least in non-specific modification. We have not succeeded yet in removing the traces of O_2 from solution and so we have no proofs of the participation of $^1O_2$ in specific modification of target (ii) by reagent (v). However the nature of products of guanine modification may be a test for singlet oxygen [20] and therefore the analysis of the products seems to be worthy of continuing.

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