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Sequence-targeted chemical modifications of nucleic acids by complementary oligonucleotides covalently linked to porphyrins

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ABSTRACT

Oligo-heptathymidylates covalently linked to porphyrins bind to complementary sequences and can induce local damages on the target molecule. In dark reactions, iron porphyrin derivatives exhibited various chemical reactivities resulting in base oxidation, crosslinking and chain scission reactions. Reactions induced by reductants, such as ascorbic acid, dithiothreitol or mercapto-propionic acid, led to very localised reactions. A single base was the target for more than 50 % of the damages. Oxidising agents such as H_2O_2 and its alkyl derivatives induced reactions that extended to a wider range of altered bases. The specificity of the chemical modifications observed in these systems is discussed from a mechanistic point of view.

INTRODUCTION

Active reagents covalently linked to oligonucleotides have been used by several groups to induce site-directed chemical modifications including chain scission reactions on complementary polynucleotides. Most of the active groups used recently consist of metal complexes such as EDTA-Fe(III) (1-4), Phenanthroline-Cu(II) (5,6) and Porphyrin-Fe(III) (7). Once linked to short oligonucleotides, these compounds exhibit nuclease activity towards complementary sequences in the presence of a reducing agent in aerated solutions. It was recently shown that the cationic porphyrin meso-tetra-(N-methylpyridyl)porphine and its metal complexes exhibited a strong affinity for DNA. When irradiated with visible light these compounds were capable of inducing chain scission reactions on double-stranded DNA (8-11). Strand breakage was also observed when transition metal complexes-mostly ferri-porphyrins-were used in the presence of reducing agents(12-14). Hybrid molecules composed of porphyrins coupled to intercalators in order to increase the affinity of the conjugate for DNA have been synthesised to mimic the antitumoral drug bleomycin. These molecules have been shown to strongly bind to DNA although in a non-specific manner and to induce strand breaks when the heme moiety was activated by reducing agents (15-20). In a previous work (7) we showed that short oli-

gonucleotides such as heptathymidylates (dT_7) coupled to porphyrin or to both a porphyrin group and an acridine derivative, one at each end, were active in cleaving complementary polynucleotides i.e., polydA or polyrA, when iron was incorporated into the porphyrin ring and the reactions induced by reducing agents in aerated solution.

In the present paper we present the results of a study on site-directed chemical modifications of nucleic acids by oligo$(dT)_7$-porphyrin derivatives. A synthetic oligonucleotide, 27 nucleotides-long, containing the target A_8 sequence, was used as substrate. Several metal-porphyrin systems were tested and the reaction was induced by redox processes at the metal center. These compounds exhibited high selectivity in inducing local damages of the target nucleic acid. Different types of damages were observed depending on the nature of the reaction involved.

MATERIALS AND METHODS

The 27-mer oligodeoxynucleotide whose sequence is presented on figure 1, was synthesised on an Applied Biosystem synthesiser and purified by polyacrylamide gel electrophoresis followed by reverse phase chromatography. End labelling (5'-end) was achieved by T_4 polynucleotide kinase (Amersham) using γ-^{32}P-ATP (Amersham).

Synthesis of oligo-thymidylates attached at the 3'-end to methylpyrroporphyrin XXI (Aldrich) or to both an acridine derivative (2-methoxy, 6-chloro, 9-amino acridine) at the 3'-end and to the porphyrin derivative at the 5'-end through various linkers (see fig. 1) was described previously (6,21).

Metallation of oligothymidylate-porphyrin conjugates was carried out by heating ($\simeq$ 80°C) under argon atmosphere an aqueous solution containing 5 mM Tris-HCl, pH 7.4, in the presence of the corresponding metal salts ($FeCl_2$, $4H_2O$; $MnCl_2$,$4H_2O$; $CoCl_2$,$6H_2O$ and $CuCl_2$,$2H_2O$) at a metal/porphyrin ratio of 20 (Co,Cu) or 60(Fe,Mn). Metal incorporation was followed by absorption spectroscopy in the spectral range : 220-700 nm. After completion of the reaction, the solution (0.5 ml) was extensively dialysed against bi-distilled water (3 x 500 ml) containing ion-exchange resin beads (Chelex 100, Biorad) to remove excess metal ions. Final concentrations of the oligo$(dT)_7$-metallated porphyrins were determined by absorption measurement at 265 nm using a molar extinction coefficient of 80.000 M^{-1} cm^{-1}, taking into account the contribution of the porphyrin at this wavelength.

Reducing agents used were : dithiothreitol, DTT (Boehringer) ; mer-

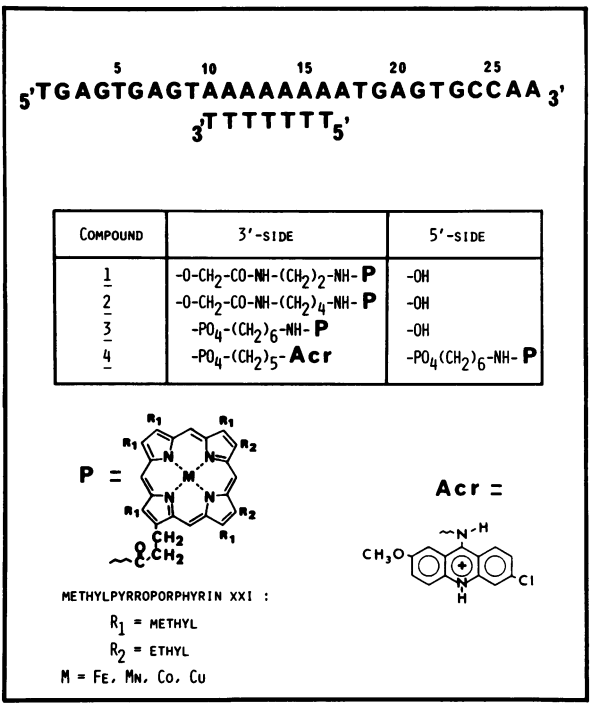

Figure 1 : Target 27-mer oligonucleotide containing an A_8 sequence complementary to oligo-$(dT)_7$ whose 3'- or 5'- end was tethered to porphyrin. In compound 4, in addition to porphyrin linked to the 5'-end, the intercalator 2-methoxy, 6-chloro, 9-amino acridine was linked at the 3'-end for stabilisation of the complex. In dark reactions, metal derivatives, M = Fe, Mn, Co,Cu of porphyrins were used.

capto-propionic acid, MPA (Aldrich) ; glutathione (Boehringer) ; cysteamine (Sigma) ; β-nicotinamide adenine dinucleotide (reduced form), NADH (Sigma). Hydrogen peroxide 30 % (v/v) in water was from Prolabo. Tertbutyl-and cumylhydroperoxides were kindly provided by Dr. M. Mansuy. Potassium peroxymonosulfate (Oxone) was purchased from Aldrich. All solutions were always freshly prepared before use in degased Tris-HCl buffer, pH 7.4.

A standard procedure consisted in successive additions of the following compounds in an Eppendorf tube : 10 nM (fragment) of the ^{32}P-labelled 27-mer, 4 to 20 µM of the oligo$(dT)_7$-porphyrin derivative and the appropriate amount of Tris-HCl, pH 7.4, (50 mM final concentration) and NaCl (usually 0.25 M final concentration). The mixture (total volume = 5 µl) was kept at 0°C for half an hour.The activating agent was then added at 0°C to start the reaction.

At different time intervals, tubes were removed, frozen and kept at - 70°C before lyophilisation. We checked that the reaction was efficiently stopped by this procedure. In order to characterise the crosslinking sites on the 27mer (see below) samples were submitted to alkaline treatment. The reacted sample was dissolved with 50 µl of a 1 M piperidine solution (pH ≈ 12) and heated at 90 °C for 20 minutes followed by 2 cycles of washing with 100 µl of water and lyophilisation. The reacted product was then redissolved in 5 µl formamide containing xylene cyanol dye, and loaded on a polyacrylamide gel (20 % acrylamide containing bis-acrylamide, 1:40 (M/M), 7M urea). Autoradiography was obtained by exposing the gel with a Kodak (X-OMat) or Fuji (X-Ray) films with an intensifying screen at - 70°C overnight. Quantitative analysis of the reaction was carried out by excising the relevant bands from the gel and counting for radioactivity.

RESULTS

Activity of oligo(dT)$_7$-porphyrin derivatives with sodium ascorbate as reducing agent :

Reactivity of iron complexes of compounds 2 (lane 2), 3 (lane 3) and 1 (lane 4) with the target 27-mer oligonucleotide in the presence of Na ascorbate is shown on figure 2. Compound 3 (FeIII) was the most active under the experimental conditions specified in the legend of figure 2. Compound 2 (FeIII) exhibited some cleavage activity at the expected site of the metal active center, i.e. around T-9 position but also at non-expected positions such as A-15 and A-20 to G-23. The most selective reaction was observed with compound 3 (FeIII) with a strong localised cleavage at G-8 and weaker cleavage bands in the A-20 to G-23 region. The chemical structure of the three compounds differs basically in the mode of linkage of the porphyrin moiety to the oligonucleotide. In compounds 1 and 2, the linker was directly attached to the 3'-OH of the terminal sugar while in compound 3 it was linked to the phosphate group of the 3'-end. The length and chemical composition of each linker were also different as shown in figure 1. The presence of amide bonds in the linkers of compounds 1 and 2 could confer a lesser flexibility of the chain and this may be account for the lower efficiency of these derivatives as compared to 3. It should be noted that slow migrating bands appeared above the band corresponding to the starting material in the case of the most active compound 3 (FeIII). Cleaved fragments appeared as doublets suggesting that the scission reaction yielded fragments with 3'-ends of different nature as already observed by Hashimoto and coworkers with haemin-based reagents (19).

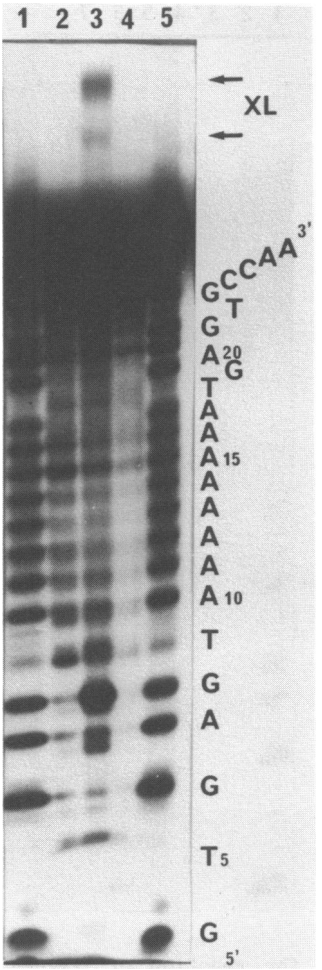

Figure 2 : Autoradiogram of the polyacrylamide gel corresponding to the reaction of oligo(dT)$_7$-porphyrin derivatives (4 µM) with 5'-end ^{32}P-labelled 27-mer (10 nM) with Na ascorbate (5 mM) in aqueous buffer solutions containing 50 mM Tris-HCl, pH 7.4 and 0.25 M NaCl. The reaction was carried out at 0°C for 18 hours. Lanes 1 and 5 : G + A ; lane 2 : compound 2 (FeIII) ; lane 3 : compound 3 (FeIII) ; lane 4 : compound 1 (FeIII). Arrows indicate the formation of crosslinked products, XL (see text).

Replacing the central Fe(III) ion by Co(III) in compound 2 led to a similar pattern of cleavage. When the temperature was raised to 20°C, homogeneous cleavage all along the 27-mer chain was observed. This was very likely due to oxidising radicals liberated in the solution by the reaction of ascorbate with

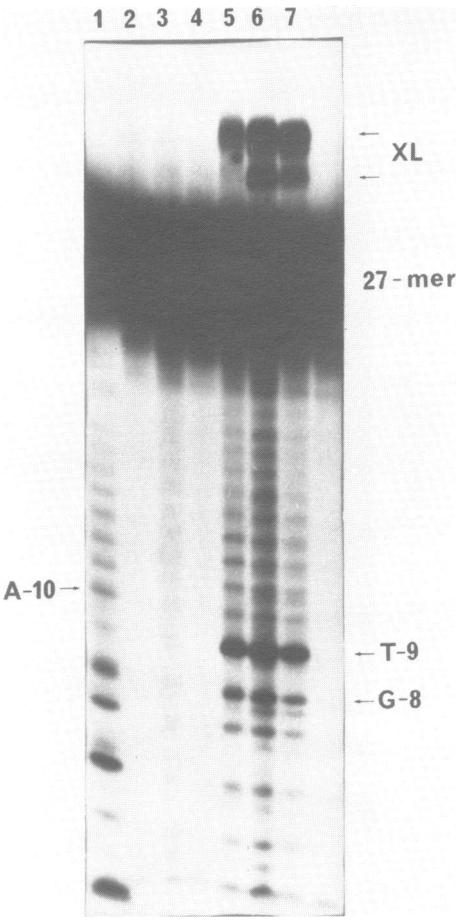

Figure 3 : Autoradiogram for reaction of compound 3 (FeIII) (20 µM) with 27-mer (10 nM) in the presence of thiol-containing reagents. All other conditions as in figure 2. Lane 1 : G + A ; lanes 2,3,4 : control experiments with thiols alone : 2 : 5 mM DTT ; 3 : 5 mM MPA ; 4 : 2 mM MPA ; lanes 5,6,7 : same thiol concentrations as in 2,3,4 but in the presence of 3 (FeIII).

the free iron-porphyrin-oligonucleotide conjugate.The Mn(III) and Cu(II) porphyrin derivatives exhibited very low activity as did metal-free porphyrin under the same conditions (results not shown).

Reductant solutions were always freshly made before use. Although great care was taken in preparing solutions, e.g., treating the water and buffer solutions with ion-exchange resins and using the same protocol for mixing the different reactants, we were faced with a poor reproducibility of

the experiments when sodium ascorbate was used to initiate the reaction. For further work, we selected compound 3 (FeIII) which appeared to be the most active (compound 4 (FeIII) had an activity comparable to 2 (FeIII)) and among reductants we used thiol-contanining compounds such as dithiothreitol or mercapto-propionic acid.

Activity of oligo(dT)$_7$-porphyrin derivatives with DTT or MPA as reducing agents :

The autoradiogram presented in figure 3 shows that metal-free derivative of 3 did not induce any cleavage reaction in the presence of 5 mM DTT (lane 2) or 2 mM (lane 3) and 5 mM (lane 4) MPA. The weak bands observed in the A-20 to G-23 region were also present in the 27-mer and reflected the presence of very small amounts of degradation products. With the iron-derivative, in the presence of DTT (5 mM, lane 5), crosslinking reaction took place concomitantly with strand scission reaction. The cleavage reaction was highly localised ; strong cleavage was observed at the T-9 base. Non-specific cleavage was also more important (as compared with the control in lane 2). It should be noted that in these experiments there was a large excess of 3 Fe(III) over the 27-mer and oxidative radicals were also produced in the bulk solution (see Discussion). They should be involved in non specific attack all along the 27-mer chain. When DTT was replaced by MPA (lanes 6 and 7) a second slow migrating band appeared but the cleavage pattern remained the same as with DTT. As expected decreasing the MPA concentration in the solution, reduced the specific and the non-specific reactions. In these systems, thymine at position 9 was the most susceptible base whereas with sodium ascorbate as activator, it was guanine at position 8. Among the several reducing agents mentioned in Materials and Methods, MPA appeared to be the most active reagent followed by DTT and β-mercaptoethanol. All the other tested reductants were inactive.

When the reaction mixture was submitted to alkaline treatment the intensity of the crosslink bands decreased (but did not disappear completely) yielding essentially three bands at G-8, T-9 and A-10 positions, 80% of the reaction being localised on the T-9 base. Counting the radioactivity of the individual bands allowed us to quantitate the different processes. At neutral pH, direct cleavage represented around 40 % of the reaction and crosslinking 60 %. After alkaline treatment, the radioactivity in the T-9 band was 5 to 6 times higher than that of the sum of the crosslink and the T-9 bands observed at pH 7. This result demonstrates that the T-9 base was oxidised during the reaction at pH 7 but the major part of the reaction was revealed only when

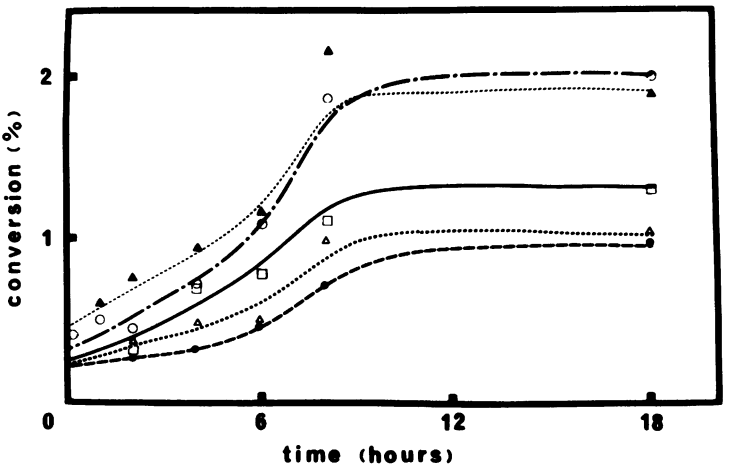

Figure 4 : Kinetics of the reaction of 3 (FeIII) (10 µM) with the 27-mer (10 nM) in the presence of MPA (2mM) under the conditions described in the legend of figure 2. Percent conversion represents the radioactivity of the T-9 band (see figure 3) over the total radioactivity of the lane after alkaline treatment. The reaction was carried out at various temperatures : 0°C (o) ; 5°C (▲) ; 10°C (□) ; 20°C (Δ). Lower curve (●) represents the non-specific cleavage at T-5 position at 20°C.

the sample was heated in the presence of piperidine. The yield of the total reaction (direct cleavage + crosslinking + base oxidation) was relatively low and represented 1-2 % of the original material. In the following experiments, we systematically treated the sample with hot piperidine and took the radioactivity of the T-9 band as representative of the whole process.

The kinetics of the reaction was investigated at different temperatures at fixed MPA concentration (2 mM). Results are shown on figure 4. It can be seen that conversion curves were sigmoidal in shape and the reaction levelled off after 8 hours of incubation. The reaction rate was higher at low temperatures (0-5°C) than at high temperatures (10-20°C) consistent with the thermal stability of the oligonucleotide-target hybrid. At 20°C the reaction was essentially non-specific as the yield of the cleavage reaction at a remote base like T-5 was very close to that of T-9 base at the same temperature.

The stability of complexes between complementary oligonucleotides depends on ionic concentration as a result of a decrease of the repulsion between the negative charges of the two molecules when ion concentration increases. Therefore, we analysed the reaction as a function of salt concentration. Results are presented on figure 5. The oxidation reactions of T-9 and the adjacent bases (G-8 and A-10) presented a maximum around 0.25 M NaCl. The

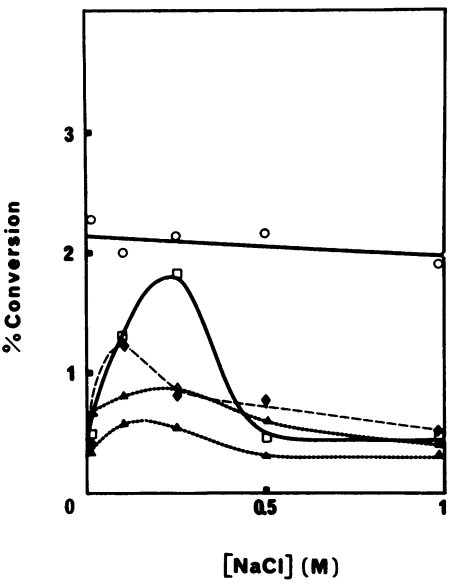

Figure 5 : Influence of ionic concentration on the reaction rate of 3 (FeIII) with 27-mer in the presence of 2 mM MPA. Yields of crosslinking and cleavage reactions versus NaCl concentrations after 8 hours incubation followed by alkaline treatment at T-9 (□) ; G-8 (Δ) ; A-10 (▲) ; G-19 + G-21 + G-23 (o) and remaining crosslink (◆). All other conditions as in figure 2.

rate dropped rapidly above 0.25 M NaCl. At 0.5 M it reached the level observed in the absence of NaCl, and then remained constant up to 1 M NaCl. The amount of crosslinks remaining after piperidine treatment seemed to have its maximum value around 0.1 M NaCl.The non-specific reaction was measured by the chemical modification of the three Gs located on the 3'-side of the target molecule. For sake of clarity the sum of the cleavage reactions at these three Gs are presented on figure 5. It can be seen that this reaction did not depend on NaCl concentration. By comparison of the cleavage reactions at the two Gs located on either side of the target A_8 sequence, one can conclude that no specific reaction occurs neither at low salt (0 M NaCl) nor at high salt concentration (0.5-1.0 M NaCl).

Oxidation reactions induced by oligo(dT)$_7$-porphyrin derivatives in the presence of oxidants :

Metallo-porphyrin derivatives are known to form oxo-derivatives with hydroperoxides including H_2O_2 (22,23). Therefore we analysed the reactions which could be induced in the target sequence when the hybrid was incubated in

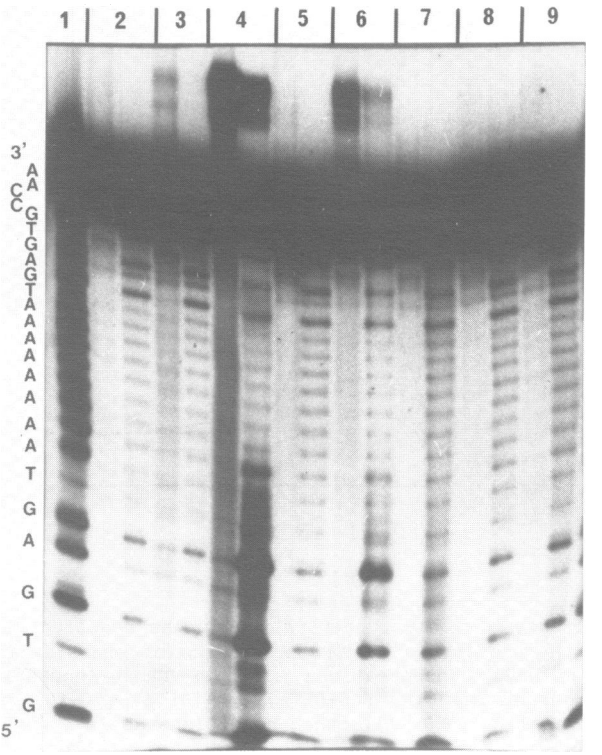

Figure 6 : Autoradiogram of the reaction of 3 (FeIII) (4 µM) with 27-mer (10 nM) in the presence of H_2O_2 (1 mM) for 18 hours at 0°C. Each numbered lane is divided: in left side = before alkaline treatment and right side = after alkaline treatment, except for lane 1 which represents the Maxam-Gilbert (G + A) sequencing reaction ; lane 2 : 27-mer alone ; lane 3 : 3 (2H) ; lane 4 : 3 (FeIII) ; lane 5 : 2 (2H) ; lane 6 : 2 (FeIII) ; lane 7 : 2 (CuII) ; lane 8 : 2 (CoIII) ; lane 9 : 2 (MnIII).

the presence of H_2O_2. In the presence of 3 (FeIII) hydrogen peroxide induced strong crosslinking reactions under neutral conditions (yield 10 %) (figure 6, lane 4, left). Alkaline treatment revealed three main fragments cleaved at G-8, G-6 and G-4 positions. The T-9 and A-10 bases were also altered giving rise to more diffuse bands. Base oxidation (mainly guanine) appeared to be the predominant process. The total radioactivity of the altered region, going from A-11 to G-4, represented 5 to 6 times that of the crosslink bands taking into account that the alkaline treatment did not cleave all the crosslinked species. Piperidine treatment was carried out at 90°C for 20 minutes. This led to weak cleavage reactions at G bases in the absence of the porphyrin-containing

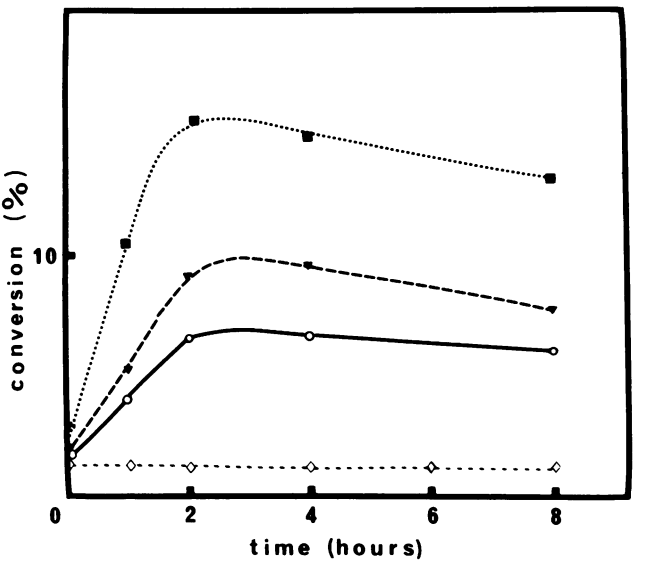

Figure 7 : Kinetics of oxidation reaction at G-8 (■) ; G-6 (▲) ; G-4 (o) and G-19 (◇) of 27-mer (10 nM) by 3 (FeIII) (10 µM) in the presence of 1 mM H_2O_2 at 0°C. The medium is the same as described in figure 2.

oligonucleotide as shown in lane 2. Prolonged piperidine treatment yielded more G cleavage without improving the conversion of crosslinked species to cleaved products. Under the same experimental conditions, 2 was less active than 3 (lane 6) and among the metals tested only the iron derivative exhibited a measurable activity (figure 6, lanes 5-9).

The yield of the oxidation reaction induced by H_2O_2 was high as compared with the reaction induced by reductants, but the reaction was less localised in the sense that a broader region of the target was altered (A-11 to G-4). The specificity of the reaction is illustrated on figure 7 where are compared the oxidation reactions of the three Gs on the 5' side of the 27-mer. It can be seen that G-8 was the most altered base among the three Gs located on the 5'-side of the target. The reaction yield decreased when the distance from the active iron center increased (G-8 > G-6 > G-4). No reaction occurred at G bases of the 3'-side of the 27-mer ; the radioactivity of the G-19 band was of the same order of magnitude as that observed in control experiments (figure 6, lane 2).The process was much faster with H_2O_2 (4 times) than that induced when MPA was used as reducing agent (figure 4). In order to examine whether both the reaction rate and the specificity of the reaction could be

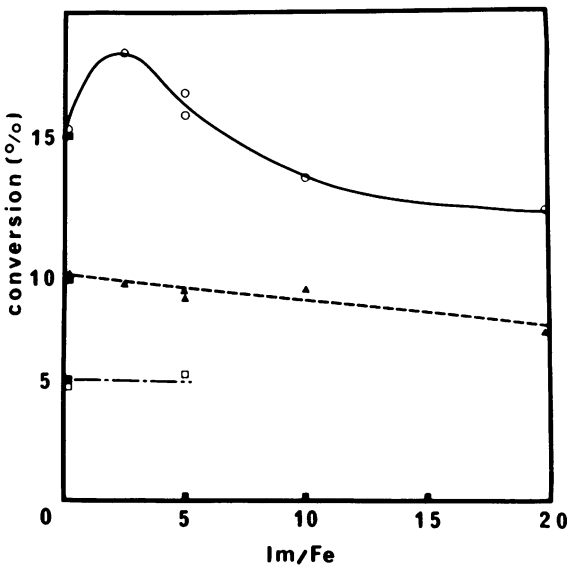

Figure 8 : Influence of imidazole on guanine G-8 oxidation in the reaction of 27-mer (10 nM) with 3 (FeIII) (10 µM) and H_2O_2 (1 mM) for 4 hours at 0°C. The yield of cleaved fragment at G-8 after alkaline treatment is represented as a function of the imidazole/iron ratio : H_2O_2 (o) ; tertbutyl-hydroperoxide (▲) ; cumyl-hydroperoxide (□).

improved, we investigated the effect of imidazole, a well-known ligand for the central Fe atom and we also replaced H_2O_2 by its alkyl derivatives : tertbutyl and cumyl hydroperoxides. Results are shown on figure 8 where a slight increase of the reaction rate was observed in the case of H_2O_2 at low imidazole : Fe ratio. As imidazole concentration increased in the medium, the reaction rate slowed down as the sixth coordination position of Fe was occupied preventing the fixation of the oxidizing agent by the metal. Alkyl hydroperoxides were less efficient than H_2O_2 ; the reactivity of the catalytic system decreased when the bulkiness of the substituent increased. A few other oxidants were also tested but no net improvement of the reaction yield or specificity were observed. The peracid $KHSO_5$ was recently shown to give high yield and specificity in olefin epoxidation reactions catalysed by Mn-porphyrins (24) . In our hands the reaction rate was found higher than with H_2O_2 but reaction yields were comparable as shown on figure 9.

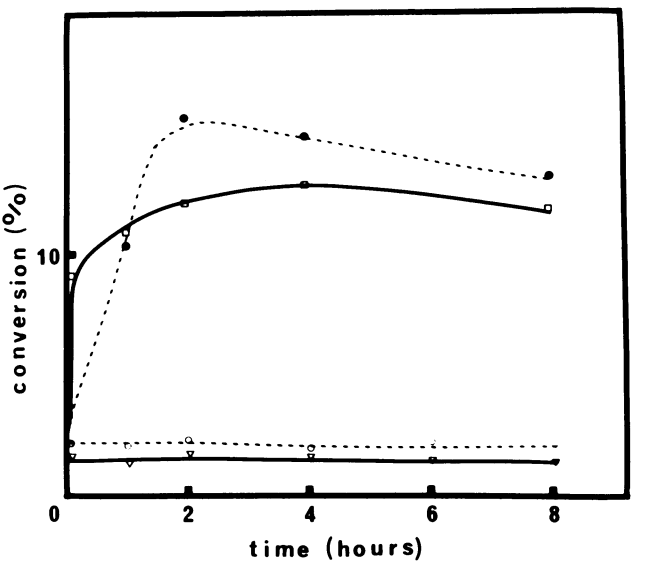

Figure 9 : Comparison of guanine G-8 (upper curves) and G-19 (lower curves) oxidation reactions in the presence of 1 mM H_2O_2 (---) and 1 mM $KHSO_5$ (——). All other conditions as in figure 8.

DISCUSSION

In previous papers from our laboratory, we showed that oligonucleotides covalently linked to acridine derivatives recognised and strongly bound to their complementary sequence (25,26). It was also shown that they could interfere in a specific way with biological processes such as messenger RNA translation as demonstrated with β-globin m-RNA (27). Hybridisation of oligonucleotides to their target sequence is a physical interaction involving association-dissociation equilibria between the two molecules. For this reason the blocking action of the oligonucleotide towards any biological process can never reach 100 % efficiency. A possible solution to this problem is to construct molecules that can bind and create irreversible damages in the target sequence. For this purpose we synthesised oligonucleotides covalently linked to porphyrins and the results presented above show that these derivatives are active in inducing a great variety of local chemical damages on the target molecule. This is due to the rich redox chemistry involved in this system (for a recent review on this topic see (28)). The production of the reduced forms

of dioxygen, namely $O_2^{-\bullet}$ and H_2O_2 in the presence of iron-porphyrin complexes occurs according to the following reactions :

(1) $2PORFe(III) + 2RSH \rightarrow 2PORFe(II) + RS\text{-}SR + 2H^+$

(2) $PORFe(II) + O_2 \rightarrow PORFe(III) + O_2^{-\bullet}$

where PORFe is an iron-porphyrin complex and RSH, a thiol or any other reductant. Hydrogen peroxide can be produced as a result of the spontaneous dismutation of $O_2^{-\bullet}$ or upon reduction by Fe(II) :

(3) $2O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$

(4) $PORFe(II) + O_2^{-\bullet} + 2H^+ \rightarrow PORFe(III) + H_2O_2$

Once H_2O_2, $O_2^{-\bullet}$ and Fe(II) are present in the medium, Fenton or Haber-Weiss reactions can take place :

(5) $PORFe(II) + H_2O_2 \rightarrow PORFe(III) + OH^- + OH^\bullet$

(6) $O_2^{-\bullet} + H_2O_2 \rightarrow O_2 + OH^- + OH^\bullet$

producing highly reactive hydroxyl radicals. These species react rapidly with components of DNA (29). $OH^\bullet$ radical addition and hydrogen abstraction reactions on DNA lead to production of radicals including peroxy-radicals :

(7) $DNA + OH^\bullet \rightarrow DNA\text{-}OH^\bullet$

(8) $DNA(H) + OH^\bullet \rightarrow DNA^\bullet + H_2O$

(9) $DNA^\bullet + O_2 \rightarrow DNA\text{-}O\text{-}O^\bullet$

Such radicals are known to undergo internal reactions leading to strand cleavage (30). Alternatively they can react with a second DNA molecule to give rise to crosslinks. The porphyrin ring itself can generate intermediate radicals which can lead to crosslinking reactions with the target sequence. The reaction of $OH^\bullet$ radicals should not exhibit any base specificity since reaction rates are high with both purines and pyrimidines as well as with sugars (29). In thiol-mediated reactions cleavage occurred at neutral pH with decreasing efficiency when the distance to the porphyrin ring increased (T-9 > G-8 > A-7, see figure 3). This observation suggests the involvement of $OH^\bullet$ radicals in the cleavage reaction. The efficiency of their reaction is expected to be maximum at the base which is located closer to the $OH^\bullet$ radical-generating porphyrin ring as experimentally observed. Involvement of $O_2^{\bullet -}$ and H_2O_2 in the process is confirmed by the inhibitory effect of superoxide dismutase and catalase as observed by Lown **et al.** (20) in reactions with haemin-based reagents. The kinetics of the reaction is slow, due very likely to the types of reactions involved. Autooxidation of iron (reaction 2) is known to be unfavourable on a thermodynamics basis (28). In addition, reaction 3 ($k = 0.35\ M^{-1}\ s^{-1}$ (28)), reaction 5 ($k = 76\ M^{-1}\ s^{-1}$ (31)), and reaction 6 ($k = 0.13\ M^{-1}\ s^{-1}$ (32)) proceed at rather low rates. When the reaction is initiated by so-

dium ascorbate the main cleavage site was observed at G-8 instead of T-9 in the presence of thiols-based reagents. The preferential cleavage at purines-rich segments of DNA has been observed in the presence of ascorbate and copper ion or copper chelates (33). Kasai and Nishimura (34) have also shown that guanine bases were hydroxylated at C-8 position when DNA was allowed to react with ascorbate and oxygen.

Alkyl hydroperoxides are known to induce free radical chain reactions catalysed by iron (III) porphyrin (35,36). In biological reactions such as the oxidative transformations catalysed by the heme-containing monooxygenase cytochrome P-450 or peroxide decomposition by peroxidases, oxygen activation and transfer to the recipient molecule occur at the metal site through a reactive iron(V)-oxo complex or some radical-type equivalent. This alternative oxidative reaction mediated by alkyl hydroperoxides should lead to a more localised reaction as it does not involve diffusive species such as $OH^{\bullet}$ radicals. Moreover, neither oxygen nor reducing agents are required, provided exogenous oxygen donors are used. Along these lines selective oxidation of hydrocarbons catalysed by metallo-porphyrins has been the subject of extensive research. Literature data are abundant on the following systems : H_2O_2-Mn(III) porphyrins (22,23) iodosylbenzene-iron-porphyrin (14,37) NaClO-Mn(III)-porphyrin (38) tertiary-alkyl hydroperoxides with molybdenum-porphyrin complexes (39) and with Mn(III)-porphyrins (40,41). The presence of ligands on the central metal atom plays a crucial role in olefin epoxidation reaction as shown with imidazole derivatives (40,41). In our system, H_2O_2 and its two tertiary-alkyl derivatives produced similar effects: guanines are selectively oxidised. H_2O_2 is more active than the two parent compounds and imidazole at a ratio of 2.5 molecules per Fe(III) produced only a slight increase of the reaction rate ($\simeq$ 20 %) (see figure 8). Very few data on porphyrin-mediated epoxidation of nucleic acid bases are available. Guanines are the most susceptible bases in the investigated reaction and to a lesser extent thymines and adenines. Hydroxyl radicals produced by the Fenton reaction (reaction 5) should not discriminate between different bases (see above). The observation that guanine bases were much more oxidised as compared to other bases when hydroperoxides were utilised suggests that an oxidative catalytic process mediated by a hypervalent iron center as discussed above may be more specific regarding guanine bases. It should be noted that hydrogen peroxide has also a destructive effect on the porphyrin macrocycle leading to bile-pigment type compound as shown by Pasternack and Halliwell (42). This concomitant reaction may help

to understand why only limited yields are reached in the oxidation and cleavage reactions.

In conclusion, our results have shown that porphyrin-oligonucleotide conjugates specifically recognise a target sequence and induce various damages including chain scission, base oxidation and crosslinking reactions. These types of DNA lesions, especially photoadducts, can be efficient blocking sites for polymerising or hydrolysing enzymes as recently reviewed (43). Photoactive derivatives such as porphyrin, proflavin and azido derivatives covalently attached to oligonucleotides have also been shown to be very efficient in site-directed crosslinking reactions (44 and results to be published). Oligonucleotides linked to metalloporphyrins or to photosensitizers are therefore good candidates for potential applications of these compounds as anti-messenger reagents.

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