Design, synthesis and cleaving activity of an abiotic nuclease based on a manganese(III) porphyrin complex bearing two acridine moieties

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The synthesis of *meso*-5,15-bis(*m*-aminophenyl)porphyrin bearing two acridine derivatives and of its manganese(III) complex was achieved; in the presence of oxygen atom donors, the metalloporphyrin was shown to cleave double-stranded DNA.

Although the development of abiotic or biomimetic systems capable of probing[1] and/or cleaving[2] DNA has been a topic of active research over the last two decades, the design and synthesis of topo- and regio-selective artificial nucleases is still a current challenge.

The cleavage of DNA may either occur under hydrolytic or oxidative conditions. Dealing with the latter case, transition metal complexes,[3] in particular metalloporphyrins[4] have been shown to act, in the presence of oxygen donor reagents, by oxidation of the sugar units. The interactions between the tetracationic manganese porphyrins complexes such as *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrinatomanganese(III) pentaacetate Mn–TMPyP[4] and DNA is believed to occur within the minor groove rich in A–T sequences by electrostatic charge/charge interactions with the negatively charged phosphodiester groups. Since the initial observation of interactions between acridine derivatives and double-stranded DNA (mainly by intercalation) by Lerman in 1961,[5] this mode of interaction between a large variety of intercalators such as acridine[6] and methidium[7] derivatives with nucleic acid oligomers has been well studied.

Our approach to the design of artificial nucleases was based on the positioning, by double intercalation using two acridine moieties, of oxidative cleaving centres such as metalloporphyrin complexes. In this vein, a class of abiotic DNA cleaving agent combining a metalloporphyrin core, two spacers and two 9-aminoacridine moieties was designed and prepared (Fig. 1). The spacer connecting the reactive centre to the intercalators may be amino acids of variable length such as aminobutyric, aminovaleric or aminocaproic acids allowing to control the distance between the porphyrin and the acridine centres. Dealing with geometrical features, *i.e.* the positioning of the acridines with respect to the porphyrin core, based on molecular models, the *meso*-5,15-diarylporphyrin bearing an amino function at the *meta* position of each phenyl group appeared to be the most appropriate choice. Analogues of bleomycin based on a porphyrin core functionalised at β-pyrrolic positions by two acridine derivatives have been reported.[8]

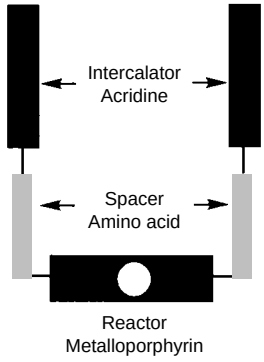
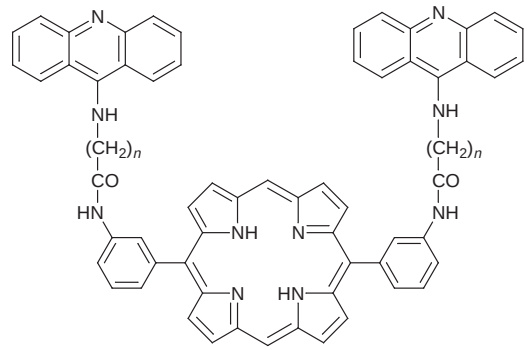
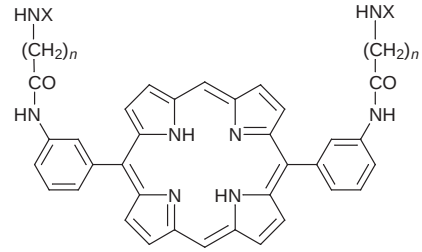


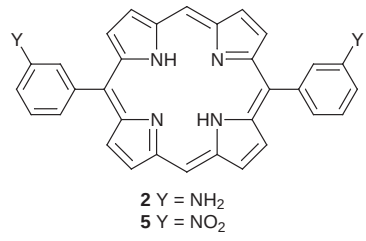
Fig. 1 Schematic representation of an abiotic nuclease based on a metalloporphyrin core functionalised by two acridine intercalators using amino acids as spacers

Here, we report the synthesis of compound **1** (Scheme 1) and the DNase activity of its manganese complex.

The synthetic strategy employed to prepare the compound **1** was based on the synthesis of bis(*m*-aminophenyl)porphyrin **2** followed by introduction of the spacer groups affording compound **3** and finally the introduction of acridine moieties. Whereas the synthesis of *meso*-arylporphyrins bearing amino groups at the *ortho* or *para* positions of the phenyl groups is well documented,[9] only few reports dealing with the preparation of the *meso*-*meta*-aminophenylporphyrins have been reported.[10,11] Compound **2** was prepared in two steps by modification of the described procedure for the bis(*o*-aminophenyl)porphyrin.[12] The condensation of 2,2-dipyrrylmethane,







Scheme 1

prepared following reported procedures,[13] with 3-nitrobenzaldehyde (*ca.* 5 mM) in CH_2Cl_2 in the presence of catalytic amounts of CF_3CO_2H (TFA) afforded the nitroporphyrinogen which was oxidised by *p*-chloranil affording the nitroporphyrin **5**. The latter, owing to its rather poor solubility, was not isolated but directly reduced at 70 °C to the amino compound **2** by treatment with $SnCl_2$/HCl.[14] Since the overall yield was rather low (2%), in order to optimise it, both the nature of the acid and the ratio of reactants were varied. The use of a Lewis acid such as $BF_3{\cdot}OEt_2$ instead of the protonic acid TFA increased the yield to 7%. However the highest yield of 12% was reached by increasing the concentration of reactants to the 20 mM range. The rather poor yield may be related to the low reactivity of the aldehyde used.

On the other hand, starting with aminobutyric acid [$H_2N(CH_2)_3CO_2H$] the NH_2 group was protected using the Boc group [$HNBoc(CH_2)_3CO_2H$][15] and then the carboxylic acid moiety was activated by treatment with ethyl chloroformate [$BocHN(CH_2)_3CO_2COEt$].[16] The condensation of the latter in the presence of Et_3N with compound **2** in a mixture of THF and toluene afforded the protected compound **4** in 60% yield. The deprotection of the Boc group was achieved in quantitative yield by treatment with AcOH–HCl[16] affording **4**·2HCl. The condensation of the latter with 9-phenoxyacridine[17] in phenol at 120 °C gave, after recrystallisation from Et_2O–MeOH, the final compound **1** in 56% yield.

The **1**–Mn^{III} complex was prepared at 120 °C by reacting ligand **1** with $Mn(OAc)_2{\cdot}4H_2O$ in DMF in the presence of 2,4,6-trimethylpyridine for 2 h. The metallation was followed in DMF by UV-VIS spectroscopy which revealed a bathochromic shift of the Soret band from 408 to 458 nm and a reduction of the number of Q bands from four to two. After precipitation by adding diethyl ether and washing the solid with water, the complex was purified by column chromatography [basic alumina, CH_2Cl_2–MeOH (9/1)] and characterised by MS (FAB positive mode: $M^+ = 1070$).

The nuclease activity of the **1**–Mn complex was tested at room temperature on the supercoiled double-stranded ΦX174 DNA (form I) at various concentrations and pH values using phosphate buffer in the presence of either $KHSO_5$[18] or magnesium monoperphthalate (MMPP),[19] two oxidants able to generate high-valent manganese–oxo species.[20] The addition of the oxidant initiated the DNA cleavage reaction. The cleavage products were analysed on agarose gel by electrophoresis as previously reported.[18,19]

As expected, no degradation of DNA was observed under the same conditions in the absence of **1**–Mn or in the presence of free ligand **1**. The cleaving activity of **1**–Mn was observed at 1 μM and 500 nM concentrations in the presence of 1 or 10 mM $KHSO_5$, respectively. Although the cleavage of supercoiled DNA in the presence of 10 mM $KHSO_5$ appeared to be more spectacular since 90% of form I was transformed into forms II and III, the efficiency of the **1**–Mn complex was found to be below that observed with Mn–TMPyP for which DNA cleavage was detected at 5 nM in the presence of 10 μM $KHSO_5$.[18] However, in marked contrast with the tetracationic Mn–TMPyP complex for which a strong salt effect due to electrostatic interactions with DNA was observed,[18] the DNA cleavage activity of **1**–Mn was not affected by modification of the ionic strength of the reaction mixture by addition of NaCl. This absence of a salt effect is in agreement with the possible intercalation of the acridine moieties of the complex between DNA base pairs.

The nuclease activity of **1**–Mn with MMPP was one order of magnitude higher than with $KHSO_5$ (the same cleavage efficiency was observed at 0.1 mM with MMPP and at 1 mM with $KHSO_5$). A similar difference has been previously found with Mn–TMPyP as cleaver.[19]

Finally, the appearance of the linear DNA form III before the complete conversion of form I indicated that **1**–Mn is probably able to perform some direct double-strand breaks (see ref. 18 for a discussion of double-strand breaks resulting from the accumulation of single-strand breaks in opposition to direct double-strand breaks). This observation is in favour of a strong interaction, probably by intercalation of the acridine moieties, between the metalloporphyrin and DNA.

In conclusion, a new porphyrin bearing two acridine intercalators was designed and prepared. Its manganese complex was shown to cleave double-stranded DNA in the presence of different oxygen atom donors involving mainly single-strand breaks with a participation of direct double-strand breaks. Work is in progress to demonstrate the role of the spacer amino acid moieties and to investigate the physico-chemical aspects of the cleaving process and the possible bis-intercalation behaviour of this DNA cleaver.

Notes and References

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Received in Basel, Switzerland, 6th February 1998; 8/01095A