Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 601–606

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SELECTIVE PHOTOCLEAVAGE OF SINGLE-STRANDED NUCLEIC ACIDS BY CYCLOBISINTERCALAND MOLECULES

A. John Blacker[a,†], Marie-Paule Teulade-Fichou[b], Jean-Pierre Vigneron[b], Mireille Fauquet[c] and Jean-Marie Lehn[a,b*].

a) Laboratoire de Chimie Supramoléculaire, ISIS, Université Louis Pasteur, 4 rue Blaise Pascal, 67000 Strasbourg, France ‡

b) Laboratoire de Chimie des Interactions Moléculaires (CNRS, UPR 285), Collège de France, 11 place Marcelin Berthelot, 75005 Paris, France ‡

c) Laboratoire Cytosquelette et Développement (CNRS, URA 2115), CHU Pitié-Salpétrière, 105 Bd de l'Hôpital, 75013 Paris, France

Received 9 December 1997; accepted 2 February 1998

Abstract: Irradiation of mixtures of a single-stranded circular plasmid and of a double-stranded supercoiled DNA in presence of the cyclobisintercaland compounds **2** or **3** shows that these reagents effect the selective photocleavage of the single-stranded entity. Furthermore, **2** also cleaves tRNAasp preferentially at single-stranded domains. © 1998 Elsevier Science Ltd. All rights reserved.

Molecular recognition of specific structural features of nucleic acids (NAs) is an actively investigated topic of both chemical and biological interest. Recognition of nucleotide sequences through hydrogen bonding may make use either of hybridization with a complementary strand in the case of single stranded (ss) NAs, or of triple helix formation and of groove binding of specially designed molecules in the case of double stranded (ds) NAs. Another mode of interaction is intercalation whereby flat molecules, usually of heterocyclic type and positively charged, insert between base pairs in ds NAs. Making use of these various types of interactions both sequence probes and structural probes may be developed.[1-4]

In addition, when NA recognition species are fitted with reactive functional groups, selective modification or cleavage at specific sites becomes possible.[1-7] In particular, photonuclease activity may be obtained by linkage with photoactivable groups and by making use of photoactive molecules or metal complexes.[5-10]

We have been interested in such processes of structural recognition and of selective modification of NAs[8] in the course of the investigation of the molecular recognition properties presented by a class of artificial receptor molecules, cyclo-intercalands, in which intercalating subunits are incorporated into a macrocyclic structure that confers defined geometrical features. In particular, cyclobisintercalands (CBIs) of type **1**, where the macrocycle contains two such intercalating units, may be expected on the one hand to bind selectively small planar substrates by insertion between the two flat walls of the macrocyclic cavity, and on the other hand to present NA recognition features, namely discrimination between ss and ds NAs with preferential binding to ss entities (Fig. 1).[9] This latter expectation is based on the idea that the binding of suitably designed CBIs to ds NAs should be severely hindered by the strain that would result from insertion of the two intercalating groups into

† Present address: ZENECA Process Technology Department, Leeds Road, Huddersfield, HD2 1FF, UK

‡ Fax: +33-388-411020 (Strasbourg), +33-144-271356 (Paris); e-mail: lehn@chimie.u-strasbg.fr

0960-894X/98/$19.00 © 1998 Elsevier Science Ltd. All rights reserved.

PII: S0960-894X(98)00085-7

two successive positions of the double strand (nearest-neighbour exclusion), as well as by the steric interaction of the bridges linking the two intercalating groups with the framework of the double helix. Such destabilizing effects should not exist in the binding of CBIs to ss NAs (Figure 1).

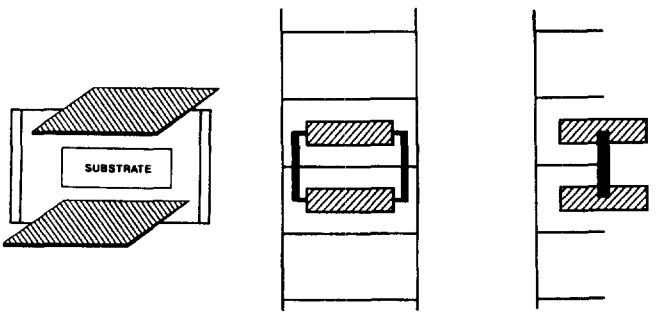
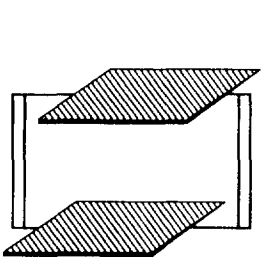


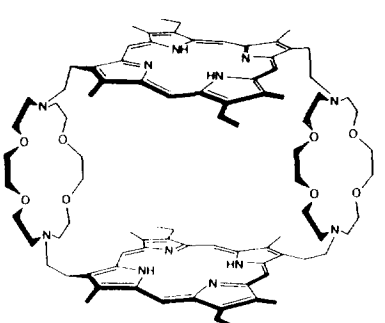
Figure 1. Schematic representation of the binding of a substrate by a cyclobisintercaland molecule **1** (left) and of its modes of interaction with double-stranded (center) and single stranded (right) nucleic acids.

Thus, CBIs containing photoactivable groups might function as photonucleases presenting selectivity for the cleavage of ss NAs and of ss domains in complex NA structures. Such features would make these molecules structure specific NA reagents as well as potential agents of interest for photodynamic therapy.

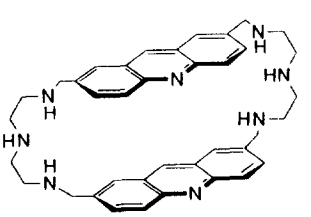
We describe here the properties of two CBI reagents **2** and **3** which combine ss NA binding selectivity with photocleavage ability. They contain respectively porphyrin and acridine subunits as photoactive intercalators linked by bridges containing amine functions whose protonation provides cationic sites for electrostatic interaction with the NA phosphate groups. Their synthesis has been reported earlier.[11,12] The receptor molecule **3** has been shown to bind small anionic substrates by insertion into its central cavity.[12,13] Furthermore, the ability of **2** and **3** to discriminate between ss and ds NAs has been established. The bisporphyrin **2** forms more stable complexes with ss than with ds polynucleotides[14] and the bisacridine **3** has been found to bind selectively to the loop of model DNA hairpins[15] and to destabilize synthetic ds polynucleotides.[16] On the basis of these properties, compounds **2** and **3** may be able to cleave NAs preferentially at ss relative to ds domains under light irradiation.



1



2



3

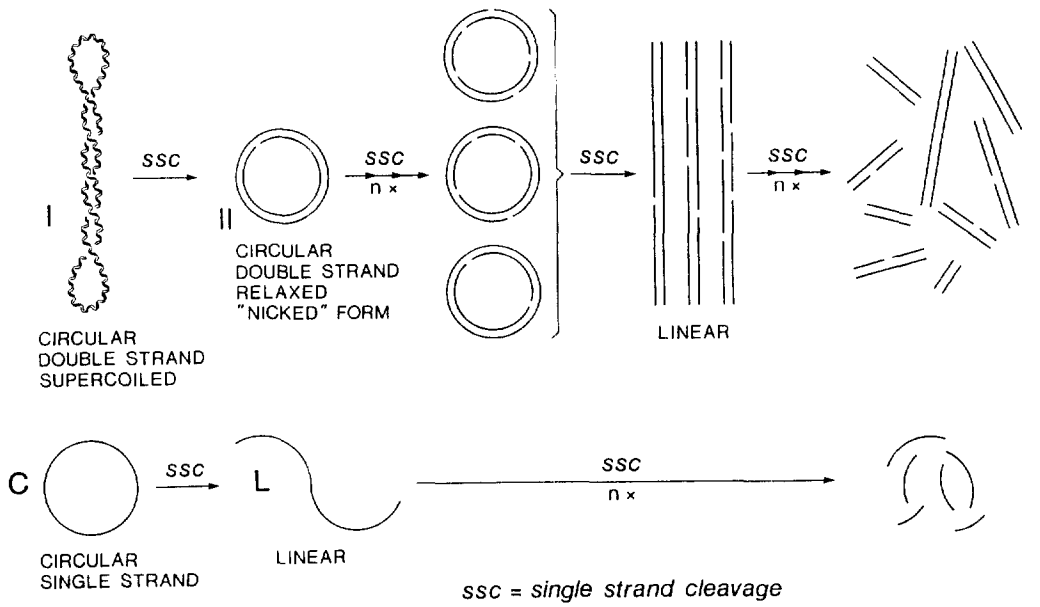


Figure 2. Types of species generated by single-strand cleavage reactions of supercoiled circular double-stranded (top) and of circular single-stranded (bottom) nucleic acids.

Photocleavage experiments were conducted on circular supercoiled ds plasmids pBR322 or pUC18 and on circular ss DNA M13mp19 or M13mp18.[17] A single cut of either type of DNA results in the formation of a new species with very different electrophoretic migration properties. The ds supercoiled DNAs yield a relaxed circular form, which may be followed by a linear ds one after multiple cleavage events. On the other hand, the ss circular DNAs give a linear ss species after a single cut (Figure 2).

Experiments with CBI **2** gave the results listed in Table 1. Whereas both ds pBR322 and ss M13mp19 are photocleaved in the higher concentration range, where **2** binds to both DNAs, marked ss cleavage selectivity takes place at lower concentrations; finally, as the concentration decreases further little cleavage is observed due to lack of binding to either DNA. Efficient photocleavage occurs at slightly acidic pH (about 4.5) where the porphyrin groups are protonated.[18]

Table 1. Photocleavage of equimolar mixtures (0.06 mM in nucleotide phosphate units) of supercoiled ds pBR322 and circular ss M13mp19 by cyclobisintercaland **2**[17]

Concentration of **2** (μM)	0.8	1.6	2	3	6.25	12.5	25	50
% ds pBR322 remaining	100	100	100	75	80	60	40	0
% ss M13mp19 remaining	90	50	12	0	0	0	0	0

Figure 3 presents results obtained for the photocleavage of supercoiled ds pUC18 and of ss M13mp18 by the bisacridine **3** (see also Fig. 2). In the concentration range examined, the ds starting material (I) (Fig. 3A) remains essentially intact except at the highest **3**/phosphate ratio (Fig. 3A, lane 5) where a slight transformation into the circular relaxed form(II) is observed. On the contrary, the circular ss DNA (C) is progressively converted into the linear form (L) (Fig. 3B, lanes 2,3) and then completely degraded as indicated by the smear of the band (Fig. 3B, lanes 4,5). This indicates that low concentrations of compound **3** are sufficient to extensively photocleave ss DNA in a selective manner. Much higher concentrations of **3** and longer irradiation times are required to induce a significant conversion of the ds supercoiled pUC18 (I) to its relaxed form (II); moreover the linear form is never observed. When mixtures of the two plasmids are treated in similar fashion, again selective cleavage of the ss DNA takes place whereas the ds DNA is little affected (Fig. 3, right).

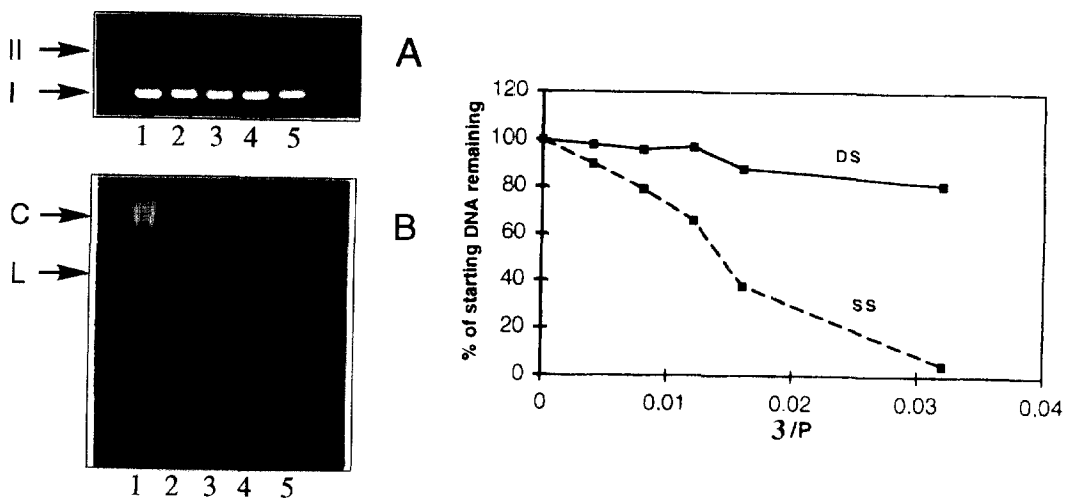


Figure 3. (Left. A, B): agarose gel electrophoresis patterns for the separate photocleavage of ds pUC18 DNA (A) and ss M13mp18 (B) by **3**; [DNA]: 0.3mM in nucleotide phosphate unit; [**3**] /[Phosphate]: lane 1, 0; lane 2, 1/250; lane 3, 1/125; lane 4, 1/60; lane 5, 1/30. (Right): photocleavage of mixtures of pUC18 and M13mp18 by **3**; [ds DNA] = [ss DNA] = 0.3mM in nucleotide phosphate unit; variation of ds and ss DNA as a function of the concentration of **3**.[17]

The same experiment (irradiation of mixtures of pUC18 and M13mp18) has been conducted in the presence of the monomeric reference compound [2,7(di-npropylamino) acridine]. A similar degradation of both ds and ss DNA was observed, thus revealing the absence of selectivity of the monomeric compound by contrast with its dimeric analog **3** (Fig. 3).

Finally, photocleavage experiments were conducted with a transfer RNA (tRNA) which presents both ds and ss domains within the same molecule. Following irradiation of mixtures of **2** (5μM) with ^{32}P, 5'-end-labelled tRNAasp (0.1mM in bases)[19], the photocleavage products were analysed by autoradiograms of electrophoretic polyacrylamide gels, and compared with controls lacking the cleavage reagent. The cleavage patterns can be related to the X-ray crystallographic structure of tRNAasp[20] and hence the preferred binding

sites of the CBI can be located. Figure 4 shows the positions where photocleavage was found to occur. Interestingly, **2** binds almost exclusively at the ss RNA domains. Photocleavage activity is highest at points involving purine bases, in agreement with other studies that have shown preferential photocleavage of oligonucleotides at guanine sites.[21]

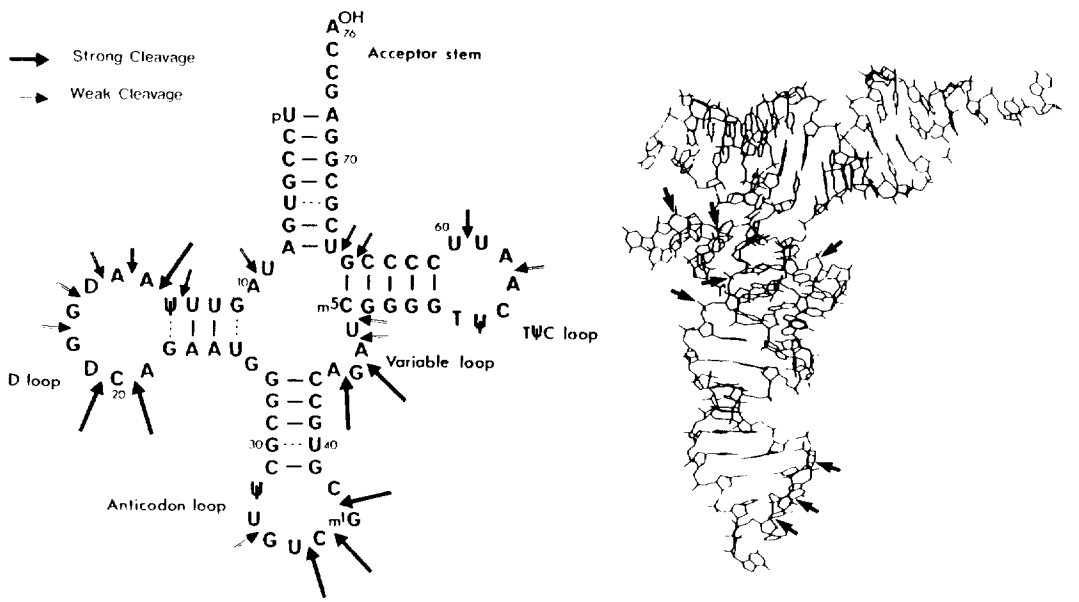


Figure 4. (Left): photocleavage of tRNAasp (cloverleaf representation) by cyclobisintercaland **2**. Full arrows indicate points of strong cleavage, hollow arrows, points of weaker cleavage. (Right): major cleavage positions indicated on a representation of the crystal structure of tRNAasp.[20]

In conclusion, the present experiments establish that the cyclobisintercalands **2** and **3** are selective reagents for the preferential photocleavage of single-stranded nucleic acids or single-stranded regions in complex nucleic acids. They indicate selective binding at such positions, in agreement with earlier observations, and confirm the basic design features of these substances. They also point to the potential of CBI reagents to function as structural probes for single-stranded domains such as loops, bulges,[22] hairpins or for local defects in complex nucleic acids. Such domains could be possible targets for eliciting therapeutic effects, for instance antiviral activity.[23] Finally, CBIs may be able to displace single strand binding proteins from their site of attachment and thus interfere with a variety of genomic processes.

References and Notes

1. Dervan, P. B., in *Nucleic Acids and Molecular Biology*, (Eds.: F. Eckstein and D. M. J. Lilley), Springer, Berlin, **1988**, *2*, 49.
2. Thuong, N. T.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666.
3. a) Knorre, D. G.; Vlassov, V. V. *Progress Nucl. Acid. Res. and Mol. Biol.* **1985**, *32*, 291; b) Knorre, D. G.; Vlassov, V. V.; Zarytova, V. F.; Lebedev, A. V.; Federova, O. S. *Design and Targeted Reactions of Oligonucleotide Derivatives*, CRC Press, Boca Raton, **1994**.
4. Nielsen, P. E. *J. Mol. Recogn.* **1990**, *3*, 1.
5. Sigman, D. S.; Bruice, T. W.; Mazumder, A.; Sutton, C. L. *Acc. Chem. Res.* **1993**, *26*, 98; Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.
6. Barton, J. K. *Comments Inorg. Chem.* **1985**, *3*, 321.
7. Hélène, C. *Photobiochem. Photobiophys.* **1987**, *Suppl.*, 3.
8. Blacker, A. J.; Jazwinski, J.; Lehn, J.-M.; Wilhelm, F. X. *J. Chem. Soc., Chem. Commun.* **1986**, 1035.
9. Blacker, A. J.; Lehn, J.-M., unpublished work; Blacker, A. J., Ph. D. Thesis, University Louis Pasteur, Strasbourg, 1988.
10. Maiya, B. G. *Current Science* **1997**, *72*, 808.
11. Hamilton, A. D.; Lehn, J.-M.; Sessler, J. *J. Am. Chem. Soc.* **1986**, *108*, 5158.
12. Teulade-Fichou, M.-P.; Vigneron, J.-P.; Lehn, J.-M. *Supramolecular Chem.* **1995**, *5*, 139.
13. Paris, T.; Vigneron, J.-P.; Lehn, J.-M.; Cesario, M.; Guilhem, J.; Pascard, C. *J. Inclusion Phenom. Mol. Recognit. Chem.* in press.
14. Slama-Schwok, A.; Lehn, J.-M. *Biochemistry* **1990**, *29*, 7895.
15. Slama-Schwok, A.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Taillandier, E.; Lehn, J.-M. *J. Am. Chem. Soc.* **1995**, *117*, 6822.
16. Slama-Schwok, A.; Peronnet, F.; Hantz-Brachet, E., Taillandier, E.; Teulade-Fichou, M.-P.; Vigneron, J.-P.; Best-Belpomme, M.; Lehn, J.-M. *Nucleic Acids Res.* **1997**, *25*, 2574.
17. *Irradiation experiments.* a) *With compound 2*. Equimolar mixtures (0.06 mM in nucleotide phosphate units) of pBR322 and of M13mp19 in NaOAc buffer at pH = 4.4 were irradiated for 30 mn with visible light (>395 nm filter) in presence of different amounts of **2**. Samples were loaded on a 1% agarose gel in a pH 8.0 Tris/EDTA buffer, submitted to electrophoresis and then stained with ethidium bromide. The DNA bands were revealed by UV light and a polaroid photograph taken; the amounts of DNA forms were quantified by reflectance densitometry. b) *With compound 3*. The DNA (pUC18 or M13mp18 or 1/1 mixtures of the two) solutions (0.3 mM in nucleotide phosphate units) were irradiated for 20 mn with 360 nm light (provided by the monochromator of a Spex Fluoromax fluorimeter) in Tris HCl buffer (0.1 M, pH 7.0, total volume 10 µl) in the presence of concentrations of **3** from 0 to 15 µM ds pUC18 was analyzed by electrophoresis on a 1% agarose gel in 1M Tris-acetate buffer, whereas a 1.4% agarose gel in 0.5M Tris-borate buffer was preferred for ss M13mp8 and for experiments on the mixtures of NAs, these conditions providing a much better separation of the circular and linear forms. In both cases the extent of cleavage has been quantified by the disappearance of the starting material (forms I or C, Fig. 3) with respect to the control.
18. J. Mauzerall *J. Am. Chem. Soc.* **1962**, *84*, 2437.
19. Romby, P.; Moras, D.; Bergdoll, M.; Dumas, P.; Vlassov, V. V.; Westhof, E.; Ebel, J.-P.; Giégé, R. *J. Mol. Biol.* **1985**, *184*, 455.
20. Moras, D.; Comarmond, M. B.; Fischer, J.; Weiss, R.; Thierry, J. C.; Ebel, J.-P.; Giégé, R. *Nature* **1980**, *288*, 669.
21. Slama-Schwok, A.; Jazwinski, J.; Béré, A.; Montenay-Garestier, T.; Rougée, M.; Hélène, C.; Lehn, J.-M. *Biochemistry* **1989**, *28*, 3227.
22. For selective strand scission at bulges by intercalating drugs, see for instance also: Williams, L.D.; Goldberg, I. H. *Biochemistry* **1988**, *27*, 3004.
23. Wilson, W. D.; Ratmeyer, L.; Cegla, M. T.; Spychala, J.; Boykin, D.; Demeunynck, M.; Lhomme, J.; Krishnan, G.; Kennedy, D.; Vinayak, R.; Zon, G. *New. J. Chem.* **1994**, *18*, 419.