1. Interaction of Double-Helical Polynuclear Copper(I) Complexes with Double-Stranded DNA

by **Bruno Schoentjes**[1]) and **Jean-Marie Lehn***

Laboratoire de Chimie Supramoléculaire, URA CNRS no 422, Université Louis Pasteur, 4, rue Blaise Pascal, F–67000 Strasbourg

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Double-helical metal complexes, the helicates $\mathbf{H_2}$–$\mathbf{H_5}$, were found to bind to double-helical DNA by spectroscopic, DNA-melting, and electrophoretic-mobility measurements. The helicates also inhibited the cleavage of DNA by two restriction enzymes, SspI and EcoRV, a property which agrees with the binding occurring in the major groove of the double helix. Visible-light irradiation of solutions containing a helicate and the pBR322 plasmid led to single-strand cleavage, indicating that these complexes could be of interest as potential probes for nucleic-acid structure.

Introduction. – The study of the interactions of small molecules with DNA has been an actively growing research field, following closely the progress of knowledge on structure and function of nucleic acids. The goals are to better understand the interactions with nucleic acids through the analysis of model systems, and to develop drugs acting on nucleic acids. Metal complexes are frequently used as nucleic-acid ligands in these investigations.

We present here results relative to the reversible binding to double-stranded DNA of one class of such compounds: the helicates, double-helical copper(I) complexes that are formed by self-assembly between two ligand strands and Cu^I ions [1]. These processes thus involve the interaction of the natural polyanionic double helix of DNA with the artificial polycationic double helix of the helicates.

The ligands L = dibp to pentabp used consist of a strand of binding subunits derived from 6,6′-dimethyl-2,2′-bipyridine linked by an oxybis(methylene) bridge (*Fig. 1*). The Cu^I ions are coordinated tetrahedrally to two bipyridine (bpy) units and act as the structure organizer. The complexes $\mathbf{H_2}$–$\mathbf{H_5}$ were characterized by UV/VIS and NMR spectroscopy and are represented schematically in *Fig. 2*. The double-helical geometry was confirmed by determination of the structure of the trinuclear complex by X-ray crystallography [1].

Such Cu^I complexes could bind in multiple ways to DNA and even effect strand cleavage like other metal complexes [2–4]. While interaction through H-bonding is very unlikely, electrostatic binding is expected to be important, because DNA is a large polyanion, and each helicate molecule bears multiple positive charges. Hydrophobic effects might also play a role since the trinuclear helicate $\mathbf{H_3}$ has a hydrophobic surface

[1]) Present address: *Sanofi Recherche*, 371, rue du Prof. Blayac, F–34184 Montpellier Cedex 04.

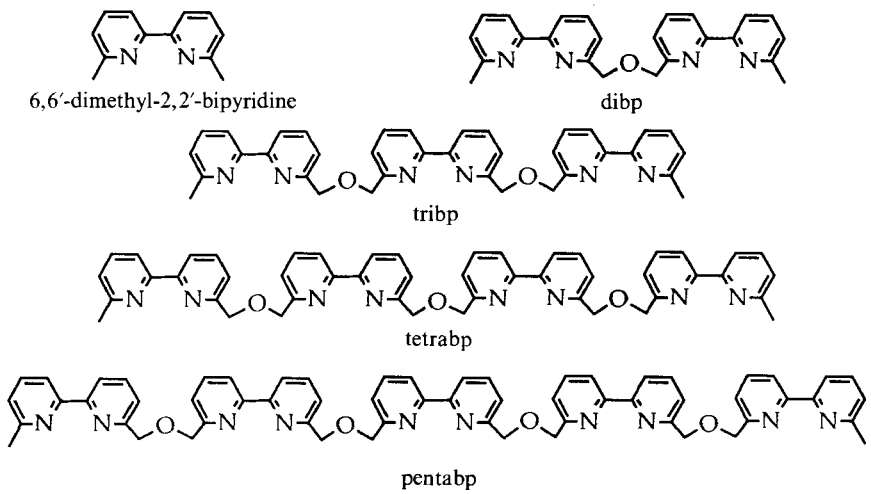

Fig. 1. *Polybipyridine ligands dibp to pentabp*

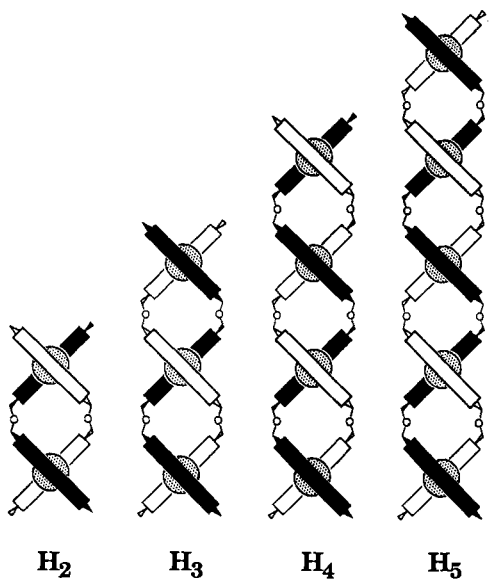

Fig. 2. *Schematic representation of dinuclear to pentanuclear helicates* $\mathbf{H_2}$–$\mathbf{H_5}$. The ligands are shown as black or white strands; the dotted circles represent the Cu^I ions.

and a size (length 17 Å, diameter 6 Å) compatible with the major groove of form B of DNA (width 11.7 Å, depth 8.8 Å) [5]. Some small hydrophobic molecules were shown to migrate towards sites inside the grooves of DNA and release bound solvent molecules.

Electronic Absorption and Emission Measurements. – The helicates contain several $Cu^I(bpy)_2$ centres characterized by a metal-to-ligand charge-transfer (MLCT) absorption

band centered around 450 nm. We measured the absorption and emission spectra of the trinuclear helicate $\mathbf{H_3}$ in the presence of an excess of calf-thymus DNA. For solubility reasons, the measurements were effected in solutions containing 30% MeOH in 25 mM *Tris*-HCl buffer (pH 7.8) with an $\mathbf{H_3}$ concentration of 20 μM. The presence of DNA induced a slight bathochromic shift of 5 nm as well as a hypochromic effect of 7% on the MLCT absorption band of the complex (*Fig. 3*). The data are summarized in *Table 1*.

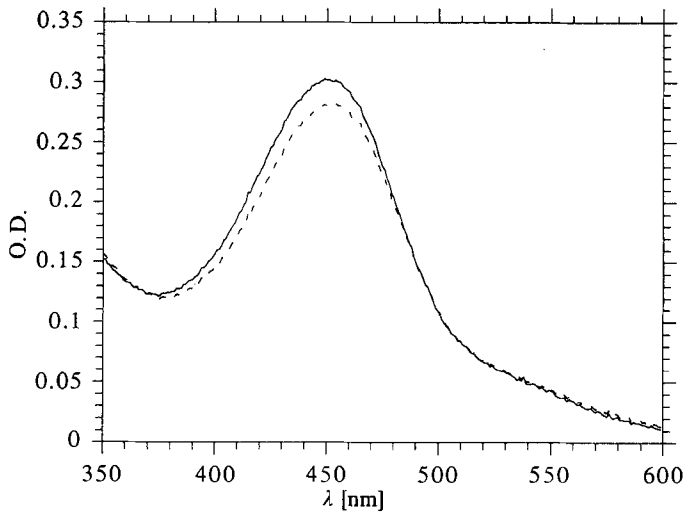

Fig. 3. *VIS Absorption spectra of helicate* $\mathbf{H_3}$ *in the presence* (----; DNA-P/$\mathbf{H_3}$ 36:1) *or absence* (——) *of calf-thymus DNA*

Table 1. *VIS Absorption Features* (λ_{max}; absorbance *A*) *of Helicate* $\mathbf{H_3}$ *in the Presence of DNA*

DNA-P/$\mathbf{H_3}$	λ_{max} [nm]	A_{449}
0:1	449.0	0.30
18:1	454.0	0.28
36:1	454.0	0.28

Under the same conditions, no significant modification was observed in the UV part of the absorption spectrum, corresponding to the π-π^* absorption bands of the pyridine units.

The bathochromic and hypochromic effects on the MLCT in presence of DNA indicate that the helicate binds to the macromolecule [6]. The weak electronic perturbation observed suggests an external mode of binding of the complex to the DNA double helix.

The luminescence of the helicates was very weak in a *Tris* buffer/MeOH solution. Indeed, the charge-transfer excited state of [Cu^I(diimine)] complexes is efficiently deactivated by polar solvents like H_2O or MeOH [7].

A strong emission band centered around 610 nm was observed under excitation at 450 nm. It was not enhanced on addition of calf-thymus DNA to the solution in a DNA-P/

helicate ratio of 100:1. These results indicate that the DNA-bound form of $\mathbf{H}_3$ is still largely accessible to solvent molecules.

DNA-Melting Measurements in Presence of Helicates. – *Calf-Thymus DNA.* The measurement of the variation of the melting temperature T_m of the DNA double helix in the presence of ligands was widely used for studying the interaction of small molecules with DNA [8] [9]. The interaction of helicates with calf-thymus DNA was investigated under conditions identical to those used for similar measurements conducted with polyamines [9b]. As is the case of spermine, the main binding mode is probably electrostatic with, nevertheless, a contribution of secondary nonelectrostatic effects. Sequence-specific binding is not expected.

We determined the effect of helicates on the melting temperature of the calf-thymus DNA double helix (B-form DNA). T_m for the fragment of calf-thymus DNA used was measured reproducibly at $66.3 \pm 0.2°$. The observed melting curve was monophasic which testifies for the homogeneity of the population of DNA molecules. *Fig. 4* presents

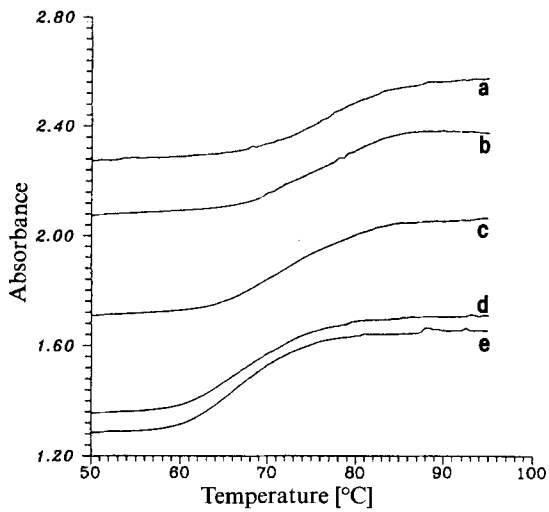

Fig. 4. *Thermal denaturation of calf-thymus DNA in the presence of* $\mathbf{H}_3$ *in an* $\mathbf{H}_3$*/base pair ratio of 0.25*(a), *0.17*(b), *0.095*(c), *0.01*(d), *and 0*(e)

an example of the melting curves obtained in the presence of increasing concentrations of the trinuclear helicate $\mathbf{H}_3$. For a DNA concentration of $9.9 \cdot 10^{-5}$ M (base pairs (bp)), the absorbance at 260 nm varied between 1.22 and 2.45 according to the concentration of $\mathbf{H}_3$ in the solution. The presence of $\mathbf{H}_3$ did not modify the melting process in a qualitative fashion. The melting curves were monophasic, indicating that the DNA-molecule population stays homogeneous. The increase in absorbance was constant, corresponding to 23% of the absorbance at 50°, and was proportional to the concentration of $\mathbf{H}_3$, indicating a stabilization of the DNA double helix by binding of $\mathbf{H}_3$. The data are summarized in *Table 2.*

Table 2. *Variation of the Melting Temperatures* T_m *of Calf-Thymus DNA with the Concentration of Helicate* $\mathbf{H_3}$ *in Solution*

$\mathbf{H_3}$/DNA (bp)[a])	0	0.01	0.014	0.095	0.173	0.246
T_m [°C]	67.3	67.4	67.4	72.3	74.4	76.7

[a]) bp = base pairs.

Similar results were obtained with the other three helicates. In all cases, the addition of helicate to the solution resulted in a stabilization of the DNA double helix. This indicates a greater affinity of helicates for the double-helical form of DNA than for the denatured form. *Fig. 5* presents the results obtained for the four helicates, standardized by plotting the increase in T_m as a function of the ratio of helicate per base pair. From these curves, we can deduce qualitatively the preferential order of binding of helicates to calf-thymus DNA. The molecule with the lowest affinity is $\mathbf{H_2}$, followed by $\mathbf{H_3}$ and $\mathbf{H_4}$ at comparable values, and then $\mathbf{H_5}$ which exhibits the highest affinity. Binding to DNA

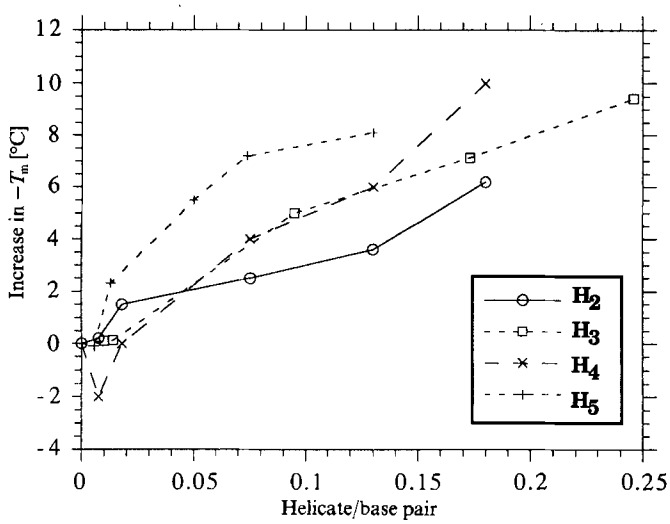

Fig. 5. *Stabilization of the calf-thymus DNA double helix by helicates* $\mathbf{H_2}$–$\mathbf{H_5}$

should increase with the size of the complex, since the number of metal centers and consequently the electrostatic contribution to binding increases. Similarly, the hydrophobic contribution to the binding should increase with the external area of the helicates and, therefore, with the number of bipyridine units in the ligand. As most polycations studied by similar methods, helicates stabilize the DNA double helix in a manner proportional to the charge of the ligand studied [9].

Poly(dA-dT)·Poly(dA-dT) and Poly(dG-dC)·Poly(dG-dC). We compared the effect of helicate $\mathbf{H_3}$ on the thermal denaturation of two different double-stranded synthetic polynucleotides: poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC). These two polynucleotides exhibit marked structural differences, especially regarding the size of the

major and minor grooves. A number of groove-binding molecules exhibit preference for (G-C)-rich sequences [10].

The DNA-melting experiments were conducted under the conditions described for calf-thymus DNA. In 5 mM sodium cacodylate and 10 mM NaCl at pH 6.5, the T_m values measured were 45° for poly(dA-dT)·poly(dA-dT) and 59° for poly(dG-dC)·poly(dG-dC). This difference in the T_m of polynucleotides of same lengths (350 base pairs) is accounted for by the difference in stability in A·T and G·C base pairs. As in the case of calf-thymus DNA, the increase in T_m in the presence of $\mathbf{H_3}$ indicates that $\mathbf{H_3}$ stabilized the double helix of both polynucleotides. This increase is plotted as a function of the ratio of $\mathbf{H_3}$ per base pair in *Fig. 6*. It was found to augment with the concentration of $\mathbf{H_3}$ in solution. In the

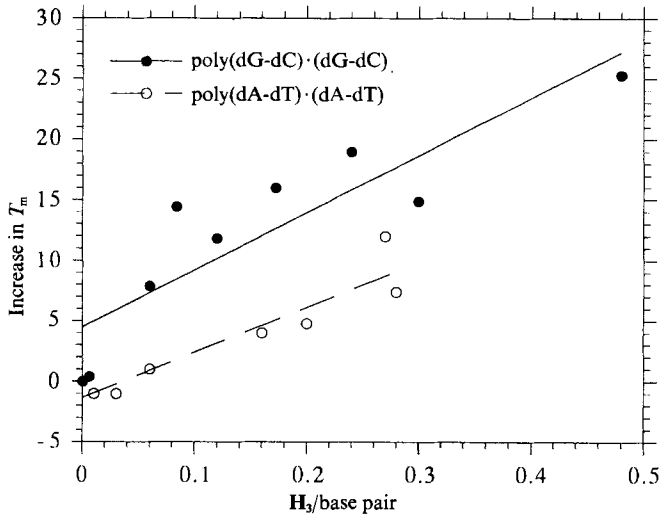

Fig. 6. *Stabilization of the poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) DNA double helix by helicate* $\mathbf{H_3}$

presence of an equal concentration of $\mathbf{H_3}$, the increase in the T_m of poly(dG-dC)·poly(dG-dC) was more important than that of poly(dA-dT)·poly(dA-dT). This may be due to the difference in the geometry of these polynucleotides as indicated by the crystallographic structure of several oligonucleotides of homogeneous or mixed sequences [11]. B-DNA consisting of G·C base pairs presents a larger minor groove than A·T homo- or hetero-polymers. The size of the major groove is inversely proportional, A·T heteropolymers possessing a wide and shallow major groove. This difference in structure between A·T heteropolymers and B-DNA or G·C heteropolymers could explain the observed difference in stabilization on the basis of the fit of the helicates into one of the grooves, probably the major one (see below).

Electrophoretic-Mobility-Shift Assays. – When performing nondenaturing polyacrylamide-gel electrophoresis (PAGE) of DNA, mobility shifts can be induced by the presence of ligands [12]. This technique was used to assess the binding of proteins to DNA [13]. We applied it to the interaction of helicates to DNA.

PAGE was performed on a ^{32}P-labelled 191-base-pairs fragment of pBR322 (situated between the restriction sites of enzymes SspI and EcoRV) in the presence of increasing concentrations of the dinuclear to pentanuclear helicates. The DNA concentration used was 0.26 μM (bp) in a solution in *Tris* (10 mM) and NaCl (50 mM) at pH 7.4. In the presence of the four helicates $\mathbf{H_2}$–$\mathbf{H_5}$, a new band appeared possessing a reduced electrophoretic mobility on the gel. The autoradiogram corresponding to an experiment conducted in the presence of $\mathbf{H_3}$ is shown in *Fig. 7*. Above a concentration of 7.3 μM $\mathbf{H_3}$, a weak band of radiolabelled material was observed with a lower mobility than the DNA alone; it increased in intensity with the concentration of $\mathbf{H_3}$ as its mobility decreased. Similar observations were made with the other three helicates. The concentration required to observe such a retarded band decreased with the nuclearity of the helicates, thus following the order of highest affinity as determined by the DNA-melting experiments. The decrease in mobility points to a family of molecules that possesses either a reduced

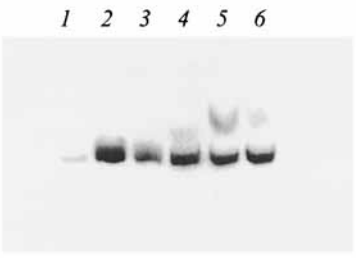

Fig. 7. *Electrophoresis of pBR322 SspI/EcoRI fragment in the presence of helicate* $\mathbf{H_3}$. All lanes: 0.25 μM DNA (bp). Lane *1*: control. Lanes *2–6*: DNA + $\mathbf{H_3}$ at 1.8 (*2*), 3.6 (*3*), 7.3 (*4*), 15 (*5*), and 23 μM (*6*). Acrylamide gel 7.5% (acryl/bis 29:1); *U* 150 V.

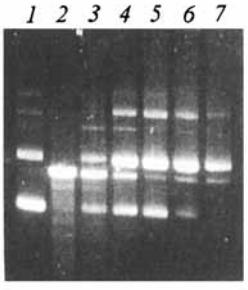

Fig. 8. *pBR322 Cleavage by restriction enzyme SspI in the presence of* $\mathbf{H_4}$. All lanes: 7 μM form I pBR322 (bp). Lane *1*: DNA alone. Lanes *2–8*: DNA + 5 units SspI + 0, 150, 112, 84, 56, and 28 μM $\mathbf{H_4}$, resp. Incubation for 60 min at 37°.

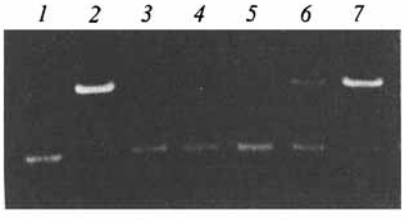

Fig. 9. *pBR322 Cleavage by EcoRV in presence of* $\mathbf{H_4}$. All lanes: 7 μM form I pBR322 (bp). Lane *1*: DNA alone. Lanes *2–8*: DNA + 5 units EcoRV + 0, 4.2, 8.5, 12.5, 17, 21, and 25 μM $\mathbf{H_4}$, resp. Incubation for 60 min at 37°.

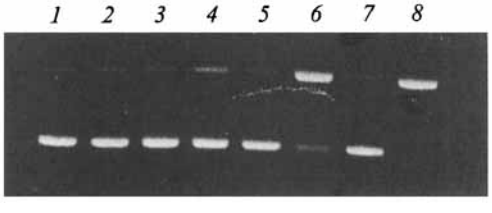

Fig. 10. *pBR322 Photocleavage by helicate* $\mathbf{H_3}$. All lanes: 250 ng form I pBR322. Lanes *1* and *2*: DNA alone. Lanes *3–8*: DNA + $\mathbf{H_3}$ at 0.02 (*3* and *4*), 0.1 (*5* and *6*) and 0.4 μM (*7* and *8*) with (even-numbered lanes) and without (uneven-numbered lanes) irradiation (1000 W Xe lamp, H_2O/*Pyrex* filter, 4°).

apparent charge or an increased hydrodynamic volume. Both of these possibilities may occur in the case of a DNA-helicate complex. The observed bands were not sharp and exhibited some streaking indicating that the species does not possess a precise stoichiometry, or that it partly dissociated during the experiment.

These experiments revealed a relatively strong interaction between DNA and helicates, maintained under a potential of 150 V during the experiment, although the DNA and the helicates tend to be pulled apart by the electrical field. DNA-Protein complexes of weakest affinity observed by this method have a dissociation constant of $6 \cdot 10^{-6}$ M [14]. Structures which have a lifetime longer than the duration of the experiment migrate generally as a discrete band, whereas shorter lived complexes form diffuse bands and are, therefore, difficult to detect. Nevertheless, the weak ionic strength of the buffer used can help to stabilize interactions possessing a non-negligible ionic component. Also experiments conducted on protein-DNA complexes indicate that accociation and dissociation reactions are not inhibited inside a polyacrylamide gel but proceed at much slower rates [15].

The results described here show the appearance of a new molecular complex during electrophoresis experiments in the presence of helicates, a noncovalent DNA-helicate complex, in agreement with the data obtained by the other methods described above.

Inhibition of Restriction Enzymes SspI and EcoRV. – Testing a number of DNA-modifying enzymes, it was found that two type-II restriction enzymes, SspI [16] and EcoRV [17], are inhibited by helicates. The activity of these two enzymes was followed by agarose-gel electrophoresis using as a test the cleavage of plasmid pBR322 and its conversion from supercoiled to linear form under the action of the enzymes.

The restriction enzyme SspI has a unique double-strand cleavage site on plasmid pBR322 [18]. Cleavage of the plasmid in the presence of the trinuclear to pentanuclear helicates $\mathbf{H_3}$–$\mathbf{H_5}$ was investigated. *Fig. 8* presents the results in the presence of the tetranuclear helicate $\mathbf{H_4}$. In this case, the cleavage reaction was not complete anymore, the enzyme producing relaxed circular DNA rather than linear DNA. Similar observations were made for $\mathbf{H_3}$ and $\mathbf{H_5}$. The production of relaxed circular DNA in the presence of helicates indicates that the enzyme effects a single-strand cut of the plasmid. The presence of helicates thus inhibits cleavage of one of the two strands of pBR322 by SspI. Unfortunately, very little information exists on the structure or the mechanism of action of this enzyme so that it is difficult to draw conclusions from these data.

EcoRV is a type-II restriction enzyme possessing a single cleavage site on plasmid pBR322 [19]. Addition of helicates caused an inhibition of the cleavage. *Fig. 9* shows an agarose-gel analysis of the reaction products of pBR322 cleavage in the presence of $\mathbf{H_4}$. In this case, conversion of pBR322 from the supercoiled to the linear form was not complete. Similar experiments were performed with $\mathbf{H_3}$ and $\mathbf{H_5}$. Partial inhibition of the enzyme was observed, the inhibition increasing with the nuclearity of the helicate employed.

EcoRV is one of the best characterized restriction enzymes [17a] [20]; three crystallographic structures were determined [21]. Two short loops of the enzyme form direct contacts in the minor and major grooves of DNA. The protein has direct contacts with phosphate groups between positions −2 and +4. The recognition features of the enzyme were also studied. In the absence of magnesium, EcoRV binds to all DNA sequences with equal affinity [22]. It was thus proposed that it binds to DNA and then proceeds to locate

the restriction site by diffusion along the double helix [23]. If the inhibition is caused by the binding of helicates to DNA, it could occur at two different steps. Helicates could act by simply inhibiting the binding of the enzyme to DNA, if their affinity constant is high enough. The other hypothesis is that helicates could inhibit the recognition of the specific cleavage sequence by EcoRV. The recognition of the cleavage site is accompanied by the formation of direct contacts between the protein and the bases inside the two grooves, as well as a conformational change of DNA producing a narrowing of the major groove. The presence of a rigid molecule in the major groove of DNA could inhibit both of these processes.

DNA Photocleavage by Helicates. – In view of the DNA cleavage effected by [Cu^I(phenanthroline)] [2] and related complexes, it appeared of interest to investigate whether helicates presented similar properties. Simple addition of helicates to DNA does not lead to damage due probably to a too high stability of these copper(I) complexes. However, helicates were found capable of photocleaving DNA. Using as a test the cleavage of plasmid pBR322, *Fig. 10* shows a DNA-cleavage experiment by helicate $\mathbf{H_3}$. Conversion of plasmid pBR322 from the supercoiled to the relaxed linear form was observed after irradiation in the presence of $\mathbf{H_3}$; this is caused by creation of single-strand nicks in the plasmid. The cleavage was dependent on the presence of light and increased with the concentration of the trinuclear helicate. The dinuclear, tetranuclear, and pentanuclear helicates were also capable of mediating the photocleavage of pBR322. The minimum concentration of each of the four reagents necessary to induce observable cleavage is given in *Table 3*. The efficiency of the cleavage was found to be inversely

Table 3. *Minimum Concentrations of Helicates* $\mathbf{H_2}$–$\mathbf{H_5}$ *Effecting pBR322* (c = 3.5 μM (bp)) *Photocleavage*

$\mathbf{H_2}$	$\mathbf{H_3}$	$\mathbf{H_4}$	$\mathbf{H_5}$
0.011 mM	0.02 mM	0.036 mM	0.04 mM

proportional to the nuclearity of the helicate. This might seem incompatible with the order of increasing affinity as determined by the melting experiments. A possible explanation would be that cleavage proceeds through degradation of helicates, the reactivity being then inversely proportional to the stability of the complexes, which increases with the size of the helicates.

Repeating the irradiation experiments with light of wavelength above 400 nm gave no noticeable change in the efficiency of the reaction. Absorption in the VIS-light region by the MLCT absorption band of the complex is thus responsible for the cleavage reaction.

Irradiation of helicate $\mathbf{H_3}$ in a sealed cell in the presence of a large excess of calf-thymus DNA (phosphate/$\mathbf{H_3}$ 100:1) and measurement of the VIS absorption spectra before and after irradiation (*Fig. 11*) showed a decrease in absorption at 449 nm indicating a degradation or a modification of the Cu^I sites. Simultaneously, a new absorption peak appeared around 418 nm pointing to the formation of a new species in solution.

This suggests that DNA photocleavage by helicates proceeds through a degradation of the helicate-metal complexes liberating Cu^I ions, probably followed by a redox reaction involving O_2 similar to that responsible for DNA-strand cleavage by [Cu^I(phenanthroline)] [2].

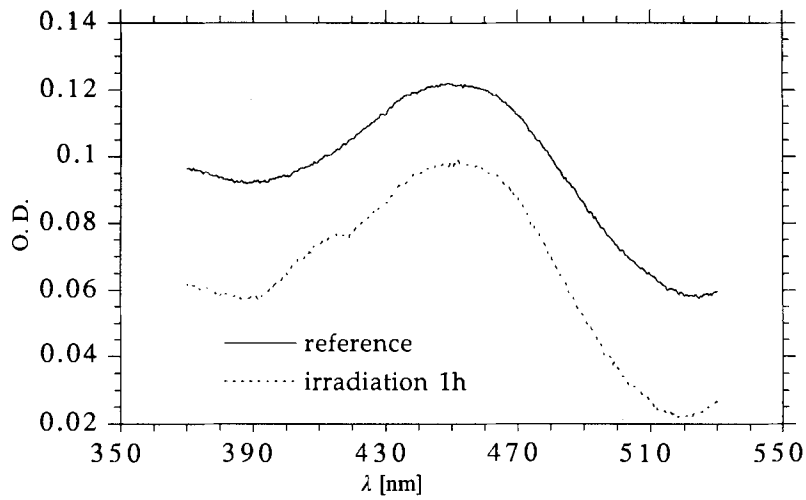

Fig. 11. *Electronic absorption spectrum of helicate* H_3 *before* (——) *and after* (· · · ·) *1-h irradiation in the presence of calf-thymus DNA*

Conclusion. – The results described here demonstrate that the double-helical Cu^I complexes, the helicates H_2–H_5, bind strongly to double-helical DNA. The binding affinity depends on the size of the complex used and on the chemical composition of the target DNA, as shown by DNA-melting experiments. Mobility-shift assays indicate the formation of slower moving species corresponding probably to a noncovalent DNA-helicate complex. The helicates are also capable of interfering with some specific restriction enzymes. These experiments have not permitted to determine precisely the binding mode of these complexes to DNA, but several elements point to major-groove binding, even if helicates have probably multiple binding sites, especially at high concentrations. Finally, helicates are capable of mediating single-strand photocleavage of DNA, while a number of other copper(I) complexes require a chemical activation [24]. The reaction is reasonably efficient, and the helicates could be potential probes for DNA and RNA structure, *via* shape-specific binding.

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Experimental Part

General. Calf-thymus DNA (type IX, sodium salt) as well as poly(dG-dC)·poly(dG-dC) and poly(dA-dT)·poly(dA-dT) were from *Sigma*, sodium cacodylate from *Prolabo*, and the restriction enzymes and their buffers from *Boehringer*. One enzyme unit is defined as the quantity of enzyme necessary to digest one microgram of DNA in 60 min under each enzyme's optimum conditions. pBR322 Plasmid (4362 bp) [25] and molecular-biology grade agarose for electrophoresis were obtained either from *Boehringer* or *Gibco BRL*. The helicates H_2–H_5 were synthesized as triflate salts by *A. Marquis-Rigault* as previously described [26]. The Cu^I complexes were dissolved in a soln. containing 5% MeOH in H_2O. The concentration of the different helicates were obtained by measurement of their absorption at 449.5 nm using the molecular absorption coefficients determined by *A. Marquis-Rigault*.

Purification of Calf-Thymus DNA. A soln. of 100 mg of calf-thymus DNA in 10 ml of 5 mM cacodylate buffer, containing 5 mM NaCl, pH 6.5, was degassed and kept at 5°. The soln. was then extracted 3 times with one volume of phenol/$CHCl_3$ 1:1 and then washed 3 times with one volume of Et_2O [27]. The sample was sonicated in a *Bransonic-220* sonicator (4 h, 4°) and centrifuged 60 min at 12000 rpm. DNA was precipitated by addition of 0.1 volume of 2.5M NaOAc and 2.5 volumes of EtOH. After centrifugation, the precipitate was washed with 90% EtOH and redissolved in sodium cacodylate buffer. The extraction and precipitation procedure was repeated until the T_m of DNA was constant and independent of DNA concentration.

Agarose-gel electrophoresis (2%) in *Tris*-acetate-EDTA buffer (50, 20, and 1 mM, resp.) in the presence of HindIII/λ molecular-weight marker (*Sigma*) showed the average length of the DNA fragments obtained to be between 500 and 700 base pairs. The concentrations of the polynucleotides in mol of base pairs per litre were obtained by measurement of the absorbance at 260 nm using molar absorption coefficients of 13200 for calf-thymus DNA and poly(dA-dT)·poly(dA-dT) [28] and 16800 for poly(dG-dC)·poly(dG-dC) [29].

Electronic Absorption and Emission Measurements. The samples were prepared by addition of aliquots of a DNA-containing buffered soln. to the helicate in soln. in 25 mM *Tris*-HCl, pH 7.8, containing 33% MeOH. Absorption spectra: at 25°; *Cary-3* spectrophotometer (*Varian*); correction by multiplication by the dilution factor due to the addition of the DNA soln. Emission spectra: *SLM/Aminco* spectrofluorimeter; excitation wavelength centered at 450 nm; spectra recorded between 400 and 800 nm.

Thermal Denaturation. Absorbance measurements were carried out on a *Cary-3* UV/VIS spectrophotometer equipped with a *Peltier*-effect thermostated six-cell holder (*Varian*) in the double-beam mode with no sample in the reference beam. Stock solns. of polynucleotides, ligands, buffer, and bidistilled H_2O were combined to obtain the final concentrations 5 mM sodium cacodylate and 10 mM NaCl with an initial absorption of *ca.* 0.6 for calf-thymus DNA, 0.3 for poly(dA-dT)·poly(dA-dT), and 0.3 for poly(dG-dC)·poly(dG-dC). The sample volume was 1.1 ml. The quartz cuvettes were sealed with tight-fitting *Teflon* stoppers. A temp. probe connected to the thermostated cell holder was placed in a reference cell containing bidistilled H_2O. The spectrophotometer and the variable-temperature accessory were driven by the *Cary 13E* software (*Varian*) on an *EpsonAX2e* personal computer. After 15 min stabilization at the starting temp., the temp. of the soln. was raised linearly by 0.5°/min. The values were not corrected for thermal expansion. The T_m of the different melting curves was calculated after smoothing using the first-derivative method (software: *H. Appel, Varian*, Germany).

Electrophoretic-Mobility-Shift Assays. Electrophoretic-mobility-shift assays were effected on the pBR322 plasmid fragment situated between the restriction sites of enzymes SspI and EcoRV (191 base pairs) [18]. This fragment was labeled on the 5′-end with ^{32}P and purified by PAGE according to described methods [30]. Product sequence was verified by sequencing according to the *Maxam* and *Gilbert* method [31]. Buffer (100 mM *Tris*, 500 mM NaCl, pH 7.4), helicates (in 5% MeOH/H_2O), and DNA solns. were combined to obtain the desired concentrations in a 10 µl volume. The mixture was incubated 15 min at 25° before loading on the gel. The electrophoresis was performed on a 7.5% polyacrylamide gel (acryl/bis 29:1) in 10 mM *Tris*, and 50 mM NaCl, pH 7.4. The electrophoresis was run at a constant voltage of 150 V for 45 min. Autoradiograms were obtained on *Kodak-XAR5* film after drying and exposing at −20° using *DuPont* intensifying screens.

pBR322 Restriction Enzymes Cleavage Assays. pBR322 Cleavage reactions were run under the following conditions: solns. of pBR322, helicate, and buffer were mixed at 4° to obtain the final concentrations 7 µM pBR322 (bp), 50 mM NaCl, 10 mM *Tris*-HCl, 10 mM $MgCl_2$, and 1 mM DTT, pH 7.6, in a 10 µl volume. To this soln. were then added 5 units of either SspI or EcoRV. The mixture was incubated 1 h at 37°. After addition of 10 µl of loading buffer (40 mM *Tris*-acetate, 10 µM EDTA, 20% glycerol, 0.1% bromophenol blue), the product was analyzed on a 1.0% agarose gel (*U* 50 V, 45 min; migration buffer: 40 mM *Tris*-acetate, 10 mM EDTA). The gels were stained with ethidium bromide and photographed under illumination at 254 nm.

Photocleavage of pBR322 by Helicates. Stock solns. and bidistilled H_2O were combined to obtain 20 µl samples of a soln. containing the desired helicate concentration in 3.5 µM pBR322 (bp), 5 mM sodium cacodylate, and 10 mM NaCl at pH 7.4. The solns. were incubated in the dark during 10 min and then irradiated during 60 min using a 1000 W Xe lamp (*Müller*, München) fitted with a H_2O/*Pyrex* filter. The solns. were maintained at 4° during irradiation. Then 4 µl of loading buffer (40 mM *Tris*-acetate, 10 mM EDTA, 20% glycerol, 0.1% bromophenol blue) were added to the samples, and the products were analyzed on a 1.0% agarose gel (*U* 60 V, 45 min; migration buffer: 40 mM *Tris*-acetate, 10 mM EDTA). The gels were stained with ethidium bromide and photographed under illumination at 254 nm.

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