Acridine-based macrocycles as potential probes for studies of the structures of nucleic acids

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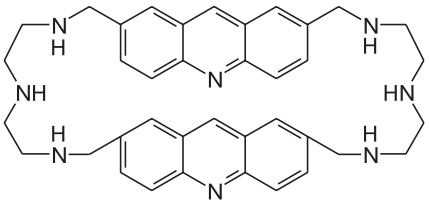
The possibility of the use of bis- and tris-acridine macrocycles as photoactivated reagents, which are sensitive to the DNA and RNA secondary structures, was examined. The bis- and tris-acridine macrocycles can induce photomodification (in the case of DNA) and photocleavage (in the case of RNA). Both processes occur predominantly at the purine residues located in loops or at the junction of the RNA and DNA hairpins.

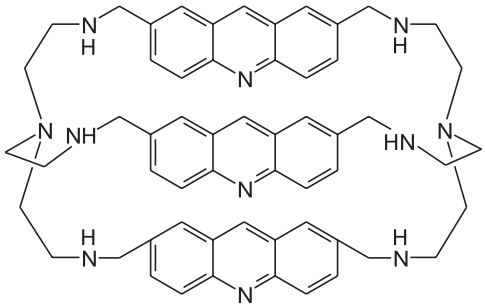
Key words: acridine-based macrocycles, photochemical modification of RNA, RNA structure.

Natural RNA have characteristic three-dimensional structures formed through hydrogen bonds between heterocyclic bases, staking interactions, and coordination of metal ions by heterocyclic bases, ribose, and phosphate residues of RNA. Heterocyclic bases in RNA can form both Watson—Crick and Hoogsteen hydrogen bonds. The involvement of bases into the above-mentioned interactions results in the fact that bases located in different regions of RNA are differently accessible to chemical reagents and, as a consequence, exhibit different reactivities. Studies of the reactivities of the nucleotide residues of RNA form the basis for the chemical modification method, which enables one to obtain information on the RNA structure from the data on the reactivities of constituent nucleotides. Investigations of the RNA structures are carried out with the use of such reagents, as dimethyl sulfate, which alkylates adenine and cytosine that are not involved in the formation of Watson—Crick pairs, the guanine bases whose N(7) atom is not involved in any interactions, and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide *p*-toluenesulfonate (CMCT), which reacts with guanine and uridine that are not involved in complementary interactions. A number of other reagents used in studies of the RNA structures were surveyed in the review.[1] The construction of probes for investigations of the DNA and RNA structures, which could enable one to register particular motifs in the structures of nucleic acids, is a topical problem. A number of chemical compounds, which react preferentially with particular elements of hairpin structures of nucleic acids, are presently available. For example, macrocyclic Ni^{II} complexes selectively react with the guanine residues located in loops.[2] Intercalating molecules, such as ethidium bromide and actinomycin D, and the methidium-propyl-EDTA-Fe^{2+} complex are bound predominantly to double-stranded regions of the molecules.[3]

Previously,[4—6] it has been demonstrated that macrocycles synthesized based on acridine, are bound to single- and double-stranded sequences of nucleic acids with different efficiency.



Mc1



Mc2

The structural features of cyclo-bis-intercalating molecules containing two acridine subunits, which are connected *via* the diethylenetriamine linkers, retard their

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interactions with the bases located in double-stranded regions of nucleic acids but do not hinder their attachment to the bases in single-stranded sequences. The ability of bis- and polyintercalators, including bis-acridine, to form complexes with various nucleotides and nucleosides, was examined in the study.[4] It appeared that bis-acridine exhibits high affinity to purine nucleotides, particularly, to the adenosine residues. In DNA, preferential binding occurs with bases located in hairpin loops rather than with those present in unstructured single-stranded regions.[5]

Previously,[7] we have studies the interaction of the bis-acridine macrocycle with the DNA hairpin by fluorometric titration. The fluorescence intensity of the bis-acridine macrocycle bound to structured DNA correlates with the size of the hairpin loop.

In the present study, we examined the feasibility of the use of bis- and tris-acridine macrocycles as photoactivated reagents sensitive to the secondary structure of DNA and RNA molecules. In the case of DNA, acridine-based macrocycles induce photomodification of the purine bases. In the case of RNA, these macrocycles cause photocleavage at the phosphodiester bonds, which are formed by purine nucleotides located in loops or at the junction of the RNA hairpin.

Experimental

Acrylamide, *N*,*N*′-methylenebisacrylamide, tetramethylethylenediamine (TEMED), Bromophenol Blue, xylenecyanol, and formamide were purchased from Sigma (USA); tris(hydroxymethyl)aminomethane was from Merck (Germany); [γ-^{32}P]ATP and 5′-[^{32}P]pCp with a specific activity of ~4000 Ci mmol^{-1} were purchased from Biosan (Russia).

The buffer solutions were prepared with the use of water purified on a Milli Q system (MilliPore, USA).

Deoxyribooligonucleotide Bc corresponding to the hairpin fragment of DNA, which is involved in retrotransposon 1731 *Drosophila* was purchased from Eurogentec. Acridine-based macrocycles were kindly supplied by Prof. J.-M. Lehn (Louis Pasteur University, Strasbourg, France). The plasmid for the the *in vitro* transcript of tRNA-like TYMV RNA (mini-tRNA) was kindly provided by Prof. R. Giege, and yeast tRNA^{Phe} was supplied by G. Keith (Institute of Molecular and Cellular Biology, Strasbourg, France).

The [^{32}P] label was introduced at the 5′-terminus of the DNA target according to a procedure described previously[8] and the [^{32}P] labeling of RNA was performed according to a known procedure.[9]

Photomodification of a DNA target. 5′-[^{32}P]-Labeled Bc ($5 \cdot 10^{-6}$ mol L^{-1}) was incubated with a macrocycle ($5 \cdot 10^{-5}$ mol L^{-1}) in a 6 *mM* Na_2HPO_4 solution at pH 7.0 containing 0.2 *M* NaCl and 0.02 *mM* EDTA at 20 °C for 16 h. Irradiation of Bc in the presence and absence of the bis-acridine and tris-acridine macrocycles (**Mc1** and **Mc2**, respectively) was carried out with condensed light using a DRK-120 mercury lamp of an OI-18A illuminator (LOMO, St. Petersburg) equipped with a BS7 glass light filter (λ_{max} — 365 nm, W = 11.5 mW cm^{-2}) at 20 °C for 20 min. After irradiation, DNA was precipitated with ethanol in the presence of a 0.3 *M* AcONa solution at pH 5.8 and a DNA carrier (1 mg mL^{-1}). The DNA precipitate was dissolved in water and treated with 1 *M* piperidine at 100 °C for 10 min followed by reprecipitation with EtOH in the presence of a DNA carrier. The cleavage products were analyzed by electrophoresis in 20% polyacrylamide gel under denaturating conditions.

Photocleavage of an RNA target. 3′-[^{32}P]-Labeled RNA ($5 \cdot 10^{-7}$ mol L^{-1}) was incubated with the macrocycle **Mc1** or **Mc2** (10^{-5} mol L^{-1}) in a 6 *mM* Na_2HPO_4 solution at pH 7.0 containing 0.2 *M* NaCl and 0.02 *mM* EDTA at 20 °C for 16 h. Irradiation of RNA in the absence or presence of **Mc1** and **Mc2** was performed at 20 °C for 30 min analogously to irradiation of the DNA target. Then the reaction mixture was successively extracted with water-saturated phenol and Et_2O, and RNA was precipitated with ethanol in the presence of 0.3 *M* AcONa (pH 5.5) and an RNA carrier (100 μg mL^{-1}). The products of RNA cleavage were analyzed by electrophoresis in 12% polyacrylamide gel under denaturating conditions. To assign the hydrolysis products, an alkaline hydrolyzate prepared under the standard conditions was analyzed in parallel.

Results and Discussion

In the present study, we used a hairpin structure involved in the long terminal repetition of retrotransposon 1731 *Drosophila melanogaster* consisting of 40 nucleotides (Bc) as a model DNA. According to the results of the study,[10] this DNA fragment represents a region of binding of the regulatory protein NssBF. Previously, it has been demonstrated that the bis-acridine macrocycle **Mc1** can be bound to the bases located in the loop of this hairpin.[7]

The DNA target (Bc) was irradiated with UV light after its preincubation in the presence of the macrocycles **Mc1** or **Mc2** for 16 h. Under these conditions, the stable DNA—macrocycle complex was formed.* Irradiation of DNA, which was preincubated with the macrocycle, with UV light afforded photomodification products of the unknown nature. Subsequent treatment of DNA, which was irradiated in the presence of **Mc1** or **Mc2**, with piperidine led to DNA cleavage at the modified bases, which made it possible to determine the positional direction of the photomodification (Fig. 1, *a*). The piperidine treatment of native 5′-[^{32}P]-DNA as well as of DNA irradiated in the absence of the macrocycles did not afford DNA degradation products, whereas the piperidine treatment of the DNA target photomodified under the action of the macrocycles led to the cleavage of Bc DNA at the sites G15, G13, and G9. The maximum total degree of photomodification of DNA isolated in the course of piperidine-dependent cleavage (~10%) was achieved upon irradiation at 20 °C for 20 min. The ar-

* A. Slama-Schwok, unpublished data.

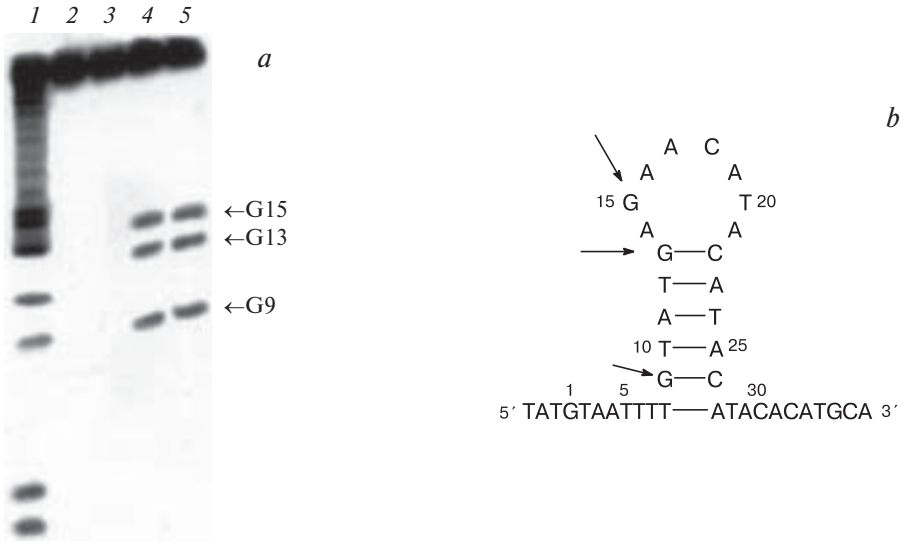

Fig. 1. *a*. Electrophoresis analysis of the piperidine-dependent cleavage products of the 5′-[^{32}P]-Bc deoxyribooligonucleotide irradiated in the presence of the macrocycles **Mc1** and **Mc2**: *1*, the A+G reaction; *2*, Bc incubated without reagents and irradiation; *3*, Bc irradiated in the absence of the macrocycles; *4*, Bc irradiated in the presence of $5 \cdot 10^{-5}$ mol L^{-1} of **Mc1**; *5*, Bc irradiated in the presence of $5 \cdot 10^{-5}$ mol L^{-1} of **Mc2**. *b*. The schematic representation of the cleavage sites in the Bc DNA molecule in the presence of the macrocycles.

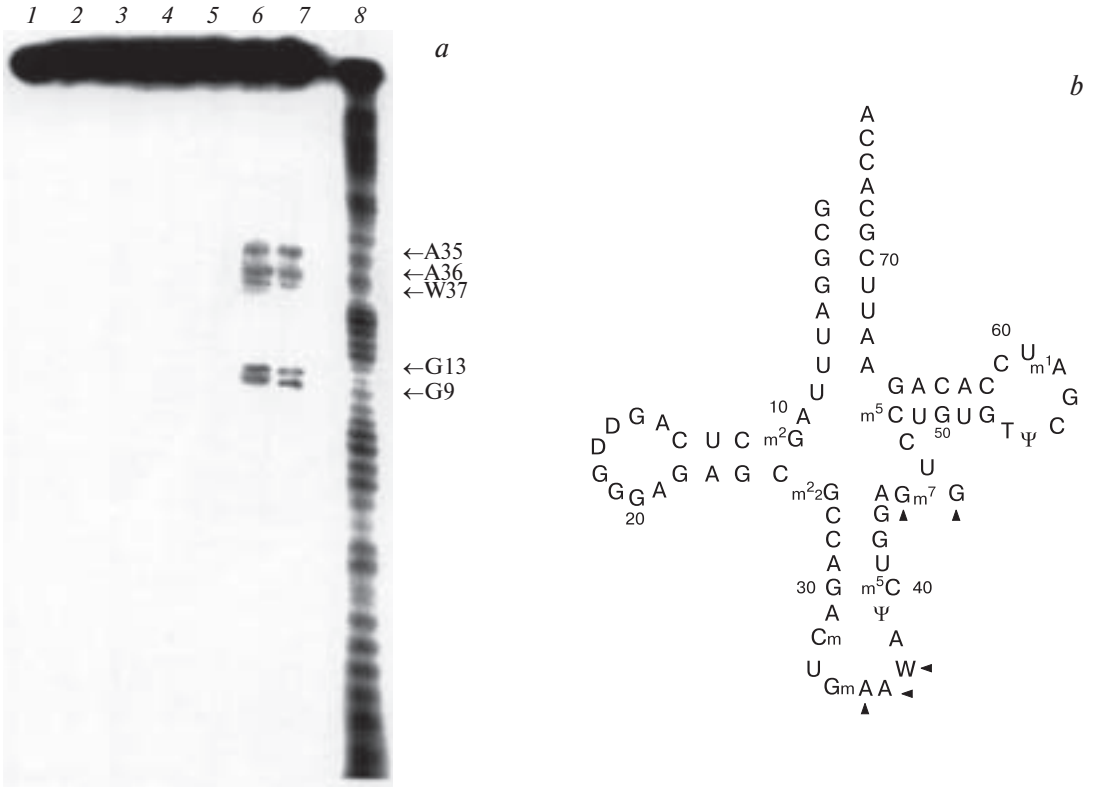

Fig. 2. *a*. Electrophoresis analysis of the cleavage products of 3′-[^{32}P]-tRNA^{Phe} irradiated in the presence of the macrocycles **Mc1** and **Mc2**: *1*, tRNA^{Phe} incubated without reagents and irradiation; *2*, tRNA^{Phe} irradiated in the absence of the macrocycles; *3*—*7*, tRNA^{Phe} extracted with water-saturated phenol and ether: *3*, tRNA^{Phe} irradiated in the absence of the macrocycles; *4*, tRNA^{Phe} preincubated in the presence of **Mc1**; *5*, tRNA^{Phe} preincubated in the presence of **Mc2**; *6*, tRNA^{Phe} irradiated in the presence of **Mc1**; *7*, tRNA^{Phe} irradiated in the presence of **Mc2**; *8*, partial hydrolyzate of tRNA^{Phe} under alkaline conditions. *b*. The schematic representation of the cleavage sites in the yeast tRNA^{Phe} molecule in the presence of the macrocycles.

rangement of the cleavage sites in the Bc DNA molecule is shown in Fig. 1, *b*. It can be seen that the photomodification proceeded at the residue G15 located in the loop of the Bc hairpin and at the residues G9 and G13 located at the junction of the DNA hairpin. This pattern of the piperidine-dependent cleavage can be attributed to high sensitivity of the G bases to photomodification under the action of the acridine-based macrocycles as well as with respect to the three-dimensional structure of the DNA target. The observed G specificity is consistent with the mechanism of the electron transfer described previously. According to this mechanism, the guanine residues of the DNA target can serve as electron donors with respect to acridine in the photoexcited singlet state. Hence, only the guanine residues can be involved in the electron transfer to photoexcited acridine due to their low redox potential. Subsequently, the resulting radical cation undergoes a series of conversions (for example, it is oxidized with oxygen present in the reaction mixture) into different products, which can be cleaved with piperidine.[11–13] It should be noted that the observed photomodification of DNA was not accompanied by the formation of covalent adducts with the macrocycle as evidence by the fact that no changes in electrophoretic mobiliy of DNA were observed under the conditions used after irradiation of the DNA complex with the macrocycle.

Yeast $tRNA^{Phe}$, whose secondary and tertiary structure was well studied and which contains elements characteristic of all natural RNAs (D, TΨC, and anticodon hairpins, double- and single-stranded fragments),[14,15] and the *in vitro* transcript of tRNA-like TYMV RNA (mini-tRNA), whose structure is a pseudoknot,[16,17] were used as RNA targets. The results of analysis of the products of photoinduced cleavage of yeast $tRNA^{Phe}$ under the action of the macrocycles are presented in Fig. 2, *a*. It can be seen that irradiation of RNA in the absence of the macrocycles, extraction of RNA with phenol or ether performed with the aim of removing the macrocycles from the samples before electrophoresis analysis, or pre-

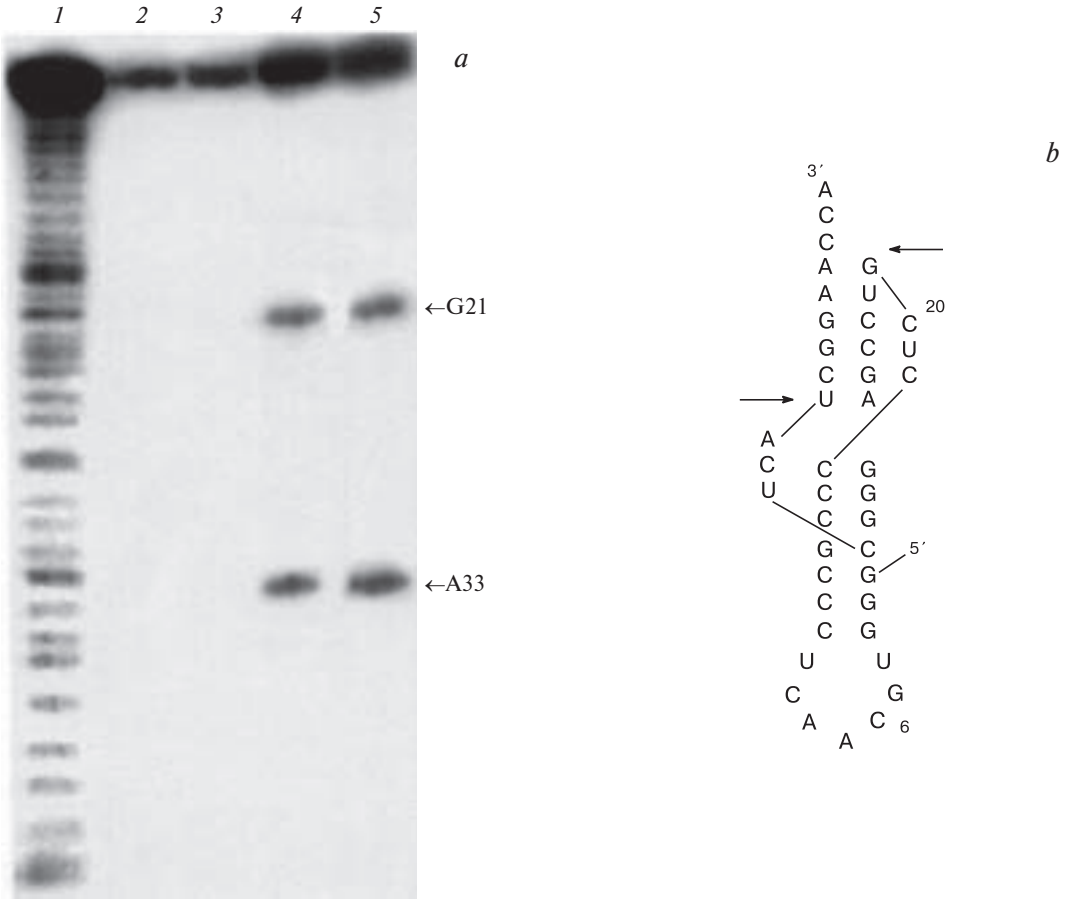

Fig. 3. *a*. Electrophoresis analysis of the cleavage products of the 3′-[^{32}P]-*in vitro* transcript of tRNA-like TYMV RNA irradiated in the presence of **Mc1** and **Mc2**: *1*, the partial hydrolyzate of RNA under alkaline conditions; *2*, incubation of RNA without reagents and irradiation; *3*, RNA irradiated in the absence of the macrocycles; *4*, RNA irradiated in the presence of **Mc1**; *5*, RNA irradiated in the presence of **Mc2**. *b*. The schematic representation of the cleavage sites in the molecule of the *in vitro* transcript of tRNA-like TYMV RNA in the presence of the macrocycles.

incubation of RNA with the macrocycles without subsequent irradiation did not lead to the cleavage of the RNA target. Accumulation of photocleavage products was observed only upon UV irradiation of RNA incubated with **Mc1** or **Mc2** for 16 h, which is necessary for binding to RNA.* The photocleavage of $tRNA^{Phe}$ most efficiently proceeded at the purine residues located in the variable (G46 and G45) and anticodon (W37, A36, and A35) loops (Fig. 2, *b*). This hydrolysis pattern results from the tertiary structure of the molecule. Thus, it is known that the bases of the D and TΨC loops are involved in the formation of the tertiary structure of $tRNA^{Phe}$ and, consequently, are less accessible to binding with the macrocycles, whereas the bases in the variable and anticodon loops remain accessible to reactions with chemical reagents. Hence, it can be said that the acridine-based macrocycles possess photoribonuclease activity. Both macrocycles hydrolyze the model RNA with approximately equal efficiency. The maximum degree of hydrolysis (~10%) was achieved upon irradiation of RNA at 20 °C for 30 min in the presence of the macrocycles in the concentration of $5 \cdot 10^{-5}$ mol L^{-1}.

Using the *in vitro* transcript of tRNA-like TYMV RNA (mini-tRNA) as an example, we carried out experiments on the determination of the cleavage specificity of the pseudoknots based on the photonuclease activity of the macrocycles. The photoinduced cleavage of mini-tRNA in the presence of **Mc1** or **Mc2** occurred only at two residues, *viz.*, G21 and A33 (Fig. 3), located at the junction of the RNA and DNA hairpins. These regions in the pseudoknots have anomalous three-dimensional structures, which differ from those of the classical single- or double-stranded regions.[16,17]

Hence, the bis- and tris-acridine macrocycles can induce hydrolytic DNA and RNA cleavage. Photomodification and photocleavage reactions proceed predominantly at the purine residues (G in the case of DNA; G, A, and W in the case of RNA) located either in loops or at the junction of the RNA and DNA hairpins. The results of the present study provide evidence that these macrocycles are the potential tool in studies of the DNA and RNA secondary structures in solutions.

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* A. Slama-Schwok, an original procedure.