DOI: 10.1002/cbic.200800235

Photocontrol of Genomic DNA Conformation by Using a Photosensitive Gemini Surfactant: Binding Affinity versus Reversibility

Marie Geoffroy,[a] Delphine Faure,[b] Reiko Oda,[c] Dario M. Bassani,[b] and Damien Baigl*[a]

In nature, genomic DNAs are long molecules that are compacted to fit within narrow spaces such as viral capsids or nuclei in eukaryotic cells. Moreover, it has been well established that gene expression is strongly affected by the higher-order structure of DNA or chromatin.[1] Several strategies have thus been developed to control DNA conformation and compaction in vitro. Most of them rely on the use of oppositely charged compounds to neutralize DNA negative charge (for example, polyamines, surfactants, polymers, nanoparticles).[2] Recently, Le Ny and Lee proposed a new methodology to control the conformation of genomic DNA by light[3] and we applied this system to the control of the DNA conformation at the single-molecule level inside cell-mimicking microenvironments.[4] This methodology consists of adding azobenzene trimethylammonium bromide (AzoTAB) to the DNA solution. AzoTAB is a cationic surfactant, which undergoes a *trans* to *cis* isomerization at 365 nm accompanied by a change of polarity. Consequently, there exists an AzoTAB concentration range for which genomic DNA is compacted under dark conditions but unfolded under UV illumination at 365 nm. However, the affinity of AzoTAB for DNA is very low, that is, a very high concentration of AzoTAB is needed to compact DNA. Herein we used for the first time a photosensitive gemini surfactant (AzoGEM)[5] to control DNA conformation by light. We found that AzoGEM interacts strongly with DNA, allowing photocontrol of conformation at a very low AzoGEM concentration. Contrary to AzoTAB, the photocontrol of DNA conformation provided by AzoGEM is not reversible in the presence of DNA.

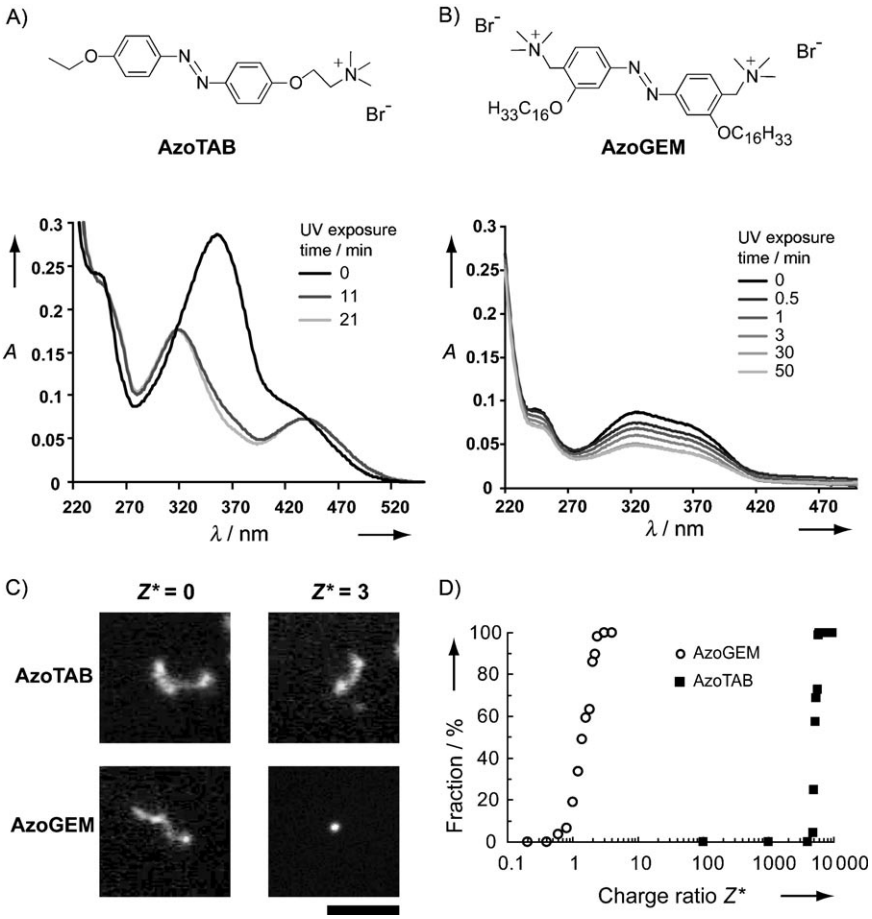

Figure 1. Molecular structure and UV/Vis absorption spectra for different UV exposure times of A) azobenzene trimethylammonium bromide (AzoTAB, 30 μM in water) and B) an azobenzene-derived gemini surfactant (AzoGEM, 10 μM in water). C) Typical fluorescence microscopy images of individual T4 DNA molecules labeled by YOYO-1 dye in the presence of AzoTAB or AzoGEM for two surfactant/DNA charge ratios Z^*. Scale bar = 5 μm. D) Fraction of individual DNA molecules in the compact state as a function of Z^* for AzoGEM and AzoTAB.

Figure 1 shows the interaction between the two photosensitive surfactants used in this study and T4 DNA (166 kb). On one hand, AzoTAB (Figure 1 A) is a photosensitive surfactant with a single ammonium group as a polar head and a short hydrophobic tail containing an azobenzene group. Under UV exposure at 365 nm in aqueous solution, it undergoes a *trans* (λ_{max} = 356 nm) to *cis* (λ_{max} = 318 nm) isomerization occurring in a few minutes (Figure 1 A). On the other hand, AzoGEM surfac-

[a] *M. Geoffroy, Dr. D. Baigl*
Department of Chemistry, Ecole Normale Supérieure
24 rue Lhomond, 75005 Paris (France)
Fax: (+33) 1-4432-2402
E-mail: damien.baigl@ens.fr

[b] *D. Faure, Dr. D. M. Bassani*
Institut des Sciences Moléculaires, CNRS UMR 5255
33405 Talence (France)

[c] *Dr. R. Oda*
CNRS UMR CBMN-5428, Institut Européen de Chimie et Biologie
2 rue Robert Escarpit, 33607 Pessac (France)

tant (Firgure 1B) presents two ammonium groups and two long hydrophobic tails separated by an azobenzene group. Under UV exposure at 365 nm in aqueous solution, the *trans* to *cis* isomerization is slower than that of AzoTAB and a stable composition is reached after approximately 30 min. It should be mentioned that UV/Vis spectra for AzoTAB were taken much below the critical micellar concentration (CMC ~ 10 mM) whereas those for AzoGEM were taken above CMC (~2.5 μM) to get enough absorbance. In the latter case, the presence of micelles can affect the absorbance properties. By using fluorescence microscopy (FM) we characterized the conformation of individual DNA molecules in the presence of AzoTAB or AzoGEM. To compare the efficiency of the two surfactants, we used the parameter Z^* defined as the charge ratio between surfactant and DNA (Z^* = [AzoTAB]/[DNA] or 2*[AzoGEM]/[DNA] where [AzoTAB], [AzoGEM], and [DNA] represent the concentrations of AzoTAB, AzoGEM, and DNA in nucleotides, respectively). Figure 1C shows typical fluorescence images of individual DNA molecules for $Z^*=0$ and $Z^*=3$. In the absence of surfactant ($Z^*=0$), DNA is in the typical elongated coil state characterized by intramolecular fluctuations and a slow translational diffusion. After the addition of a small concentration of surfactant ($Z^*=3$), all DNA molecules remain in the coil state with AzoTAB whereas they are in the fully compact state with AzoGEM. By using FM, we analyzed systematically the conformation of a large number of DNA molecules and calculated the fraction of molecules in the full compact state as a function of Z^* (compaction curve). Figure 1D shows that AzoGEM interacts much more strongly with DNA than AzoTAB does. Whereas the transition for AzoGEM occurs at $Z^*=1$–2, it is in the range $Z^*=5000$–6000 for AzoTAB. It has been well established that the folding transition of single-chain DNA by purely electrostatic interactions occurs when DNA total charge is neutralized by 88–89%.[6] This neutralization is mainly driven by the counter-ion condensation on the DNA chain and ionic exchange between DNA counter ions and cationic ions in the medium. As the neutralization rate increases with the counter-ion valency (e.g., according to the Manning–Oosawa condensation theory), from a purely electrostatic point of view, AzoGEM (divalent) is more efficient than AzoTAB (monovalent) to neutralize and therefore compact DNA. Furthermore, both AzoGEM and AzoTAB contain a hydrophobic tail. It is thus energetically favorable to exchange the highly soluble DNA counter ions by AzoGEM or AzoTAB. The binding of surfactants to DNA is thus accompanied by a hydrophobization of the chain itself, which becomes less water soluble and more prone to folding even at low neutralization. As the apolar part of AzoGEM is much more hydrophobic than that of AzoTAB, for a given concentration of surfactant, AzoGEM binds much more strongly to DNA and for a given binding rate AzoGEM induces more easily the folding of DNA. We thus conclude that the two effects act simultaneously and result in a much stronger activity for AzoGEM than AzoTAB to compact DNA.

Then we studied the possibility to control DNA conformation by light using AzoGEM. AzoGEM was exposed under UV for 50 min prior to DNA introduction. Figure 2A shows the compaction curves for AzoGEM initially exposed to UV (◇) or without UV exposure (○). There is a clear difference in the DNA

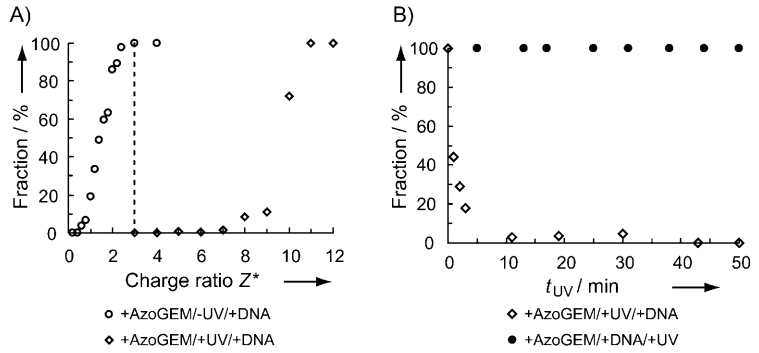

Figure 2. Fraction of individual DNA molecules in the compact state in the presence of AzoGEM. A) Effect of AzoGEM/DNA charge ratio Z^* in the absence (○) and in the presence (◇) of UV illumination. UV was applied for 50 min before DNA introduction. The dashed line indicates $Z^*=3$. B) Effect of UV illumination time t_{UV} at $Z^*=3$ for UV applied before (◇) or after (●) DNA introduction.

compaction activity of AzoGEM depending on light treatment. More AzoGEM is needed to compact DNA if AzoGEM has been exposed to UV prior to interaction with DNA. This can be interpreted as a consequence of the photoisomerization of AzoGEM under UV. Molecular modeling of the *trans* and *cis* isomers indicates that the relative area occupied by the polar head groups with respect to the nonpolar alkyl chains is smaller for the *trans* form.[5] This suggests that the *trans* isomer is more hydrophobic than the *cis* isomer, which can result in a stronger activity of the *trans* isomer to compact DNA. Figure 2A also shows that there is an AzoGEM concentration range ($Z^*=2$–7) for which the fraction of DNA molecules in compact state is 100% for AzoGEM without UV exposure and less than 2% when AzoGEM was exposed to UV for 50 min prior to interaction with DNA. It is thus interesting to study the possibility to go from one state to the other by varying the UV exposure time at a fixed AzoGEM concentration. Figure 2B shows the fraction of DNA molecules in the compact state as a function of UV illumination time t_{UV} for $Z^*=3$ (dashed line in Figure 2A). When AzoGEM was exposed to UV before DNA addition (◇), the fraction of DNA molecules in the compact state decreased markedly with an increase in UV illumination time, from 100% at $t_{UV}=0$ min to less than 3% at $t_{UV}=11$ min, and no compact DNA molecules were observed after $t_{UV}=40$ min. It can be interpreted as the consequence of the progressive decrease of the ratio between *trans* and *cis* isomers in the bulk solution as shown by UV/Vis spectra (Figure 1B). In contrast, if DNA was first compacted by AzoGEM and then exposed to UV, it was impossible to unfold DNA, even after 50 min of illumina-

tion time (black disks). This shows that DNA compaction might affect the *trans* to *cis* isomerization of AzoGEM with UV, which is the trigger of DNA unfolding.

Finally we studied the possibility of unfolding compact DNA by UV light for AzoGEM and AzoTAB photosensitive surfactants. Figure 3A shows typical FM images of individual DNA molecules initially in the elongated coil state ($Z^*=0$) and then in the compact state after addition of AzoTAB ($Z^*=6000$) or AzoGEM ($Z^*=3$). These charge ratios were chosen so that all DNA molecules were in a compact state without UV but unfolded when the surfactant was exposed to UV for 50 min before DNA introduction. Figure 3A shows that when UV was applied to DNA in the compact state, DNA progressively unfolds with AzoTAB whereas it stays in a compact state with AzoGEM. Under the conditions shown in Figure 3A, we systematically analyzed the effect of UV exposure time on the fraction of DNA in a compact state for a large number of DNA molecules. Figure 3B shows that DNA unfolds only in the case of AzoTAB for which the fraction of DNA molecules in the compact state decreases to approximately 20% for $t_{UV}=50$ min, which is in agreement with previous studies.[4] It seems that there is a direct correlation between the reversibility of the DNA folding transition and strength of molecular interaction between DNA and the photosensitive binding molecule. AzoTAB is a weak binder; a very large concentration of AzoTAB is necessary to compact DNA ($Z^*\sim5000$–6000, Figure 1D), which suggests that most AzoTAB molecules remain in the bulk solution after DNA compaction. Under UV illumination, bulk AzoTAB molecules isomerize into the *cis* form (Figure 1A) and can dynamically exchange with AzoTAB bound to DNA (*trans* or *cis* form). In contrast, AzoGEM interacts very strongly with DNA even at a very low concentration (DNA compaction at $Z^*\sim1$–2, Figure 1D), which means that most of AzoGEM molecules are probably bound to DNA during the compaction process. The fraction of AzoGEM in bulk solution, which isomerizes into the *cis* form (Figure 1B) under UV, is probably too low to enable DNA unfolding by ion exchange. Moreover, compacted DNA molecules are very dense condensates with a typical diameter of 100–150 nm.[2b,6b] Therefore, we can hypothesize that AzoGEM bound into compact DNA cannot fully photoisomerize into the *cis* form, which prevents from DNA unfolding even with a long UV illumination time.

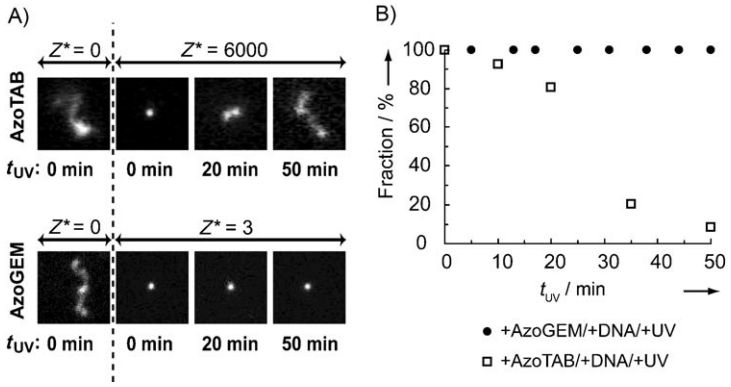

Figure 3. Effect of UV illumination time t_{UV} on DNA (0.1 μM in 10 mM Tris-HCl) initially compacted by AzoTAB ($Z^*=6000$) or AzoGEM ($Z^*=3$). UV was applied after DNA compaction. A) Typical fluorescence microscopy images of individual DNA molecules. Each image has a size 5 μm × 5 μm. B) Fraction of DNA molecules in the compact state for AzoTAB (□) and AzoGEM (●).

In this communication, we have demonstrated that a photosensitive gemini surfactant can be used to control the conformation of individual genomic DNA molecules at a surfactant-to-DNA charge ratio of near unity, which is three orders of magnitude smaller than that for the systems reported up to now. We found that this very strong interaction with DNA hindered DNA unfolding by light, whereas a weak DNA–surfactant interaction allowed one to unfold DNA with light. The compromise between sufficiently high binding activity and the possibility of unfolding compact DNA by light will open a route to the in vitro and in vivo photoswitching of DNA conformation and gene activity by a minimally invasive chemical perturbation.

Experimental Section

Materials: Bacteriophage T4 DNA (166 kb) was from Wako Chemicals, YOYO-1 iodide was from Molecular Probes. All other chemicals were purchased from Sigma. Deionized water (Millipore, 18 MΩ cm^{-1}) was used for all experiments.

AzoTAB synthesis: Azobenzene trimethylammonium bromide (AzoTAB) synthesis was adapted from the method described by Hayashita et al.[7]

AzoGEM synthesis: The photosensitive gemini surfactant (AzoGEM) was synthesized according to a procedure described elsewhere.[5]

Preparation of DNA samples: Water, Tris-HCl buffer, YOYO-1 iodide, and photosensitive surfactant were mixed in this order prior to careful T4 DNA introduction (under low shear conditions to avoid DNA breakage). For all experiments, we used T4 DNA at a final concentration of 0.1 μM (concentration in nucleotides) in Tris-HCl buffer (10 mM, pH 7.4) with YOYO (0.01 μM) as a DNA fluorescent dye. For all steps except UV illuminations, DNA samples were kept under dark conditions. Samples were equilibrated for 15 min prior to DNA characterization by fluorescence microscopy. All experiments were performed at room temperature.

UV illumination: UV exposure was performed by placing the sample at 6 cm distance from an 8W UVLMS-38 UV lamp (UVP, Upland, CA) working at 365 nm. Immediately following UV illumination and before further characterization, the sample was cooled to room temperature by using an ice bath.

Fluorescence microscopy (FM): Fluorescence microscopy was performed on an Axiovert 200 inverted microscope (Carl Zeiss), equipped with a 100x oil-immersed objective lens. Images were acquired by using a highly sensitive EM-CCD camera (Photonmax 512B, Princeton Scientific) and Metavue image acquisition software (Molecular Devices). DNA molecules were observed in 20 μL microdroplets deposited on a clean glass cover slide. For each condition, a minimum of 200 individual DNA molecules were characterized to determine the fraction of molecules in the compact state.

UV/Vis absorption: UV/Vis absorption spectra were acquired at 20 °C in 2×10 mm quartz cells (Hellma) by using a double beam

UV/Vis spectrophotometer (Uvikon 941, Kontron instruments). Concentrations were 30 μM and 10 μM for AzoTAB and AzoGEM, respectively. Water was used as a solvent for both molecules.

Acknowledgements

We thank Prof. M. Sollogoub, S. Guieu, and E. Lepeltier for help in the AzoTAB synthesis, spectral analyses, and fruitful discussions.

Keywords: compaction · DNA · gemini surfactant · photocontrol · unfolding

[1] a) B. Dorigo, T. Schalch, A. Kylangara, S. Duda, R. R. Schroeder, T. J. Richmond, *Science* **2004**, *306*, 1571–1573; b) N. J. Francis, R. E. Kingston, C. L. Woodcock, *Science* **2004**, *306*, 1574–1577.

[2] a) V. A. Bloomfield, *Curr. Opin. Struct. Biol.* **1996**, *6*, 334–341; b) L. C. Gosule, J. A. Schellman, *Nature* **1976**, *259*, 333–335; c) A. A. Zinchenko, K. Yoshikawa, D. Baigl, *Adv. Mater.* **2005**, *17*, 2820–2823; d) S. M. Mel'nikov, V. G. Sergeyev, K. Yoshikawa, *J. Am. Chem. Soc.* **1995**, *117*, 2401–2408; e) W.-H. Huang, A. A. Zinchenko, C. Pawlak, Y. Chen, D. Baigl, *ChemBioChem* **2007**, *8*, 1771–1774; f) A. A. Zinchenko, K. Yoshikawa, D. Baigl, *Phys. Rev. Lett.* **2005**, *95*, 228101; g) A. A. Zinchenko, T. Sakaue, S. Araki, K. Yoshikawa, D. Baigl, *J. Phys. Chem. B* **2007**, *111*, 3019–3031.

[3] A.-L. M. Le Ny, C. T. Lee, Jr., *J. Am. Chem. Soc.* **2006**, *128*, 6400–6408.

[4] M. Sollogoub, S. Guieu, M. Geoffroy, A. Yamada, A. Estévez-Torres, K. Yoshikawa, D. Baigl, *ChemBioChem* **2008**, *9*, 1201–1206.

[5] D. Faure, J. Gravier, T. Labrot, B. Desbat, R. Oda, D. M. Bassani, *Chem. Commun.* **2005**, 1167–1169.

[6] a) R. W. Wilson, V. A. Bloomfield, *Biochemistry* **1979**, *18*, 2192–2196; b) D. Baigl, K. Yoshikawa, *Biophys. J.* **2005**, *88*, 3486–3493.

[7] T. Hayashita, T. Kurosawa, T. Miyata, K. Tanaka, M. Igawa, *Colloid Polym. Sci.* **1994**, *272*, 1611–1619.

Received: April 8, 2008
Published online on September 18, 2008

