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Magnetizing DNA and Proteins Using Responsive Surfactants

*Paul Brown, Asad M. Khan, James P. K. Armstrong, Adam W. Perriman, Craig P. Butts, and Julian Eastoe**

One of the major challenges in biotechnology is the effective control over transport and delivery of biomolecules for the regulation of gene suppression,[1] targeted drug delivery,[1] and protein separation.[2] To date, magnetic field-based approaches using heterogeneous dispersions of magnetic particles have been used with some success,[3] although for such systems to be employed efficiently, they must be able to inhibit sedimentation, be biocompatible, and nontoxic.[4] The synthesis of ultrafine particles with these attributes is challenging, and interactions between particle surfaces and the associated biomolecules can often disrupt the native conformation and subsequent functions of the biomolecules.[5] The advantages of employing magnetic surfactants[6] over nanoparticles include their facile fabrication (one-step synthesis), fast and effective binding (which can be readily tuned via alteration of the surfactant aliphatic groups and counterion),[7] their good dispersibility and stability in solution, and the possibility of DNA-surfactant conjugate decompaction using either cyclodextrins[8] or via the formation of mixed micelles.[9] In this study, we demonstrate unprecedented low-strength magnetic field-induced migration of DNA and proteins via magnetic surfactant conjugation. Moreover, near-native conformations of the biomolecules are maintained by careful control over the biomolecule: surfactant stoichiometry.

The magnetic cationic surfactants DTAF[6] ($[C_{15}H_{34}N]^+[FeCl_3Br]^-$), DTAG ($[C_{15}H_{34}N]^+[GdCl_3Br]^-$) and DTAH ($[C_{15}H_{34}N]^+[HoCl_3Br]^-$) were synthesized (*see Materials and Methods* in the Experimental Section) using commercially available compounds to contain high-spin Fe(III), Gd(III) and Ho(III)-based counterions, resulting in high effective spin-only magnetic moments of 5.09 B.M., 7.42 B.M. and 10.60 B.M. respectively (*see Materials and Methods*). Addition of these surfactants to DNA (from herring sperm < 50 bp) and exposure to a magnetic field (0.44 T) resulted in effective migration of the DNA-surfactant complex to the magnet surfaces. This in turn increased the local concentration of the complexes beyond the solubility limit, which resulted in deposition at the point of highest magnetic field density (Figure S1 in the Supporting Information).

UV-vis spectroscopy revealed a small reduction in the intensity of the characteristic absorbance maximum at 260 nm on mixing very low concentrations of DTAG (50 μM) and DNA (150 μM) in the absence of a magnetic field, but importantly there was no evidence of aggregation over 96 hours (**Figure 1**a). Significantly, applying a small magnetic field (0.44T, gradient ~36 mT mm^{-2}) to stable aqueous solutions of DNA-surfactant complexes resulted in a notable reduction in the 260 nm peak intensity (Figure 1b), which equated to a decrease in DNA concentration of 48% over four days. This was accompanied by a concomitant elevation of the baseline, which was indicative of aggregation due to the increased concentration of the complexes adjacent to the magnet surfaces. At higher DNA or magnetic surfactant concentrations, the strong attraction between DNA and DTAG (micelles) resulted in the formation of overcharged aggregates that led to macroscopic phase separation in less than one hour. These aggregates could be manipulated with small magnets (0.2T) (**Figure 2** and Figure S6, Supporting Information), which, for in vitro applications may be useful to increase sedimentation of the complexes and the rate of transfection.[1]

The synchrotron radiation circular dichroism (SRCD) spectrum of DNA (**Figure 3**) showed the characteristic *β*-form with a negative peak at 248 nm due to helicity of the DNA, a positive peak at 285 nm due to base stacking, and a crossover peak at approximately 260 nm corresponding to the wavelength maximum for normal absorption. The inert CTAB (cetyltrimethylammonium bromide $C_{16}H_{33}N(CH_3)_3Br$) and magnetic DTAG bound to the DNA duplex with no change in the peak positions, which indicated that the *β*-form persisted. Native DNA has no CD spectrum above 300 nm, however, the DNA-surfactant complexes still exhibited a positive signal up to 320 nm, which was indicative of the formation of globular structures (condensation).[10] Moreover, the reduction in the peak intensity at 285 nm observed for the DNA- and DTAG-DNA complexes was consistent with alterations in the degree of helix hydration in the vicinity of phosphate and ribose ring due to changes in the local dielectric constant,[11] i.e., exchange of the native sodium counterion with the hydrophobic cationic surfactant DTAG would have lead to such changes in the hydration near the phosphate group of the DNA helix.[12] The decreasing intensities of the 248 nm and 285 nm peaks also suggested that DNA compaction had occurred, resulting from a reduction in the number of base pairs per helical period as the helical pitch shortens.

It is well established that the helical structure of DNA and its effective compaction can be achieved by the addition of

P. Brown, J. P. K. Armstrong, A. W. Perriman, C. P. Butts, Prof. J. Eastoe
School of Chemistry
University of Bristol, Cantock's Close
Bristol, BS8 1TS, UK
E-mail: Julian.Eastoe@bristol.ac.uk
A. M. Khan
Department of Chemistry
Armacost Science Building
Forman Christian College, Lahore, 54600, Pakistan

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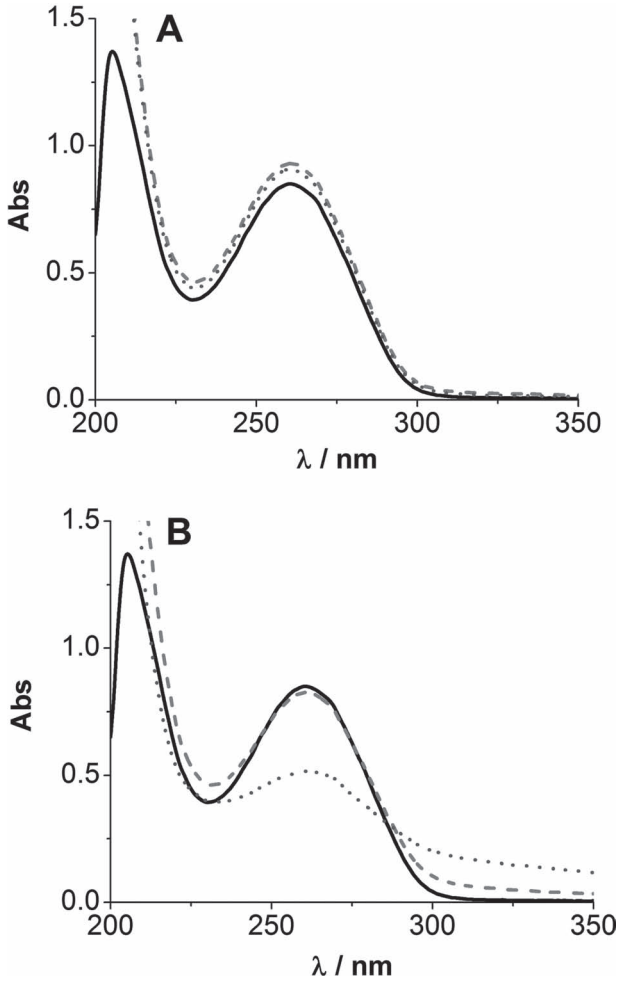

Figure 1. UV-vis spectra of the stable DNA-DTAG complexes in (A) the absence of a magnetic field and (B) in the presence of an applied magnetic field. t = 0 (black solid line), t = 24 h (dashed line) and t = 96 h (dotted line).

complexing cationic agents such as CTAB,[13] whereby isolated DNA chains undergo a discrete coil–globule transition in solution, at even very low surfactant concentrations (well below the critical micelle concentration, cmc).[14] Initially the cationic surfactants interact with DNA by electrostatic interactions, and it has been postulated that CTAB binds to DNA with one CTAB molecule bound per DNA phosphate group.[15] This is followed by hydrophobically-driven cooperative binding leading to a region of coexistence between unbound DNA chains and those saturated with surfactant.[16] This surfactant-induced compaction of DNA leads to a decrease in hydrodynamic radius, thereby changing its translational diffusion coefficient. Dynamic light scattering (DLS) measurements probe this change directly, showing coil-globule coexistence in bulk solution. Here, DNA extended coils diffuse slowly compared to surfactant-compacted DNA. At surfactant to DNA base pair ratios (X = [surf]/[PO_4^-]) greater than 1 (CTAB 5.2–36.3; DTAB 1.33–6.66) and relatively low DNA concentration (0.150 mM) (Figure S2 and Table S1, Supporting Information) CTAB reduced the hydrodynamic

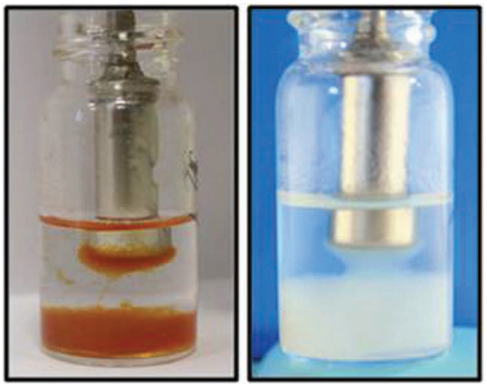

Figure 2. The magnetization of DNA by DTAF (left) and DTAG (right), with an NdFeB magnet (0.2 T).

radius of DNA by around 53% (232 nm to 108 nm). This was similar to results reported by Cardenas et al.,[17] who showed that CTAB induced a transition of the DNA hydrodynamic radius from about 100 nm to about 50 nm (~50%). In comparison, DTAF compacted DNA even more efficiently, resulting in a 72% reduction in hydrodynamic radius (64 nm), which was almost half that of CTAB. Solutions of DNA-DTAG were not stable for long periods at this concentration, and stability studies showed that the CTAB and DTAF complexes of DNA also precipitated after 1 day. Lower surfactant to DNA base pair ratios (0.006–1) yielded DNA-surfactant complexes that were stable in solution over five days, and DLS again showed a maximum in surfactant-induced compaction at X = 0.33 for all surfactants (Figure S3, Supporting Information).

The ability to control migration of DNA using magnetic surfactants was also extended to myoglobin (**Figure 4**) and green fluorescent protein (GFP) (Figure S8, Supporting Information). The UV-vis spectrum of the myoglobin surfactant complexes (Figure 4) gave an intense Soret band at 409 nm, as well as Q_α

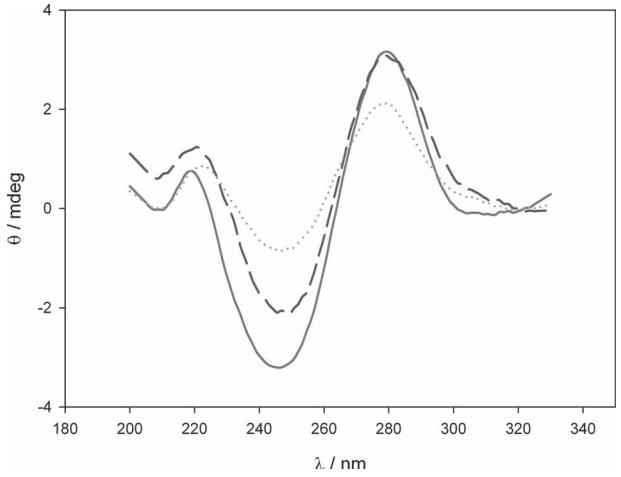

Figure 3. Synchrotron radiation circular dichroism (SRCD) spectra showing the effect of cationic surfactant addition on the conformation of herring sperm DNA. [DNA] = 150 μM; [surfactant] = 50 μM. Native DNA (solid line), DNA-CTAB (dashed line), DNA-DTAG (dotted line).

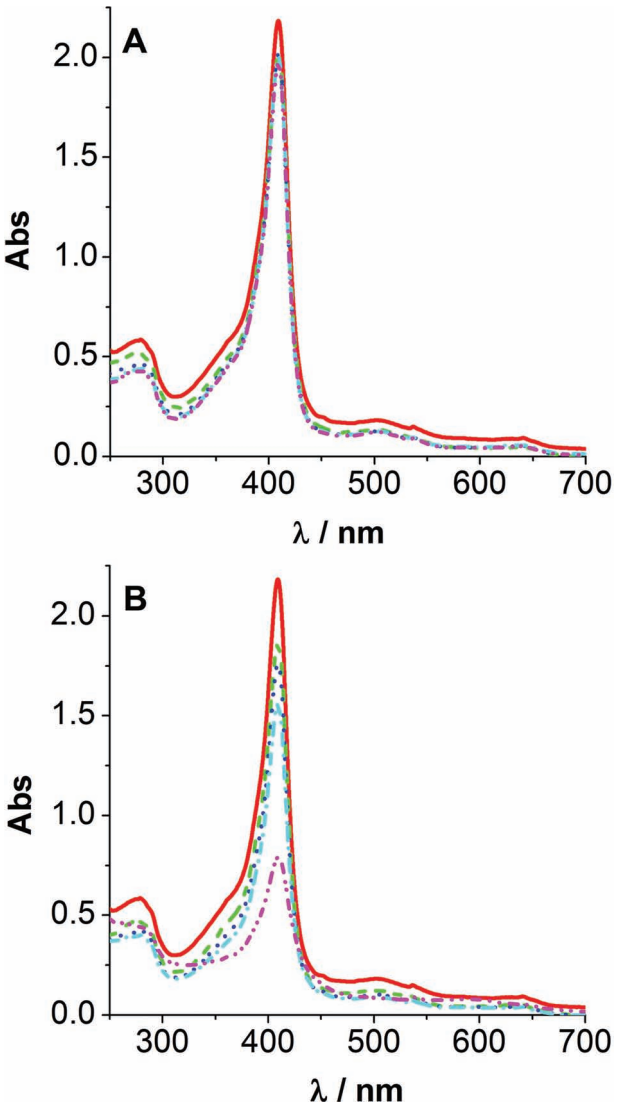

Figure 4. UV-vis spectra of myoglobin-DTAH in (A) the absence of a magnetic field and in (B) the presence of a magnetic field. [Mb] = 11.6 μM; [DTAH] = 3.03 mM. t = 0 (red solid line), t = 12 h (green dashed line), t = 36 h (light blue dot-dashed line), t = 50 h (dark blue dotted line), t = 120 h (pink double dot-dashed line).

and Q_β bands at 641 nm and 502 nm respectively. These three adsorption bands were indicative of metmyoglobin with a low-spin (diamagnetic) heme group and a six-coordinate geometry with a strongly bound water molecule.[18] It is well known that ionic surfactants can denature myoglobin via strong binding to the charged and hydrophobic side chains,[19] but adding DTAH below the cmc did not result in a shift in the positions of the characteristic adsorption bands, suggesting that there were no significant changes in the tertiary structure in the vicinity of the heme prosthetic group. Similar findings have been made after addition of anionic surfactants to myoglobin without denaturation.[20] With no magnetic field present, the myoglobin surfactant complex concentration remained constant, but after application of the magnetic field there was a reduction in concentration by approximately 60% (11.6 μM to 4.64 μM) over five days.

The UV-vis spectra from the Cat-GFP-DTAG complex (Figure S8) also showed no variation from the native protein, exhibiting the characteristic fluorophore absorbance at 277 nm (due to aromatic residues in the polypeptide side chain) and a broad band at around 500 nm (resulting from absorption at the Cat-GFP chromophore), which indicated that the presence of surfactant corona had little or no effect on the protein structure. Exposure of the complexes to a magnetic field resulted in aggregation (seen by a rise in the peak at 277 nm). This aggregation occurred concomitantly with migration towards the magnet surface lowering the overall Cat-GFP at concentration by an estimated 25% over 150 h (as seen by a reduction in 277 nm peak beginning after 110 hrs).

Exposure of the complexes to a magnetic field also resulted in migration, as evidenced by a 25% reduction in the 277 nm peak intensity over 150 h. The concentration gradient also resulted in aggregation (as can be seen by a rise in the peak at 277 nm). Aggregates and unimers are then drawn towards the magnet surface lowering the overall GFP concentration after 150 h (c.f. decrease in 277 nm peak after 110 h).

In conclusion, novel magnetic surfactants compact DNA and bind to proteins just like conventional surfactants, but now importantly allow DNA-surfactant and protein-surfactant complexes to be manipulated by an external magnetic field. This new paradigm for controlling biomolecules is significant as the studies suggest a strategy that can be applied to a diverse range of systems cheaply and non-invasively, and that could be readily developed for a wide range of biological structures and techniques.

Experimental Section

Materials and Methods: Dodecyltrimethylammonium bromide (99%, DTAB), cetyltrimethylammonium bromide (99%, CTAB), iron trichloride (99.9%), gadolinium (III) trichloride hexahydrate, holmium (III) trichloride hexahydrate and Trizma base (99.9% titration) were purchased from Sigma Aldrich and used without further purification. Dodecyltrimethylammonium trichloromonobromoferrate (DTAF) was synthesized by mixing equimolar amounts of DTAB with iron trichloride in methanol and stirring overnight at room temperature. The solvent was then removed and the product dried at reduced pressure at 80 °C overnight yielding brown/red solid. Dodecyltrimethylammonium trichloromonobromogadolinate (DTAG) and dodecyltrimethylammonium trichloromonobromoholminate (DTAH) were prepared in the same way yielding a white and pink solid respectively.[6]

DNA (oligonucleotides from herring sperm, D-3159, <50 bp) was purchased from Sigma. On preparing solutions, the final concentration of nucleic acid was 0.150 mM with respect to the phosphate groups. The DNA concentration was calculated according to the absorbance at 260 nm by using ε_{DNA} = 6600 M^{-1} cm^{-1}.[21] In all experiments, the DNA concentration was held constant at 0.150 mM and the surfactant to DNA base pair ratio was varied from X = 0 to X = 1.

Myoglobin (Mb, from equine heart) was purchased from Sigma Aldrich. Concentrations were calculated according to the absorbance at 409 nm by using ε_{Mb} = 171000 M^{-1} cm^{-1}.[22] Cat-GFP was synthesized as follows: Escherichia coli host was transformed with a pET45b(+) plasmid vector, and used to express polyhistidine-tagged enhanced green fluorescent protein (eGFP), which was subsequently purified using a nickel nitriloacetic acid (Ni-NTA) column. The acidic residues of eGFP were covalently modified with DMPA, in a carbidiimide-mediated

cationisation process to yield a protein with up to 44 cationic residues. Concentrations were calculated according to the absorbance at 277 nm by using ε_{GFP} = 55000 M^{-1} cm^{-1}.[23] Purified water was used: resistivity 18.2 MΩ cm and surface tension of 71.9 mN m^{-1} at 25 °C, in good agreement with literature values.

Critical micelle concentrations were determined though electrical conductivity measurements (Supporting Information): CTAB (0.8 mM, disscociation constant β = 0.14), DTAF (13.6 mM, β = 0.81), DTAG (11.9 mM, β = 0.59). DTAH (11.6 mM, b = 0.76). pH measurements of 0.1 M surfactant solutions in H_2O gave DTAF (pH 3.98), DTAG (pH 7.25) and DTAH (pH 7.27). SQUID magnetometry shows that these compounds have effective magnetic moments lying close to the values expected for high-spin d^5 Fe^{3+} (spin-only value: 5.09 B.M., lit = 5.92 B. M.),[24] Gd^{3+} (spin-only value: 7.42 B.M., lit = 7.94 B.M.)[25] and Ho^{3+} (spin-only value: 9.53 B.M., lit = 10.60 B.M.)[25]

Stability Studies: The bulk behavior of aqueous mixtures of DNA (150 mM) and surfactant was investigated (by visual inspection) at 25 °C in order to determine appropriate concentrations for magnetic experiments (Supporting Information). Stock solution of DNA was prepared by dissolving commercial nucleic acid in water and fragmenting by sonic vibration with a sonic probe (MSE Soniprep 150 (UK), 23 KHz, 1 min sonication time). The required amount of surfactant solution was then slowly added to avoid aggregation.

Dynamic Light Scattering (DLS): DLS measurements were taken to define the boundaries at which DNA compaction occurred. Measurements were performed on a Malvern Instruments Zetasizer Nano ZS (Malvern, UK) using a constant scattering angle of 90° at 25 °C. All solutions were made dust-free by filtration through cellulose acetate membranes of 0.45 mm pore size (Millipore, USA).

4 mL of DNA solution containing appropriate amounts of surfactant were allowed to equilibrate for 5 hours. Measurements were taken on the same day as preparation for samples that had not precipitated.

Circular Dichroism: Synchrotron radiation circular dichroism (SRCD) spectra were collected at the Diamond Light Source on beamline B23 over a wavelength range of 330 to 200 nm with an integration time of 2 s and 1 nm data intervals. Samples were run in 1.0 cm quartz cuvette cells with 4 accumulations. Concentrations were chosen below the point of precipitation (50 μM surfactant and 150 μM DNA).

UV-visible Spectrometry: UV-vis absorption spectra were recorded on a Nicolet Eco 300 UV-vis spectrometer. An aqueous solution (60 mL) of DTAG (0.05 μM) and DNA (150 μM) was placed in two specially built glass vials. One vial also contained a NdFeB magnet (12 mm × 3 mm, N52) with a magnetic field density of 0.44 T on the surface and a gradient of about 36 mT mm^{-2}. The top 1.5 mL was removed for analysis over time and replaced carefully each time.

The UV-vis experiments were also repeated using myoglobin and cationized green fluorescent protein (cat-GFP). For the myoglobin solutions DTAH was used was used and for cat-GFP, DTAG was used. [myoglobin] = 11.6 μM, [DTAH] = 3.03 mM; [cat-GFP] = 38 μM, [DTAG] = 687 μM.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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