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Micelle-Sequestered Dissociation of Cationic DNA-Intercalated Drugs: Unexpected Surfactant-Induced Rate Enhancement

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Abstract

Detergent-sequestration using micelles as a hydrophobic sink for dissociated drug molecules is an established technique for determination of dissociation rates. The anionic surfactant molecules are generally assumed not to interact with the anionic DNA and thereby not to affect the rate of dissociation. By contrast, we here demonstrate that the surfactant molecules sodium dodecyl sulphate (SDS), sodium decyl sulphate and sodium octyl sulphate all induce substantial rate enhancements of the dissociation of intercalators from DNA. Four different cationic DNA intercalators are studied with respect to surfactant-induced dissociation. Except for the smallest intercalator ethidium, the dissociation rate constant increases monotonically with surfactant concentration both below cmc and (more strongly) above cmc, much more than expected from electrostatic effects of increased counterion concentration. The rate enhancement, most pronounced for the bulky, multicationic and hydrophobic DNA ligands in this study, indicates a reduction of the activation energy for the ligand to pass out from a deeply penetrating intercalation site of DNA. The discovery that surfactants enhance the rate of dissociation of cationic DNA-intercalators implies that rate constants previously determined by micelle-sequestered dissociation may have been overestimated. As an alternative, more reliable method, we suggest instead the addition of a large amount of dummy DNA as an absorbent for dissociated ligand.

Introduction

Understanding the mechanisms by which drug molecules interact with DNA and correlating them to biological effects, has been a focus of interest for a long time. In the study of interactions between DNA and small drugs, the association and dissociation kinetics are of great diagnostic importance. For example, for a drug to be efficient as a cancer therapeutic, an extremely slow rate of dissociation from DNA is considered one of the most important properties.[1]

There are various ways to study the rate of dissociation; for example a modification of the foot-printing technique has been used to study dissociation from specific binding sites[2] and relaxation methods such as T-jump may be used to measure fast kinetics.[3] The detergent-sequestration technique, i.e. using surfactant micelles, such as sodium dodecyl sulphate (SDS), as a hydrophobic sink for the dissociated drugs, first described by Müller and Crothers 1968,[1] is a well established method to study dissociation of cationic, hydrophobic drugs from DNA.[3-9] The micelles are in this technique supposed to drive the equilibrium from DNA-bound drug towards dissociated drug by dumping the concentration of free drug by quantitative absorption into the micelles. Due to their highly negative charge, the micelles are thought not to be interacting with the negatively charged DNA or the drugs bound to DNA, i.e. not disturbing the process when the drugs leave DNA.[4-6] The rate-limiting step is generally considered to be the step when the drug leaves its binding site on DNA, while the sequestration of the drug by the surfactant micelles is thought to be diffusion controlled, and thereby considerably faster than the first step. Some studies have indicated certain concentration effects, increasing surfactant concentration slightly speeding up or slowing down the dissociation.[6,10] The negatively charged surfactant monomers, in the bulk outside the micelles, are also thought to be inert and

not to interact with DNA due to electrostatic repulsion. As will be shown, however, from systematic studies of surfactant-induced dissociation of cationic DNA-intercalators, there are strong indications for direct interactions between the surfactant molecules and the DNA complexes that influence the dissociation mechanism.

Aromatic ruthenium complexes and their interactions with DNA have been extensively studied due to their interesting photophysical properties when bound to DNA.[11-15] When studying the dissociation from DNA, and the recently discovered extremely slow rearrangement from groove binding to intercalation, of Δ,Δ-[μ-(11,11'-bidppz)$(phen)_4Ru_2]^{4+}$ (11,11'-bidppz=11,11'-bi-(dipyrido[3,2-a:2'3'-c]phenazinyl))[19] (**1** in Figure 1), we noticed that the dissociation was faster than the rearrangement from groove binding to intercalation, an observation which is formally inconsistent with the final binding mode being the thermodynamically most stable one.

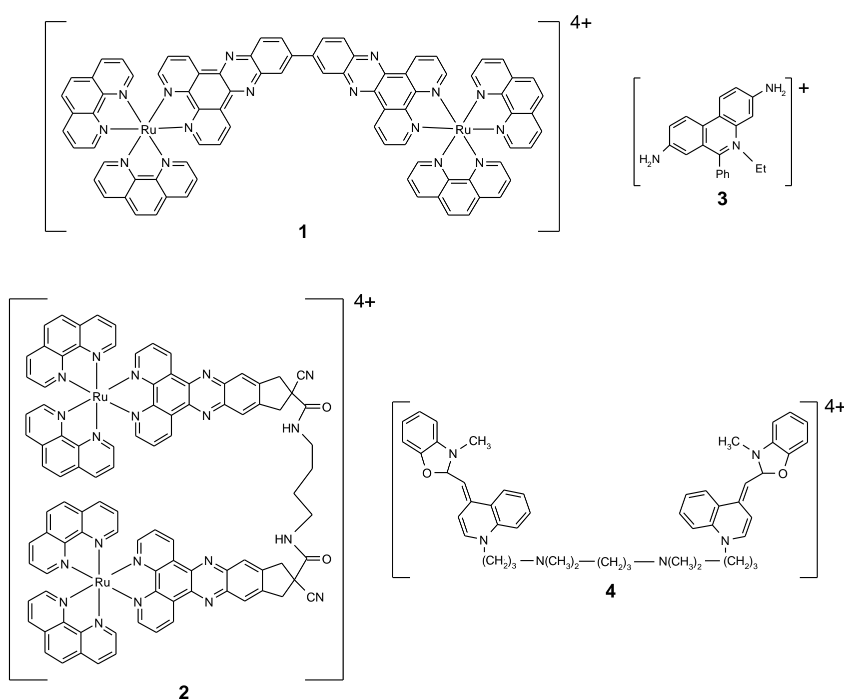

Figure 1. Structures of DNA-intercalators: [μ-(11,11'-bidppz)$(phen)_4Ru_2]^{4+}$ (**1**) [μ-c4$(cpdppz)_2(phen)_4Ru_2]^{4+}$ (**2**) ethidium^{1+} (**3**) and YOYO-1^{4+} (**4**).

The association and dissociation processes for the two binding modes are schematically represented by state A, B and C in Figure 2.

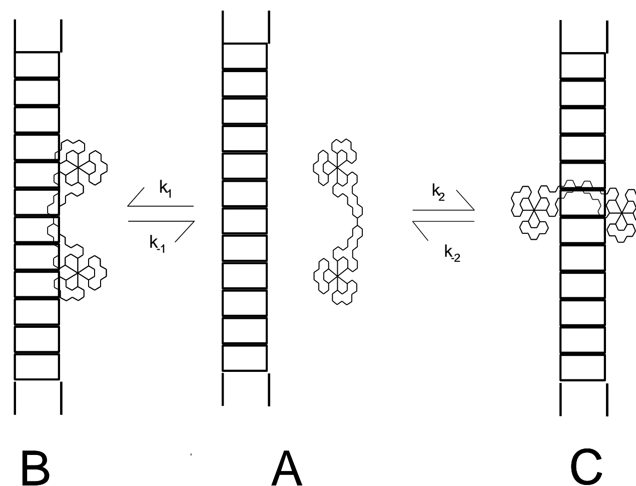

Figure 2. A schematic representation of observed rearrangement processes for [μ-(11,11'-bidppz)(phen)$_4$Ru$_2$]$^{4+}$ with respect to DNA. State A corresponds to a state with the ruthenium complex as a loosely bound ion pair in the ionic atmosphere of DNA. State B represents an initial tight binding state, before rearrangement, with the ruthenium complex bound in a groove of the DNA. State C is the final binding mode in which the ruthenium complex is intercalated by threading through the DNA.

DNA and **1** are initially a loosely bound ion pair with the ruthenium complex in the ionic atmosphere of DNA (state A). From earlier studies it is known that groove binding (state B) occurs rapidly and much faster than intercalation (state C).[19] This means that k_1 is larger than k_2. Because groove binding is much faster than intercalation there is a pre-equilibrium between A and B and the rate with which C is formed is:

$$\frac{d[C]}{dt} = k_2 \frac{k_{-1}}{k_1}[B] - k_{-2}[C] \qquad (1)$$

where k_2k_{-1}/k_1 is the rate of rearrangement from B to C. Furthermore it is known from earlier studies that the most stable binding mode is the intercalation mode,[19] i.e. the equilibrium constant for the process from A to C is larger than that from A to B, and thus $k_2/k_{-2} > k_1/k_{-1}$. Rearranging, this implies that $k_{-2} < k_2k_{-1}/k_1$, i.e. that the dissociation should be slower than the rearrangement, in conflict with the measurements on **1**.[19] However, what is not included in this simple kinetic model is the surfactant, giving us an indication that the surfactant molecules are somehow involved in the process by increasing the rate of dissociation.

We have found that the apparent rate of dissociation of **1** from DNA at a given total surfactant concentration, above cmc, varies with the length of the alkyl chain of the amphiphilic molecule, using octyl and decyl sulphate instead of dodecyl sulphate micelles. In order to investigate if these effects are general for intercalating DNA drugs, or an effect unique for **1**, we also studied Δ,Δ-[μ-c4(cpdppz)$_2$(phen)$_4$Ru$_2$]$^{4+}$ (**2** in Figure 1), ethidium (**3** in Figure 1), and YOYO-1 (**4** in Figure 1). Compound **2** has been shown to bis-intercalate in DNA by threading, and to exhibit a slow dissociation from DNA.[17,18] Ethidium, a small DNA-intercalating drug[20,21] used to stain electrophoresis gels, exhibits a very fast dissociation from DNA. YOYO-1 is a strong-binding bis-intercalator that has often been used in gel-electrophoresis experiments due to its excellent properties for detection and quantification of DNA fragments.[22,23]

Further, we present a method to determine the true rate of dissociation from DNA using added extra DNA, containing no bound drug molecules, as an absorbent for the dissociated drug instead of micelles. For example exploiting the fact that the fluorescence quantum yield varies for **2** when it is bound to ct-DNA and to poly(dA-dT)$_2$, one can monitor the drug leaving poly(dA-dT)$_2$ for ct-DNA as a decrease in

fluorescence. In this way it was demonstrated that the surfactants could enhance the dissociation rate by more than an order of magnitude.

Materials and Methods

Chemicals. Except where otherwise noted, all experiments were performed in 100 mM NaCl, 1 mM sodium cacodylate buffer, pH=7. The ruthenium complexes (**1** and **2**) were synthesised as described elsewhere,[17,24] ethidium (**3**) was purchased as its bromide salt from Sigma-Aldrich and YOYO-1 (**4**) was purchased as its iodide salt in DMSO from Molecular Probes. Calf thymus DNA (ct-DNA) was purchased from Sigma-Aldrich. Poly(dA-dT)$_2$ was purchased from Amersham Biosciences. Sodium dodecyl sulphate, sodium decyl sulphate and sodium octyl sulphate were purchased from Sigma-Aldrich and stock solutions were made in cacodylate buffer.

Preparation of DNA stock solution. Ct-DNA stock solution was prepared by dissolving lyophilised ct-DNA in buffer to a concentration of about 10 mM bases. The solution was stirred over night and then filtered three times through a 0.7 μm polycarbonate filter. Stock solution of poly(dA-dT)$_2$ was made at a concentration of ca 3mM in buffer.

Sample preparation. For **1**, **2** and **3** the concentration in all experiments was 20 μM and the DNA concentration was 160 μM in bases, for **4** the concentration was 0.5 μM and the DNA concentration was 8 μM in bases. However, in the studies of the dissociation of **2** from poly(dA-dT)$_2$ into an excess of ct-DNA the concentration of **2** was lowered to 0.5 μM and the DNA concentration was equally lowered to 4 μM. The concentrations of all duplex nucleic acid samples were confirmed by measuring the absorbance on a Cary 4B spectrophotometer, using ε_{260}=6600 cm^{-1} M^{-1} (ct-DNA) and ε_{262}=6600 cm^{-1} M^{-1} (poly(dA-dT)$_2$). Since all four drugs (**1-4**) have a higher fluorescence quantum yield when bound to DNA than to micelles, the kinetics of the dissociation from DNA to the micelles was studied by monitoring the decrease in luminescence intensity.

Fluorescence measurements. The dissociation kinetics of the ruthenium complexes (**1** and **2**) was studied using fluorescence spectroscopy on a SPEX fluorolog τ3 spectrofluorimeter. For **2** the excitation wavelength was 440 nm and the emission was recorded at 620 nm. The temperature was held constant at 25 °C by a water thermostat. For **1** the excitation wavelength was 410 nm and the emission was recorded at 615 nm. Due to the extremely slow dissociation of **1** from DNA, the temperature was raised to 50°C to speed up the dissociation process. When studying the kinetics of the rearrangement of **2,** from poly(dA-dT)$_2$ to a large excess of ct-DNA, the excitation wavelength was 440 nm and the emission was recorded at 620 nm. Due to the slowness of the dissociation the kinetics were studied at 50°C.

Stopped-flow measurements. The dissociation kinetics for YOYO-1 (**3**) and ethidium (**4**) was measured on a computer controlled stopped-flow instrument from Bio-Logic. For YOYO-1 the sample was excited at 457 nm and the emission was collected through a 500 nm cut-off filter. For ethidium the excitation wavelength was 480 nm and no cut-off filter was used. Typically five decay-spectra were averaged for each output file. The delay time between mixing and data collection was 5.2 ms. Syringes, cell and mixing chamber were held at constant temperature (25 °C) by a water thermostat.

Analysis of the dissociation data. In all the fluorescence studies the spectra are normalized with 1 being the maximum fluorescence in each measurement. In Figure 3 and 4 k is determined by mono-exponential fittings of the kinetic data. In Figure 7 and 8, k is determined by taking the time it takes for the fluorescence to reach half its initial intensity and then invert that time, to get a rate constant.

Results

In Figure 3 the apparent rate constant of the dissociation of **2** from ct-DNA is shown as a function of surfactant concentration for three different surfactants: sodium dodecyl sulphate (SDS), sodium decyl sulphate (SDeS) and sodium octyl sulphate (SOS), at three different ionic strengths.

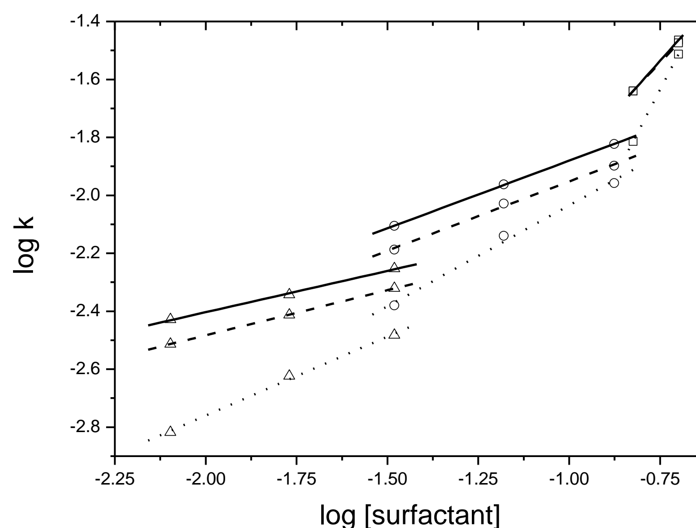

Figure 3. Dependence of the rate constant (k) for the dissociation of Δ,Δ-[μ-c4(cpdppz)$_2$(phen)$_4$Ru$_2$]$^{4+}$ from ct-DNA versus surfactant concentration. Measurements made in 1 mM sodium cacodylate buffer including 100 mM (dotted line), 150 mM (dashed line) or 200 mM NaCl (solid line) for three surfactants: sodium dodecyl sulphate (Δ), sodium decyl sulphate (o) and sodium octyl sulphate (□). All measurements refer to ambient temperature (25 °C). The cmc values for sodium dodecyl sulphate, sodium decyl sulphate and sodium octyl sulphate at 100 mM NaCl are 1.5 mM, 14 mM and 97 mM respectively.[25]

Due to the comparatively high cmc for SOS and solubility problems for SDS, all three surfactants could not be studied in the same concentration interval. However, the

concentration intervals of the three surfactants approach each other at the borders and there the effects of the surfactants may be compared. Increasing the surfactant concentration gives a much larger rate enhancement than increasing the sodium concentration to the same extent by adding salt. As an example, changing the surfactant concentration by 50 mM, from 33 to 83 mM, for SDeS at 150 mM NaCl (dashed line, open circles) increases the rate of dissociation by 54 %, whereas the difference between 150 mM (dashed line, open circles) and 200 mM added NaCl (solid line, open circles) at 33 mM SDeS is only 17 %. Clearly, the presence of surfactant increases the dissociation rate much more than what could be ascribed to the ionic strength effect.

Figure 4 compares the apparent rates of dissociation (k) of the four drugs (**1-4**) from ct-DNA, determined by detergent-sequestering at different concentrations of the three different surfactants.

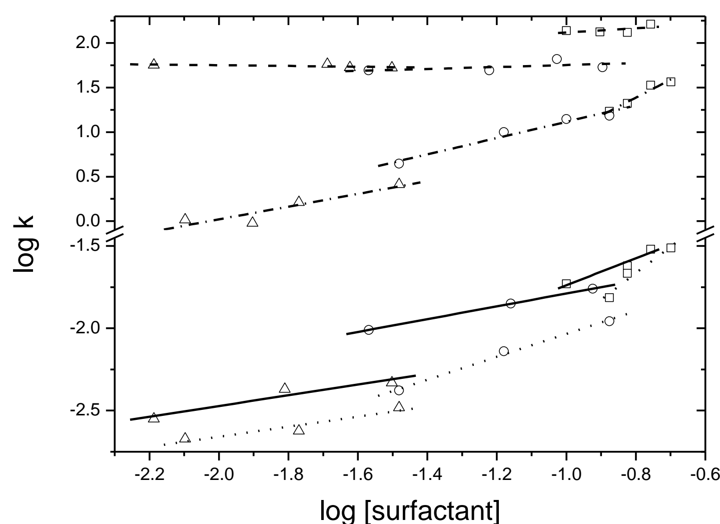

Figure 4. Dependence of the dissociation rate constant (k) on surfactant concentration for the dissociation of ethidium (dashed line), YOYO-1 (dash-dotted line), Δ,Δ-[μ-c4(cpdppz)$_2$(phen)$_4$Ru$_2$]$^{4+}$ (dotted line) and Δ,Δ-[μ-(11,11'-bidppz)(phen)$_4$Ru$_2$]$^{4+}$

(solid line) from ct-DNA, with SDS (Δ), sodium decyl sulphate (o) and SOS (□). Buffer was 100 mM NaCl with 1 mM sodium cacodylate. Measurements made at room temperature for **2**, **3** and **4**, but at 50°C for **1**.

The measurements were done at room temperature, except when studying **1** where the temperature, due to the slow kinetics, was raised to 50°C. Ethidium (**3**) shows negligible SDS concentration dependence in agreement with earlier findings.[21] A similar behaviour is noticed with sodium decyl sulphate. However, with SOS the ethidium dissociation rate appears to exhibit some enhancement. The remaining three drugs exhibit, to varying extents, concentration and surfactant dependent dissociation rate enhancements, as shown by the sloping lines and by the discontinuities between different surfactants (Figure 4).

Looking for an alternative to surfactant micelles as a method to accommodate the dissociated molecules, redistribution of the drug from one kind of DNA to a large excess of another kind of DNA was considered an attractive solution as it would eliminate any effects that interactions between the DNA and the surfactant, either as monomers or as micelles, might have on the dissociation process. Since it is known that the fluorescence quantum yield of the ruthenium complexes (**1,2**) differs significantly between poly(dA-dT)$_2$ and ct-DNA[19] (and Önfelt, unpublished results), we chose to study the dissociation of **2** from poly(dA-dT)$_2$ to an added excess of "dummy" ct-DNA using fluorescence detection. Gradually increasing the excess of ct-DNA was found to affect the emission change rate until a point where further addition of ct-DNA did not further change the rate (Figure 5). Above this point the method can be assumed to monitor the true kinetics of dissociation. Thus, the results in Figure 5 show that an excess of 50 times ct-DNA is sufficient for this purpose. It is furthermore justified to assume that the ct-DNA does not interact with the poly(dA-

dT)$_2$ and that the rate of association to ct-DNA is very fast compared to the rate of dissociation from poly(dA-dT)$_2$. Thus the trajectories (>50 times excess) in Figure 5 correspond solely to compound **2** leaving its binding sites on poly(dA-dT)$_2$.

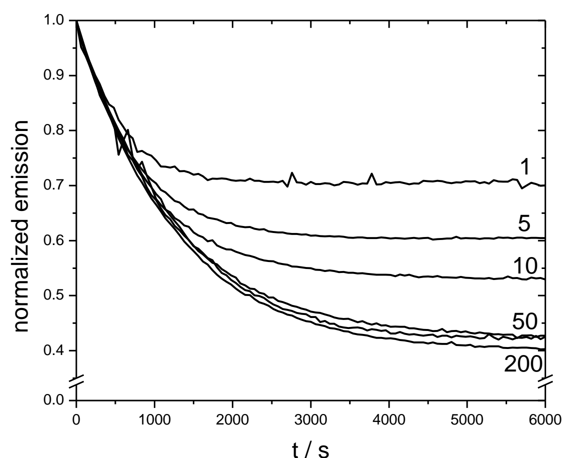

Figure 5. Fluorescence intensity decay monitoring the dissociation of **2** from poly(dA-dT)$_2$ upon an added excess of ct-DNA. The excess of absorbent DNA ranges from 200 (lowest curve) 100, 50, 10 and 5 times excess down to equal amounts of ct-DNA and poly(dA-dT)$_2$ (top curve). Buffer was 100 mM NaCl with 1 mM sodium cacodylate. Measurements performed at 50°C.

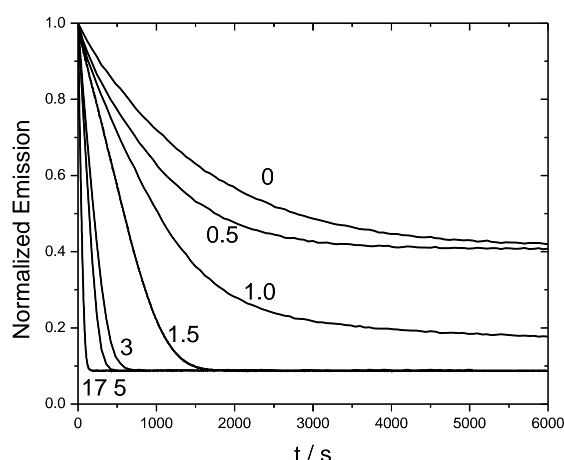

Figure 6. Fluorescence intensity decay monitoring the dissociation of **2** from poly(dA-dT)$_2$ upon addition of 50 times excess of ct-DNA (200 mM), together with

surfactant (SDS). SDS concentration ranges from 0, 0.5, 1, 1.5, 3 and 5 mM to 17 mM. Buffer was 100 mM NaCl with 1 mM sodium cacodylate. Measurements performed at 50°C.

The results in Figure 6 show what happens when 50 times excess of ct-DNA and various amounts of SDS are simultaneously added to a solution of compound **2** bound to poly(dA-dT)$_2$. Three kinds of relaxation phases are obvious from the figure. Firstly, at low surfactant concentration, all of the dissociated molecules move over to the ct-DNA, indicated by the final fluorescence having the same value as for the sample without surfactant. The next kind of behaviour is when the ruthenium compound finally ends up in the micelles, but their concentration is so low that the association to ct-DNA may still compete with that to the micelles. Since this process involves the association to ct-DNA and subsequent dissociation of some of the ruthenium complex from ct-DNA for further transport to micelles, it may take a long time, more than 50000 s at 20 mM SOS (data not shown), with the drug eventually ending up in the micelles, as indicated by the final fluorescence intensity being the same as for the pure micelle system. The third kind of dissociation behaviour is when all of the drug molecules directly end up in the micelles because the association to the micelles, in large excess, is much faster than the association to ct-DNA: the final fluorescence is then independent of the total surfactant concentration (at concentrations >1.5 mM). Corresponding measurements, using SDeS and SOS instead of SDS (data not shown), also exhibited three characteristic types of behaviour in consistency with the behaviour in Figure 6. In Figure 7 a-c these results, together with those presented in Figure 6, are summarized by plotting the inverse of the time it takes for the fluorescence to reach half of its final value as a function of surfactant concentration.

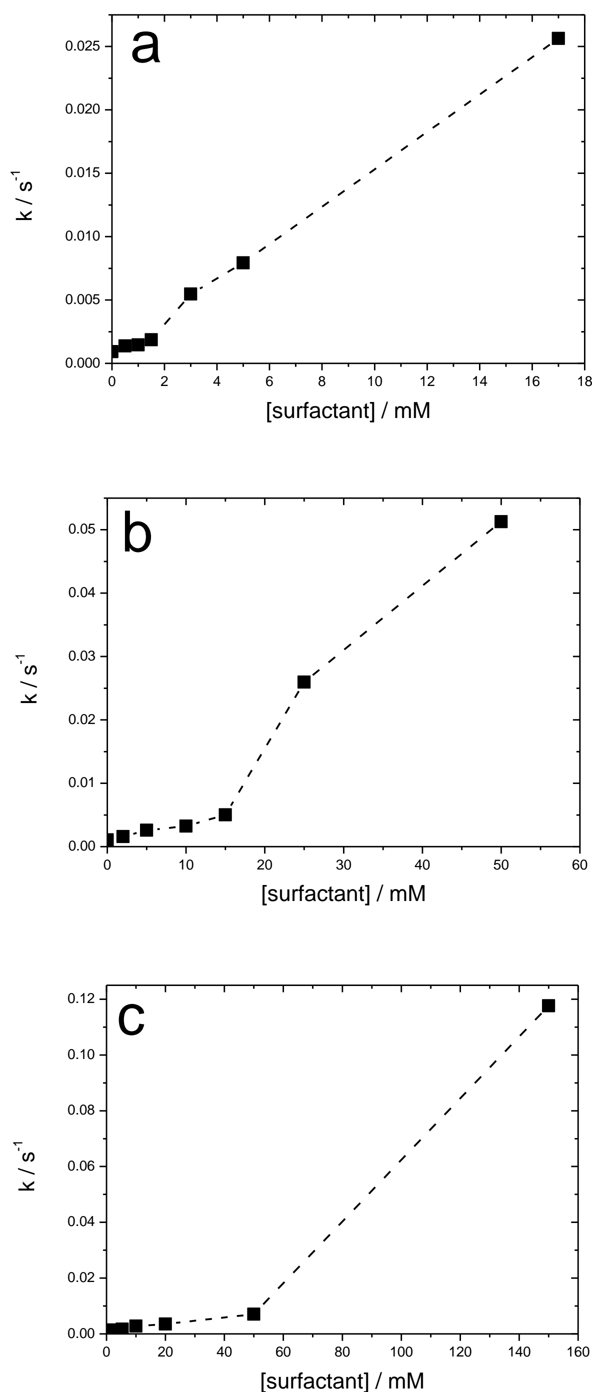

Figure 7. k_{diss} for dissociation of **2** from poly(dA-dT)$_2$ upon addition of 50 times excess of ct-DNA, and with surfactants sodium dodecyl sulphate (a), sodium decyl sulphate (b), and sodium octyl sulphate (c) added at varying concentrations. The buffer was 100 mM NaCl with 1 mM sodium cacodylate. Measurements performed at 50°C. The dashed lines are drawn only to guide the eye.

For all three surfactants, the "DNA-monitored" dissociation of the ruthenium compound from the poly(dA-dT)$_2$ is observed to significantly increase its rate with increasing surfactant concentration, also in the concentration range below cmc where only surfactant monomers should be present. We shall return to the implications of these results in the Discussion, but to further illuminate this point we have in Figure 8 compared the efficiencies of the three different surfactant monomers to enhance the dissociation rate: obviously the most hydrophobic surfactant monomer, SDS, has the greatest effect upon the rate of dissociation, followed by SDeS and SOS.

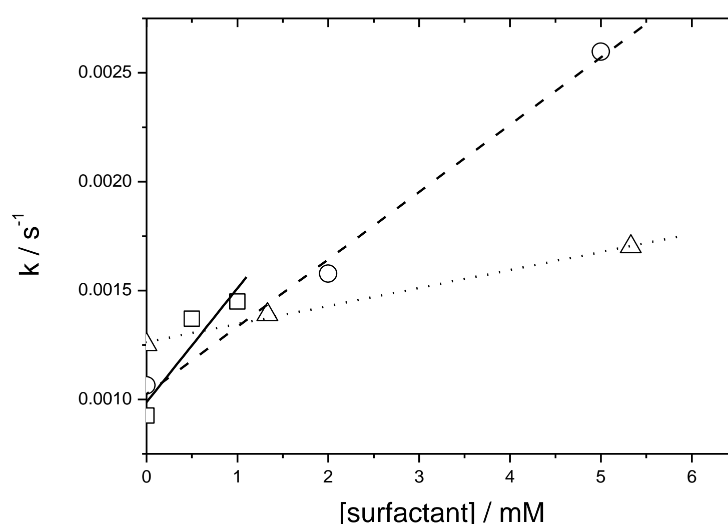

Figure 8. Monomeric surfactant effect. k_{diss} for the dissociation of **2** from poly(dA-dT)$_2$ upon addition of 50 times excess of ct-DNA, , as a function of surfactant concentration (below cmc). The slopes of the linear fits were 0.5 s^{-1} for sodium dodecyl sulphate (□), 0.3 s^{-1} for sodium decyl sulphate (o), and 0.08 s^{-1} for sodium octyl sulphate (Δ). The buffer used was 100 mM NaCl with 1 mM sodium cacodylate. Measurements performed at 50°C

Discussion

We here report the discovery that the presence of anionic surfactant species may enhance the rate of dissociation of cationic DNA-intercalating molecules from the anionic polyelectrolyte DNA. Whereas it is known that increasing ionic strength may increase the rate of dissociation of **2** from DNA,[18] it is clear from our results (e.g. in Figure 3) that the increment in the rate of dissociation cannot be explained solely by the increment in the sodium ion concentration that follows with the addition of the surfactant. Nor can the ionic strength effect explain the difference in rate of dissociation for different surfactants at the same total surfactant concentration. Thus, in contrast to a general assumption of all earlier investigations, these data demonstrate that the surfactant molecules indeed may affect the rate of dissociation of cationic DNA-bound species. The rate-enhancing effect of the surfactants can also be seen in Figure 4. When studying this figure, one should keep the conventional description in mind: i.e. that the micelles may only bind totally dissociated molecules, that the rate of dissociation should be independent of the micelle concentration and that the surfactant monomers cannot affect the rate. Such behaviour would then result in straight horizontal lines, i.e. concentration-independent dissociation rates for all of the surfactants, with no discontinuities when changing surfactant. The only effect anticipated with the conventional description would be a small positive slope due to an increasing ionic strength that follows with the addition of surfactant.

The effects studied in Figure 3 and 4 are all at concentrations well above cmc for each surfactant. However, as can be seen from Figure 6, the rate of dissociation increases even when SDS is added to the system at concentrations well below cmc (e.g. second decay curve from the top in Figure 6). The effect is also significant for SDeS and SOS, as can be seen clearly in Figure 8. Thus, we can conclude that there is a

substantial effect on the rate of dissociation also below cmc and that the effect grows with increasing surfactant monomer concentration. The observation that negatively charged surfactant monomers affect the rate of dissociation of DNA ligands is remarkable, since it implies that they have to bind or at least get very close to the strongly negatively charged DNA polyelectrolyte. The large aromatic ring systems common to all the studied DNA ligands as well as their positive charges, however, may provide an attractive environment for the association of surfactant molecules with their negative head group and hydrophobic tail, and this could make it easier for the surfactant monomers to get close to the DNA. The fact that ruthenium complex ions alone in solution can form aggregates with SDS-monomers, even below cmc, has been reported,[26] showing that these kinds of hydrophobic cationic molecules may provide an environment promoting the binding of anionic, amphiphilic monomers. Another noticeable effect is that the longer the chain of the surfactant monomer the greater the rate enhancing effect per surfactant. In Figure 8 it can be seen that the rate enhancement after addition of low concentrations of surfactant is significantly larger for SDS than for SDeS and SOS. The rate enhancement, estimated by linear fits to the data, per molar of added SDS, SDeS, and SOS is 0.5, 0.3, and 0.08 s^{-1} respectively.

So, if the monomers really increase the rate of dissociation, as indicated by our results, why does the rate continue to increase also well above cmc (Figures 3-4, 6, and 7 a-c), where the monomer concentration is generally believed to be constant or even decreasing,[27] when adding more surfactant? In the literature it is claimed that micelles and negatively charged polyelectrolytes do not get in close proximity to each other, but rather phase separate above a critical surfactant concentration.[28] Despite this view, the indisputable observation of a stronger enhancement of the rate of dissociation for high surfactant concentrations than for concentrations below cmc

clearly suggests an effect of the micelles. The total rate constant may, thus, be phenomenologically described as:

$$k_{total} = k_0 + k_{monomer}[monomer] + k_{micelle}[micelle] \qquad (2)$$

where k_0 is the natural dissociation constant and $k_{monomer}$ and $k_{micelle}$ refer to the rates in the monomer and micellar regions, respectively. Here [monomer] refers to the bulk concentration of monomer and is constant above cmc. The rate enhancement is clearly demonstrated in Figure 4 where the rate constants rapidly increase with concentration of surfactant above cmc, as in the four fastest decays of Figure 6, again all of which being above cmc.

However, as mentioned above, it is not likely that the micelle itself actually is involved in the rate enhancing mechanism but instead we suggest that this observation may be explained in terms of a dynamic model, in which micelles and monomers are in fast exchange, micelles constantly being dissolved and reformed again.[29] Thus, a higher micelle concentration will correspond to a higher probability of suddenly having a transiently high local concentration of monomers, $[monomer]_l$, anywhere in the solution. An enhancing factor of increasing the local concentration of monomers near DNA may come as a result of dispersion forces,[30-33] which are anticipated to be substantial between the polarizable DNA and the polarizable micelles, and which fall off rather slowly with distance. Dispersion forces may thus lead to an accumulation of micelles at a certain distance from the DNA helix and outside the counterion (Na^+) layer. With this approach the number density of surfactant molecules near the DNA helix would be expected to increase with total surfactant concentration, also above cmc. This provides the basis for a model we wish to propose, namely that the total

rate constant does not involve the micelle concentration, but instead depends on the dynamic local monomer concentration. The number of surfactant molecules, i, that are involved in the transition state, we suggest are aggregated as a transient "plaque" around the cationic ligand in the intercalation pocket. With this formalism a potential rate enhancing contribution from a single surfactant molecule bound to the DNA-intercalator site, i.e. i=1, is included as well as contributions from complexes that involve more than one surfactant molecule ("plaque") ranging from dimers (i=2) to a maximum limiting size (i=N).

$$k_{total} = k_0 + \sum_{i=1}^{N} k_i [monomer]_l^i \qquad (3)$$

Below cmc, $[monomer]_l$ may be regarded equal to the bulk monomer concentration (rate k_1) whereas above cmc, with increasing surfactant concentration, we may assume it to be a monotonically increasing quantity, but without knowledge of its size. It is reasonable that the rate-enhancing effect (i.e. the size of k_i) will increase with the number of surfactant molecules (i) to reach an optimum at a certain size of the "plaque".

We may only speculate in the details about the mechanism of dissociation of the DNA ligands and how the surfactant molecules bring about a reduction of the activation-barrier. As we have already mentioned, one part is the increasing hydrophobic environment that may favour transient openings of the otherwise quite compact DNA duplex structure and by providing a hydrophobic recipient for the exposed hydrophobic moieties of the intercalated ligands. The observation of an increased

efficiency of added detergent above cmc suggests that more than one detergent molecule could bind to the transition state.

The present system with DNA-intercalating cationic drugs whose dissociation undergoes a rate enhancement by "soap" molecules may seem rather artificial from a biological perspective. However, it is highly likely that the surfactant-induced rate enhancement that we here report may indeed have significance also in biological processes. Amphiphilic molecules are abundant at relatively high concentrations all around and in the living cell: from the phospholipid surfactant molecules in the cell membrane to polyamines such as spermine and spermidine in the nucleus. It is also well known that many enzymatic processes are based on catalytic effects in hydrophobic environments. More specifically, with nucleic acids, the base-base separation and the subsequent base matching in transcription and translation processes as well as the catalytic activity of RNA polymerase and other polymerases may be related to the formation of hydrophobic patches.

Conclusions

The following has been learnt from the present study of rates of dissociation of cationic hydrophobic DNA intercalators from duplex DNA by the presence of anionic micelle-forming surfactants:

1. The rates of dissociation are markedly enhanced by the surfactant molecules, increasing with increasing surfactant concentration both below cmc and (more strongly) above cmc, much more than expected from the electrostatic effect of increased counterion concentration.
2. The rate enhancing effect is stronger the longer the hydrophobic, alkyl tail of the surfactant molecule.
3. The rate enhancing effect is more pronounced for multicationic, strongly hydrophobic DNA ligands that require extensive conformational rearrangement of the DNA (large activation barrier) for the dissociation to occur.
4. The discovery that surfactants enhance the rate of dissociation of cationic DNA-intercalators implies that rate constants earlier determined from micelle-sequestered dissociation may be prone to errors. An alternative method, based on an added excess of dummy DNA as an absorbent for dissociated ligand, is presented.

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