

## 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 sulfate (SDS), sodium decyl sulfate, and sodium octyl sulfate 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 constants increase 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, 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 excess 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 have 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, a slow rate of dissociation from DNA is considered one of the most important properties.<sup>1</sup>

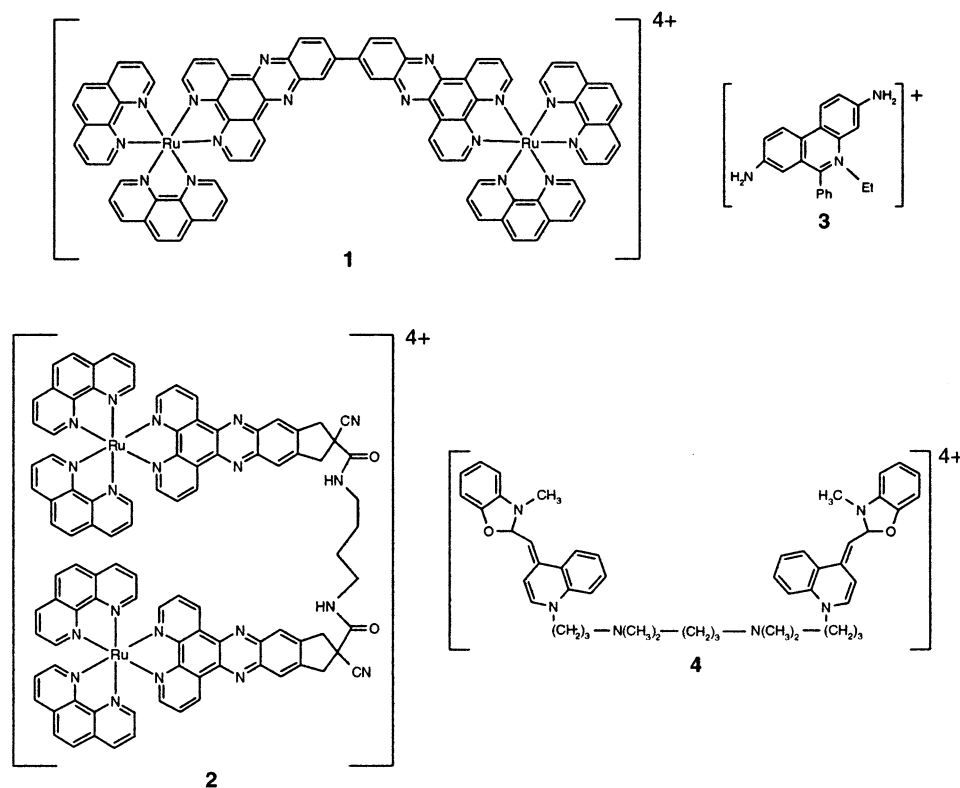
There are various ways to study the rate of dissociation; for example, a modification of the foot-printing technique has been used to study dissociation from specific binding sites,<sup>2</sup> and relaxation methods such as T-jump may be used to measure fast kinetics.<sup>3</sup> The detergent-sequestration technique, i.e., using surfactant micelles, such as sodium dodecyl sulfate (SDS), as a hydrophobic sink for the dissociated drugs, first described by Müller and Crothers 1968,<sup>1</sup> is a well-established method to study dissociation of cationic, hydrophobic drugs from DNA.<sup>4–9</sup> The

micelles are in this technique supposed to drive the equilibrium from DNA-bound drug toward 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 drug leaves DNA.<sup>4–6</sup> 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 speeds up or slows down the dissociation.<sup>6,10</sup> The negatively charged surfactant monomers, in the bulk outside the micelles, are 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

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**Figure 1.** Structures of DNA intercalators:  $[\mu\text{-(11,11'-bidppz)(phen)}_4\text{Ru}_2]^{4+}$  (**1**),  $[\mu\text{-c4(cpdpzp)}_2(\text{phen})_4\text{Ru}_2]^{4+}$  (**2**), ethidium (1+) (**3**), and YOYO-1 (4+) (**4**).

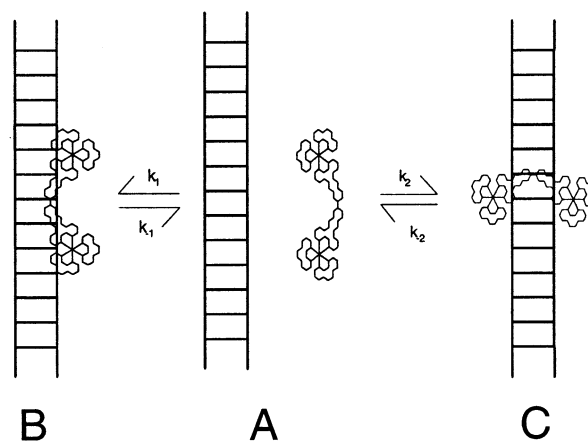
photophysical properties when bound to DNA.<sup>11–18</sup> When studying the dissociation from DNA and the recently discovered extremely slow rearrangement from groove binding to intercalation of  $\Delta, \Delta\text{-}[\mu\text{-(11,11'-bidppz)(phen)}_4\text{Ru}_2]^{4+}$  (11,11'-bidppz = 11,11'-bis(dipyrido[3,2-a:2'3'-c]phenaziny))<sup>19</sup> (**1** in Figure 1), we noticed that the dissociation was faster than the rearrangement from groove binding to intercalation, an observation that is formally inconsistent with the final binding mode being the thermodynamically most stable one.

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

From earlier studies it is known that groove binding (state B) occurs rapidly and much faster than intercalation (state C).<sup>19</sup> This means that  $k_1$  is larger than  $k_2$ . Because groove binding is much faster than intercalation, there is a preequilibrium between A and B and the rate with which C is formed is

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

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**Figure 2.** A schematic representation of observed rearrangement processes for  $[\mu\text{-(11,11'-bidppz)(phen)}_4\text{Ru}_2]^{4+}$  with respect to DNA. State A corresponds to the ruthenium complex being bound in the ionic atmosphere of DNA. State B represents an initial tight binding state, 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.

where  $k_2 k_{-1} / k_1$  is the rate of rearrangement from B to C. Furthermore, the most stable binding mode is intercalation,<sup>19</sup> i.e., the equilibrium constant for the process from A to C is larger than that from A to B, and thus  $k_2 / k_{-2} > k_1 / k_{-1}$ . This implies that  $k_{-2} < k_2 k_{-1} / k_1$ , i.e., that the dissociation should be slower than the rearrangement, in conflict with the measurements on **1**.<sup>19</sup> 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.

We have found that the apparent rate of dissociation of **1** from DNA at a given total surfactant concentration, above the

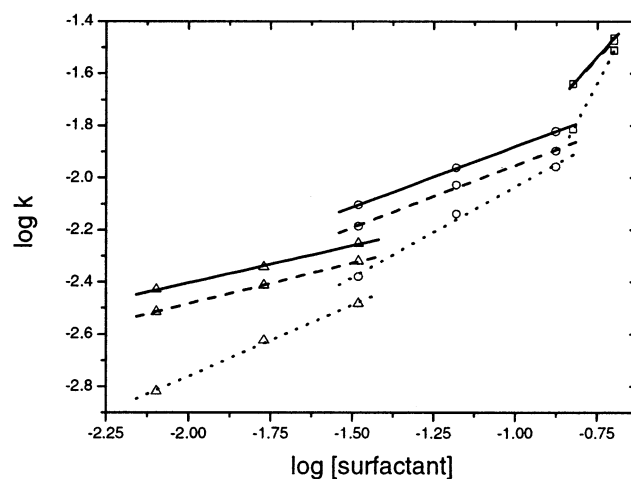
cmc, varies with the surfactant concentration and with the length of the alkyl chain of the amphiphilic molecule, using decyl and octyl sulfate instead of dodecyl sulfate micelles. To investigate if these effects are general for intercalating DNA drugs or an effect unique for **1**, we also studied  $\Delta, \Delta$ -[ $\mu$ -c4(cpdpz)<sub>2</sub>(phen)<sub>4</sub>Ru<sub>2</sub>]<sup>4+</sup> (**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.<sup>17,18</sup> Ethidium, a small DNA-intercalating drug<sup>20,21</sup> 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.<sup>22,23</sup> Further, we present a method to determine the true rate of dissociation from DNA by adding an excess of a different DNA, as an absorbent for the dissociated drug instead of micelles.

## Materials and Methods

**Chemicals.** Except where otherwise noted, all experiments were performed in 100 mM NaCl, 1 mM sodium cacodylate buffer, pH = 7. The pH of the solutions with the highest concentrations of surfactant did not significantly deviate from that in buffer alone. The ruthenium complexes (**1** and **2**) were synthesized as described elsewhere,<sup>17,24</sup> ethidium (**3**) was obtained as its bromide salt from Sigma-Aldrich, and YOYO-1 (**4**) was obtained as its iodide salt in DMSO from Molecular Probes. Calf thymus DNA (ct-DNA) was purchased from Sigma-Aldrich and poly(dA-dT)<sub>2</sub> from Amersham Biosciences. Sodium dodecyl sulfate (SDS), sodium decyl sulfate (SDeS), and sodium octyl sulfate (SOS) were purchased from Sigma-Aldrich and stock solutions made in cacodylate buffer.

**Sample Preparation.** Stock solution of ct-DNA was prepared by dissolving lyophilized ct-DNA in buffer to a concentration of about 10 mM bases. The solution was stirred overnight and then filtered three times through a 0.7  $\mu$ m polycarbonate filter. A stock solution of poly(dA-dT)<sub>2</sub> was made at a concentration of ca. 3 mM in buffer. For **1**–**3** the concentration in all experiments was 20  $\mu$ M and the DNA concentration was 160  $\mu$ M in bases, for **4** the concentration was 0.5  $\mu$ M and the DNA concentration was 8  $\mu$ M in bases. However, in the studies of the dissociation of **2** from poly(dA-dT)<sub>2</sub> into an excess of ct-DNA, the concentration of **2** was lowered to 0.5  $\mu$ M and the DNA concentration was equally lowered to 4  $\mu$ M. The concentrations of all duplex nucleic acid samples were confirmed by measuring the absorbance on a Cary 4B spectrophotometer, using  $\epsilon_{260} = 6600 \text{ cm}^{-1} \text{ M}^{-1}$  (ct-DNA) and  $\epsilon_{262} = 6600 \text{ cm}^{-1} \text{ M}^{-1}$  (poly(dA-dT)<sub>2</sub>). 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 could be monitored as 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  $\tau$ 3 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



**Figure 3.** Influence of surfactant concentration on the rate constant ( $k$ ) for the dissociation of  $\Delta, \Delta$ -[ $\mu$ -c4(cpdpz)<sub>2</sub>(phen)<sub>4</sub>Ru<sub>2</sub>]<sup>4+</sup> intercalated in ct-DNA in 100 mM (dotted line), 150 mM (dashed line), or 200 mM NaCl (solid line) for three surfactants: sodium dodecyl sulfate ( $\Delta$ ), sodium decyl sulfate ( $\circ$ ), and sodium octyl sulfate ( $\square$ ). All measurements refer to ambient temperature (25 °C) and 1 mM sodium cacodylate buffer. The cmc values at 1 mM sodium cacodylate buffer for sodium dodecyl sulfate, sodium decyl sulfate, and sodium octyl sulfate are 1.5, 14, and 97 mM, respectively.<sup>25</sup>

speed up the dissociation process. When studying the kinetics of the rearrangement of **2**, from poly(dA-dT)<sub>2</sub> to a large excess of ct-DNA, the excitation wavelength was 440 nm and the emission was recorded at 620 nm. Due to the extreme slowness of the dissociation at room temperature, the kinetics was studied at 50 °C. In all the fluorescence studies the spectra are presented normalized with respect to the maximum fluorescence in each measurement. In Figures 3 and 4 the rate constant  $k$  is determined by monoexponential fitting of the kinetic data, while in Figures 7 and 8,  $k$  is just represented as  $(\tau_{1/2})^{-1}$ .

**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 cutoff filter. For ethidium the excitation wavelength was 480 nm and no cutoff 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, the cell, and the mixing chamber were held at constant temperature (25 °C) by a water thermostat.

## 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 sulfate (SDS), sodium decyl sulfate (SDeS), and sodium octyl sulfate (SOS), at three different ionic strengths.

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 surfactants approach each other at the borders, where 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,

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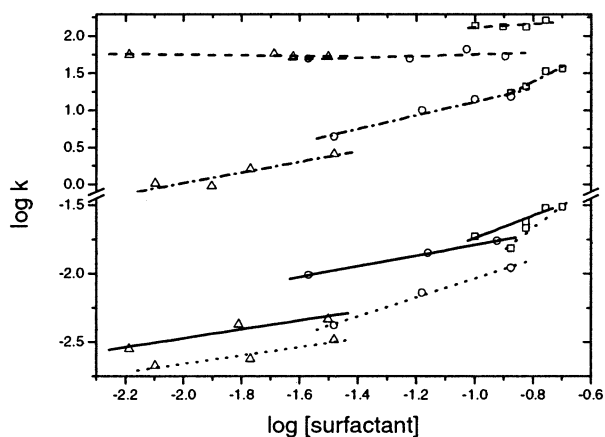
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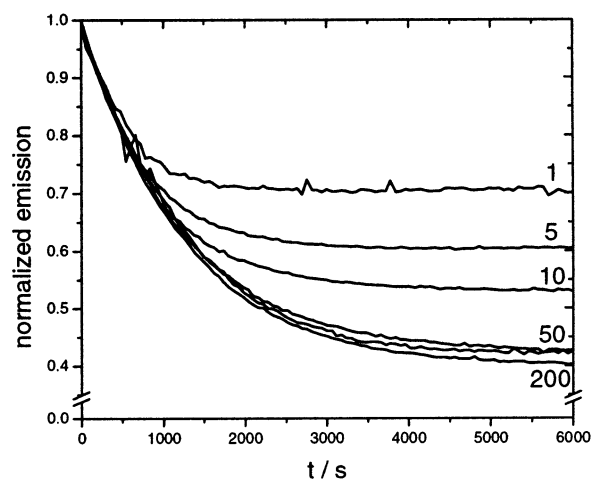
**Figure 4.** Dependence of the dissociation rate constant ( $k$ ) on surfactant concentration for the dissociation of ethidium (dashed line), YOYO-1 (dash-dotted line),  $\Delta, \Delta$ - $[\mu$ -c4(cpdpz) $_2$ (phen) $_4$ Ru $_2$ ] $^{4+}$  (dotted line), and  $\Delta, \Delta$ - $[\mu$ -(11,11'-bidppz)(phen) $_4$ Ru $_2$ ] $^{4+}$  (solid line) from ct-DNA, with sodium dodecyl sulfate ( $\Delta$ ), sodium decyl sulfate ( $\circ$ ), and sodium octyl sulfate ( $\square$ ). Buffer was 100 mM NaCl with 1 mM sodium cacodylate. Measurements were made at room temperature for 2, 3, and 4, but at 50 °C for 1.

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.

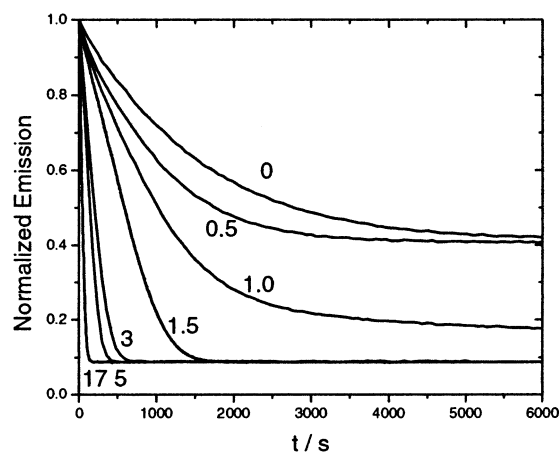
Figure 4 compares the apparent rates of dissociation ( $k$ ) of the four drugs (1–4) from ct-DNA, determined by detergent sequestering at different concentrations of the three different surfactants.

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.<sup>21</sup> A similar behavior is noticed with SDeS. 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 2 differs significantly between poly(dA-dT) $_2$  and ct-DNA (Önfelt, personal communication), 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 decays (>50 times excess) in Figure 5 correspond solely to compound 2 leaving its binding sites on poly(dA-dT) $_2$ .



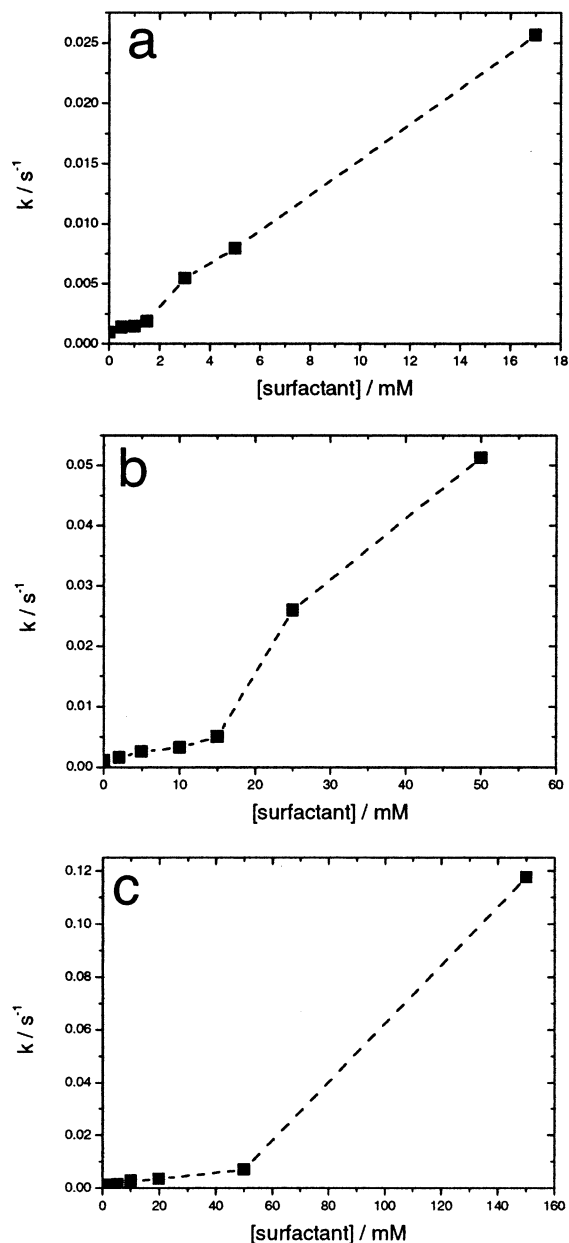
**Figure 5.** Fluorescence intensity decay monitoring the dissociation of 2 from poly(dA-dT) $_2$  upon an added excess of ct-DNA. The excess of absorbent DNA ranges from 200 (lowest curve), 100, 50, 10, and 5 times excess down to equal amounts of ct-DNA and poly(dA-dT) $_2$  (top curve). Buffer was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



**Figure 6.** Fluorescence intensity decay monitoring the dissociation of 2 from poly(dA-dT) $_2$  upon addition of a 50 times excess of ct-DNA (200 mM), together with surfactant (SDS). SDS concentration range was 0, 0.5, 1, 1.5, 3, 5, and 17 mM. Buffer was 100 mM NaCl with 1 mM sodium cacodylate. Measurements were performed at 50 °C.

The results in Figure 6 show what happens when a 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. First, 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 behavior is when the ruthenium compound finally ends up in the micelles, but the concentration of micelles being 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 50 000 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 behavior is when all of the drug molecules directly end up in the micelles, because

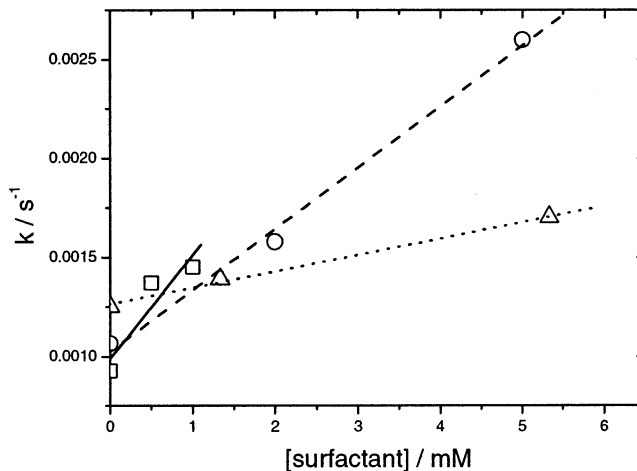


**Figure 7.**  $k_{\text{diss}}$  for dissociation of **2** from poly(dA-dT)<sub>2</sub> upon addition of a 50 times excess of ct-DNA, and with surfactants sodium dodecyl sulfate (a), sodium decyl sulfate (b), and sodium octyl sulfate (c) added at varying concentrations. Buffer was 100 mM NaCl with 1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

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 (>1.5 mM).

Corresponding measurements, using SDeS and SOS instead of SDS (data not shown), also exhibited three characteristic types of behavior, consistent with the behavior in Figure 6. In Figure 7a–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.

For all three surfactants, the “DNA-monitored” dissociation of the ruthenium compound from poly(dA-dT)<sub>2</sub> is observed to significantly increase its rate with increasing surfactant concentration, also in the concentration range below the cmc, where



**Figure 8.** Monomeric surfactant effect.  $k_{\text{diss}}$  for the dissociation of **2** from poly(dA-dT)<sub>2</sub> upon addition of a 50 times excess of ct-DNA, as a function of surfactant concentration (below cmc). The slopes of the linear fits were 0.5  $\text{s}^{-1}$  for sodium dodecyl sulfate ( $\square$ ), 0.3  $\text{s}^{-1}$  for sodium decyl sulfate ( $\circ$ ), and 0.08  $\text{s}^{-1}$  for sodium octyl sulfate ( $\triangle$ ). Buffer used was 100 mM NaCl with 1 mM sodium cacodylate. Measurements were performed at 50 °C.

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 rate of dissociation: obviously, the most hydrophobic surfactant monomer, SDS, has the greatest effect upon the rate of dissociation, followed by SDeS and SOS.

## Discussion

We report the discovery that anionic surfactants enhance the rate of dissociation of cationic DNA–intercalating molecules from DNA. Whereas it is known that increasing ionic strength may increase the rate of dissociation of **2** from DNA,<sup>18</sup> it is clear (e.g. in Figure 3) that the increased rate of dissociation is not solely an effect of increased sodium ion concentration. Nor can ionic strength effects explain differences in rates in the presence of 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 is further demonstrated in Figure 4: if the micelles had only been able to bind totally dissociated drug molecules, the rate of dissociation should be independent of the micelle concentration and thus resulted in straight horizontal lines 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 Figures 3 and 4 are all at concentrations well above the 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 the 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 the cmc and that the effect grows with increasing surfactant monomer concentration.

The observation that negatively charged surfactant monomers can 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 the large DNA ligands in combination with their positive charges, however, may provide an attractive environment for the association of surfactant molecules with their negative headgroup and hydrophobic tail, and this could make it easier for the surfactant monomers to get close to the DNA. The effects is also expected to be larger with increasing charge on the ligand, because a high charge would neutralize the negative charge on DNA more and thereby facilitate the approach of the negatively charged surfactant monomers. This can be seen in Figure 4, where the effect is smallest for ethidium, which has only one positive charge, and is of similar magnitude for the three compounds that have four positive charges. That ruthenium complex ions alone in solution may form aggregates with SDS monomers, even below the cmc, has been reported,<sup>26</sup> showing that these kinds of hydrophobic cationic molecules may provide an environment promoting the binding of anionic, amphiphilic monomers. The electrostatic effect can also be noticed in Figure 3, where the slope is steeper for 100 mM NaCl than for the higher ionic strengths. This is because each added sodium ion that follows with the surfactant gives a higher relative contribution to the total electrostatic effect at lower salt concentration. 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 mole of added SDS, SDeS, and SOS is 0.5, 0.3, and 0.08 s<sup>-1</sup> M<sup>-1</sup>, 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 the cmc (Figures 3, 4, 6, and 7a–c), where the monomer concentration is generally believed to be constant or even decreasing,<sup>27</sup> 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.<sup>28</sup> Despite this view, the indisputable observation of a stronger enhancement of the rate of dissociation for high surfactant concentrations than for concentrations below the cmc clearly suggests an effect of the micelles. The total rate constant may, thus, be phenomenologically described as

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

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

However, as mentioned, it is not likely that the micelle itself is actually 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.<sup>29</sup> Thus, a higher micelle concentration will also correspond to a higher probability of suddenly having a transiently high local concentration of monomers, [monomer]<sub>local</sub>, at positions in the solution where a micelle happens to disrupt. An enhancing factor of increasing the local concentration of monomers close to DNA may come as a result of dispersion forces,<sup>30–33</sup> which are anticipated to be substantial between the polarizable DNA and the polarizable micelles. Dispersion forces may thus lead to an accumulation of micelles at a certain distance from the DNA helix outside the counterion (Na<sup>+</sup>) layer and lead to the number density of surfactant molecules near the DNA helix increasing with total surfactant concentration, also above the cmc.

The model we wish to propose is that the mechanism of the surfactant-induced increased rate of dissociation does not involve the micelles, as such, but instead depends on the concentration peaks of the fluctuating 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 clusters of surfactant molecules (“plaque”) up to a maximum limiting size ( $i = N$ ).

$$k_{\text{total}} = k_0 + \sum_{i=1}^N k_i [\text{monomer}]_{\text{local}}^i \quad (3)$$

Below the cmc, [monomer]<sub>local</sub> may be regarded equal to the bulk monomer concentration (rate  $k_1$ ), whereas above the cmc, with increasing surfactant concentration, we may assume it to be a monotonically increasing quantity, though without knowledge of its size. It is reasonable that the rate-enhancing effect (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 how the surfactant molecules bring about a reduction of the activation barrier for dissociation of the DNA ligands. One part could be that a hydrophobic environment may favor transient openings of the otherwise quite compact DNA duplex structure, providing a recipient for exposed hydrophobic moieties of the intercalated ligands. The observation of an increased efficiency of added detergent above the 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

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enhancement that we here report may indeed have significance also in biological processes. Amphiphilic molecules are abundant at relatively high concentrations around and in the living cell: from the phospholipids 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 and (more strongly) above the 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 and 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 proposed.

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