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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · JULY 2009

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Kinetic Study of the Cetyltrimethylammonium/DNA Interaction

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Received: December 12, 2008; Revised Manuscript Received: April 23, 2009

A kinetic study of the interaction of the surfactant cetyltrimethylammonium (CTA^+) with DNA was carried out in water and in salt (NaCl) solutions. The results can be explained in terms of a reaction mechanism involving two consecutive reversible steps. The first step corresponds to the union/separation of the surfactant with/from the DNA. The second step corresponds to a conformational change of the surfactant/DNA complex. The equilibrium constant, calculated from the forward and reverse rate constants of these steps, agrees with the results of a previous thermodynamic study.

Introduction

The complexation of DNA with cationic surfactants has recently been of interest because of the relationship of this type of process to DNA purification[1] and particularly to gene transfer[2] and gene therapy[3] applications. These applications are based on the fact that surfactants produce a reduction of the DNA charge, together with a compaction of the DNA. These effects facilitate the uptake of nucleic acids through the cellular membrane.[4] It is generally assumed that, at concentrations of surfactant lower than the critical aggregation concentration (cac), DNA adopts extended coil conformations similar to those observed in the absence of surfactants.[5] Above the cac, the union of surfactants with DNA does not occur uniformly across the population of DNA molecules in solution. This union involves both electrostatic interactions of the polar heads of the surfactants with the phosphate groups and hydrophobic interactions between the hydrophobic tails of the surfactants. Under these circumstances, a part of the DNA in solution remains as extended coils, and the rest adopts a globular (compact) state.[6] When the surfactant concentration is high enough to produce complete compaction of the DNA, further addition of the surfactant induces an aggregation of the globules, leading to the precipitation of DNA/surfactant aggregates. This view of surfactant−DNA interactions is supported by a large amount of structural and thermodynamic data obtained from fluorescence techniques,[7] calorimetry,[8] UV−vis and IR spectroscopies,[9,10] NMR spectroscopy,[10] dynamic light scattering,[11] simulation techniques,[12] fluorescence microscopy,[13] potentiometry,[14] and linear and circular dichroism.[9,15]

In previous research in this field[16] using pyren-1-carboxyaldehyde as a fluorescence probe, our group carried out a systematic study of the interactions of a surfactant series of the alkyltrimethylammonium type, $R-N(CH_3)_3$, with DNA, where $R = CH_3$, C_2H_5, C_4H_9, C_8H_{17}, $C_{12}H_{25}$, $C_{14}H_{29}$, and $C_{16}H_{33}$. This study permitted the electrostatic and nonelectrostatic free energies of binding to be separated. Moreover, it was established that the nonelectrostatic free energy is linearly related to the number of carbon atoms in R, when this number is greater than or equal to 4. The results can be interpreted by taking as a basis a two-state model of DNA (in the presence of surfactants). That is, the results support the idea that DNA, when present in solutions also containing surfactants, appears as a mixture of two conformational states (the extended coil and the globular state) in a proportion that depends on the surfactant concentration. In other words, the free energy of binding of the probe to DNA, in the presence of surfactants, is the weight average of the free energies corresponding to binding to the two different states of DNA. These states are present in the solution in proportions depending on the concentration of the surfactant, which produces the change from one to the other.

From the preceding discussion, it is clear that the effects of the interactions between surfactants and DNA on the structure of the latter are reasonably well-known. However, the mechanism of the compaction process is poorly understood. This information is particularly necessary in relation to gene transport, because the release of DNA from the vectors, which obviously has kinetic implications, is one of the parameters that controls this process.[17] For this reason, we conducted a kinetic study on surfactant−DNA interactions to learn more about the factors controlling the compaction/decompaction process of DNA by surfactants, a field where kinetic studies are rather scarce.[18]

Experimental Details

Materials. Calf thymus DNA was purchased from Pharmacia and used without further purification, as preliminary experiments showed that purification does not produce any changes in the experimental results. The average number of base pairs per DNA molecule was 3000 bp.

Polynucleotide concentrations were determined spectrophotometrically from molar absorbance (6600 mol^{-1} dm^3 cm^{-1} at 258 nm in order to have the DNA concentration in phosphate units).[19] High-molecular-weight poly(vinyl chloride) (PVC), cetyltrimethylammonium bromide (CTABr), and tetrahydrofuran (THF) were products of Fluka. The plasticizer, dioctyl phthalate, was purchased from Aldrich, Milwaukee, WI. NaCl and sodium tetraphenylborate were obtained from E-Merck, Darmstadt, Germany. All reagents were used as purchased. The water used in the preparation of solutions had a conductivity of less than 10^{-6} S m^{-1}.

Methods. A new cetyltrimethylammonium-cation- (CTA^+-) selective membrane was prepared employing CTA^+-tetraphenylborate neutral ion-selective material. The carrier complex was synthesized by mixing equimolecular amounts of CTABr

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10.1021/jp810966n CCC: $40.75
Published on Web 05/27/2009

SCHEME 1: Basic Cell Layout

	Reference	CTA^+-selective	Test	
Ag/AgCl	Solution	membrane	solution	Ag/AgCl

and sodium tetraphenylborate in solution. The resulting white precipitate of the complex was washed repeatedly with water and then recrystallized in THF. Membranes were prepared following a procedure similar to that described previously.[20] The membrane was placed on one end of a PVC tube, using PVC paste and THF as glue. It was conditioned for 3−4 h with the reference CTABr solution (1 mmol dm^{-3} containing 10 mmol dm^{-3} NaCl) and then used.

The basic layout of the cell is depicted in Scheme 1.

The salt bridges used were KCl saturated in agar gel. The electromotive force (emf) of the system was measured with a custom-built electrometric amplifier using an INA 116 ultra-low input bias current instrumentation amplifier, followed by a unity-gain Sallen & Key low-pass filter [$n = 4, f_c = 15$ Hz (−3 db)]. The stability of the readings was 0.1 mV.

The response was monitored with a PROTEK 6800 DMM instrument, interfaced to a PC through an RS232 interface. In all cases, the membrane employed had a nernstian response to CTA^+ concentrations, with slopes ranging from −0.057 to −0.060 V.

All kinetic experiments were carried out at 298.2 ± 0.1 K, at fixed initial concentrations of the surfactant and salt, and at different concentrations of DNA, ranging from 5×10^{-6} to 1×10^{-3} mol dm^{-3}, for each salt concentration. Changes of the free CTA^+ concentration were found to be biexponential with respect to time.

Preliminary kinetic studies at a fixed concentration of DNA (5×10^{-4} mol dm^{-3}) revealed that the relaxation times are independent of CTAB concentration within the limits of experimental error, in the concentration range from 7.5×10^{-6} to 5×10^{-5} mol dm^{-3}. This implies that there is no evidence of cooperative effects on the kinetics of this reaction. It should be noted that, in the presence of such effects, the binding constants (and thus the corresponding rate constants) would be dependent on the relation between the concentrations of DNA and surfactant.[21]

Results

Figure 1 displays a typical plot of one kinetic experiment. As mentioned previously, variations of the CTA^+ concentration with time are described by a biexponential function

$$[CTA^+] = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} \quad (1)$$

characterized by two time constants, τ_1 and τ_2. This behavior is consistent with a two-step process, as is frequently observed in kinetic studies corresponding to the binding of other species to DNA[22]

$$\text{DNA} + \text{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\text{DNA/S})_\text{I} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (\text{DNA/S})_\text{II} \quad (2)$$

For an excess of DNA, as in our experiments, it can be shown that[23]

$$\frac{1}{\tau_1} = k_1[\text{DNA}] + k_{-1} \quad (3)$$

Thus, k_1 and k_{-1} can be obtained from the plot of $1/\tau_1$ vs [DNA], and an example is shown in Figure 2.

On the other hand, $1/\tau_2$ is given by[23]

$$\frac{1}{\tau_2} = \frac{k_2 K_1[\text{DNA}]}{1 + K_1[\text{DNA}]} + k_{-2} \quad (4a)$$

with

$$K_1 = \frac{k_1}{k_{-1}} \quad (4b)$$

Consequently, k_2 and k_{-2} can be obtained from a fit of $1/\tau_2$ to eq 4a, once k_1 and k_{-1} have been obtained, so that that K_1 is a known parameter (see Figure 3).

The values of k_1, k_{-1}, k_2, and k_{-2}, obtained as previously described, are reported in Table 1.

Discussion

As can be seen in Table 1, all of the rate constants decrease when the ionic strength, I (NaCl), of the solution containing the DNA and surfactant increases.

The decrease of k_1 as the concentration of NaCl increases is in line with the idea, mentioned in the Introduction, that the driving force for the union of the surfactant and the DNA is electrostatic in nature. Hence, according to the classical theory of salt effects, this is an expected result. In fact, the logarithm of the rate constant is expected to decrease proportionally to the factor $2A|Z_+Z_-|I^{1/2}/(1 + I^{1/2})$, according to the Brönsted−Debye equation[24]

$$\log k = \log k_0 - \frac{2A|Z_+Z_-|\sqrt{I}}{1 + \sqrt{I}} \quad (5)$$

In this equation, k_0 is the rate constant at zero ionic strength, A is the Debye−Hückel constant (∼0.5 $L^{1/2}$ $mol^{-1/2}$ in water at 298.1 K), and Z_+ and Z_- are the charges of the ions participating

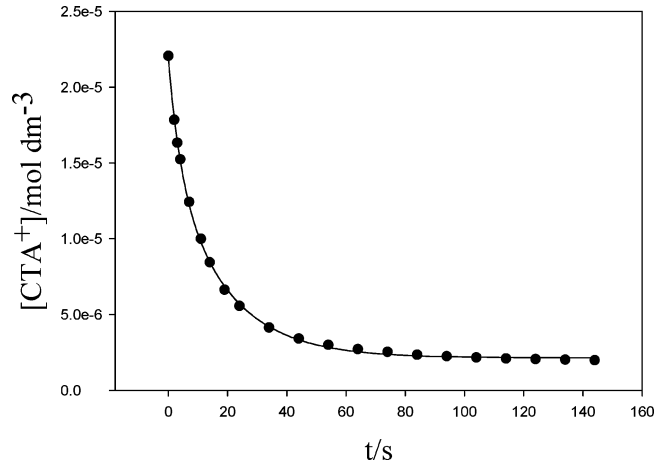

Figure 1. Plot of $[CTA^+]$ vs time, t, in a typical kinetic experiment. The dots represent experimental values, and the curve is the best fit of eq 1 to the experimental data.

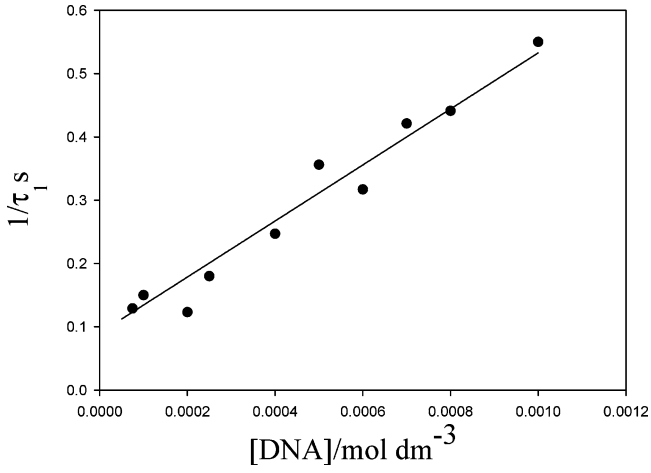

Figure 2. Dependence of the reciprocal fast relaxation time, τ_1, on the DNA concentration (0.01 mol dm^{-3} NaCl).

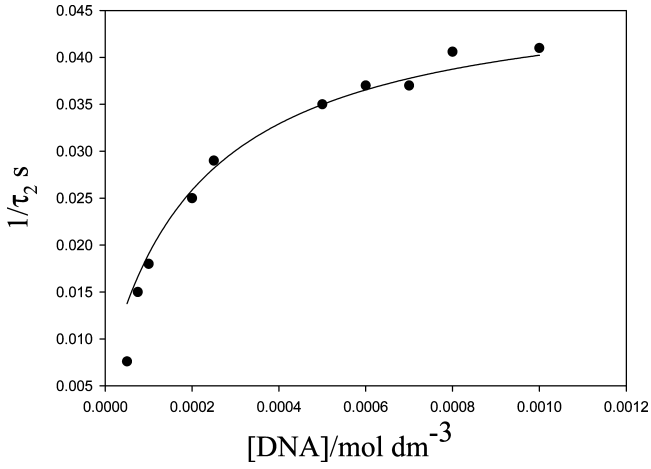

Figure 3. Dependence of the reciprocal slow relaxation time, τ_2, on the DNA concentration (0.01 mol dm^{-3} NaCl).

in the reaction ($Z_+ = +1$ for the surfactant). In the case of DNA, given its polyelectrolytic character, Z_- is difficult to establish. In any case, one can see in Figure 4 that there is a linear relationship between log k and $I^{1/2}/(1 + I^{1/2})$. Thus, the driving force for the DNA/surfactant union (the forward process of the first step of the mechanism given by eq 2) seems to be electrostatic in nature. We obtained the effective charge of the DNA in the presence of the surfactant molecules from the slope of the line in Figure 4. This results in $Z_- = 9$, which is reasonable. Notice that, despite the large charge of the DNA molecule, the screening effect of the supporting electrolyte, at a relatively high concentration in the close vicinity of DNA (see below), will permit the incoming surfactant molecule to feel the effects of only the charges of a reduced region of the DNA close to the union point.

Regarding the values of k_{-1}, it can be seen in Table 1 that this rate constant decreases when the ionic strength increases,

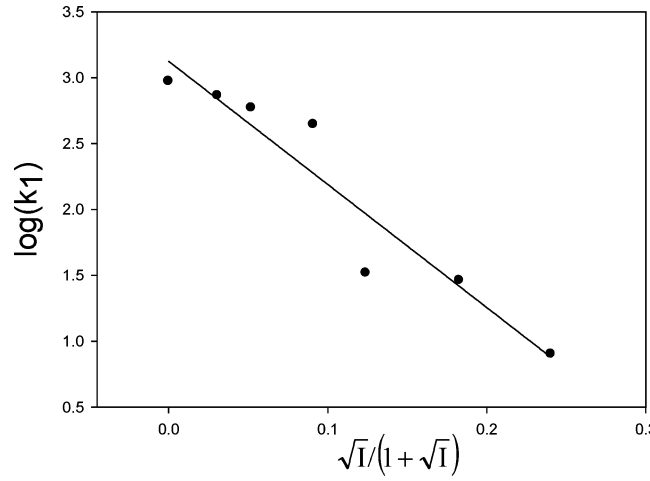

Figure 4. Plot of log k_1 vs $I^{1/2}/(1 + I^{1/2})$, where I represents the ionic strength of the solution.

as does k_1 as well. However, the decrease of k_1 is more marked than the decrease of k_{-1}, being 2 and 1 orders of magnitude, respectively. This produces a decrease in K_1, the equilibrium constant of the first step given in eq 2, as can be seen in Table 1. The decrease of k_{-1} when ionic strength increases is, in some sense, counterintuitive if one considers the first step of the mechanism as merely representing the union (forward) or separation (reverse) of charges of opposite sign, namely, that of the polar head of the surfactant and the negative charge of the phosphate groups. According to this view, a positive salt effect would be expected for k_{-1}. Notice, however, that, in the state corresponding to the product of the first step, $(DNA/S)_I$, in eq 2, hydrophobic interactions between the hydrophobic tails of the surfactant, as well as between the tails and the hydrophobic moieties in the DNA, will be operative.[25] This would imply an additional stabilization of the final state, through hydrophobic interactions, and more so when the ionic strength increases, because an increase of the ionic strength, as a consequence of the increase of the concentration of the salt, produces an enhancement of hydrophobic interactions due to the salting-out of the hydrophobic solutes, as has been known for a long time.[26] It is interesting to note that, according to this explanation of the decrease of k_{-1} as the salt concentration increases, hydrophobic interactions must be absent in the transition state or at least less important than in the final state. Therefore, the transition state would correspond to the surfactant bound to DNA mainly by electrostatic interactions.

The rate constant k_2 must correspond to a conformational change of the DNA/surfactant complex formed in the first step, $(DNA/S)_I$, toward the final conformation of the complex $(DNA/S)_{II}$. Obviously, k_{-2} corresponds to the reverse configurational change.[27] These configurational changes, as a whole, are relatively slow, but they involve microscopic changes corresponding to small portions of DNA that must be sufficiently rapid, given that the final configuration is reached within a

TABLE 1: Equilibrium and Rate Constants for the Interaction of DNA with CTA^+

[NaCl] (M)	K ($\times 10^{-4}$ M^{-1})	K_1 ($\times 10^{-3}$ M^{-1})	K_2	k_1 (M^{-1} s^{-1})	k_{-1} ($\times 10^3$ s^{-1})	k_2 ($\times 10^4$ s^{-1})	k_{-2} ($\times 10^3$ s^{-1})
0.000	3.6	6.5	4.6	939	140	866	19.00
0.001	3.5	6.0	4.8	732	115	720	15.00
0.003	3.5	6.0	4.8	597	100	591	12.20
0.010	3.4	4.5	6.7	443	90	415	6.10
0.020	3.1	1.8	17.4	33	19	96	0.55
0.050	2.7	1.3	20.0	29	22	20	0.10
0.100	1.5	0.4	32.0	8	19	17	0.06

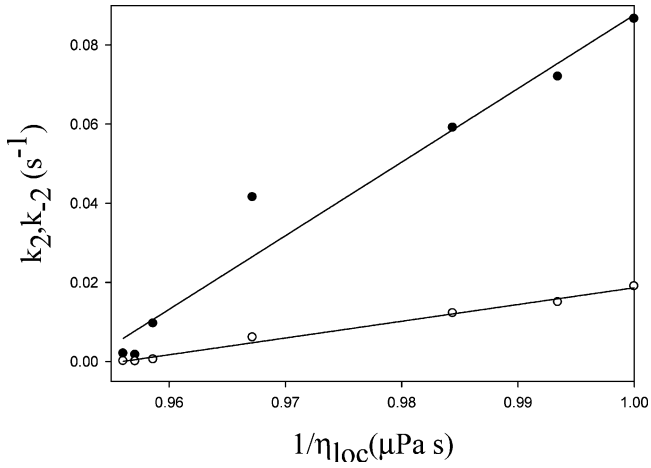

Figure 5. Forward and reverse rate constants, k_2 (●) and k_{-2} (○), respectively, corresponding to the second step (see eq 2) vs $1/\eta_{loc}$. η_{loc} is the local viscosity estimated as described in the text.

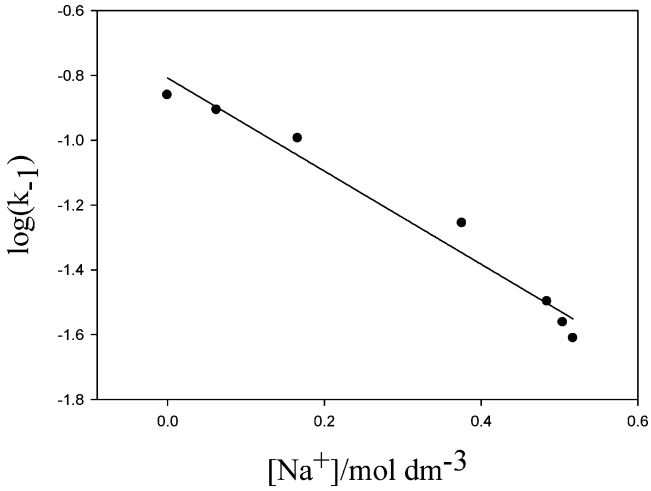

Figure 6. Plot of log k_{-1}, the reverse rate constant of the first step in eq 2, vs the local concentration of sodium cation.

reasonable time. If these rapid microconfigurational changes are diffusion-controlled, then an increase in the salt concentration, which produces an increase in the viscosity, would make both the forward and reverse processes in step 2 more difficult. According to the Smoluchowsky[28] treatment of diffusion-controlled reactions, the rate constant for these kind of processes are inversely proportional to the viscosity of the reaction medium (η).[29] There are difficulties in supporting this idea, because k_2 and k_{-2} should correlate with the *local* viscosity, η_{loc}. However, we were able to estimate this local viscosity as follows: The concentration of counterions (Na^+) in the close vicinity of the DNA was calculated by assuming a Boltzmann distribution of these counterions

$$\frac{[Na^+]_{DNA}}{[Na^+]_{bulk}} = e^{-\Psi F/RT} \tag{6}$$

where Ψ is the electrostatic potential difference between the DNA and the bulk. The (negative) values of Ψ were taken from ref 30. From the values of $[Na^+]_{DNA}$, we estimated the local viscosity, assuming that it is the same as the viscosity of the solution of NaCl of the same concentration. This assumption is in agreement with the fact that the contribution of the chloride ion to the viscosity is small, as it corresponds to the low value of the B (Jones−Dole) coefficient for this ion.[31] In other words, changes in the local viscosity are mainly due to changes in the local concentration of Na^+ ions.

Once the values of the local viscosity had been obtained, the k_2 and k_{-2} rate constants were plotted against the values of $1/\eta_{loc}$. There is a linear relationship between these rate constants and $1/\eta_{loc}$, as can be seen in Figure 5, which supports the idea that k_2 and k_{-2} correspond to diffusion-controlled configurational changes.

Once the local concentrations of Na^+ ions had been estimated, the variations of k_{-1} were considered again. According to the previous discussion of this parameter, its variations are related to an increase of the hydrophobic interactions of the surfactant tails caused by the salt. This increase in the hydrophobic interactions is, of course, a consequence of the salting-out effects of the salt on the hydrophobic solutes. These salting-out effects would imply a linear relationship between log k_{-1} and the local concentration of ions. Thus, the Brönsted equation in this case would be

$$\log k_{-1} = \log(k_{-1})_0 + \log \frac{\gamma_{(DNA/S)_I}}{(\gamma \neq)_1} \tag{7}$$

and according to Setchenow's equation[32]

$$\log \gamma_i = K_S[NaCl] \tag{8}$$

Consequently

$$\log k_{-1} = \log(k_{-1})_0 + \Delta K_S[NaCl] \tag{9a}$$

$$\Delta K_S = (K_S)_{(DNA/S)1} - (K_S)_{\neq 1} \tag{9b}$$

As can be seen in Figure 6, eq 9a holds true. This gives support to our arguments about the cause of changes in k_{-1} when the salt concentration is changed. Moreover, the decrease of k_{-1} with increasing salt concentration ($\Delta K_S < 0$) implies that hydrophobic (stabilizing) effects are greater in the initial state of this reaction, $(DNA/S)_I$, than in the transition state, in agreement with previous qualitative explanations.

With respect to the equilibrium constants for steps 1 and 2 in eq 2, K_1 decreases as the salt concentration increases. This, clearly, is a consequence of the fact that changes in this equilibrium constant are mainly controlled by k_1, which, in turn, depends on the electrostatic interactions between the polar head of the surfactants and the phosphate groups. On the other hand, K_2 increases with increasing salt concentration. This compensates for variations of K_1, so that variations of K

$$K = K_1(1 + K_2) \tag{10}$$

are relatively minor. It is interesting to note that the value of the global equilibrium constant K

$$DNA + S \overset{K}{\rightleftharpoons} (DNA/S)_{II} \tag{11}$$

agrees, in the absence of the salt, with the value obtained for this parameter in a previous work[16] ($K = 3.2 \times 10^4$ mol dm^{-3}) where the value of K was directly obtained from a purely thermodynamic method. This fact gives support to the mechanism proposed here because eq 10 is a consequence of this mechanism.

In conclusion, a two-step reversible mechanism is proposed for the union of surfactants with DNA. The forward process for the first step is controlled by the electrostatic interactions between the polar head of the surfactant and the phosphate groups on the DNA. The reverse process is dependent on the hydrophobic interactions between the tails of the surfactant molecules, once they are bound to the DNA. The second step, in the forward and reverse directions, is a diffusion-controlled conformational change.

Acknowledgment. This work was financed by the D.I.GYT (CTQ-2008-00008/BQU) and the Consejería de Educación y Ciencia de la Junta de Andalucía.

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