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PROBING THE MICELLE/WATER INTERFACE BY A RAPID LASER-INDUCED PROTON PULSE

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Summary

The laser-induced pH jump (Gutman, M. and Huppert, D.J. (1979) Biochem. Biophys. Methods 1, 9—19) has a time resolution capable of measuring the diffusion-controlled rate constant of proton binding. In the present study we employed this technique for measuring the kinetics of protonation-deprotonation of surface groups of macromolecules.

The heterogeneous surface of proteins excludes them from serving as a simple model, therefore we used micelles of a neutral detergent (Brij 58) as a high molecular weight structure. The charge was varied by the addition of a low concentration of sodium dodecyl sulfate and the surface group with which the protons react was an adsorbed pH indicator (bromocresol green or neutral red).

The dissociation of a proton from adsorbed bromocresol green is slower than that from free indicator. This effect is attributed to the enhanced stabilization of the acid form of the indicator in the pallisade region of the micelle. The pK shift of bromocresol green adsorbed on neutral micelles is thus quantitatively accounted for by the decreased rate of proton dissociation. Indicators such as neutral red, which are more lipid soluble in their alkaline form, do not exhibit such decelerated proton dissociation in their adsorbed state nor a pK shift on adsorption to neutral micelles.

The protonation of an indicator is a diffusion-controlled reaction, whether it is free in solution or adsorbed on micelles. By varying the electric charge of the micelle this rate can be accelerated or decelerated depending on the total

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Abbreviations: SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide.

charge of the micelle. The micellar charge calculated from this method was corroborated by other measurements which rely only on equilibrium parameters.

The high time resolution of the pH jump is exemplified by the ability to estimate the diffusion coefficient of protons through the hydrated shell of the micelle.

Introduction

Protons are involved in a multitude of chemical equilibria with biochemical systems, thus in principle, a rapid pH jump can be used as an efficient perturbant of a biochemical reaction. The effects of pH on a biochemical reaction have been used in equilibrium studies, yet the kinetic aspect was limited to a few reaction which are slower than the mixing time of the rapidly mixing systems used for changing the pH [1—3].

In order to observe a kinetic response a perturbed system, the perturbing event must be faster than the resulting response. Thus, if we wish to extend the pH perturbation to a fast reaction, such as coupling between a redox reaction and proton translocation in mitochondrial membranes, the change of pH must be faster than the response of the measured system. Recent studies in our laboratory [4,5] and by Campillo et al. [6] utilized an intense and short laser pulse to induce a fast sub-nanosecond dissociation [7] of protons from excited naphthol of hydroxypyrene derivatives. The protonation of small molecules in solution has been described in our previous publication [5]. In the present study, we investigate the rapid kinetics of protonation of macromolecules. The complexity of protein surfaces prevents their use as a model system, however, micelles are well recognized structures and their physical properties can be easily measured, calculated and accounted for [8] during the quantitative evaluation of the results. The ability to form mixed micelles of neutral and charged detergents allows the modification of the electric charge of the micelles [9]. Finally, the high affinity of adsorption of some pH indicators to micelles enables us to attach these 'reporter' groups to the micellar surface [9—11]. All these properties render the micellar system a good model for studying the kinetics of protonation of surface groups of high molecular weight structures.

In the present study, we could discriminate between two effects of the micelle/water interface on the kinetic properties of the adsorbed indicator. The first effect is due to the selective dissolution of one form of the indicator (HIn) in the hydrophobic core of the micelle. The consequence of this stabilization is the deceleration of the proton dissociation from HIn, accompanied by an alkaline shift of the pK. This observation furnishes the kinetic evidence for the equilibrium studies of Tong and Glesmann [12] who studied the pK shift of compounds adsorbed on neutral micelles of Triton X-100.

The second effect we studied was the effect of the surface charge on the kinetics of protonation of the adsorbed indicator. As will be documented, the charge effect on the rate of protonation is fully accounted for by Debye's theory for diffusion-controlled reactions [13,14]. The number of effective charges measured by this kinetic analysis was corroborated by thermodynamic

analysis of the equilibrium parameters (pK shift) [15] or according to Gouy-Chapman equations [16,17].

The ability of a charged surface to concentrate solutes at its interface and thus to accelerate the rate of the reaction has been known for a long time. Still, the rates of measured reactions (see Ref. 18) were 4—6 order of magnitude slower than diffusion-controlled rate constants. In the present report, we extended these studies to the range of a diffusion-controlled reaction in which the reacting species are a proton and an adsorbed indicator. The advantage of this high time-resolving technique is the possibility of investigating the properties of the hydration shell and the proton conductance at the water/micelle interface. Finally, the fact that the rate of protonation is a precise function of the macromolecules charge opens a new possibility to utilize the rate of protonation for measuring the kinetics of events leading to changes in the total charge of macromolecules, such as those encountered during a redox reaction or proton translocation.

Materials and Methods

The pH jump was induced by a short pulse of the second harmonic frequency of a Q-switched ruby laser (30 ns full-width at half intensity, 30—50 mJ per pulse) or a nitrogen laser (2 ns, approx. 0.2 mJ per pulse). For details of the optical arrangement see Ref. 4. The ejected protons were detected by the absorbance change of a pH indicator present in the pulsed solution. A continuous interrogating light was passed either colinearly or perpendicular to the excitation pulse, collimated and focussed into the entrance slit of a Jerral-Ash 250 mm monochromator. The output of the photomultiplier (RCA 1P-28) was either recorded on an oscillioscope and photographed, or amplified and digitized by a Biomation 8100 transient recorder (10 ns/address) and averaged by a Nicolet 1170 signal averager.

The solution of the indicator, micelles and proton emitter was continuously gassed by N_2 and circulated from a reservoir through a flow cell (1 mm optical path). The pH in the reservoir was monitored continuously during the experiment.

The proton emitter, 2-naphthol-3,6-disulfonate, was a Merck, Sharp & Dome preparation, recrystallized before use. Detergents used in this study were purchased from Sigma and used without further purification (Brij 58 (polyoxyethylene 20-cetyl ether), lot No. 42 1080; SDS, 45C-0084; and CTAB, Li8B-220).

Results

Determination of the rate constants of protonation and deprotonation of adsorbed pH indicator

Fig. 1 demonstrates a typical recording of a pH jump in the absence of micelles. The proton emitter was excited by a 2 ns pulse of a nitrogen laser operating at a repetition rate of 30 Hz. The ejected protons were monitored by their reaction with bromocresol green (pK 4.7; k_{on} (I = 10 mM) = $6.9 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$). The changes in absorbance due to protonation of the indicator

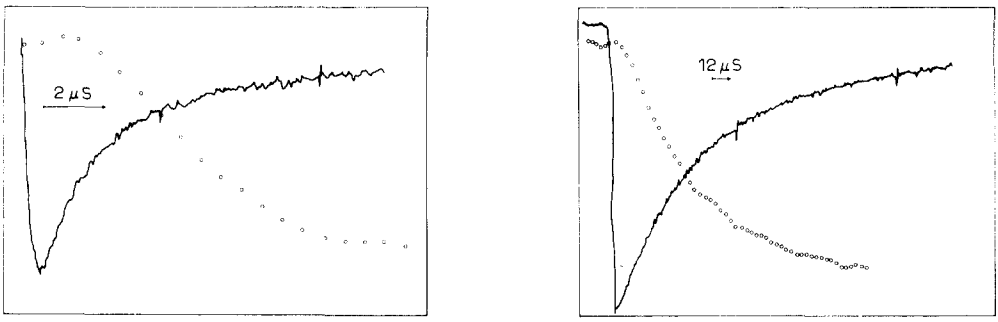


Fig. 1. The kinetics of protonation and deprotonation of bromocresol green after a proton pulse. The irradiated solution of the pH indicator (35 μM) and 3 mM of the proton emitter, 2-naphthol-3,6-disulfonate (pK^0 = 9.3, pK^* = 0.5), pH 7.5, were kept flowing through a flow cell with an optical path of 1 mm. The proton emitter was excited by repeating pulses of a nitrogen laser (2 ns, 0.2 mJ per pulse) operating at 30 pulses per s. The changes in absorption due to protonation of the indicator were monitored by following the transmittance of the 632.8 line of a continuous HeNe laser. Changes in transmittance were measured using an RCA 1p-28 photomultiplier and the output was amplified, digitized and accumulated as described in Materials and Methods. The figure represents the accumulation of 1024 events. The initial decrease in absorbance represents the formation of the protonated indicator. This section of the kinetics is expanded in the dotted presentation in which the time interval between the dots corresponds to 20 ns. The maximal deflection corresponds to protonation of 6.5 μM indicator following each laser pulse. The recovery of the signal to its prepulse level represents the redissociation of the protonated indicator. The time scale for this reaction is given in the figure.

Fig. 2. The kinetics of protonation and deprotonation of bromocresol green adsorbed on Brij 58 micelles. The experiments were carried under conditions identical to those in Fig. 1 (3 mM 2-naphthol-3,6-disulfonate, 35 μM indicator, pH 7.4, accumulation of 1024 events) except that the pulse solution contained also 4 mg/ml of Brij 58 (corresponding to 50 μM micellar concentration). The continuous line represents a full cycle of protonation-deprotonation while the initial phase of protonation of the indicator is extended by the dots. The time difference between the two dots is 20 ns.

were measured at 632 nm ($\epsilon = 33 \cdot 10^3\ M^{-1} \cdot cm^{-1}$), using a continuous HeNe laser for interrogation. The photomultiplier (RCA 1P-28) output was amplified and then digitized by a Biomation 8100 transient recorder and processed by a Nicolet 1170 signal averager. The sum of 1024 excitation pulses is documented in Fig. 1. As can be seen in this figure, the protonation of the indicator is very fast, the initial velocity (V_i) corresponds to $30\ M \cdot s^{-1}$ and the maximal concentration of HIn, measured approx. 0.5 μs after the excitation pulse, corresponds to 6.5 μM. The dissociation of the protonated indicator observed in this tracing is a first-order reaction with a rate constant $k_{off} = 1 \pm 0.2 \cdot 10^6\ s^{-1}$.

The rate of protonation calculated from this experiment ($k_{on} = k_{off}/K_{diss} = 5 \pm 0.2 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$) is identical to the value calculated according to Debye [13,14]. Using this value, the concentration of the ejected proton was calculated from the initial velocity [5]. $[H^+] = V_i/[In^-]$ (k_{on}) and found to be 13.8 μM.

Fig. 2 depicts a similar experiment in which the indicator was adsorbed on Brij 58 (50 μM with respect to micellar concentration). While the initial velocity of the reaction ($20\ M \cdot s^{-1}$) is nearly unchanged, the rate of proton dissociation is much slower ($k_{off} = 1.64 \cdot 10^4\ s^{-1}$). This slower dissociation is fully reflected in the pK value of the adsorbed indicator (pK' = 6.4). The

TABLE I

KINETIC PARAMETERS OF PROTON INTERACTION WITH BROMOCRESOL GREEN ADSORBED ON MICELLES

The kinetics were measured as described in Materials and Methods and the pK was determined experimentally at 25°C (I = 0.01 M). The pH was modulated by pulsing a 2.7 mM solution of 2-naphthol-3,6-disulfonate. The detergent concentrations were adjusted to give comparable (30—40 μM) concentrations of micelles. (Tween 80, 10 mM; Triton X-100, 10 mM; Brij 58, 4 mg/ml and CTAB, 7.1 mM).

Micelle	pK	$pK_{micelle} - pK_{H_2O}$	k_{off}	$\log \frac{k_{off(H_2O)}}{k_{off(micelle)}}$
None (H_2O)	4.8	—	$1 \cdot 10^6$	—
Tween 80	6.0	1.2	$1.35 \cdot 10^4$	1.8
Triton X-100	6.2	1.4	$2.45 \cdot 10^4$	1.6
Brij 58	6.45	1.65	$16 \cdot 10^4$	1.8
CTAB	3.1	−1.7	$43 \cdot 10^4$	1.3

rate of protonation (k_{on}), calculated as $k_{off}/K_{diss} = 4.6 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$, is the same as that calculated from the measured initial velocity and the known concentrations of In^- and H^+.

$$k_{on} = \frac{V_i}{[H^+]\,[In^-]} = 4.1 \cdot 10^{10}\ M^{-1} \cdot s^{-1} \quad (1)$$

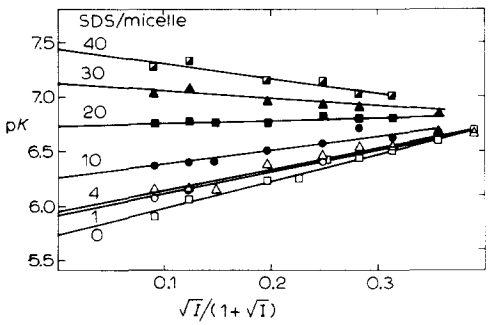
As seen in Table I, adsorption of the same indicator on different micelles slows the rate of deprotonation. The magnitude of the change in k_{off} measured for the neutral micelles accounts for the change of the pK.

The effect of micellar charge on the pK value of adsorbed indicator

As shown in Table I (bottom line), in contrast to neutral micelles, there is a huge discrepancy between the pK shift of indicator adsorbed on positively charge micelles such as CTAB and that calculated from the slowdown of k_{off}. This 1000-fold discrepancy is attributed to electrostatic repulsion of the proton by the positive charge of the micelle.

In order to quantitate the effect of micellar charge on the rate of protonation we used the uncharged indicator, neutral red (pK = 6.8; k_{on} (I = 10 mM) = $4.2 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$), adsorbed on micelles of Brij 58 (40 μM indicator in the presence of Brij 58 at a concentration equivalent to 50 μM with respect to the micellar concentration). The electric charge of the micelles was modified by a low concentration (0.2—2 mM) of SDS, well below the critical micellar concentration of SDS itself [8,18]. Even at a high salt concentration where the critical micellar concentration of SDS is lower than 2 mM, the presence of Brij 58 micelles ensures that no pure micelles of SDS will be formed.

The charging of the micellar surface by SDS alters the ion concentration in the diffused double layer [16,17]. At low ionic strength, the local proton concentration at the micellar surface will be different from that of the bulk. As we can measure only the pH of the bulk [19]; we observe an apparent pK shift of the indicator [8,9,11]. This apparent pK shift diminishes and converges to the true value given that the ionic strength is high enough to



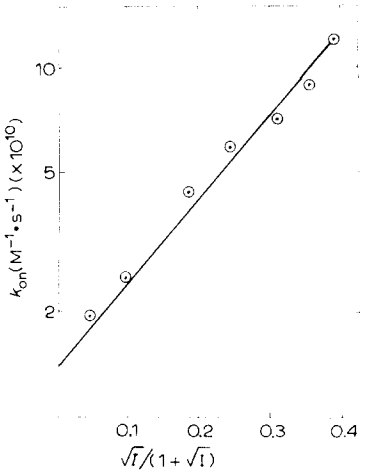


Fig. 3. The effect of ionic strength and micellar composition on the pK of neutral red adsorbed on micelles of Brij 58. The pK of the indicator was determined by spectrophotometric titration of a solution containing neutral red (40 μM), Brij 58 (4 mg/ml corresponding to 50 μM micellar concentration) and SDS at the ratios indicates in the figure, supplemented by 10 mM Tris acetate, all at 25°C. The pH was varied by addition of small volumes of either NaOH (1 M) or HCl (1 M). The pH was determined and the absorbance measured at 540 nm. The ionic strength was varied by $NaClO_4$. The lines intercept in a single point corresponding to pK = 6.8.

Fig. 4. The dependence of the second-order, diffusion-controlled rate constant of protonation of adsorbed neutral red on the ionic strength of the solution. Neutral red (40 μM) was adsorbed on mixed micelles of Brij 58 (4 mg/ml, corresponding to 50 μM micellar concentration) and SDS (200 μM) in the presence of 3 mM 2-naphthol-3,6-disulfonate. The kinetics of protonation and deprotonation were monitored at 540 mm (using a xenon lamp as light source for the interrogation beam). The second-order rate constant of protonation was calculated as described above.

screen the micellar charge. This behavior is demonstrated in Fig. 3 which relates the pK of neutral red, adsorbed on Brij 58, supplemented by varying amount of SDS, to the ionic strength. Extrapolation of the lines to the point of convergence yields pK = 6.8, identical to that of free neutral red.

The effect of micellar charge on the rate of protonation of adsorbed neutral red

The dependence of a second-order rate constant on the ionic strength is given in Eqn. 2:

$$\log k'_{on} = \log k^0_{on} + 1.02 Z_1 Z_2 \times \frac{\sqrt{I}}{1+\sqrt{I}} \qquad (2)$$

where k^0_{on} is the rate constant at $I = 0$ and Z_1 and Z_2 are the electric charges on the reactants. As the proton carries a single positive charge, we can deduce the effective charge of the micelle by measuring the slope of the dependence of $\log k'_{on}$ on the ionic strength. As neutral red is uncharged in its alkaline form, the charge effect we measure is the electrostatic interaction between the micellar charge and the proton.

In order to determine the effective charge of the micelle, k'_{on} was measured as a function of ionic strength. A typical set of measurements drawn according to Eqn. 2 is given in Fig. 4 and the slope of the line as well as k^0_{on} was calcu-

TABLE II

DETERMINATION OF MICELLAR CHARGE OF MIXED MICELLES OF SDS AND BRIJ 58 USING NEUTRAL RED AS INDICATOR

The results were taken from the experiment as described in Fig. 4. Z_1Z_2 was calculated from the slope of the line as exemplified in Fig. 4. The effective charge is calculated as ΔZ_1Z_2 divided by the average number of SDS molecular per micelle.

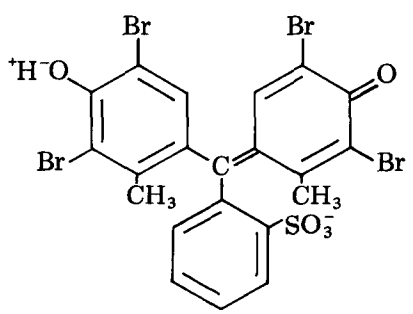
SDS (μM)	SDS (molecules/micelle)	k_{on} ($M^{-1} \cdot s^{-1}$)	Z_1Z_2	ΔZ_1Z_2	Effective charge (%)
00	0	$0.77 \cdot 10^{10}$	+3.44	0	—
200	4	$1.4 \cdot 10^{10}$	+2.35	1.09	27
500	10	$4.17 \cdot 10^{10}$	+0.958	2.48	24.8
1000	20	$14.6 \cdot 10^{10}$	—0.708	4.15	20.7

lated. The results of such experiments repeated at ratios of 0, 4, 10 and 20 SDS molecules per micelle are summarized in Table II.

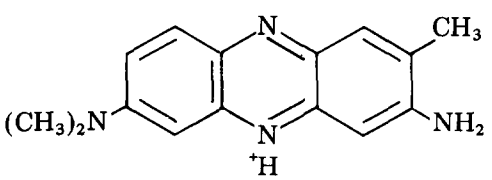
Discussion

The purpose of this report is first to demonstrate that the kinetic parameters of protonation of a surface group can be accurately measured and additionally, to utilize this information to evaluate the properties of the water/surface interface.

As demonstrated in Figs. 1 and 2 and summarized in Table I, the adsorption of bromocresol green on micelles affects the rate of proton dissociation. This effect is explained by the nature of the phenolic ring of bromocresol green (and other indicators of the sulfonophthalin group) (Scheme I). In the acidic state, both the quinoid and phenolic rings will favor the hydrophobic region int he pallisade region of the micelle, while in the alkaline state, due to the resonance between the phenolate and quinone forms, the lipid solubility of both rings will be lowered. Consequently, the enhanced solubility of the proton-bearing structure in the hydrophobic environment of micelles slows the rate of proton dissociation. Using a model system, Tong and Glesmann [12] reported a pK shift of about 1—2 units for naphthol or phenol derivatives



Bromocresol Green



Neutral Red

adsorbed on Triton X-100. The decrease in the rate of proton dissociation we measured thus accounts for the pK change of the adsorbed indicator. The small difference between ΔpK and $\log(k_{off}\ (H_2O)/k_{off}$ (micelle)) (0.2 log units) can be attributed to a few charges being adsorbed on the micelles (see below).

In order to study the effect of electrostatic interaction between protons and a micelle on the rate of protonation we used the uncharged indicator, neutral red (Scheme I), which does not change the micellar charge. This indicator is water soluble only in its protonated form. The uncharged alkaline form precipitates in water but is soluble in organic solvents. In the presence of micelles, no such precipitation takes place which indicates that the indicator is well adsorbed or dissolved in the micelles.

In contrast to neutral red, the proton emitter, 2-napththol-3,6-disulfonate, was not adsorbed to the micelle as was determined by Sephadex G-25 chromatography or by a pH titration. Consequently, the kinetics reported in this study represent a proton transfer from the bulk of the solution to the surface of the micelle.

The rate constant of a diffusion-controlled reaction is given by Debye's equation [13,14]:

$$k_{DC} = 4\pi DR \cdot \frac{N\sigma}{1000} \cdot \frac{\delta}{e^{\delta} - 1} \cdot e^{\delta[R\kappa/(1+R\kappa)]} \tag{3}$$

where R is the radius of the encounter complex, σ a steric factor, D the sum of the diffusion coefficients of the reactants and $\kappa = 3.28 \cdot 10^7 \cdot \sqrt{I}$ (cm^{-1}) is the reciprocal of the ionic atmosphere radius (the distance at which the electrostatic interaction is equal to the thermal energy, kT). δ is given by:

$$\delta = \frac{Z_1 Z_2 e^2}{\epsilon R k T}$$

where e is the electronic charge and ϵ the dielectric constant. For neutral red, in which $R = 6$ Å, $\delta = 1.18\, Z_1 Z_2$.

In the following text, we shall first examine to what extent our kinetic results can be described by Eqn. 3. Once the applicability of this equation to the experimental system is established, we can use the equation for calculating the parameters affecting the rate of protonation of adsorbed groups.

The contribution of the electrostatic interactions to the measured rate constant is most easily evaluated according to Eqn. 2, in which the product, $Z_1 Z_2$, can be estimated directly from the slope of the line as shown in Fig. 4. The micellar electric charges, calculated from this kinetic method and k^0_{on} at $I = 0$, are listed in Table II. The cause for the positive charge measured for Brij 58 micelles (supplemented by 0.8 molecules of neutral red per micelle) is unknown. * Still, upon addition of SDS, the effective charge of the micelle decreases as a function of the concentration of added SDS. The ratio of the micellar charge to the amount of SDS incorporated into the micelle is nearly constant, decreasing slightly from 27 to 21% (final column, Table I).

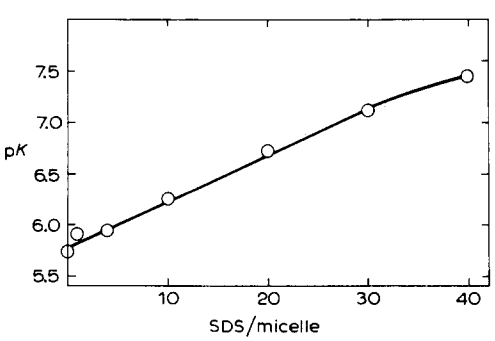
* A possible contamination of the preparation (lot No. 42-1080) by cationic surfactants should be considered.

The micellar charge calculated from the kinetic studies can be corroborated by two independent methods which rely on equilibrium parameters. As shown in Fig. 3, the pK value of adsorbed neutral red varies with the ratio of SDS molecules added per micelle. According to the theory dveloped for pH titration of proteins [15], this pK shift represents the electrostatic interaction of protons with the total charge of the macromolecules or the micelle in our case. As discussed by Tanford [15], the Brij 58/SDS mixed micelle can be regarded as an impenetrable sphere without a radius of exclusion (see Eqn. 16 in Ref. 18). This approximation is permitted because the volume of the polyoxyethylene side chains is less than 25% of the total volume occupied by this 25—45 Å thick hydrated shell. For such an impenetrable sphere without a radius of exclusion, the slope of the line relating the change of the pK of a titrable group to the effective charge of the macromolecules is given by $\Delta pK/\Delta Z = 0.867W$, where ΔZ is the increment of micellar charge and W [15] is given in Eqn. 4:

$$W = \frac{e^2}{2\epsilon r_0 kT} \cdot \frac{1}{1 + r_0\kappa} \tag{4}$$

where r_0 is the radius of the impenetrable sphere (17 Å, as estimated according to Tanford [8]).

At $I = 0$, the second term of Eqn. 4 becomes unity and we can calculate the magnitude of W for a mixed micelle of Brij 58 and SDS to be $W = 0.202$. The accuracy of such calculations is determined by the possible error of r_0 or the deviation of the micelle from a spherical configuration. Thus, as long as the molar contents of SDS in the micelle is low, the physical shape of the micelle is relatively constant and we can assume W to be invariable. Being aware of these limitations we restricted our quantitative analysis to the linear section in Fig. 5 only.



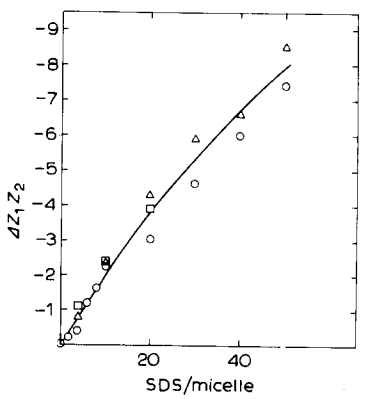


Fig. 5. The dependence of the pK of adsorbed neutral red on the average SDS content of the mixed micelle. The pK values presented are the extrapolated values (I = 0) from Fig. 3.

Fig. 6. Comparison between the effective charge of adsorbed molecules of SDS and their average ratio as added per micelle of Brij 58. The decrement of the micellar charge $\Delta Z_1 Z_2$ was calculated according to the Gouy-Chapman equation (△) from the ΔpK values of adsorbed neutral red measured at I = 10 mM (data taken from Fig. 3). (The surface of the impenetrable sphere of the micelle was estimated to be 6000 Å [8]). Alternatively, the change of micellar charge was estimated from the second-order rate constant of protonation of bound neutral red according to Eqn. 3, using the data given in Fig. 7 (I = 0; □) or Fig. 8 (I = 10 mM; ○).

The experimental slope (Fig. 5) yields $W = 0.0674$, which is only 33% of that expected. These results indicate that either 66% of the added SDS was not adsorbed on the micelle or that 66% of the SDS molecules are neutralized by counterions. Of these two interpretations, the second is the more plausible. Though the critical micellar concentration of SDS is rather high (9 mM) in the presence of pre-existing neutral micelles of Brij 58, the free energy of dissolution of SDS in the Brij 58 micelles is $\Delta G < -10$ kcal/mol [8]. Consequently, the partial expression of the SDS charge is attributed to neutralization by counterions. The measured value (33%) is very close to that derived from the kinetic analysis (see Table II).

The pK shift caused by SDS for adsorbed neutral red can also be interpreted according to another physical model, the Gouy-Chapman diffused double layer. The electrostatic attraction (or repulsion) between the macromolecule and ions in the solution alters the proton concentration bulk. This local proton concentration is detected as an apparent shift in the pK of the titrable group [9,19]. Increasing the ionic strength of the solution will decrease the width of the diffused double layer with consequent screening of the protons from the micellar charge. This account for the fact that at high ionic strength, the pK of the adsorbed neutral red becomes independent of the SDS content of the micelle (Fig. 3). If we equate the Gouy-Chapman potential with the pK shift [9,19], $\psi_G = 60\Delta pK$, we can use the Gouy-Chapman equation [16] to calculate the charge density of the micellar surface. From the charge density and the estimated surface of the impenetrable sphere, we can calculate the effective charge of the micelles. The results of such calculations (carried out for $I = 10$ mM), expressed as the decrement of the effective

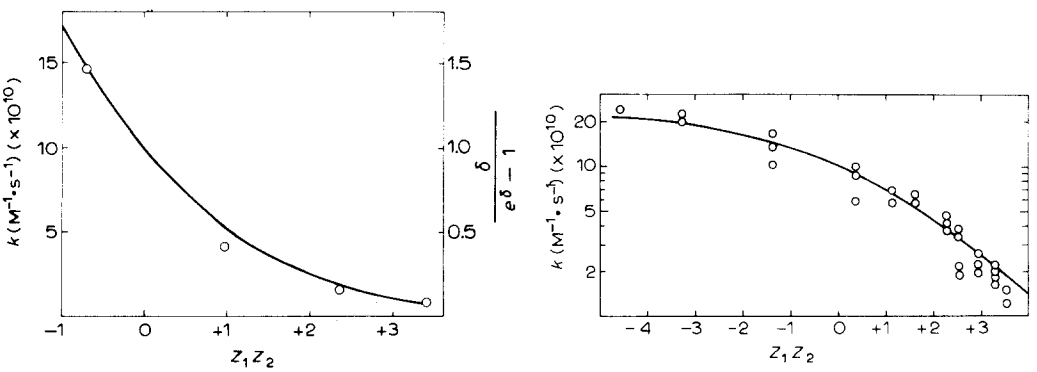


Fig. 7. The correlation between the measured rate constants of protonation and the theoretical curve according to Debye. The continuous line (right-hand ordinate) represents the function of electrostatic interaction between charged particles in water with a radius of encounter of 6 Å in the absence of of ionic atmosphere. The experimental points are the rate constants of extrapolated to zero ionic strength as detailed in Table II. The continuous function was calculated for the corresponding values of Z_1Z_2, determined experimentally for the appropriate ratios of SDS molecules per micelle.

Fig. 8. Comparison between experimental results and the values predicted according to Debye's equation for a diffusion-controlled reaction. The experimental points were measured at $I = 10$ mM using 40 μM neutral red, 4 mg/ml Brij 58, 3 mM 2-naphthol-3,6-disulfonate and varying concentrations of SDS (0—2 mM). The continuous line was calculated according to Eqn. 3 using rate of the encounter $k_{en} = 4\pi N/1000 \cdot RD = 10^{11}$ $M^{-1} \cdot s^{-1}$, as estimated from Fig. 7, considering both electrostatic interaction and screening by the ionic atmosphere according to Eqn. 3.

micellar charge compared to the average number of SDS molecules added per micelle are given in Fig. 6. It is evident that the same effect is calculated whether based on themodynamic considerations or kinetic analysis according to Debye. It is of interest to point out that the calculated effective charge decreases with increasing the content of SDS. Thus, the effective charge of pure SDS micelles (15%) measured by Feinstein and Rosano [20] can be taken as the limiting value for high SDS content.

Once we have determined the micellar charge and corroborated the values based on kinetic studies by other methods, we can examine to what extent the effect of the micellar charge on the rate of protonation is in accord with Eqn. 3. For this purpose, we relate the measured rate constant k_0 to the estimated micellar charge and as seen in Fig. 7, the points fit the function, $\delta/(e^{\delta} - 1)$ (represented by the continuous line, right-hand coordinate), calculated for the Z_1Z_2 values of the abcissa.

The contribution of the last exponential term in Eqn. 3 represents the increased screening of the micellar charge by the ionic atmosphere. At a constant salt concentration of the magnitude of the last term varies with the micellar charge. The screening will increase the rate of encounter between similarly charged species ($Z_1Z_2 > 0$) and decrease that between unlike charges ($Z_1Z_2 < 0$). A micelle carrying many negative charges will be better screened from the proton by the ionic atmosphere than one with low charge density. The correction for the ionic strength in the calculation of k_{DC} according to Eqn. 3 is given in Fig. 8 as a continuous line. The accuracy of the equation in predicting the experimental measured rate constant is self-evident.

In the experiments described above, we have used a simple model for studying the kinetics of interaction of protons with high molecular weight structures. The model we selected was such as to permit evaluation of the experimental results by alternative methods. Furthermore, it can be subjected to rigorous analysis accounting for the electrostatic interaction between the reacting species, the screening ionic atmosphere and the variation of the diffusion coefficient in the micellar interface. From Fig. 7, we can estimate the magnitude of the rate of encounter between a proton and an indicator. The measured rate of encounter (10^{11} $M^{-1} \cdot s^{-1}$) is surprisingly high for protonation of the small uncharged neutral red molecule immobilized on an uncharged micelle. This fast reaction can be attributed either to an increased radius of encounter or, alternatively, to a faster diffusion of protons on the micelle surface. As the micelle volume is very large we can neglect its own diffusion so that:

$$D = D_{H^+} = 9.35 \cdot 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$$

Using this figure, we have calculated for an adsorbed neutral red molecule an encounter radius of 14 Å, approximately the radius of the micellar inner core (17 Å [8]), which is most unlikely *. In order to change the absorption spectrum of neutral red, the proton must form a covalent bond with the

* In these calculations, we assume the steric factor, σ, to be practically unity. If $\sigma < 1$ the effective encounter radius will be even bigger. The same assumption was also applied when estimating D_{H^+} for the micellar surface.

indicator. Thus, the encounter radius cannot exceed the sum of the van der Waals' radii of the reactants (6 Å in our case). The other explanation to account for fast encounter is to assume that the diffusion of the proton through the hydrated shell of the micelle is faster than in bulk water. The fast diffusion of the proton can be due to two-dimensional diffusion on the surface of the micelle, enhanced proton transfer in the ordered water molecule region at the interface [21], or a combination of both effects. Using $R = 6$ Å, we estimate the effective diffusion coefficient of protons through the hydrated shell of the micelle to be $D_{H(\text{micellar surface})} = 22.5 \cdot 10^{-5}$ cm$^2 \cdot$ s^{-1}. It should be stressed that as in this experiment we measured the diffusion of the proton through two environments (bulk water and the hydration shell) the $D_{H(\text{micelle})}$ value does not have a precise physical meaning and is an apparent one.

The effect of total charge on the rate of protonation may be used in the study of reactions which alter the charge of an enzyme. Any event such as a redox reaction, conformation change or binding of ligand which changes the total charge of the enzyme will also alter the rate of protonation of the surface groups. As protonation is an extremely fast diffusion-controlled reaction, following the kinetics of the state of protonation of a surface group (such as adsorbed or covalently bound indicator with a proper pK) can be employed to study the kinetics of the event which affects the enzyme charge. Experiments in this direction are currently being performed in our laboratories.

The ability of the physicochemical treatment to describe quantitatively the experimental results demonstrates that the pH-jump technique is an accurate tool for studying properties of macromolecules. Application of this technique in the study of proteins and membranes may lead to better understanding of the macromolecule-water interaction.

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