Color Stabilization of Anthocyanins: Effect of SDS Micelles on the Acid−Base and Hydration Kinetics of Malvidin 3-Glucoside (Oenin)

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The origin of the substantial stabilization of the color of the natural anthocyanin malvidin-3-glucosyl chloride (Oenin) in aqueous micellar SDS solutions has been elucidated by employing laser flash photolysis and pH-jump techniques to determine all four rate constants involved in the acid−base and hydration reactions of Oenin. These rate data show that the pronounced stabilizing effect of SDS micelles on the color of aqueous solutions of oenin, as measured by the shift in the bleaching constant ($pK_{1/2}$) from moderately acid to near-neutral pH, cannot be attributed just to the difference in local pH between the aqueous and micellar phases. Thus, the major contribution to the SDS-induced shift in the acid−base equilibrium constant arises from the large decrease in the deprotonation rate constant in SDS micelles ($k_d = 2 \times 10^5\ s^{-1}$) with respect to water ($k_d = 5.0 \times 10^6\ s^{-1}$). Although the hydration rate constant also decreases in SDS micelles ($k_h = 4.6 \times 10^{-3}\ s^{-1}$ versus $k_h = 8.5 \times 10^{-2}\ s^{-1}$ in water), the major contribution to the observed shift in the hydration equilibrium constant comes from the back reaction ($k_{-h} = 7.1 \times 10^3\ l{\cdot}mol^{-1}{\cdot}s^{-1}$ in SDS versus $k_{-h} = 34\ l{\cdot}mol^{-1}{\cdot}s^{-1}$ in water). The present results conclusively demonstrate that color stabilization by anionic SDS micelles involves significant preferential stabilization of the cationic form of oenin with respect to the neutral base, hemiacetal and *E*-chalcone forms, resulting in profound changes in the energetics of deprotonation and hydration, the two key equilibria that affect color.

Introduction

Anthocyanins are natural, water-soluble pigments responsible for the color of many fruits and flower petals. Although these compounds are potentially interesting as natural colorant additives in foods, this application requires improvement of both their chemical and their photochemical stability.[1]

The structural changes that anthocyanins undergo in aqueous solution in the pH range between 2 and 5 are complex.[1−5] Oenin, or malvidin 3-glucoside, is a representative, naturally occurring anthocyanin that is found, for example, in European casts of red grapes. Deprotonation of the red flavylium cation form, AH^+, of oenin can lead to a blue quinonoidal base, A (Scheme 1). On the other hand, hydration of the flavylium cation results in the formation of two colorless diastereoisomeric hemiacetals, B, that can subsequently open to the *E*-chalcone, C_E, and *Z*-chalcone, C_Z, forms.[6,7] At pH 2, the colored flavylium cation is the predominant species present in aqueous solutions of oenin. At pH values above ca. 4, however, the colorless hemiacetal and chalcone forms dominate, resulting in bleaching.

For oenin, the kinetics of the major reaction pathways involving the flavylium cation have been studied in aqueous solution.[4,8−10] The rate constants for deprotonation of AH^+ ($k_d = 5 \times 10^6\ s^{-1}$) and for protonation of A ($k_p = 2.5 \times 10^{10}\ l{\cdot}mol^{-1}{\cdot}s^{-1}$) are large and, depending on the pH, the acid−base equilibrium ($pK_a = 3.7$) is typically established in the μs to sub-μs time range.[10] The rate constants for hydration ($k_h = 8.5 \times 10^{-2}\ s^{-1}$) and dehydration ($k_{-h} = 34\ l{\cdot}mol^{-1}{\cdot}s^{-1}$) are much smaller than those for proton transfer and the hydration equilibrium state ($pK_h = 2.6$) is approached much more slowly (several seconds).[7,8] Although the subsequent tautomerization to the *E*-chalcone is fast[7] (complete within a second), chalcone isomerization is slow $k_i = 4.5 \times 10^{-5}\ s^{-1}$ and $k_{-i} = 3.8 \times 10^{-4}\ s^{-1}$)[7,8] and several hours are required to attain the global equilibrium state following an increase of the pH of the solution. In the case of oenin, the concentration of the flavylium cation is controlled by the hydration equilibrium and, since the pK_h is only 2.6, aqueous solutions of oenin rapidly lose their color even at relatively acidic pH values (≥3).

Several strategies have been proposed[11] for color stabilization at higher pHs. These include: (i) self-association of the colored flavylium cations or anhydrobases;[12−19] (ii) intermolecular copigmentation, involving association between the colored form and an appropriate colorless molecule (copigment);[20,21] (iii) intramolecular copigmentation, as occurs in anthocyanins possessing cinnamoyl residues covalently linked to the glucosyl moieties;[22−25] and (iv) incorporation of anthocyanins in aggregates of amphiphilic molecules, in cyclodextrins or in polyelectrolytes.[26−28] (The authors acknowledge one of the referees for calling attention to ref 28.) Most of these color stabilization strategies rely on the formation of molecular complexes capable of inhibiting or hindering the nucleophilic attack of water on the flavylium cation, thereby provoking a

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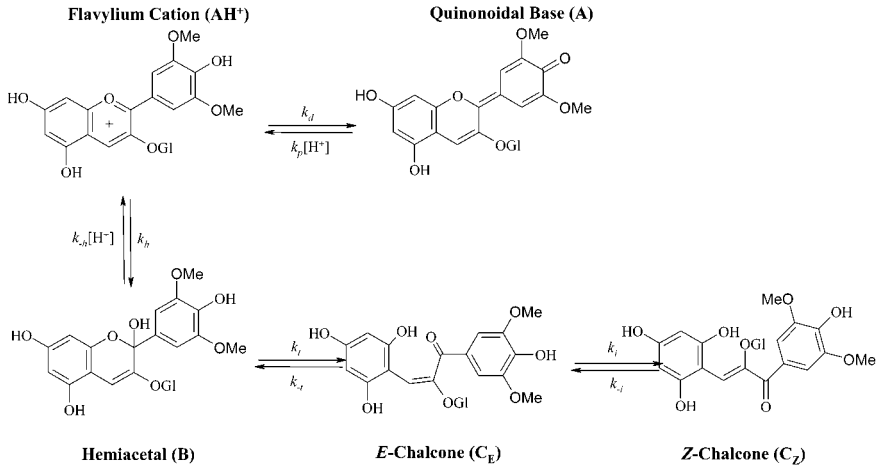
SCHEME 1



decrease in the equilibrium constant for hydration, K_h, which is the controlling factor for color loss.

In this work, we demonstrate that incorporation of anthocyanins into anionic sodium dodecyl sulfate (SDS) micelles results in pronounced color stabilization (to our knowledge, the strongest yet found). A comprehensive study of the modification of the reactivity of oenin in the presence of micellar SDS permits identification of the origin of the color stabilization of oenin by the anionic micelles.

Experimental Section

Reagents. HPLC grade malvidin-3-glucosyl chloride (Oenin), malvidin-3,5-diglucosyl chloride, (Malvin), cyanidin-3-glucosyl chloride (Kuromanin) and cyanidin-3,5-diglucosyl chloride (Cyanin) were purchased from Extrasynthése. Sodium dodecyl sulfate (SDS), polyoxyethylene[10]dodecyl ether ($C_{12}E_{10}$), Triton X-100 (TX100) and sodium phosphate and chloride, p.a., from Sigma and sodium 1,2-bis-(2-ethylhexyl)-1-sulfosuccinate (AOT), p.a., from Fluka, were used without further purification in the equilibrium measurements. Ultrapure bioreagent grade SDS from Mallinckrodt Baker, Inc., was employed for all kinetics measurements. Organic solvents were spectroscopic or HPLC grade from Merck.

Sample Preparation. Aqueous solutions were prepared in either Elgastat or Millipore Milli-Q quality water. Sodium phosphate buffer (typically 10 mM) was employed to maintain the required pH and ionic strength. Titrations were performed by mixing solutions in which all of the components except the pH were the same. The final pH was measured using an ORION 720A pH meter with a combined RedRod electrode. Aqueous micellar SDS solutions and AOT lamellar phases were obtained by direct dissolution of the desired amount of surfactant in water (containing the appropriate amount of added salt). Oenin containing solutions where prepared by placing the surfactant solution in contact with a dried film of oenin obtained by evaporation of the desired amount of an oenin stock solution in methanol.

Measurements. The pH-jump experiments were performed on a Hewlett-Packard 8452A Diode Array Spectrometer. Solutions were prepared either by adding 5.0 μL of a 6.8 mM solution of oenin in methanol to 2.00 mL of a 0.10 M solution of SDS buffered at the desired pH (final concentration of oenin 17 μM) or by adding 2.00 mL of the buffered SDS solution to a dried film of oenin in the cuvette. The moment of mixing was taken as time zero and the initial absorption spectrum of the resultant mixture measured 10 s after mixing. The spectra obtained at later times were analyzed by subtraction of the initial spectrum after normalization.

Mole fractions of the acid, base, hemiacetal and chalcone forms in 0.10 M aqueous SDS solutions were calculated as follows. Using the spectrum of the flavylium cation, AH^+, measured at pH = 2.65 ($\epsilon_{max}(536) = 27\,000$ l·mol^{-1}·cm^{-1}), the spectrum of the base form, A ($\epsilon_{max}(546) = 15\,400$ l·mol^{-1}·cm^{-1} and $\epsilon(610) = 7200$ l·mol^{-1}·cm^{-1}), was obtained by global decomposition of the spectra measured at $t = 0$ in solutions with final pH values of 5.11, 5.78, 6.17, and 6.60, where only AH^+ and A are present. These spectra were subsequently employed to obtain the mole fractions of AH^+ and A at longer times and in the final equilibrated solutions. Thus, for each pH, the spectrum measured at $t = 0$ (only AH^+ and A present) was normalized and subtracted from the spectra measured at later times to obtain the contribution to the absorption from the hemiacetal B, and chalcones C, products as a function of time. At times shorter than ca. 360 s, the products are B ($\epsilon_{max(276)} = 19\,600$ l·mol^{-1}·cm^{-1}) and the *E*-chalcone, C_E ($\epsilon_{max(345)} = 13\,000$ l·mol^{-1}·cm^{-1}). At longer times, the absorption of the *Z*-chalcone, C_Z ($\epsilon_{max(360)} = 13\,000$ l·mol^{-1}·cm^{-1}), can be detected. The values of the molar extinction coefficients of the hemiacetal and chalcones, which are identical to those obtained previously from coupled ^{1}H NMR and UV−vis absorption data[29,30] in water, were then used to determine the mole fractions of B, C_E and C_Z at equilibrium.

Flash photolysis experiments were carried out with an Edinburgh Analytical Instruments LP900 laser flash photolysis system equipped with a Surelite I-10 Nd:YAG laser for excitation and a 450 W high pressure xenon lamp for monitoring transient absorption. The second harmonic (532 nm) of the Nd:YAG laser was used for excitation, and 10 laser shots were averaged to obtain the transient absorption decays, the solution being stirred between each laser shot. The standard exponential decay routines of the LP900 system software were employed to analyze the decays of the transient and obtain the lifetimes of the excited species. Lifetimes shorter than 30 ns were deconvoluted using the pulse shape of the laser (obtained by monitoring the Raman scattering of the laser pulse in water in the fluorescence mode, i.e., with the monitoring beam shutter closed). Lifetimes longer than 30 ns were obtained from direct fits of the decay (from the maximum of the laser pulse on) without deconvolution. In all cases, a single exponential adequately described the decay. Transient absorption spectra

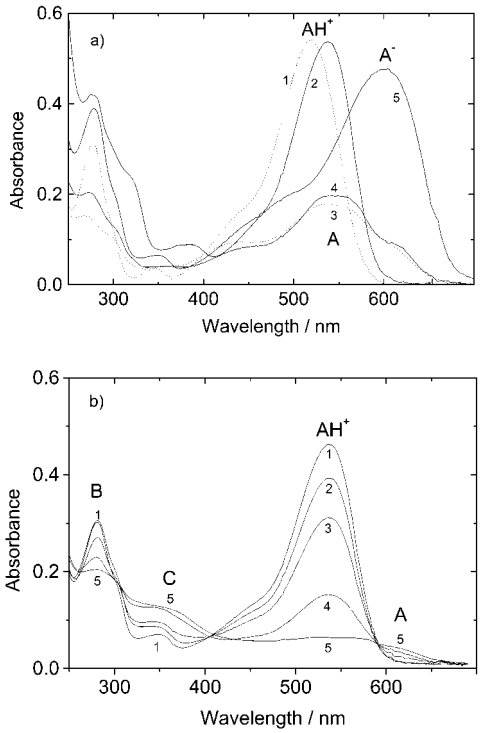


Figure 1. (**a**) Absorption spectra of malvidin 3-glucosyl chloride (2×10^{-5} M): Curve 1, equilibrated solution in water at pH = 1.1; Curve 2, equilibrated solution in 0.1 M SDS at pH = 2.64; Curve 3, 10 s after dissolution in water at pH 5.6; Curve 4, 10 s after dissolution in 0.1 M SDS at pH=6.17; Curve 5, 10 s after dissolution in 0.1 M SDS at pH = 10. (**b**) Absorption spectra of malvidin 3-glucosyl chloride as a function of pH in 0.10 M SDS, at 20 °C. Curves 1−5 correspond to pHs 2.64, 3.76, 5.11, 5.78 and 7.05 and AH^+, A, B, and C to the flavylium cation, the quinonoidal base, the hemiacetal and the chalcone forms, respectively.

were determined point-by-point and, in all cases, the lifetime of the transient showed no significant variation with wavelength.

Results

Equilibria in Microheterogeneous Media. Figure 1a shows the absorption spectra of the flavylium cation form, AH^+, of malvidin 3-glucoside (oenin) in water at pH = 1.1 and in 0.10 M aqueous SDS at pH = 2.64. The red-shift of the $S_0 \rightarrow S_1$ transition of AH^+ upon going from water (519 nm) to SDS (536 nm) is indicative of incorporation of the cationic chromophore into the anionic detergent micelles. Upon raising the pH to 7 (Figure 1b), the absorption band of AH^+ at 536 nm is replaced by those of the base A (shoulder at 610 nm), the hemiacetals B (276 nm), and the chalcones C (360 nm). At pHs above 7, the ionized form of the base A^-, is also observed (the spectrum of A^-, obtained 10 s after adding oenin to a pH 10 SDS solution, is shown in Figure 1a). The absorption spectra of A in water and in SDS, obtained by spectral decomposition (see the Experimental Section) are also shown in Figure 1a.

The data in SDS reveal three major differences with respect to water. First, the onset of bleaching of the AH^+ form (which occurs at pH $\approx$ 3 in water) is shifted to much higher pH values in the presence of SDS. Second, the absorption bands of the base A, can be clearly observed in equilibrated SDS solutions, whereas in water in the absence of SDS only absorption bands due to the colorless hemiacetal, B, and chalcone forms C, can be observed at equilibrium. Finally, the absorption due to the *Z*-chalcone (350 nm) at basic pH is much more intense in micellar SDS than in water (see also Figure 5b).

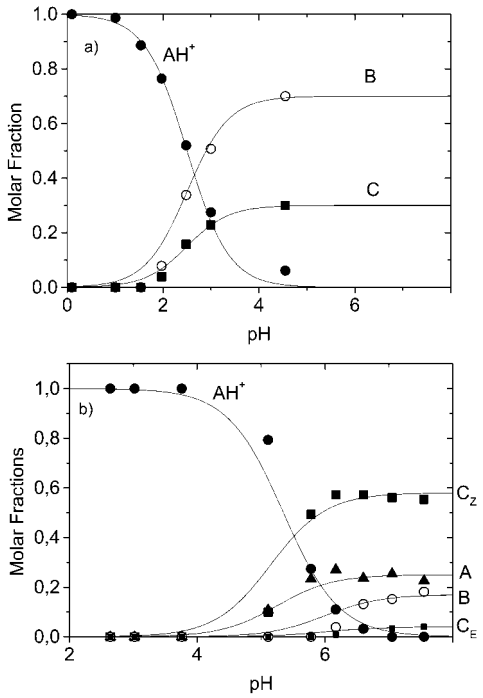


Figure 2. Mole fractions of the flavylium cation form (AH^+), hemiacetal forms (B) and chalcone forms (C) of malvidin 3-glucosyl chloride (Oenin) at 20 °C. as a function of the aqueous phase pH in (**a**) water and (**b**) 0.1 M SDS,

Figure 2, parts a and b, shows the pH dependence of the mole fractions of each form in water and 0.10 M SDS solutions, respectively. Operationally, it is convenient to define an apparent bleaching constant, $pK_{1/2}$, defined as the pH value at which half of the total oenin is present in the form of AH^+ and half as the other forms (A, B, and C). Thus defined, the apparent bleaching constant for oenin is found to increase from $pK_{1/2} = 2.5$ in water to $pK_{1/2} = 5.4$ in 0.10 M SDS. Figure 2b also provides estimates of the apparent (expressed in terms of the pH in the intermicellar aqueous phase rather than the local pH at the micelle surface) ionization and hydration constants: $pK_a = 6$ and $pK_h = 6.0$, respectively.

For comparison, we investigated briefly the stabilization of the AH^+ form of several other naturally occurring anthocyanins by SDS and of oenin by other types of interfaces, including anionic lamellar phases and micelles of nonionic surfactants. Figure 3 shows the pH dependence, in water and in aqueous micellar SDS, of the mole fractions of the AH^+ form of three other naturally occurring anthocyanins: malvin, kuromanin and cyanin (diglucosylated malvidin chloride and mono- and diglucosylated cyanidin chloride, respectively). In all cases, incorporation into the SDS micelle stabilizes the AH^+ form. The increase in $pK_{1/2}$ with respect to water, $\Delta pK_{1/2}$, is smaller for the diglucosylated anthocyanins than for the monoglucosides and is smaller for the cyanidin derivatives than for the malvidin derivatives (Table 1). AOT/water lamellar phases, containing up to 10% AOT by weight, also stabilize the AH^+ form of oenin ($pK_{1/2}$ ca. 4), though to a lesser extent than SDS. In contrast, in micellar solutions of the nonionic surfactants $C_{12}E_{10}$ and TX-100 (0.20 M detergent) the value of $pK_{1/2}$ decreases by ca. 0.6 pH units relative to water.

Laser Flash Photolysis Experiments. We have previously employed laser flash photolysis to measure the ground-state rate constants for deprotonation of the acid form and reprotonation of the base form of oenin in water.[10] This method takes advantage of the enhanced acidity of the singlet excited state

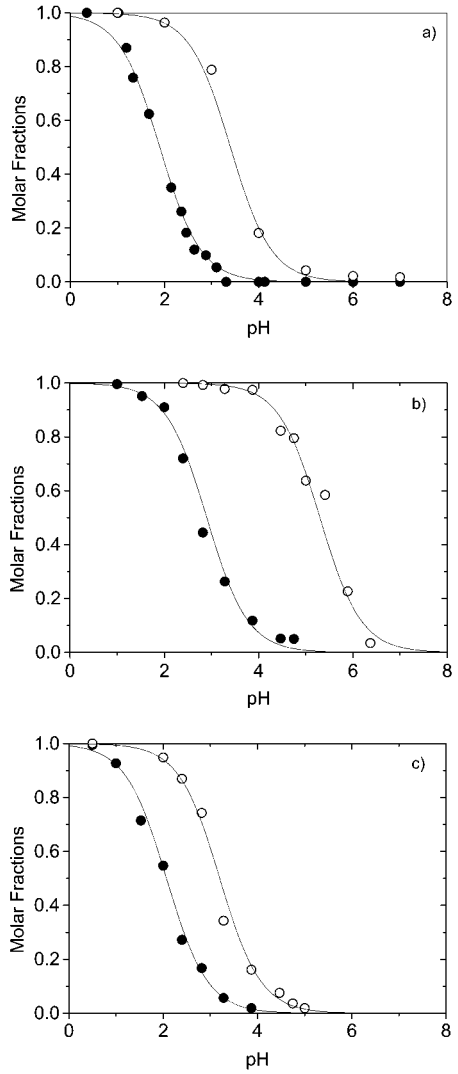


Figure 3. Mole fractions of the flavylium cation form of (**a**) malvidin 3,5-diglucosyl chloride (Malvin), (**b**) cyanidin 3-glucosyl chloride (Kuromanin), and (**c**) cyanidin 3,5-diglucosyl chloride (Cyanin), in water (●) and in 0.10 M aqueous SDS solutions (○) at 20 °C as a function of the aqueous phase pH.

of oenin, which results in ultrafast adiabatic deprotonation of excited AH^+ followed by subnanosecond decay of excited A to its ground state. Thus, a 5 ns laser pulse in effect instantaneously perturbs the ground-state acid−base equilibrium toward the base. The kinetics of relaxation back to equilibrium in the ground state can then be followed with nanosecond time resolution by monitoring either the disappearance of A or the recovery of AH^+.

In the present work, this technique was employed to investigate the kinetics of the ground-state acid−base equilibrium of oenin in micellar SDS. Figure 4a shows the differential absorption spectrum of the transient generated upon flash photolysis (532 nm laser pulse) of a solution of oenin in 0.10 M SDS at an intermicellar aqueous phase pH of 5.54. In addition to the depletion of AH^+ centered at 530 nm, a positive transient absorption signal is observed at ca. 610 nm, where A is known to absorb in micellar SDS (Figure 1a). The decay time of the absorption at 600 nm, τ, is equal to the recovery time of AH^+ at 500 nm and both increase with increasing pH. On this basis, the absorption band at 610 nm is assigned to the base form, A, and its decay is assigned to the relaxation of A back to AH^+ via protonation, as observed in water.[10]

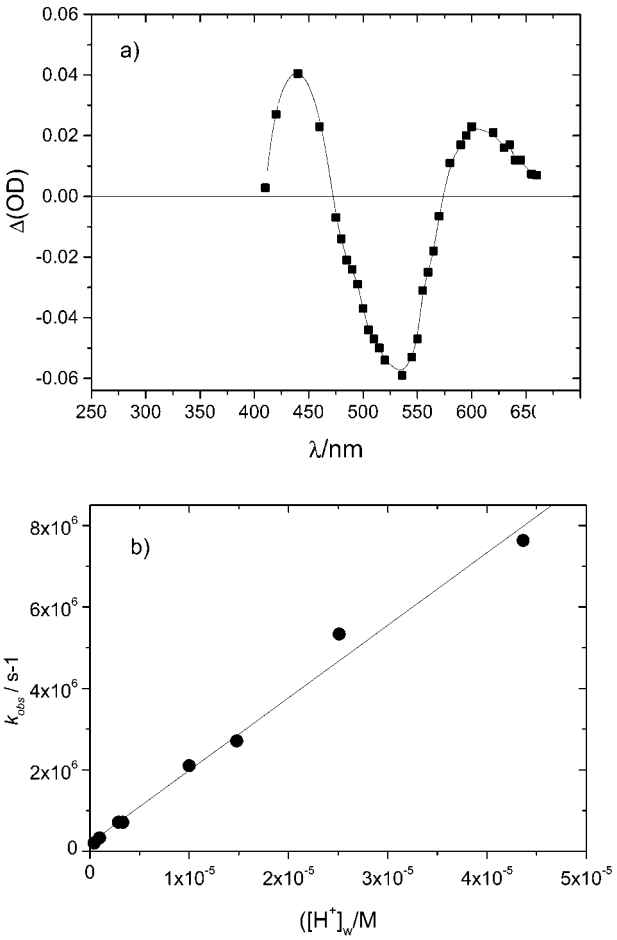


Figure 4. (**a**) Transient absorption spectrum of malvidin 3-glucosyl chloride (Oenin) in 0.10 M aqueous SDS solution at pH = 5.54 and 20 °C, measured at time zero after excitation with a 5 ns laser pulse at 532 nm. (**b**) Reciprocal decay time (k_{obs}) of the base form, A, of malvidin 3-glucosyl chloride (Oenin) at 600 nm as a function of the aqueous phase proton activity, $[H^+]_w$, in 0.10 M aqueous SDS solution at 20 °C.

As previously shown,[10] the acid−base equilibrium of anthocyanins (including oenin) in water is established in the submicrosecond time range, i.e., ca. 6 orders of magnitude faster than the hydration, tautomerization or isomerization reactions. Consequently, the acid−base equilibrium is kinetically uncoupled from these other, much slower equilibria. Therefore, in the sub-microsecond time range, only the acid−base kinetics need be considered. Conversely, when the other reactions are followed on the time scale of seconds to hours, the AH^+/A pair can be treated kinetically as a single species.

Under our nanosecond laser flash photolysis conditions, the transients exhibit first-order decay kinetics[10] and the reciprocal of the transient decay time, $1/\tau = k_{obs}$, is a simple function of the forward (k_d) and back reaction (k_p) rate constants (eq 1)

$$k_{obs} = k_d + k_p[H^+] \qquad (1)$$

Figure 4b shows a plot of the reciprocal decay time, k_{obs}, for reprotonation of the base form of oenin, A, generated by the laser pulse, as a function of the measured proton activity in the intermicellar aqueous phase, $[H^+]_w$. As found in water in the absence of micelles, the plot is linear. The intercept and the slope of this plot provide the values of $k_d = 2.0\ (\pm 1.3) \times 10^5\ s^{-1}$ and $k_p = 1.79\ (\pm\ 0.09) \times 10^{11}\ l.mol^{-1}.s^{-1}$. The value of k_p is obtained with acceptable accuracy, but the k_d value has a

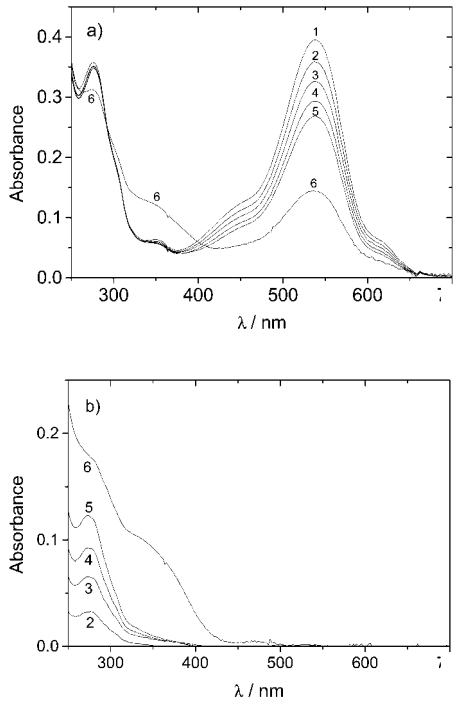


Figure 5. (**a**) Absorption spectra of malvidin 3-glucosyl chloride (Oenin) at 20 °C as a function of time after dissolution in a 0.10 M SDS solution buffered at pH 5.78. (**b**) The same absorption spectra after subtraction of the flavylium (AH^+) and base (A) contributions. Curves 1−6 correspond to times of 10, 30, 60, 120, 600, and 10 800 s, respectively.

TABLE 1: Bleaching Constants, $K_{1/2}$, of Malvidin-3-glucosyl Chloride (Oenin), Malvidin-3,5-diglucosyl Chloride (Malvin), Cyanidin-3-glucosyl Chloride (Kuromanin), and Cyanidin-3,5-diglucosyl Chloride (Cyanin) in Water and 0.10 M Aqueous SDS Solutions, at 20 °C

	$pK_{1/2}$		
	water	SDS, 0.1 M	$\Delta pK_{1/2}$
Oenin	2.5	5.4	2.9
Malvin	1.8	3.4	1.6
Kuromanin	2.9	5.3	2.4
Cyanin	2.1	3.3	1.2

much larger error. Nevertheless, from these two values, one can calculate an apparent pK_a in SDS of 6.0, a value in good agreement with that estimated from equilibrium data (Figure 2b).

pH-Jump Experiments. Figure 5a shows the initial absorption spectrum, registered 10 s after addition of oenin (17 μM final) to a 0.10 M SDS solution buffered at pH 5.78, together with spectra at subsequent times up to 1×10^4 s. The absorption bands of AH^+ (536 nm) and A (610 nm) decrease with time, being replaced in the initial phase of reaction (ca. 10−360 s) by the hemiacetal and the *E*-chalcone absorptions at 275 and 345 nm, respectively (Figure 5b). During the slower phase of the reaction (beyond ca. 360 s), the *Z*-chalcone absorption can be observed at 360 nm (Figure 5b). The AH^+/A absorbance ratio is independent of time, indicating that AH^+ and A are always in equilibrium, consistent with the known μs time scale of proton transfer.

The kinetic data for the disappearance of AH^+ (absorbance vs time) are nicely fit by the sum of two exponentials plus a constant (the final absorbance when equilibrium is reestablished at the new final pH), as shown in Figure 6a. Representative decay times and preexponential coefficients derived from fits of

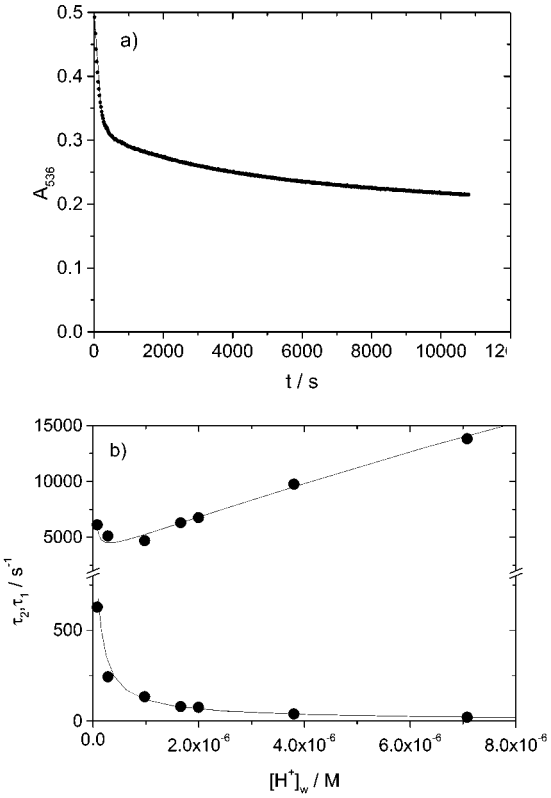


Figure 6. (**a**) Time dependence of the absorbance of malvidin 3-glucosyl chloride (Oenin) at 536 nm after dissolution at 20 °C in 0.10 M SDS solution buffered at pH = 6.01. The solid curve represents the best-fit to a double exponential function. (**b**) Variation of τ_1 ands τ_2 (eq 2 and Table 2) as a function of the final proton activity in the intermicellar aqueous phase in 0.10 M SDS solution.

TABLE 2: pH-Jumps of Malvidin-3-glucosyl Chloride (Oenin) in 0.10 M Aqueous SDS Solutions, at 20 °C. Final pH, Decay-times (τ_1, τ_2), Normalized Preexponential Coefficients (a_1, a_2) and Constant Term (a_0) from Double Exponential Best-Fits of the Absorbance Decays at 536 nm after pH-jumps

pH	τ_2/s	τ_1/s	a_2	a_1	a_0
5.15	21.7	13800	0.059	0.219	0.722
5.42	39.5	9750	0.156	0.218	0.626
5.70	76.3	6760	0.301	0.214	0.448
5.78	80.3	6300	0.337	0.217	0.446
6.01	134	4690	0.403	0.202	0.395
6.55	244	5120	0.459	0.218	0.322
7.09	628	6120	0.507	0.245	0.248

kinetic data obtained at a series of pHs are collected in Table 2. With increasing pH, the value of the shorter decay time, τ_2, increases, whereas the longer one, τ_1, initially decreases, goes through a minimum around pH 6, and then increases (Figure 6b).

The kinetic analysis of the hydration-tautomerization-isomerization multiequilibria of oenin in SDS is, a priori, more complex than the acid−base equilibrium. Although there are four kinetically coupled species and six rate constants, the fact that the reactions occur on quite different time scales permits considerable simplification. Thus, in the seconds-to-hours time scale of the pH-jump experiments, the AH^+/A pair can be considered as one single kinetic species, as evidenced by the fact that the $[AH^+]/[A]$ ratio of oenin in SDS is independent of time after the pH-jump. In addition, previous work has shown that the tautomerization reaction is considerably faster (sub-

SCHEME 2

$$(A + H^+ / AH^+) \underset{k_{-h}'[H^+]}{\overset{k_h'}{\rightleftharpoons}} B/C_E \underset{k_{-i}}{\overset{k_i'}{\rightleftharpoons}} C_Z$$

second range) than the hydration (minutes) and isomerization reactions (hours).[7,31,32] Therefore, it is reasonable to assume that, under our conditions, the hemiacetal and the *E*-chalcone are also in equilibrium, as indeed observed during the initial 300 s after the pH-jump (Figure 5b). These conditions reduce the kinetic scheme to a system with three species and four rate constants (Scheme 2).

The time dependence of the concentration of AH^+ is predicted to be a double exponential function of time plus a constant (see Appendix), as experimentally found (eq 2)

$$[AH^+] = a_0 + a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2} \quad (2)$$

The reciprocal decay times, $\lambda_{2,1} = 1/\tau_{1,2}$, are related to the four rate constants by eqs 3−5

$$\lambda_{2,1} = \frac{S \pm \sqrt{D^2 + 4k_i' k_{-h}' [H^+]}}{2} \quad (3)$$

$$S = k_h' + k_{-h}'[H^+] + k_i' + k_{-i} \quad (4)$$

$$D = k_h' + k_{-h}'[H^+] - k_i' - k_{-i} \quad (5)$$

The apparent hydration constant k_h', is related to the hydration rate constant k_h, through the fraction of (A/AH^+) that is in the form of AH^+ at each pH

$$k_h' = k_h \frac{[H^+]}{[H^+] + K_{ap}} \quad (6)$$

where K_{ap} is the apparent acidity constant of AH^+ in micellar SDS.

The same applies to the apparent dehydration rate constant k_{-h}', that depends on the fraction of B/C_E in the form of B, and isomerization rate constant k_i', which depends on the fraction of B/C_E that is in the form of C_E (eqs 7 and 8)

$$k_{-h}' = k_{-h} \frac{1}{1 + K_t} \quad (7)$$

$$k_i' = k_i \frac{K_T}{1 + K_t} \quad (8)$$

The tautomerization equilibrium constant, K_t, can be obtained directly from the spectra of B and C_E (Figure 5b) during the first 300 s of reaction by using the experimentally determined extinction coefficients of B (19 600 $l \cdot mol^{-1} \cdot cm^{-1}$ at 275 nm) and C_E (13 000 $l \cdot mol^{-1} \cdot cm^{-1}$ at 345 nm). The value of 0.22 found here for K_t in micellar SDS is very close to that determined previously in water ($K_t = 0.26$).[29]

The data analysis was carried out as follows. It can be shown (from eqs A8−A10 and A14−A15 of the Appendix) that k_h' is a function of the normalized preexponential coefficients, a_1 and a_2, and the reciprocal decay times, λ_1 and λ_2

$$k_h' = a_2\lambda_2 + a_1\lambda_1 \quad (9)$$

Combining this equation with eq 6 indicates that a plot of $1/(a_2\lambda_2 + a_1\lambda_1)$ vs $1/[H^+]_w$ should be linear, as confirmed in Figure 7a. The reciprocal of the intercept provides a value of $k_h = 4.6 (\pm 0.6) \times 10^{-3}\ s^{-1}$ and the slope/intercept ratio a value of $K_{ap} = 6.3 \pm 0.3$ (as compared to 6.0 derived from the absorption spectral data). Because the sum of the reciprocal decay times is equal to the sum of all of the rate constants, $\lambda_2 + \lambda_1 = S$ (eqs 3 and 4), $(S - k_j')$ should be a linear function of $[H^+]_w$, as experimentally verified in Figure 7b. The slope provides the value of $k_{-h}' = 5.8 (\pm 0.2) \times 10^3\ l \cdot mol^{-1} \cdot s^{-1}$. Substitution of this value, together with $K_t = 0.22$, in eq 7 gives $k_{-h} = 7.1 (\pm 0.3) \times 10^3\ l \cdot mol^{-1} \cdot s^{-1}$. The value of 6.2 ± 0.2 for pK_h, calculated from the ratio of the values of k_h and k_{-h}, is also in good agreement with the value of 6.0 estimated from equilibrium data (Figure 2b). Finally, a rough estimate of the values of k_i' and k_{-i} was obtained by fitting the variation of the decay times τ_1 and τ_2 as a function of pH employing eqs 3−7 and the known values of the other rate and equilibrium constants (Table 3).[33] The best fit curves (Figure 6b), corresponding to $k'_i = 7 \times 10^{-4}\ s^{-1}$ and $k_{-i} = 3.8 \times 10^{-5}\ s^{-1}$, nicely reproduce the pH dependence of both τ_1 and τ_2, including the minimum of τ_1.

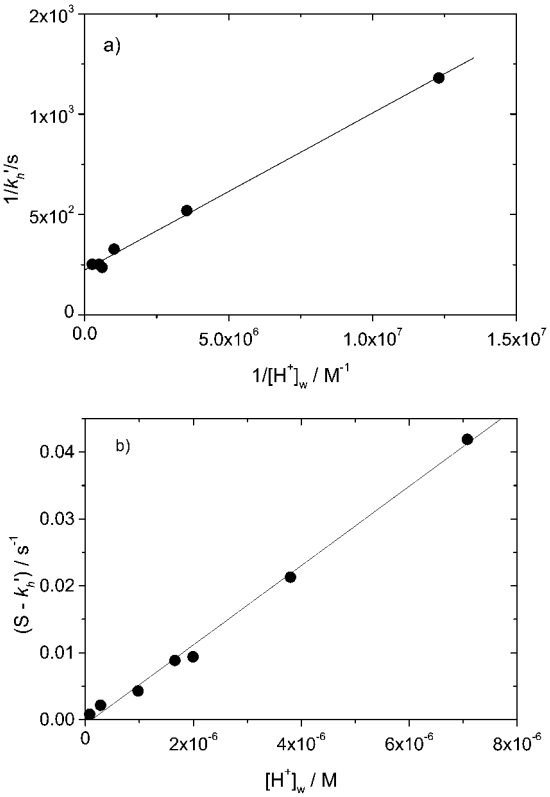


Figure 7. (**a**) Double reciprocal plot of k_h' as a function of $[H^+]_w$ (eqs 6 and 9) for malvidin 3-glucosyl chloride (Oenin) in 0.10 M aqueous SDS solution at 20 °C. (**b**) Plot of the data as $S - k_h'$ vs. $[H^+]_w$.

TABLE 3: Kinetic Data for Oenin in Water and SDS Micelles

	SDS (0.1 M)	water
k_d/s^{-1}	2×10^5	5.0×10^6
$k_p/l \cdot mol^{-1} \cdot s^{-1}$	1.8×10^{11}	2.5×10^{10}
pK_a	6.0	3.7
k_h/s^{-1}	4.6×10^{-3}	8.5×10^{-2} [a]
$k_{-h}/l \cdot mol^{-1} \cdot s^{-1}$	7.1×10^3	3.4×10^1 [a]
pK_h	6.2	2.6
pK_t	0.66	0.59

[a] From refs 8 and 9.

Discussion

The pronounced red-shift of the flavylium absorption spectrum upon addition of SDS (Figure 1) clearly points to strong interaction of the cationic form of the anthocyanin with the anionic micelle. It is also indicative of an environment less polar than water, because a decrease of solvent polarity is known to red-shift the first (π,π^*) absorption band[30] (the electric dipole moment of oenin decreases upon going from the ground to the lowest excited state). At the relatively high SDS concentration employed in this work (0.10 M), there is no evidence for significant partitioning of oenin or any of the forms between the aqueous and micellar phases, i.e., for all practical purposes, all forms are completely incorporated into the SDS micellar phase.

The interaction of AH^+ with SDS results in significant color stabilization, i.e., to an increase of the bleaching constant, $pK_{1/2}$. The apparent bleaching constant is related to the equilibrium constants of Scheme 1 by the relationship

$$pK_{1/2} = -\log (K_a + K_h + K_hK_t + K_hK_tK_i)$$

For oenin in water, the observed $pK_{1/2}$ of 2.5 is largely determined by the hydration constant ($pK_h = 2.7$), with only a modest contribution from the acidity constant ($pK_a = 3.7$) of the flavylium cation. The three-pH-unit increase in $pK_{1/2}$ upon going from water to SDS is due to the micelle-induced increase of both the apparent pK_h and the pK_a in the presence of SDS (Table 3). The obvious interpretation for the increase in the apparent values of pK_h and pK_a relative to water is the fact that the local proton concentration, $[H^+]_m$, is much higher at the negatively charged interface of the SDS micelle[34−36] than that in the aqueous phase, $[H^+]_w$. However, interpretations based solely on equilibrium data are dangerous and often erroneous.

Ionic micelle-induced shifts of the apparent pK_a of weak acids relative to water are typically attributed to two factors:[36] (1) micellar charge control of the effective proton concentration at the micelle surface; (2) medium-induced changes in the intrinsic pK_a of the weak acid at the micellar surface, due to the lower effective dielectric constant at the micellar surface. This latter effect is often estimated by comparing the shift of the apparent pK_a in the micelle of the ionic detergent of interest to that in a micellar solution of an appropriate nonionic detergent micelle such as Triton-X100 or Brij 35. Our results show that equilibrium data are inadequate for understanding the origin of the SDS-induced shift in the apparent pK_a of oenin.

The flash photolysis technique employed in the present work permits determination of the individual rate constants for the ground-state acid−base equilibrium of oenin at the SDS micellar surface, the ratio of which determines the apparent pK_a. The apparent rate constant for protonation of the base form of oenin in micellar SDS (1.8×10^{11} $l \cdot mol^{-1} \cdot s^{-1}$) is larger than that ($2.5 \times 10^{10}$ $l \cdot mol^{-1} \cdot s^{-1}$) in water (see Table 3). Because the protonation of the base form of anthocyanins is diffusion-controlled in water and the magnitude of the apparent rate constant in SDS is consistent with known values for entry of counterionic species into ionic micelles, this suggests that protonation occurs via diffusion-mediated encounter of the micelle-solubilized base with protons entering from the aqueous phase.[37] Whatever the diffusion mode involved in proton-base encounters, the increase of k_p relative to water is only 7-fold and can thus account for less than half of the observed change in the apparent pK_a in the presence of SDS.

In this context, the truly interesting result is the directly determined rate constant for unimolecular deprotonation of the flavylium cation, k_d. Despite being independent of the local pH, the value of this rate constant *decreases 25-fold* upon going from water ($k_d = 5.0 \times 10^6$ s^{-1}) to the SDS micellar surface ($k_d = 2 \times 10^5$ s^{-1}). Thus, it is the decrease of k_d, and not the effective proton concentration at the micelle surface, that is the major contributor to the stabilization of the flavylium cation with respect to its base form in the presence of micellar SDS.

As to the origin of this stabilization, a significant contribution from changes in the properties of the medium at the micelle surface (e.g., local dielectric constant, water activity, etc.) can be ruled out on the basis of data for the model anthocyanin 4-methyl-7-hydroxyflavylium chloride (HMF). For this compound, which exhibits only the acid−base equilibrium in aqueous solution without suffering significant hydration, the deprotonation rate constant, k_d, decreases 50-fold upon going from water to anionic SDS micelles, but is unchanged in cationic CTAC micelles and even increases slightly in nonionic Triton X-100 micelles.

If the observed shift in the apparent pK_a of oenin in SDS cannot be attributed to either the local pH or a local medium effect, it must be due to preferential stabilization of the flavylium cation form of oenin relative to the neutral base due to the strong interaction of the former with the negatively charged SDS micelle. Because the hemiacetal and chalcone forms of oenin are also neutral species, selective stabilization of the flavylium cation relative to these should provoke a large shift in the pH-dependent hydration equilibrium, yet leave the pH-independent equilibrium constant, K_t, for the hemiacetal to *E*-chalcone tautomerization relatively unchanged. Indeed, the overall shift in the apparent hydration equilibrium constant, pK_h, is even larger than that of the apparent pK_a, whereas K_t is essentially unaffected by the presence of SDS (Table 3). The individual rate constants determined from the pH-jump experiments show that the shift in K_h is only partially due to the increase in the local pH at the SDS micelle surface with respect to water. Thus, $k_{-h}[H]_w$ increases 1 60-fold, as compared to the 75−80-fold increase expected on the basis of an increase in the local pH as the only factor responsible for the rate acceleration. Again, it is the pH-independent rate constant, k_h, for hydration of the flavylium cation that decreases significantly (15-fold) upon going from water to the anionic micellar surface.

In summary, the pronounced stabilizing effect of SDS micelles on the color of aqueous solutions of oenin, as measured by the shift in the bleaching constant ($pK_{1/2}$) from moderately acid to near-neutral pH, cannot be simplistically attributed to either a local pH effect or to a change in local medium dielectric properties. The present results conclusively demonstrate that the determining factor is instead the preferential stabilization of the cationic form of oenin with respect to the neutral base, hemiacetal and *E*-chalcone forms, which provokes profound—and concomitant—changes in the energetics of the two key equilibria that affect color, deprotonation and hydration.

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Appendix

The time dependence of the concentrations of A/AH^+, B/C_E and C_Z is ruled by the differential eq A1

$$\frac{d}{dt}\begin{bmatrix}[A/AH^+]\\ [B/C_E]\\ [C_Z]\end{bmatrix} = \begin{bmatrix}-k'_h & k'_{-h}[H^+] & 0\\ k'_h & -(k'_i + k'_{-h}[H^+]) & k_{-i}\\ 0 & k'_i & -k_{-i}\end{bmatrix} \times \begin{bmatrix}[A/AH^+]\\ [B/C_E]\\ [C_Z]\end{bmatrix} \quad \text{(A1)}$$

The solutions of eq A1 are double exponential functions

$$[A/AH^+] = a_0 + a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t} \quad \text{(A2)}$$

$$[B/C_E] = b_0 + b_1 e^{-\lambda_1 t} + b_2 e^{-\lambda_2 t} \quad \text{(A3)}$$

$$[C_Z] = c_0 + c_1 e^{-\lambda_1 t} + c_2 e^{-\lambda_2 t} \quad \text{(A4)}$$

in which the decay constants λ_2 and λ_1 are given by

$$\lambda_{2,1} = \frac{S \pm \sqrt{D^2 + 4k'_i k'_{-h}[H^+]}}{2} \quad \text{(A5)}$$

with

$$S = k'_h + k'_{-h}[H^+] + k'_i + k_{-i} \quad \text{(A6)}$$

and

$$D = k'_h + k'_{-h}[H^+] - k'_i - k_{-i} \quad \text{(A7)}$$

The preexponential coefficients (a_i, b_i, and c_i) are interrelated by eqs A8 through A11

$$a_i = \alpha_i \times b_i \quad \text{(A8)}$$

$$c_i = \gamma_i \times b_i \quad \text{(A9)}$$

$$\alpha_i = \frac{k'_{-h}[H^+]}{k'_h - \lambda_i} \quad \text{(A10)}$$

$$\gamma_i = \frac{k'_i}{k_{-i} - \lambda_i} \quad \text{(A11)}$$

and obey the initial conditions

$$\sum_i^3 a_i = [AH^+]_0 \quad \text{(A12)}$$

$$\sum_i^3 b_i = 0 \quad \text{(A13)}$$

$$\sum_i^3 c_i = 0 \quad \text{(A14)}$$

where $[AH^+]_0$ is the initial concentration of the acid form and a_0, b_0, and c_0 are equal to the equilibrium concentrations of AH^+, B and C, respectively.

Rearrangement of eqs A8−A14, results in eqs A15−A21 for the normalized ($[AH^+]_0 = 1$) preexponential coefficients

$$a_0 = \frac{\alpha_0(\gamma_1 - \gamma_2)}{P} \quad \text{(A15)}$$

$$a_1 = \frac{\alpha_1(\gamma_2 - \gamma_0)}{P} \quad \text{(A16)}$$

$$a_2 = \frac{\alpha_2(\gamma_0 - \gamma_1)}{P} \quad \text{(A17)}$$

$$b_0 = \frac{\gamma_1 - \gamma_2}{P} \quad \text{(A18)}$$

$$b_1 = \frac{\gamma_2 - \gamma_0}{P} \quad \text{(A19)}$$

$$b_2 = \frac{\gamma_0 - \gamma_1}{P} \quad \text{(A20)}$$

$$P = \frac{k'_h\lambda_1(\gamma_2 - \gamma_0) + k'_h\lambda_2(\gamma_0 - \gamma_1) + \lambda_2\lambda_1(\gamma_2 - \gamma_0)}{k'_h(k'_h - \lambda_1)(k'_h - \lambda_2)} \quad \text{(A21)}$$

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(33) Although the sum of $k_i' + k_{-i}$ can, in principle, be determined from the intercept of Figure 7b, the intercept is close to zero and the error so large that it provides only an indication of the order of magnitude of $k_i' + k_{-i}$.

(34) Since the selectivity coefficient for proton/sodium counterion exchange at the SDS micellar surface is essentially unity, the ratio of local concentrations of protons in the micellar and aqueous phases should be equal to that of sodium.[35] Taking the degree of counterion dissociation of the micelle to be $\alpha = 0.25$ and the molar volume of SDS to be $V_m = 0.25$ l.mol^{-1}, the local concentration of sodium ion at the micelle surface is found to be 3.0 M.[35,36] Using standard PPIE mass balance relationships,[36,37] the concentration of sodium ion in the aqueous phase is estimated to be 0.035−0.040 M. This corresponds to an 80-fold concentration of protons at the micellar surface relative to the aqueous phase, corresponding to a shift in the apparent pK_a of 1.9 units relative to water.

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(37) Assuming an average micellar aggregation number, N_{ag}, of 75,[38] less than 2% of the micelles should have a bound proton at an aqueous phase pH of 5 (the midpoint of the data of Figure 4b), requiring that protonation of the base occur by entry of a proton from the aqueous phase. Likewise, application of the Infelta-Tachiya equation[39] to the decay of the base produced by the nanosecond laser pulse leads to the conclusion that $k_p = k_{+H}k_{rH}/(k_{rH} + k_{-H})$, where k_{+H} and k_{-H} are the rate constants for entry and exit of the proton from the micelle and k_{rH} the pseudo-unimolecular rate constant for protonation of the base by the proton after entry into the micelle.

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