Kinetics and Thermodynamics of the Structural Transformations of Thiamine in Aqueous Media. Part 5. Interaction of Thiamine with SDS Micelles

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In basic micellar sodium dodecyl sulphate (SDS) media, the transformation of thiamine into its yellow form is slowed down, but still occurs *via* the intramolecular attack of the 4'-amino at the 2-position of A^+ to generate the σ-adduct **D**, with apparent second-order rate constants varying from 25.50 to 39.50 $dm^3\ mol^{-1}\ s^{-1}$ at SDS concentrations ranging from 0.1 to 0.05 mol dm^{-3}, respectively. This allows the measurement of the A^+–micelle dissociation constant $K_A = 1.6 \times 10^{-4}$ mol dm^{-3} which indicates that one micelle interacts with one thiamine molecule and that the σ-adduct formation occurs in extramicellar water with a second-order rate constant ($k_{14} = 99\ dm^3\ mol^{-1}\ s^{-1}$) and in the micelle with a rate constant $k'_{14} = 10.9\ dm^3\ mol^{-1}\ s^{-1}$. The fast transformation of intermediate **D** into J^- (second-order rate constant $5.15 \times 10^6\ dm^3\ mol^{-1}\ s^{-1}$) competes with the interaction of **D** with the micelle. **D** seems to be instantly and partially solubilized by the SDS micelle, yielding J^- after the micelle dissociates into its monomers with a relaxation time of 150×10^{-3} s. Moreover, the hydroxylation of thiamine to its pseudobase in the micellar medium is slower than in water. This transformation occurs mostly in extramicellar bulk water with a second-order rate constant $k_{12} = 19.6\ dm^3\ mol^{-1}\ s^{-1}$. It also occurs in the micelle where it is considerably retarded with an endomicellar second-order rate constant $k'_{12} = 0.85\ dm^3\ mol^{-1}\ s^{-1}$. **D** is the most hydrophobic thiamine species, its interaction with the SDS micelle may be of importance in the transport of the thiamine molecule through natural membranes.

Thiamine, or vitamin B1, is a coenzyme responsible for some of the metabolic processes involved in the tricarboxylic acid cycle,[5] *e.g.* pyruvate decarboxylation and benzoin condensation.[6,7] It exists in aqueous media above pH 7 as six to nine different structures connected by more-or-less fast equilibria all of which are acid and/or base promoted, equations (1)–(6) and Figure 1.[1–4]

$$A^+ \rightleftharpoons B + H^+ \quad (1)$$

$$A^+ + OH^- \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} B \quad \text{(rate-limiting)} \quad (2)$$

$$B + OH^- \rightleftharpoons C^- \quad (3)$$

$$A^+ \rightleftharpoons D + H^+ \quad (4)$$

$$A^+ + OH^- \underset{k_{41}}{\overset{k_{14}}{\rightleftharpoons}} B \quad \text{(fast)} \quad (5)$$

$$D^+ + OH^- \underset{k_{54}}{\overset{k_{45}}{\rightleftharpoons}} D^- \rightleftharpoons J^- \quad \text{(very fast)} \quad (6)$$

Micelles in water are aggregates of surfactants resulting from an equilibrium between hydrophobic interactions of the organic tails and electrostatic repulsions of the polar heads of the amphiphiles.[8,9] Micelles are dynamic species with lifetimes in the tenth of a second range.[9,10] They have been extensively studied,[8,9,11] and their structure is most commonly considered to be a droplet of oil with a polar coat the average radius of which is the length of the detergent tail.[8,9] Micelles are known to interact with and to solubilize a great number of organic and inorganic species.[8–11]

Although some experiments involving thiamine have been performed in micellar media,[12] to our knowledge there is no direct evidence for any thiamine–micelle interaction. This, and the fact that in equations (1)–(6) all species other than A^+ and C^- are kinetic products[1–4] which can be possibly solubilized in micelles, led us to this physicochemical study of the chemical relaxation[13] of the structural transformations of thiamine (STT) in sodium dodecyl sulphate (SDS) micellar media.

Experimental

Thiamine (Merck) was kept under vacuum and used without further purification, m.p. 248–250 °C (lit.[1]). NaOH, HCl (Merck Titrisol) KCl, (Merck très pur) and SDS (Janssen) were used without further purification. Water was distilled twice under argon.

Stock solution. Fresh solutions of thiamine–SDS were used for kinetic measurements with thiamine concentrations ranging from 5 to 10^{-4} mol dm^{-3} and 0.1 to 0.05 mol dm^{-3} SDS. Ionic strength was adjusted to 0.2 mol dm^{-3} with KCl.

pH Measurements. pH values were measured with a radiometer pH-meter equipped with a 'Metrohm EA 125' combined electrode at (15 ± 0.5) and at (25 ± 0.5) °C. Buffers used for pH standardization were pH 6.86 and pH 10.01 NBS standards (Beckman).

^{1}H *NMR spectroscopy.* A Bruker 500 MHz NMR spectrometer was used to acquire the proton NMR spectrum of AH^{2+} [A^+ protonated at (N)1'] in D_2O ($c = 1 \times 10^{-3}$ mol dm^{-3}) in the presence of 0.2 mol dm^{-3} SDS. In order to generate AH^{2+}, the pH is rendered slightly acidic (pH *ca.* 4) by microinjections of HCl (Merck).

Kinetic Measurements.—Slow. For the J^- to C^- transformation, neutral thiamine–SDS solutions were perturbed by microinjections of 10 mol dm^{-3} NaOH according to previously

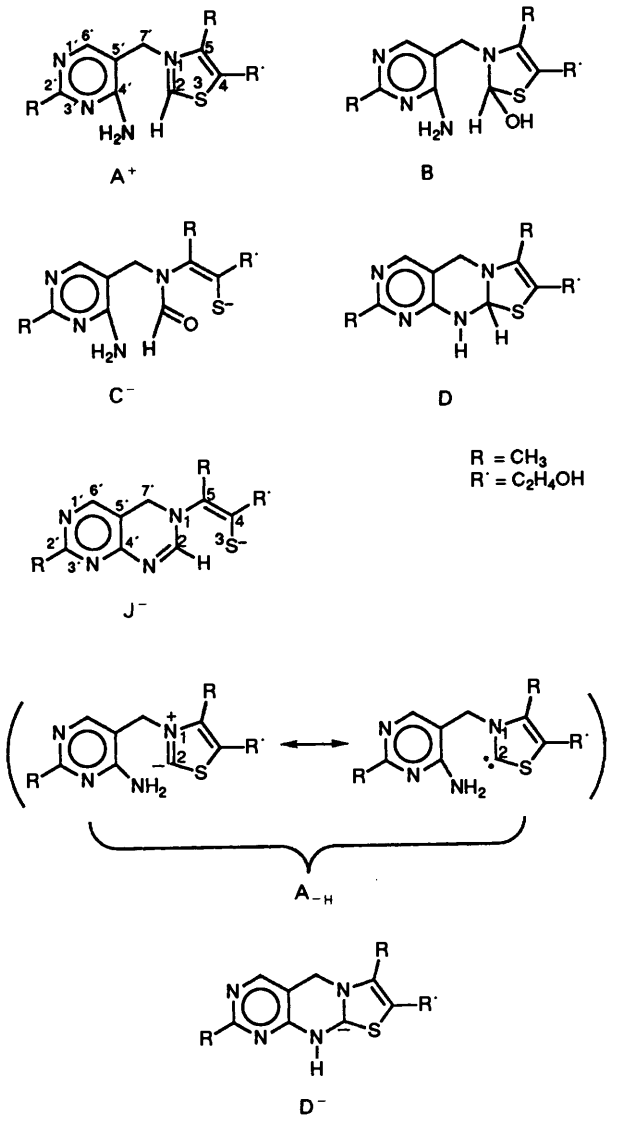

Figure 1.

Table 1. Chemical shifts (δ) of AH^{2+} in a purely aqueous medium, and in the presence of SDS micelles.

Proton	aqueous δ(ppm)	micellar δ(ppm)
2	9.83	9.80
6′	8.19	8.18
6	3.30	3.13
7	3.90	3.85
8′	2.69	2.53
8	2.79	2.62
7′	5.69	5.50

described methods.[1,2] Kinetic measurements were performed on a Cary C 210 spectrophotometer in a sample cell thermostatted at (25 ± 0.5) °C.

Stopped-flow. Kinetic measurements were performed under argon on a SF-4 series Hi-Tech stopped-flow spectrophotometer, equipped with a thermostatted bath held at 25 ± 0.5 °C, according to previously described methods.[3,4] Volumes of thiamine–SDS solutions were injected with equal volumes of NaOH (4 × 10^{-2}–0.4 mol dm^{-3}) in the mixing chamber (mixing time 3 × 10^{-3} s). The final concentrations ranged from 5 × 10^{-5} to 10^{-4} mol dm^{-3} for thiamine and from 0.1 to 0.05 mol dm^{-3} for SDS. Experimental signals were acquired and treated by known procedures.[1-4]

T-Jump. A Messanlagen Studiengesellschaft Joule effect *T*-jump spectrophotometer equipped with an external reservoir [thermostatted at (15 ± 0.5) °C] connected to the *T*-jump cell *via* a peristaltic pump[3] was used to analyse the transformation of J^- into **D** in aqueous micellar media. The micellar SDS solution (0.20 mol dm^{-3}) was introduced into the reservoir and into the cell. At thermal equilibrium, the medium was rendered basic by microinjection of a solution of 10 mol dm^{-3} NaOH into the reservoir. J^- was then produced by microinjection of a 10^{-1} mol dm^{-3} thiamine solution (final thiamine concentrations: 0.7–1 × 10^{-3} mol dm^{-3}). Once J^- was generated (after a mixing time of 30 s)[3] a temperature jump of 6 to 7 °C was produced by discharging a 0.05 μF condenser charged at 23 kV. The absorbance at 300–400 nm was recorded as a function of time on a PDP 11 computer and treated as previously.[3] The relaxation signals were acquired from pH 11 to pH 11.5. Their range of uncertainty varied from 15 to 25%. This is because, in order to avoid the precipitation of SDS, our experiments were performed at 15 °C where, in the vicinity of pH 11.5, the lifetime of J^- is considerably shortened, rendering the amplitude of these signals unstable and weak.

Results

SDS micelles leave the absorbance spectra of A^+, C^- and J^- practically unaffected. However, in the NMR spectrum of AH^{2+} (thiamine protonated at the N-1′ position) in the presence of SDS micelles, most of the proton signals are shifted slightly upfield (Table 1) indicating a possible interaction between AH^{2+} and the SDS micelles.[8,9,14] However, it should be noted that under our experimental conditions the concentration of micelles [M] in the medium is only about 2 to 3 times that of thiamine {[M] *ca.* 2.4 × 10^{-3} mol dm^{-3} and c = 9.5 × 10^{-3} mol dm^{-3}}.* Above these micelle concentrations, the proton signals become much too weak to be detected correctly. Nevertheless, if A^+ and SDS micelles do interact, this would take place in the water-rich area of the micelle, probably at the water–micelle interface in the so-called Stern layer or Gouy–Chapman double layer.[8,9] Otherwise, upfield shifts would be more important.[8]

Kinetic and Thermodynamic Study of STT in the Presence of SDS.—When a neutral thiamine–SDS solution is subjected to a fast increase in basicity (pH > 11), two kinetic phenomena are detected in the range 300–350 nm (Figure 2). The first kinetic process is a fast stopped-flow time-resolved exponential increase of absorbance, the amplitude of which becomes pH independent above pH 12.5. As for the second phenomenon, it is a slow exponential decay of absorbance which practically disappears at t_∞. Thus, when A^+ is placed in a basic micellar medium (11 < pH < 12.5), it is partially and rapidly transformed into J^- which in turn yields C^- *via* **D**, A^+ and **B**.[2] Although slowed down by SDS, the rates of the transformation of J^- into C^- are still—as in a purely aqueous medium[2]—proportional to pH up to a certain pH value after which they become inversely proportional to pH (Figure 3).

* SDS micelle concentrations [M] (Table 2) were approximated by the use of the following equation,[15] in which cmc is the critical micelle

$$[M] = ([SDS] - cmc)/n$$

concentration and n is the micelle aggregation number. At 25 °C and 0.2 mol dm^{-3} ionic strength, cmc and n are reported as < 1.5 × 10^{-3} mol dm^{-3} and 85, respectively.[15,16]

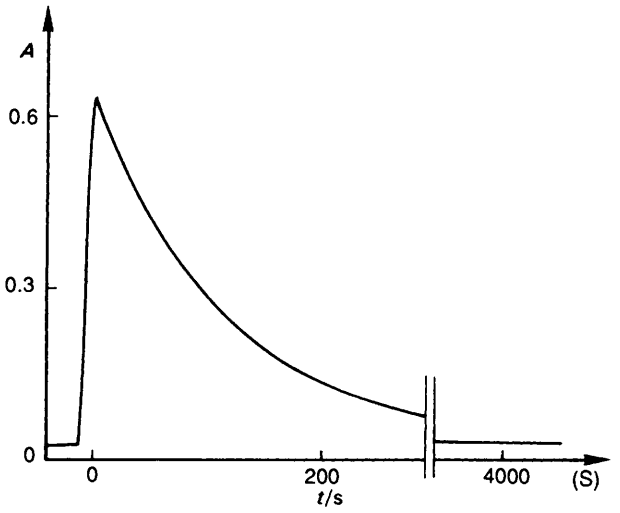

Figure 2. Absorbance change in a thiamine–SDS solution at 340 nm after a pH jump from neutral to basic (pH = 12.42) at 25 °C, 0.2 mol dm^{-3} ionic strength, [SDS] = 0.1 mol dm^{-3}, and $c = 1.3 \times 10^{-4}$ mol dm^{-3}.

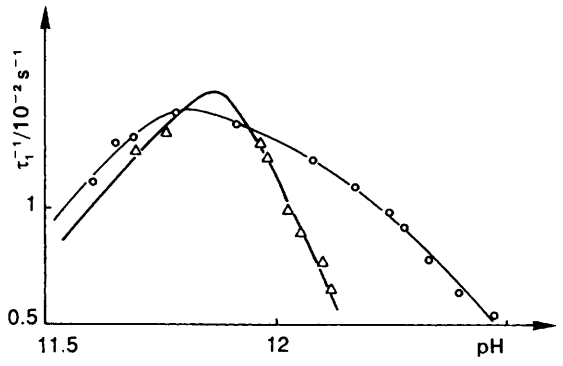

Figure 3. Plot of τ_1^{-1} *vs.* pH: △, [SDS] = 0.05 mol dm^{-3}; ○, [SDS] = 0.1 mol dm^{-3}.

In order to perform the *T*-jump experiments in water, the $A^+ \rightarrow J^-$ and the $J^- \rightarrow C^-$ transformations were slowed by a temperature drop to 2 °C.[3] However, with SDS, the temperature cannot be lower than 15 °C, where the lifespan of J^- is only a few minutes. This renders the *T*-jump measurements difficult with larger experimental uncertainties. Nevertheless, when the micellar J^- solution is subjected to a *T*-jump, a single relaxation process is observed in the 300 to 400 nm range. This single phenomenon occurs with a relaxation time of (150 ± 40) × 10^{-3} s which is independent of pH and of the thiamine concentration. In purely aqueous media, the **D** to J^- transformation [reaction (6)] occurs as a fast, pH-dependent phenomenon with a second-order rate constant $k_{15} = 5.15 \times 10^6$ dm^3 mol^{-1} s^{-1}.[3] It should be noted, moreover, that the single *ca.* 0.1 s kinetic process detected in a micellar J^- solution is also observed with probes known to be solubilized in SDS micelles.[10,17]

Thermodynamics of the $A^+ \rightarrow$ **D** *and of the* **D** $\rightarrow J^-$ *transformations in an SDS micellar medium.* Equilibrium constants $K_b = [A^+][OH^-]^2/[J^-]$, $K_{3b} = [A^+][OH^-]/[D]$ and $K_{4b} = [D][OH^-]/[J^-]$ were measured in water by spectrophotometric methods.[2] These methods were utilized to measure the apparent equilibrium constants K'_b, K'_{3b}, and K'_{4b} at three different micelle concentrations (Table 2). Only apparent K'_{3b} values are affected by the presence of micelles, while K'_{4b} values are always practically equal to K_{4b}, which means that the formation of the yellow form J^- from the σ-adduct **D** [reaction (6)] seems to occur only in extramicellar bulk water. This may be the consequence of a non-interaction of J^- with the micelle, probably because of electrostatic repulsion by the negative heads of SDS surfactants.

Kinetics of the $A^+ \rightarrow$ **D** *transformation; the stopped-flow kinetic process.* As in purely aqueous media, the single stopped-flow relaxation observed in basic micellar SDS was ascribed to the A^+ to J^- transformation which is rate-limited by the A^+ to **D** step.[3,4] We first assumed that the base-promoted intramolecular attack of the 2-position of thiazolium by 4′-NH_2 to yield the σ-adduct **D** occurs both in bulk water [reaction (5)] and in the micelle [reaction (8)], that A^+ and **D** interact with only one micelle each [reactions (7) and (9)], that the **D** to J^- transformation occurs only in extramicellar water, as shown by the pK_4 value, and that J^- does not interact with the negatively charged SDS micelle.

$$A^+ + M \rightleftharpoons AM \quad K_A = [A^+][M]/[AM] \qquad (7)$$

$$AM + OH^- \underset{k_{41}}{\overset{k_{14}}{\rightleftharpoons}} DM \qquad (8)$$

$$D + M \rightleftharpoons DM \quad K_D = [D][M]/[DM] \qquad (9)$$

M, **AM** and **DM** are written without any formal charges for the sake of simplicity, **AM** is the micelle–A^+ complex and **DM** is the micelle–**D** complex.

Micelle–small-substrate interactions usually take place in the microsecond range,[9,10,17] and the **D**→J^- transformation is very fast compared with σ-adduct (**D**) formation.[3,4] Therefore, before the $J^- \rightarrow C^-$ transformation and during the A^+ to **D** step, reactions (6), (7), and (9) can be considered to be in a constant state of equilibrium.[13] This can be expressed as equations (10)–(12).

$$\Delta[J^-] = [OH^-]\Delta[D]/K_{4b} + [D]\Delta[OH^-]/K_{4b} \qquad (10)$$

$$\Delta[DM] = [M]\Delta[D]/K_D + [D]\Delta[M]/K_D \qquad (11)$$

$$\Delta[AM] = [M]\Delta[A^+]/K_A + [A^+]\Delta[M]/K_A \qquad (12)$$

At the end of the $A^+ \rightarrow J^-$ transformation and before J^- yields C^-, conservation of mass implies equation (13).

$$\Delta[A^+] + \Delta[D] + \Delta[J^-] + \Delta[AM] + \Delta[DM] = 0 \qquad (13)$$

From equations (10) to (13), and with $[OH^-] \gg [M]$, $[A^+]$, [AM], [D], [DM] and $[J^-]$, we can derive the reciprocal relaxation time equation (14) associated with reactions (7) and (5):

$$\tau_2^{-1} = (k_{14}K_A/[M] + k'_{14})[OH^-] + (k_{41} + k'_{41}[M]/K_D)K_{4b}/[OH^-] \qquad (14)$$

which can be expressed as equation (15).

$$[OH^-]\tau_2^{-1} = (k_{14}K_A/[M] + k'_{14})[OH^-]^2 + (k_{41} + k'_{41}[M]/K_D)K_{4b} \qquad (15)$$

If $[OH^-]$ varies at constant micelle concentration, $(k_{14}K_A/[M] + k'_{14})$ should remain constant with $k_{14} = 99$ dm^3 mol^{-1} s^{-1}.[3,4] This would permit the measurement of K_A and k'_{14}. Three linear least-squares regressions of the data employing equation (9) gave for [SDS] = 0.1, 0.075, and 0.05 mol dm^{-3}, $(k_{14}K_A/[M] + k'_{14})$ values of 25.60, 28.55 and 39.44 dm^3 mol^{-1} s^{-1}, respectively, with $(k_{41} + k'_{41}[M]/[D])K_{4b}$ too small to be measured. From these slopes and k_{14}, equilibrium constant $K_A = (1.60 \pm 0.15) \times 10^{-3}$ mol dm^{-3}, and second-order rate constant $k'_{14} = (10.90 \pm 1.5)$ dm^3 mol^{-1} s^{-1} are determined.

Table 2. pK', pK'_3 and pK'_4 at different micelle concentrations.

[SDS]/mol dm^{-3}	[M]/mol dm^{-3}	pK'	pK'_3	pK'_4
0	0	11.50	12.30	10.90
0.05	5.60×10^{-4}	11.73 ± 0.02	12.50 ± 0.05	10.95 ± 0.05
0.075	8.70×10^{-4}	11.81 ± 0.02	12.70 ± 0.05	10.95 ± 0.05
0.1	1.16×10^{-3}	11.86 ± 0.02	12.85 ± 0.05	10.95 ± 0.05

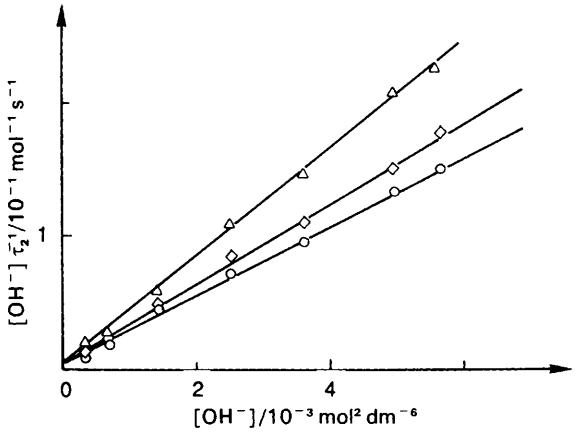

Figure 4. Plot of $[OH^-]\tau_2^{-1}$ *vs.* $[OH^-]^2$: ○, [SDS] = 0.1 mol dm^{-3}, intercept (4.20 ± 5.05) × 10^{-3} s^{-1}, slope 25.60 ± 1.5 dm^3 mol^{-1} s^{-1}, r = 0.998 59; ◇, [SDS] = 0.075 mol dm^{-3}, intercept (4.65 ± 4.05) × 10^{-3} s^{-1}, slope 28.55 ± 1.25 dm^3 mol^{-1} s^{-1}, r = 0.999 57; △, [SDS] = 0.05 mol dm^{-3}, intercept (4.45 ± 4.10) × 10^{-3} s^{-1}, slope 39.45 ± 1.65 dm^3 mol^{-1} s^{-1}, r = 0.995 79.

If more than one thiamine molecule interacts with a single

$$n\mathbf{A}^+ + \mathbf{M} \rightleftharpoons \mathbf{MA}_n \quad K_{An} = [\mathbf{A}^+]^n[\mathbf{M}]/[\mathbf{MA}_n] \quad (16)$$

$$n'\mathbf{D} + \mathbf{M} \rightleftharpoons \mathbf{MD}'_n \quad K_{Dn} = [\mathbf{D}]^{n'}[\mathbf{M}]/[\mathbf{MD}_n] \quad (17)$$

micelle, the reciprocal relaxation time associated with reaction (16) can then be expressed as equation (18).

$$\tau_2^{-1} = k_{14}(\text{obs})(K_{An} + [\mathbf{A}^+]^n)/(K_{An} + [\mathbf{A}^+]^n + n^2[\mathbf{A}^+]^{n-1}[\mathbf{M}]) + k_{41}(\text{obs})K_{4b}/[OH^-] \quad (18)$$

A kinetic analysis of the data by equation (18) cannot be achieved without K_{Dn} and K_{An}. However, the very good correlation coefficients of the lines of Figure 4, and the micelle concentrations used in our experiments (not more than ten times the concentration of thiamine) allow us to infer that under our experimental conditions, only one micelle interacts with one molecule of thiamine, and that the intramolecular attack of the 4′-NH_2 at C-2 is both extramicellar and endomicellar with an endomicellar rate inhibition factor of *ca.* 10.

Kinetics of the $\mathbf{J}^- \rightarrow \mathbf{C}^-$ *transformation.* As in purely aqueous media, the single slow relaxation of Figure 2 [the transformation of $\mathbf{J}^-$ into $\mathbf{C}^-$ in basic micellar SDS] is ascribed to the rate-limiting step in STT which is the hydroxylation of cation $\mathbf{A}^+$ to give the pseudobase **B**. Although water is involved in pseudobase formation,[1,18] we cannot assume that reaction (2) occurs only in extramicellar water. We will, therefore, take into account possible pseudobase formation in the micelle. However, we will consider, as was done for the $\mathbf{D} \rightarrow \mathbf{J}^-$ transformation, that that of **B** into $\mathbf{C}^-$ occurs in bulk water [reaction (3)] and that only one micelle interacts with pseudobase **B** in which **BM** is the pseudobase–micelle complex.

$$\mathbf{B} + \mathbf{M} \rightleftharpoons \mathbf{BM} \quad K_B = [\mathbf{B}][\mathbf{M}]/[\mathbf{BM}] \quad (19)$$

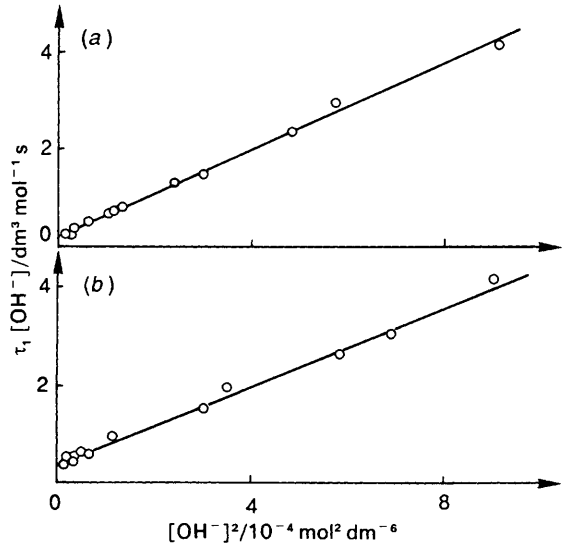

Figure 5. Plot of $[OH^-]\tau_1 = [OH^-]^2/k_{12}(\text{obs})$ K_6: (*a*), [SDS] = 0.1 mol dm^{-3}, intercept 0.35 ± 0.20 mol dm^{-3} s, slope (4.05 ± 0.15) × 10^3 dm^3 mol^{-1} s, r = 0.997 29; (*b*), [SDS] = 0.05 mol dm^{-3}, intercept 0.20 ± 0.2 mol dm^{-3} s, slope (4.40 ± 0.20) × 10^3 dm^3 mol^{-1} s, r = 0.996 57.

$$\mathbf{AM} + OH^- \underset{k'_{21}}{\overset{k'_{12}}{\rightleftharpoons}} \mathbf{BM} \quad (20)$$

If we assume that, besides pseudobase formation, all the other reactions involved in the $\mathbf{J}^- \rightarrow \mathbf{C}^-$ transformation are in a constantly equilibrated state, we can write equations (21)–(23),

$$\Delta[\mathbf{D}] = [OH^-]\Delta[\mathbf{A}^+]/K_{3b} + [\mathbf{A}^+]\Delta[OH^-]/K_{3b} \quad (21)$$

$$\Delta[\mathbf{BM}] = [\mathbf{M}]\Delta[\mathbf{B}]/K_B + [\mathbf{B}]\Delta[\mathbf{M}]/K_B \quad (22)$$

$$\Delta[\mathbf{C}^-] = [\mathbf{B}]\Delta[OH^-]/K_{2b} + [OH^-]\Delta[\mathbf{B}]/K_{2b} \quad (23)$$

in which $K_{2b} = [\mathbf{B}][OH^-]/[\mathbf{C}^-] \ll K_{3b}, K_{4b}$.[1,2]

Conservation of mass implies equation (24), whereas our

$$\Delta[\mathbf{A}^+] + \Delta[\mathbf{B}] + \Delta[\mathbf{C}^-] + \Delta[\mathbf{D}] + \Delta[\mathbf{J}^-] + \Delta[\mathbf{AM}] + \Delta[\mathbf{DM}] + \Delta[\mathbf{BM}] = 0 \quad (24)$$

experimental conditions imply that $[OH^-] \gg [\mathbf{M}] > [\mathbf{A}^+]$, $[\mathbf{B}]$, $[\mathbf{C}^-]$, $[\mathbf{D}]$, $[\mathbf{J}^-]$, $[\mathbf{AM}]$, $[\mathbf{BM}]$ and $[\mathbf{DM}]$.

The kinetic equation of rate-limiting pseudobase formation in the presence of SDS micelles can be written as equation (25).

$$-d[\mathbf{C}^-]/dt = -k_{12}[OH^-][\mathbf{A}^+] + k_{21}[\mathbf{B}] - k'_{12}[OH^-][\mathbf{AM}] + k'_{21}[\mathbf{BM}] \quad (25)$$

From equations (10)–(12) and (21)–(25), we can derive the reciprocal relaxation equation of pseudobase formation in the presence of micelles [equation (26)] in which $k_{12}(\text{obs}) = k_{12} +$

$$\tau_1^{-1} = \frac{k_{12}(\text{obs})K_{3b}K_bK_AK_D[OH^-]}{K_{3b}K_bK_AK_D + [OH^-]K_bK_AK_D + [OH^-]^2K_{3b}K_AK_D + [M]K_DK_{3b}K_b + [M][OH^-]K_AK_b} + k_{21}(\text{obs})K_{2b}/[OH^-] \quad (26)$$

$k'_{12}[M]/K_A$ and $k_{21}(\text{obs}) = k_{21} + k'_{21}[M]/K_B$. Since K_{2b} *ca.* 8×10^{-6} mol dm^{-3},[1] under our experimental conditions the term in $k_{21}(\text{obs})$ is negligible compared with that in $k_{12}(\text{obs})$ and to τ_1^{-1}. The use of equation (26) requires K_D. However, at a constant micelle concentration, this can be avoided by equation (27) which is directly derived from equation (26) without the term in $k_{21}(\text{obs})$.

$$[OH^-]\tau_1 = 1/k_{12}(\text{obs}) + [OH^-](1/K_{3b} + [M]/K_{3b}K_D)/k_{12}(\text{obs}) + [OH^-]^2/k_{12}(\text{obs})K_b + [M]/k_{12}(\text{obs})K_A \quad (27)$$

Two double linear least-squares regression of $[OH^-]\tau_1$ against $[OH^-]$ and $[OH^-]^2$ at [SDS] = 0.1 and 0.05 mol dm^{-3}, respectively, show that the term in $[OH^-]$ can be neglected. Two least-squares linear regressions of $[OH^-]\tau_1$ against $[OH^-]^2$ at the same SDS concentrations were, therefore, performed (Figure 5). These gave $k_{12}(\text{obs}) = 25.0 \pm 1.50$ and 22.5 ± 1.5 dm^3 mol^{-1} s^{-1}, respectively. This allows the determination of $k'_{12} = 0.85 \pm 0.2$ mol^{-1} dm^3 s^{-1} which is about 5% of the k_{12} value. This shows that thiamine pseudobase formation occurs mostly in extramicellar water.

Discussion

Table 3, we summarize our findings and introduce the general mechanism of the structural transformations of thiamine in aqueous SDS micellar media.

Thiamine–Micelle Interactions.—We have shown that a single thiamine molecule interacts with one micelle [reaction (7)]. The stability constant of **AM** $K_A^{-1} = 6.25 \times 10^3$ dm^3 mol^{-1} is rather high for water-soluble molecules like thiamine.[8,9] Moreover, the electrostatic interactions that may occur between positive thiazolium and negative SO_4^- at the SDS micelle–water interface are reported to be weak and cannot alone explain the K_A value.[8,9] However, it should be noted that pyrimidines are known to interact with micelles where they are considered as being anchored in the hydrophobic core of the aggregate.[19] This anchoring of the pyrimidine, added to electrostatic interaction between thiazolium and the heads of the SDS surfactants at the surface of the micelle, can explain the K_A value and give the thiamine–micelle complex the same stability as that with more hydrophobic molecules.[8]

Table 3. Mechanism of the structural transformations of thiamine in basic aqueous SDS micellar media.

Reactions	Second-order rate constants/ dm^3 mol^{-1} s^{-1}	Reverse rate constants/ s^{-1}
$A^+ + M \rightleftharpoons AM$	—	—
$A^+ + OH^- \rightleftharpoons B$	19.2–19.6	1.15×10^{-4}
$AM + OH^- \rightleftharpoons BM$	0.85	—
$B + M \rightleftharpoons BM$	—	—
$B + OH^- \rightleftharpoons C^-$	6.74×10^4	8.85×10^{-2}
$A^+ + OH^- \rightleftharpoons D$	9.9	1.5
$AM + OH^- \rightleftharpoons DM$	10.90	—
$D + M \rightleftharpoons DM$	—	—
$O + OH^- \rightleftharpoons C^-$	5.15×10^6	5.25×10^3

D is stabilized by alcohol,[20] and can be considered as the most hydrophobic species of thiamine. This is not sufficient to explain the endomicellar $A^+ \rightarrow D$ transformation, because the nucleophilic attack of the 2-site of thiamine by the 4′-amino, to form **D**, is an intramolecular OH^--promoted process which is expected to occur in water. Moreover, pseudobase formation occurs either by covalent hydration immediately followed by proton loss, or by direct attack of the hydroxide ion.[1,18] Both nucleophilic attacks require, therefore, the presence of water and would probably occur in the water-rich area at the micelle–water interface.[8,9] Moreover, micelle cores cannot be considered to be totally hydrophobic; they are wet and contain a certain amount of water[11] which would contribute to endomicellar **D** formation, which may explain the endomicellar pseudobase formation.

The $D \rightarrow J^-$ *Transformation in SDS Media.*—The formation of a micelle in aqueous media is a two-step process. The first step is extremely fast and lasts only a few microseconds, while the second takes a tenth of a second.[10]

$$S_{(n-1)} + S \longrightarrow M \quad \text{(very fast)}$$

$$M \longrightarrow (n-1)S \quad \text{(slow)}$$

S is the surfactant.

The first very fast step is ascribed to the equilibrium between a micelle and one of the surfactant molecules composing it. The second process is attributed to the total breakdown of the micelle into its components.[10,17] We have shown that the rate of the transformation of **D** into J^- is controlled by this second process (micelle breakdown and formation). Thus **D**, the most hydrophobic thiamine species, seems to be at least partially trapped in the SDS micelle. Such a trapping of a small molecule like **D** would have a rate similar to that of the monomer–micelle equilibrium and would occur in a few microseconds.[9,10,17] The control of the **D** to J^- step by the life-time of the micelle (0.1 s) can be interpreted in terms of a probable competition between the **D**–micelle interaction and the **D** to J^- transformation which occurs in water with a second-order rate constant of 5.15×10^6 dm^3 mol^{-1} s^{-1}. Therefore, **D** is probably partially and very rapidly solubilized by the micelle and when the micelle dissociates into its monomers **D** is released and transformed into J^- through the fast $D \rightarrow J^-$ step [Reaction (6)]. Thus **D** would play the role of a probe for the rate of micelle breakdown and formation. The increases of pK'_3 in the SDS micellar medium (Table 2) are independent of the stabilization of **D** by the micelle. Both pK'_3 and pK'_4 are measured in a semi-equilibrated state after the formation of J^- and before the slow transformation of this species into C^- when **D** and **DM** are practically absent from reactional media. pK'_3 is therefore not directly related to the stability constant of **DM** formation [reaction (9)].

Conclusions

For a concentration of SDS micelles about 10 times that of thiamine, cation A^+ interacts with a single micelle to form a rather stable complex. It is still, however, attacked by OH^- and 4′-NH2 to form pseudobase **B** and σ-adduct **D**. **D** is partially trapped and kinetically stabilized by SDS micelles indicating that this most hydrophobic thiamine species would probably be preferentially trapped and stabilized by a less labile aggregate

structure such as a liposome or a vesicle or even a natural membrane.[9] In this case, the biological role of the **D** species would be important not only for the C-2 deprotonation into $\mathbf{D}^-$, which we regard as a possible biocatalyst for the metabolic activity of the vitamin,[3,4] but also for its transport through cell membranes.

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