Retinylidene Schiff-base Protonation in Surfactant-solubilized Water Pools in Heptane

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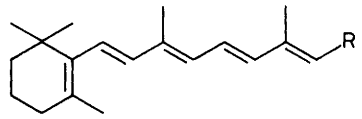
All-*trans*-*N*-retinylidene-n-butylamine (**4**) and its 3-chloropropionic acid (CPA) protonated analogue (**5**) remained stable in sodium bis(2-ethylhexyl) sulphosuccinate, reversed-micelle-solubilized water pools in heptane, and the extent to which (**4**) protonated depended on the [CPA] to [(**4**)] ratio and on the amount of solubilized water; hence, this system mimicked the behaviour of rhodopsins.

Retinyl Schiff-base protonation is central to our understanding[1] of the visual process and energy transduction. The chromophore of rhodopsin[2] is a retinylidene Schiff base (**2**) formed between retinal (**1**) and the ε-amino group of a lysine residue of the apo-protein *via* a Schiff-base (SB) linkage (**2**). Remarkably, the relatively weak acids available at the active site of the protein (glutamic and aspartic acids) are capable of fully protonating the chromophore and providing the environment necessary for stabilizing retinylidene SB (**2**) and its protonated analogue (**3**). In contrast to (**2**), synthetic retinyl-



(1) R = CHO
(2) R = CH=N-Lys-apo-protein
(3) R = CH=$\overset{+}{N}$(H)-Lys-apo-protein
(4) R = CH=NBu^n
(5) R = CH=$\overset{+}{N}$(H)Bu^n

Table 1. Protonation of all *trans-N*-retinylidene-n-butylamine (**4**) by 3-chloropropionic acid (CPA) in AOT (0.01 M) in heptane.[a]

ω[b]	Protonation[c] /%	Equilibrium protonation[d]/%
	<0.50[e]	
0.1	0.60	46(210)
8.0	6.0	51(60)
24.0	15.0	61(6)
48.0	34.0	68(6)
72.0[f]	67.0	76(3)

[a] At room temperature, protonations (%) were calculated from the absorption decreases at 357 nm, taking ε 4.96 × 10^4 mol^{-1} dm^3 cm^{-1} for (**4**) and assuming quantitative conversion of (**4**) to (**5**). [b] ω = $[H_2O]$/AOT]. [c] [**4**] = [CPA] = 1.8 × 10^{-5} M. [d] Values of [CPA] at which equilibrium protonation is reached are in parentheses. [e] In the absence of AOT. [f] Solution is highly metastable.

idene Schiff bases, *e.g.* (**4**), require strong acids ($pK_a \leq 2.0$) fo. complete protonation in organic solvents.[3] Furthermore, synthetic protonated retinylidene Schiff bases, *e.g.* (**5**), are highly unstable in water, even in the presence of moderate concentrations of acid. We report here the protonation of all-*trans*-*N*-retinylidene-n-butylamine (**4**) by relatively weak 3-chloropropionic acid (CPA; pK_a in water 3.99) in sodium bis(2-ethylhexyl) sulphosuccinate (Aerosol-OT or AOT; Eastman)-solubilized water pools in heptane and that both (**4**) and its protonated counterpart (**5**) remain stable in these reversed micelles.[4]

Typical absorption spectra of (**4**) and (**5**) in AOT (0.01 M) reversed micelles in heptane are shown in Figure 1. The absorption of (**4**) in reversed micelles ($\lambda_{max.}$ 357 nm, ε 5.0 × 10^4 mol^{-1} dm^3 cm^{-1}) is identical to that observed in pure heptane and remains unaffected by increasing the $[H_2O]$/[AOT] ratios (ω values) from 0 to 72 (at constant [AOT] of 0.01 M). In contrast, increasing ω values in AOT (0.01 M) in heptane resulted in a gradual shift of the absorption maximum of (**5**) from 433 to 419 nm. Being apolar, (**4**) is predominantly located in the bulk heptane phase. Conversely, the protonated SB (**5**) is incorporated into the AOT micelles by ion-pairing with the sulphosuccinate head-groups of AOT. Reversed micelle formation, solubilization therein, and the protonation of (**4**) are, of course, all dynamic processes.[4]

CPA was found to protonate (**4**) in heptane in the presence of AOT reversed micelles. The extent of protonation depended on the molar ratio of CPA to (**4**) and on the amount of water present in the reversed micelles. At a given ω value, increasing amounts of CPA increased the extent of protonation up to a point beyond which the addition of further CPA had no effect. In the absence of added water (ω 0.1), 200-fold

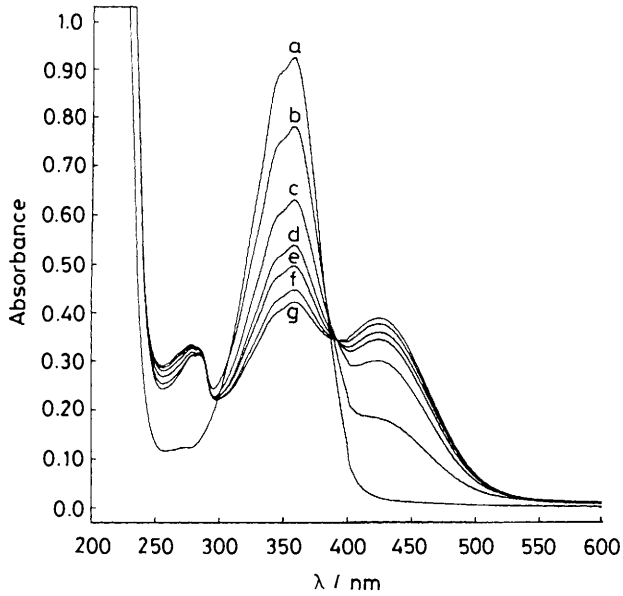


Figure 1. Absorption spectra for the protonation of 1.8 × 10^{-5} M (**4**) in 0.01 M AOT/n-heptane (ω 24) at 25 °C. [CPA] = 0 (a), 1.8 × 10^{-5} (b), 3.6 × 10^{-5} (c), 5.4 × 10^{-5} (d), 7.2 × 10^{-5} (e), 9.0 × 10^{-5} (f), and 10.8 × 10^{-5} M (g).

excess CPA was needed to reach the equilibrium protonation of (**4**), whereas with a ω value of 24, it took only a six-fold excess to protonate (**4**) to a much higher extent! The data obtained are summarized in Table 1. Comparing the extent of protonation at [(**4**)] = [CPA] = 1.8 × 10^{-5} M is, perhaps, the most informative. Availability of protons, rather than acid strength, is of over-riding importance in Schiff-base protonation. Proton availability depends on the extent of CPA dissociation. This, in turn, is favoured by the availability of water in higher concentrations in the reversed micelles. It is most significant that (**4**) and (**5**) remain stable even in the presence of water (0.25 M) and AOT, whereas they promptly decompose if more than 0.1% of aqueous acid is added to the neat organic solvent. Apparently, Schiff-base protonation and stability require finely tuned interactions, and a delicate balance between the right amount and 'right kind' of water molecules. These requirements are eminently fulfilled in the protein and appear to be well mimicked in surfactant-solubilized water pools in apolar solvents.

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