Biochimica et Biophysica Acta, 1036 (1990) 34–40
Elsevier

BBAGEN 23372

Retinylidene Schiff bases in alkylammonium carboxylate reversed micelles

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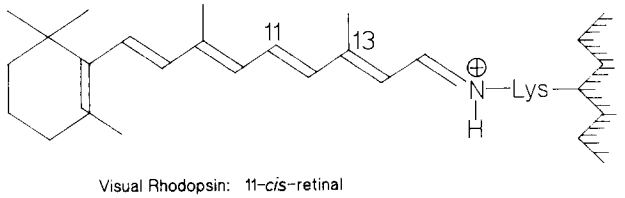
(Received 22 December 1989)
(Revised manuscript received 29 March 1990)

Key words: Retinylidene; Schiff base chromophore; Reversed micelle; Micelle, reversed; Rhodopsin; Alkylammonium carboxylate surfactant

All-*trans*-retinal, incorporated into dodecylammonium propionate, dodecylammonium 3-chloropropionate and dodecylammonium trifluoroacetate reversed micelles in cyclohexane containing different amounts of solubilized water, gave all-*trans*-*N*-retinylidene-*n*-dodecylamine. Formation of all-*trans*-*N*-retinylidene-*n*-dodecylamine was complete within a few minutes when all-*trans*-retinal was solubilized along with L-lysine in reversed-micellar dodecylammonium propionate in cyclohexane as compared to the several hours required for the comparable reaction to occur in the absence of lysine. In situ formed all-*trans*-*N*-retinylidene-*n*-dodecylamine was not protonated by the acidic moiety (i.e., propionate, 3-chloropropionate or trifluoroacetate anions) of dodecylammonium propionate, dodecylammonium 3-chloropropionate or dodecylammonium trifluoroacetate reversed micelles. Addition of propionic or 3-chloropropionic acid did not cause observable protonation of the reversed-micelle-incorporated all-*trans*-*N*-retinylidene-*n*-dedecylamine. Addition of strong trifluoroacetic acid to reversed micellar solutions of all-*trans*-*N*-retinylidene-*n*-dodecylamine caused protonation as well as hydrolysis to retinal. Retinylidene Schiff bases are protected from protonation by strongly held surfactant-ion pairs which may model proton channels whose function is controlled by protein conformational changes.

Introduction

Rhodopsins [1,2] the intrinsic membrane-bound proteins of vertebrate retina and halobacteria, exhibit several striking properties. An important feature of the active site of rhodopsin is the present of a protonated retinylidene Schiff-base chromophore (Fig. 1). The Schiff-base chromophore is believed to be involved in intimate interactions with the surrounding apoprotein [3]. Both polar and non-polar microenvironments have been suggested for the Schiff-base chromophore in rhodopsin [4–8].



Visual Rhodopsin: 11-*cis*-retinal
Bacteriorhodopsin: all-*trans* retinal (light adapted)

Fig. 1. Retinal attached to lysine via a protonated Schiff-base linkage in rhodopsins.

Protonation of the rhodopsin chromophore in vivo is assumed to be caused by relatively weak acids. Glutamic (pK_a in water = 4.25, side-chain COOH) and aspartic (pK_a in water = 3.86, side-chain COOH) acid residues are the likely proton donors, since they are located in the vicinity of the Schiff-base linkage [9–12]. Equilibrium between the non-protonated and protonated Schiff base is believed to be delicately maintained by water molecules [7,12–14] and counterion interactions. Furthermore, in natural environment, the protonated Schiff base remains stable.

Investigations of retinylidene Schiff bases in organic solvents have provided a considerable insight into the structural and functional properties of rhodopsins [13,15]. These models had, however, many shortcomings. Most importantly, in contrast to the situation in vivo, weak acids have been found to be incapable of fully protonating the retinylidene chromophore and the protonated Schiff base decomposed in the presence of aqueous acids.

An ideal model for the Schiff-base chromophore in vivo would provide a means for constricting the retinylidene Schiff base and the proton donor to close

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proximity and to allow for the control of the microscopic polarity and acidity at the site of protonation. Surfactant-solubilized water pools, reversed micelles, approximate this ideal requirement better than water. We have initiated studies, therefore, on retinylidene Schiff-base protonation in reversed micelles and have reported that sodium bis[2-ethylhexyl]sulfosuccinate-solubilized Schiff bases showed reasonable stabilities and could be protonated by weak 3-chloropropionic acid (pK_a in water = 3.99) [16]. The extent of protonation depended on the ratio of Schiff base to 3-chloropropionic acid and on the amount of solubilized water.

The present work extends our investigations to alkylammonium carboxylate reversed micelles [17]. Variation of the acid strength of the carboxylate anion allowed the examination of the protonation of retinylidene Schiff base in water pools solubilized by dodecylammonium propionate, dodecylammonium 3-chloropropionate and dodecylammonium trifluoroacetate reversed micelles in cyclohexane.

Experimental procedures

All experiments using retinal and its Schiff base were performed in dim red light. All of the chemicals were obtained from either Aldrich or Sigma. Sonications were done by a Fisher Sonic membrane disruptor (Model 300). UV-Vis absorption spectra were recorded on a Varian Carey 16 spectrophotometer. Infrared spectra were measured on a Nicolet 5DXB Fourier transform spectrophotometer. ^{1}H-NMR spectra were recorded on a Brucker 400 MHz spectrometer in $CDCl_3$ using TMS as internal standard.

Dodecylammonium propionate was prepared and purified as described in the literature [18,19].

*Solubilization of all-trans-retinal (**8**) and L-lysine in dodecylammonium propionate reversed micelles in cyclohexane.* L-Lysine (5 mg) was stirred in 5 ml of $2 \cdot 10^{-4}$ M dodecylammonium propionate (**5**) in cyclohexane containing $2 \cdot 10^{-3}$ M H_2O ($\omega = 10$ where $\omega = [H_2O]/[DAP]$). Undissolved lysine was filtered and all-*trans*-retinal ($2 \cdot 10^{-5}$ M) was added to the clear solution. The mixture was briefly sonicated (2 min, 90 W) and kept in the dark at ambient temperature. The progress of the reaction was monitored by UV-Vis absorption measurements at different time intervals.

*All-trans-N-retinylidene-n-dodecylamine (**9**).* All-*trans*-retinal (**8**) and dodecylamine (**1**, 1.5-fold excess) were mixed in dry methanol in the presence of anhydrous sodium sulfate. The reaction mixture was kept under N_2 in the dark at 4° C for 24 h. The solution was filtered and the solvent was evaporated. The solid was dissolved in dry ether and slowly passed through a neutral alumina column. The column was eluted by 5% ethyl acetate/hexane. The solvent was completely removed from the dark yellow liquid by high vacuum. The resulting brown solid showed physico-chemical data characteristic of the desired Schiff base (**9**): UV-Vis, λ_{max} (cyclohexane); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.85 (3 H, t, $-CH_3$), 1.02 (6 H, s, C_1-CH_3), 1.25 (20 H, s, $-CH_2$), 1.70 (3 H, s, C_5-CH_3), 1.97 (3 H, s, C_9-Me), 2.07 (3 H, s, C_{13}-Me), 3.48 (2 H, br, t, $-CH_2$), 6.16 (1 H, d, J, 10 Hz, C_{14}-H), 6.11 (1H, d, J, 16 Hz, H_8), 6.21 (1 H, d, J, 16 Hz, H_7), 6.34 (1 H, d, J, 15 Hz, H_{12}), 6.81 (1 H dxd, J, 12 and 15 Hz, H_{11}), 6.95 (1 H, d, J, 12 Hz, H_{10}), 8.28 (1 H, d, J, 10 Hz, C_{15}).

*Dodecylammonium trifluoroacetate (**7**).* Dodecylamine (**1**, 9.25 g, 50 mM) was dissolved in 200 ml of *n*-hexane/benzene (1 : 1). Trifluoroacetic acid (8.5 ml) was added dropwise (approx. 15 min) to the whitish amine solution. The clear, colorless solution was stirred for 2 h at ambient temperature. The solvent was removed from the solution on a rotary evaporator. The white waxy solid was crystallized from hexane to obtain shiny fine crystalline solid. It was dried over P_2O_5 under vacuum overnight; yield, 70%; m.p., 65–66° C; IR ($CHCl_3$), ν, 2929 (s), 2927 (s), 2857 (s), 2800-2400 (m-w), 1690 (s, CF_3COO^-) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ; 0.88 (3 H, t, CH_3-), 1.10-1.20 (18 H, br, s, $-CH_2$-), 1.64 (2 H, m, $-CH_2$-), 2.89 (2 H, t, $-CH_2$-$\overset{+}{N}H_3$).

*Reactions of all-trans-retinal (**8**) in dodecylammonium trifluoroacetate (**7**) reversed micelles in cyclohexane.* (1) A solution of dodecylammonium trifluoroacetate in cyclohexane ($1 \cdot 10^{-2}$ M) containing all-*trans*-retinal (**8**, $1.5 \cdot 10^{-5}$ M) was allowed to stand in the dark at ambient temperature. The absorption spectra of the solution was recorded over a period of time. The initial absorption band at 372 nm shifted to 356 nm after 24 h. Addition of trifluoroacetic acid to this solution caused deep yellow coloration with absorption maximum at 470 nm.

(2) A saturated solution of L-lysine (50 mg) in 10^{-2} M dodecylammonium trifluoroacetate in cyclohexane (25 ml) was prepared by vigorous stirring, followed by the removal of undissolved amino acid. This mixture (100 μl) was added to a solution of all-*trans*-retinal (**8**, $1.5 \cdot 10^{-5}$ M) in 10^{-2} M dodecylammonium trifluoroacetate-cyclohexane (4 ml). The entire mixture was sonicated (90 W, 5 min) and then left at ambient temperature in the dark. Absorption spectra was measured over a period of time. An initial absorption band at 372 nm shifted to 356 nm within 1 h. Addition of trifluoroacetic acid caused deep yellow coloration with absorption maximum at 470 nm.

(3) The micellar solutions of L-lysine and all-*trans* retinal (**8**) were prepared in dodecylammonium trifluoroacetate-cyclohexane containing 0.01, 0.05, 0.10, and 0.15 M H_2O. In all of the cases, the initial 372 nm peak shifted to 356 nm. However, in solutions containing relatively small amounts of water (e.g., 0.01 or 0.05 M), the reaction was complete in few minutes, while it took

2 h for the complete development of the absorbance at 356 nm in the presence of a large amount of water (e.g., greater than 0.1 M). In all of the cases, addition of trifluoroacetic acid caused the 356 nm band to shift to 470 nm.

(4) All-*trans*-retinal (**8**, $1.5 \cdot 10^{-5}$ M) was allowed to react in dodecylammonium trifluoroacetate-reversed micelles in cyclohexane ($1 \cdot 10^{-2}$ M) in the absence and in the presence (approximately equimolar amount and excess) of L-lysine. The initial absorption maximum at 372 nm shifted to 356 nm in times depending on the amount of lysine present in the solutions.

Dodecylammonium 3-chloropropionate (6). Dodecylamine (**1**, 1.85 g, 0.01 mol) was stirred (Teflon) n 80% *n*-hexane-benzene (10 ml). A solution of 3-chloropropionic acid (**3**, 1.08 g, 0.01 mol) in benzene (1 ml) was slowly added to amine solution with stirring at ambient temperature. Addition of the acid caused the contents of the flask to become totally transparent. The mixture was stirred for 2 h. Removal of the solvent on a rotavapor yielded a white microcrystalline solid. It was recrystallized from warm (35 °C) *n*-hexane. Colorless crystals (2.80 g) were dried in vacuum for 6 h; m.p. 57–58 °C; IR, ν_{max}, nujol, 2800–2300 (m), 2596, 2681, 2760 (continuous, ν_{as} $\overset{+}{N}H_3$, ν_s $\overset{+}{N}H_3$), 1650 (m), 1580 (m), 1542 (s), 1468 (m), 1452 (m, $\nu_{C\text{-}O}$ antisym. and sym.), 986 (m), 937 (m), 919 (m), 837 (w), 793 (w), 763 (m), 699 (s) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ, 0.87 (3 H, t, J, 7 Hz, CH_3), 1.25 (20 H, s, CH_2), 1.63 (2 H, m, CH_2), 2.58 (2 H, t, J, 6.40 Hz, CH_2COO), 2.81 (2 H, t, J, 7.5 Hz, -CH_2 $\overset{+}{N}H_3$), 3.72 (2H, t, J, 6.40 Hz, Cl-CH_2-).

Reaction of all-trans-retinal (8) in dodecylammonium 3-chloropropionate reversed micelles in cyclohexane. All-*trans*-retinal (**8**, $1.8 \cdot 10^{-5}$ M) was added to a cyclohexane solution of dodecylammonium 3-chloropropionate (**6**, $4 \cdot 10^{-2}$ M). The mixture was briefly sonicated (5 min, 60 W) and kept in the dark at ambient temperature. The initial absorption band at 368 nm shifted to 357 nm during 24 h. Addition of trifluoroacetic acid resulted in the formation of a new red-shifted absorption band at 470 nm.

All-trans-N-retinylidene-n-butylamine (10) in dodecylammonium 3-chloropropionate-cyclohexane reversed micelles. Dodecylammonium 3-chloropropionate solutions ($4 \cdot 10^{-2}$ M) containing 0.00 M, 0.20 M, and 0.40 M water were prepared in cyclohexane. The Schiff base (**10**) was added to these solutions and the mixture was sonicated for 10 min (60 W) in the dark at ambient temperature. Absorption spectra of all of the samples were measured. Protonation of the micellar Schiff base was effected by adding aliquots of 3-chloropropionic (**3**) or trifluoroacetic acid in cyclohexane.

Retinal-lysine Schiff base. L-Lysine (0.035 g) was added to a sodium bis[2-ethylhexyl]sulfosuccinate solution (10 ml, $1 \cdot 10^{-2}$ M in *n*-heptane, 0.08 M H_2O). The suspension was sonicated (90 W, 10 min) at ambient temperature. Undissolved lysine was removed to obtain a clear solution. All-*trans*-retinal was added to this solution and the mixture was sonicated again (90 W, 1 min) at ambient temperature in the dark. The resulting solution was kept at room-temperature in the dark. The Schiff base showed an absorption band at 356 nm. Addition of trifluoroacetic acid caused this band to shift to 430 nm.

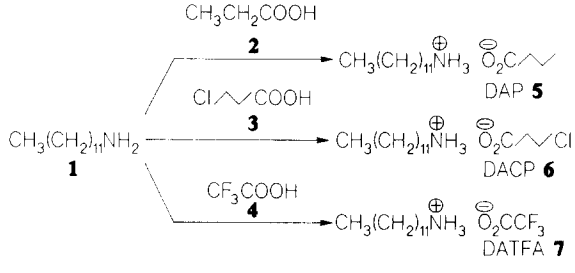
Results and Discussion

Alkylammonium carboxylates

Alkylammonium carboxylates of propionic (**2**, pK_a in water = 4.87), 3-chloropropionic (**3**, pK_a in water = 3.99) and trifluoroacetic (**4**, pK_a in water = −0.26) acids were prepared by allowing the respective acid to react with dodecylamine (**1**) at ambient temperature (Scheme I). The new alkylammonium carboxylates (Scheme I), namely, dodecylammonium-3-chloropropionate (**6**), and dodecylammonium-trifluoroacetate (**7**) were characterized by Infrared and ^{1}H-NMR analyses. The most characteristic features of these salts included continuous Infrared absorption in the 2800–2300 cm^{-1} region due to ν_{as} $\overset{+}{N}H_3$ and ν_s $\overset{+}{N}H_3$, and strong absorptions in the region of 1500–1600 cm^{-1} due to the COO^- group. Dodecylammonium trifluoroacetate showed a very strong Infrared band at 1690 cm^{-1}, characteristic of a trifluoroacetate group. Proton NMR of these compounds indicated characteristic down field (in comparison to -CH_2 CH_2 NH_2) triplets in δ 2.8–2.9 range due to methylene protons adjacent to the $\overset{+}{N}H_3$ group.

Incorporation of all-trans-retinal (8) in alkylammonium carboxylate reversed micelles in cyclohexane

Alkylammonium carboxylates [17] form reversed micelles in non-polar solvents, with their polar groups concentrated in the interior of the aggregate and hydrophobic moieties extended into, and surrounded by, the bulk apolar solvent. Controlled amounts of water can be entrapped in the reversed micelles. Such reversed micellar solutions are homogeneous and optically trans-



Scheme I.

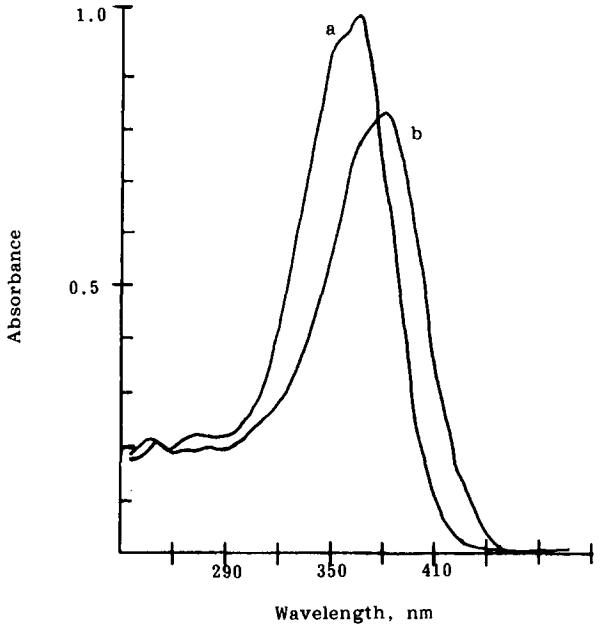


Fig. 2. Absorption spectra showing (a) formation (20 h) of Schiff base of retinal ($2 \cdot 10^{-5}$ M) in reversed micelles of dodecylammonium propionate ($2 \cdot 10^{-4}$ M) in cyclohexane and (b) protonation of the micellar Schiff base by trifluoroacetic acid.

parent. All three dodecylammonium carboxylates gave homogeneous solutions (approx. 10^{-2} M) when dissolved in cyclohexane. Water to the extent of $\omega = 10$ (approx. 0.1 M) could be incorporated into these micelles without affecting the homogeneity of the system.

All-*trans*-retinal (**8**, approx. $2 \cdot 10^{-5}$ M) could be incorporated into approx. 10^{-2} M alkylammonium carboxylate reversed micelles in cyclohexane containing an appropriate amount of water by sonication. It was anticipated that retinal would react with co-solubilized amine (e.g., lysine) in the reversed micelle to give the corresponding Schiff base. However, retinal was found to react preferentially with the dodecylammonium part of the micelle, giving the corresponding Schiff base **9** which showed its absorption band at 357 nm (Figs. 2 and 3).

The Schiff base formed (in situ) was not protonated by the acidic moiety of the micelles (i.e., propionate, 3-chloropropionate or trifluoroacetate anions). Addition of propionic or 3-chloropropionic acid to the corresponding micellar solution did not cause observable protonation of the reversed-micelle-incorporated **9**. Trifluoroacetic acid, however, was able to protonate Schiff base **9** up to 60%, but it destabilized the reversed-micellar system, as indicated by the appearance of turbidity and hydrolysis of the Schiff base to retinal.

When L-lysine was co-solubilized along with retinal in reversed micellar dodecylammonium propionate or dodecylammonium trifluoroacetate in cyclohexane, the initial retinal absorption rapidly shifted from 368 nm to 357 nm, apparently forming a Schiff base which could

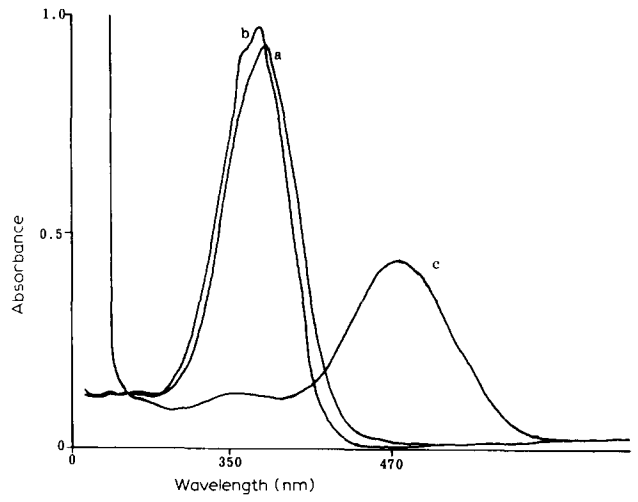


Fig. 3. Absorption spectra of $1.5 \cdot 10^{-5}$ retinal (a), its Schiff base (b) and its protonated Schiff base (c) in reversed micelles of dodecylammonium trifluoroacetic in cyclohexane. Protonation was effected by trifluoroacetic acid.

only be protonated by the addition of trifluoroacetic acid (Fig. 3). The protonated Schiff base showed its absorption band at 470 nm. Schiff-base formation was complete within a few minutes when retinal was solubilized along with lysine, as compared to the several hours required for the comparable reaction to occur in the absence of lysine (Figs. 4–6). Lysine renders the dodecylammonium group to be more nucleophilic and thus aids in Schiff-base formation. The fact that alkylammonium carboxylates react with retinal in the absence of lysine does not rule out the possibility of Schiff-base formation between retinal and lysine in alkylammonium carboxylate reversed micelles. Indeed, formation of both **9** and **11** could be rationalized (Scheme II). Thin-layer chromatographic analysis (re-

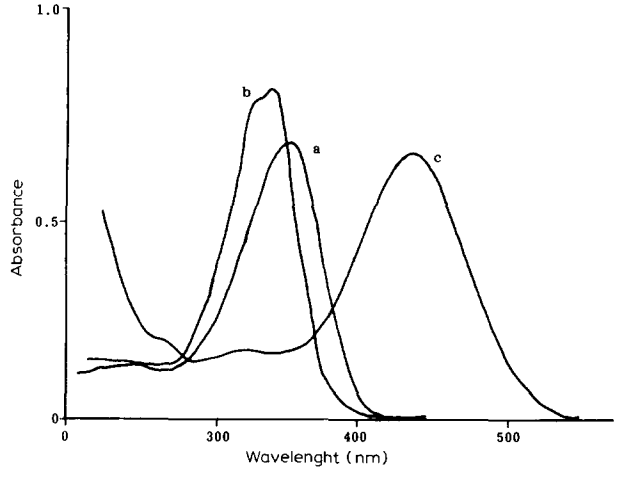


Fig. 4. Absorption spectra of $2.0 \cdot 10^{-5}$ M retinal (a), its Schiff base (b) and its protonated Schiff base (c) in reversed micelles of dodecylammonium propionate ($2 \cdot 10^{-4}$ M) containing lysine in cyclohexane. Protonation was effected by trifluoroacetic acid.

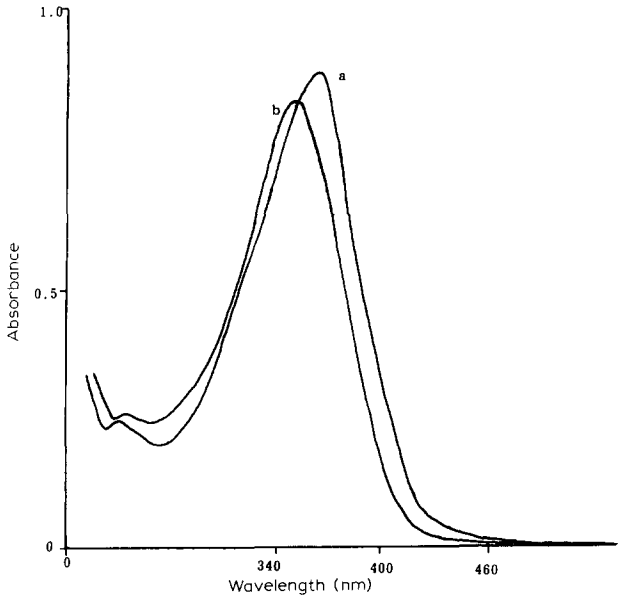


Fig. 5. Absorption spectra of $2.0 \cdot 10^{-5}$ retinal in $1 \cdot 10^{-2}$ M AOT in *n*-heptane (a) and its Schiff base with $2 \cdot 10^{-5}$ M lysine (b).

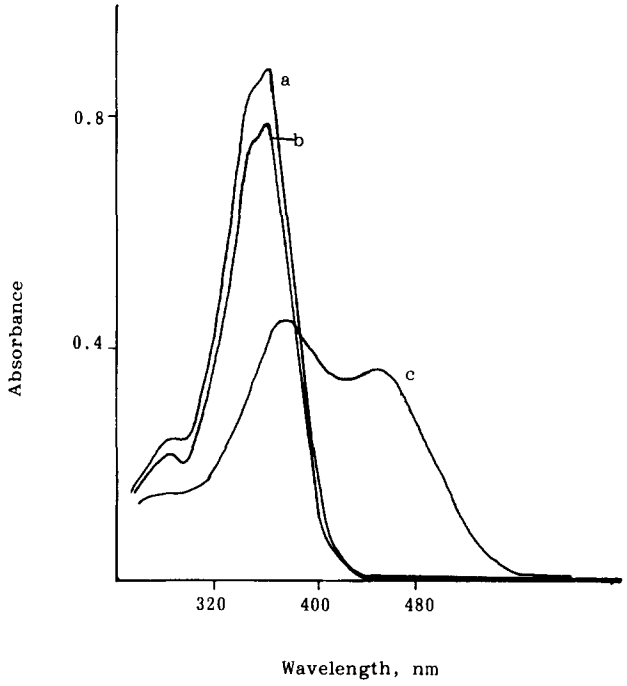
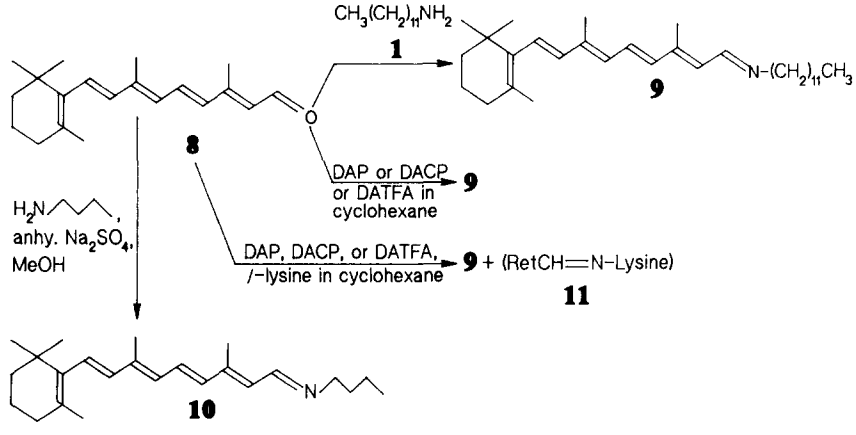


Fig. 6. Absorption spectra of (a) all-*trans*-*N*-retinylidene-*n*-butylamine ($1.7 \cdot 10^{-5}$ M) solubilized in dodecylamine-3-chloropropionate ($4 \cdot 10^{-2}$ M) in cyclohexane containing 1·0.2 M water; (b) spectrum after addition of 3-chloropropionic acid ($1.7 \cdot 10^{-3}$ M); and (c) spectra after addition of trifluoroacetic acid ($1.7 \cdot 10^{-5}$ M).

versed phase, silanized silica, methanol) of the mixture showed the presence of Schiff base **9** in the micellar mixture as major compound. The presence of water molecules in excess of $\omega = 6$ in reversed micelles delayed the formation of the Schiff base.

An authentic sample of Schiff base **9** was prepared by reacting retinal (**8**) with dodecylamine (**1**) in dry methanol. A cyclohexane solution of the corresponding Schiff base **9** showed an absorption band at 356 nm which underwent red shift to 470 nm after the addition of trifluoroacetic acid. The Schiff base formed between retinal (**1**) and L-lysine in a sodium bis[2-ethylhexyl]sulfosuccinate-heptane micelle had an absorption band with a maximum at 356 nm which shifted to 430 nm upon protonation by trifluoroacetic acid (Fig. 5).

Schiff-base protonation by 3-chloropropionic acid was studied in detail. No internal protonation was observed in dodecylammonium 3-chloropropionate reversed micelles, even when acid was present in over 2000-fold excess of the Schiff base (Fig. 6). Similar observations were made in dodecylammonium 3-chloropropionate reversed micelles in cyclohexane which contained 0.20 to 0.40 M water. However, addition of strong trifluoroacetic acid to reversed micellar solutions of **9** caused protonation as well as hydrolysis to retinal (Fig. 6c). There was never complete protonation of the Schiff base by equimolar trifluoroacetic acid added to the reversed micelles. A maximum of 60% protonation was observed in dodecylammonium 3-chloropropionate reversed micelles in the presence of 0.20 M water when



Scheme II.

trifluoroacetic acid was in 1000-fold excess over the Schiff base. Further addition of trifluoroacetic acid caused the micellar solution to become turbid.

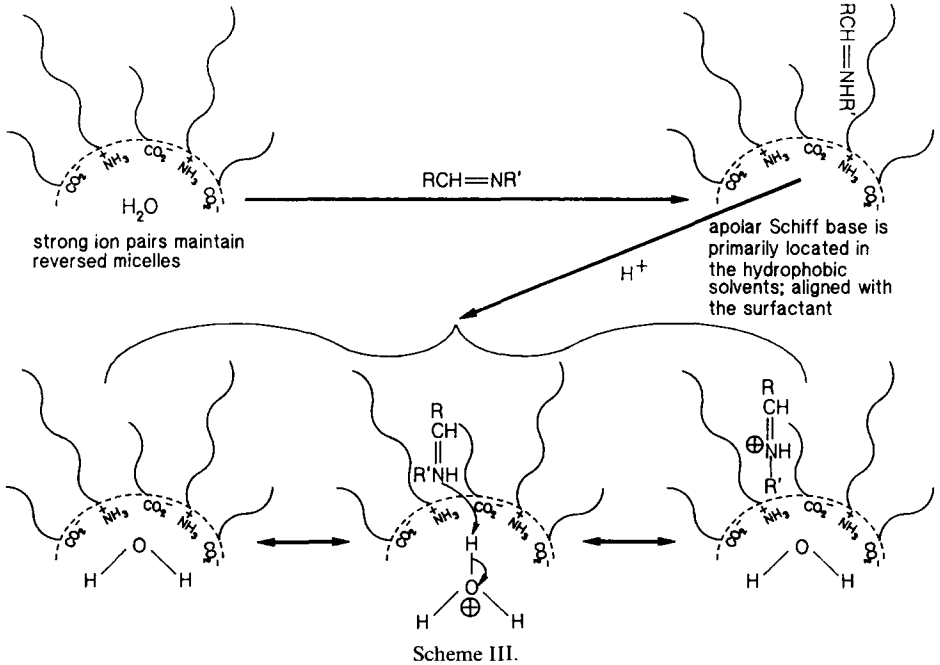
Dodecylammonium 3-chloropropionate reversed micelles in cyclohexane remained stable when 10% neutralized by 10 M hydrochloric acid. No protonation was observed, however, even in this solution. Addition of more acid caused turbidity and eventual precipitation. It is known [19] that alkylammonium carboxylates an be titrated by the addition of external acid.

Schiff base **10** could be incorporated into dodecylammonium 3-chloropropionate reversed micelles in cyclohexane which contained water up to $\omega = 10$. It could also be micellized in 10% neutralized dodecylammonium 3-chloropropionate in cyclohexane ($\omega = 5$ and $\omega = 10$). However, there was no observable protonation of **10**. Instead, some hydrolysis was noted.

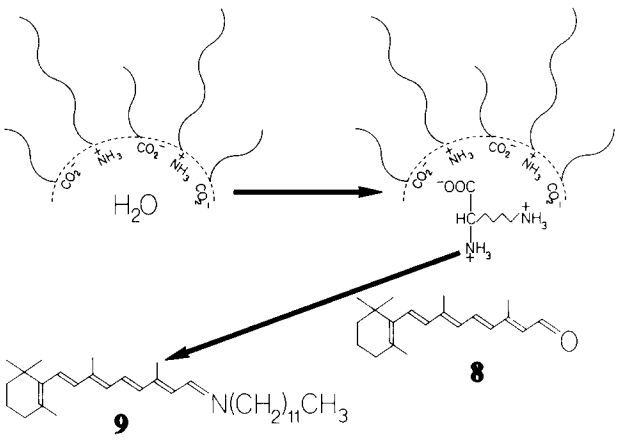
Stability of retinylidene Schiff bases in alkylammonium carboxylate reversed micelles in cyclohexane and their resistance to protonation, the most significant findings of the present work, are entirely in accord with the known structure of these systems [17]. Strong dipole-dipole attractions between the alkylammonium cation and the carboxylate anion provides the primary driving force for micelle formation in apolar solvents. Hydrated surfactant headgroups surround a number of water molecules whose properties are distinctly different from bulk water. Importantly, reversed micelles are dynamic structures, they aggregate and disaggregate on the 10^{-3} to 10^{-5} s time scale. Also, there is a rapid exchange between the water pools and their contents. Being apolar, the retinylidene Schiff base is predominantly located in the organic solvent where hydrophobic attractions are likely to align it along the hydrocarbon chain of the surfactant.

Addition of an acid to the Schiff-base-containing reversed micelle has a number of consequences (Scheme III). The C = N group of the Schiff base, the carboxylate ion of the surfactant and the solubilized water all compete for the proton. The extent of protonation of these species depends on the strength of the acid introduced and on the basicity of the different acceptors. Protonation equilibria of the Schiff base, the alkylammonium carboxylate surfactant and water are profoundly influenced, of course, by the unique microenvironment provided by reversed micelles. Specifically, alterations of the type and concentration of the surfactant, of the nature of the bulk organic solvent, and of the ratio of solubilized water to surfactant (i.e., the co-value) are expected to shift the various protonation equilibria. The condition for partial Schiff-base protonation became favorable only in the presence of excess trifluoroacetic acid. The protonated Schiff base (RCH $= N^+HR$) is drawn toward the polar cavity of the reversed micelle. In excess, the strong acid (CF_3COOH) and/or the protonated Schiff base interfere with the alkylammonium carboxylate ion pairs and, hence, they destabilize the reversed micelle. Destabilized micelles cannot protect the protonated (and the non-protonated) Schiff base. This is then manifested in hydrolysis to retinal.

Being ionic, lysine is primarily located in the polar core of reversed-micellar alkylammonium carboxylate (Scheme IV). It undergoes preferential proton donation to the carboxylate ion of the surfactant in preference to reacting with the organic solvent solubilized Schiff base.



Scheme III.



Scheme IV.

Concomitant with carboxylate group protonation, the alkylammonium group becomes an alkylamine, which, in turn, is a stronger nucleophile and, hence, it is more prone to react with retinal to give the Schiff base. Thus, the faster formation of the dodecylamine Schiff base is not unexpected.

Evidence has been presented in this work for the importance of ion pairs in influencing Schiff-base stability and protonation equilibria. The retinylidene Schiff base is protected from protonation by strongly held surfactant ion pairs. Separation of these ion pairs is a requirement of proton transfer. Such a separation may be mediated by polar groups (e.g., water) in rhodopsins. The ion pairs may be visualized as proton channels or gates whose functioning may well be controlled by protein conformational changes (Fig. 7). It may be noted that site-specific mutagenesis experiments [20,21] have shown the presence of carboxylate moieties near the active site of bacteriorhodopsin. Also, non-invasive biophysical experiments [22] have revealed the significance of ion pairs at the active site of bacteriorhodopsin.

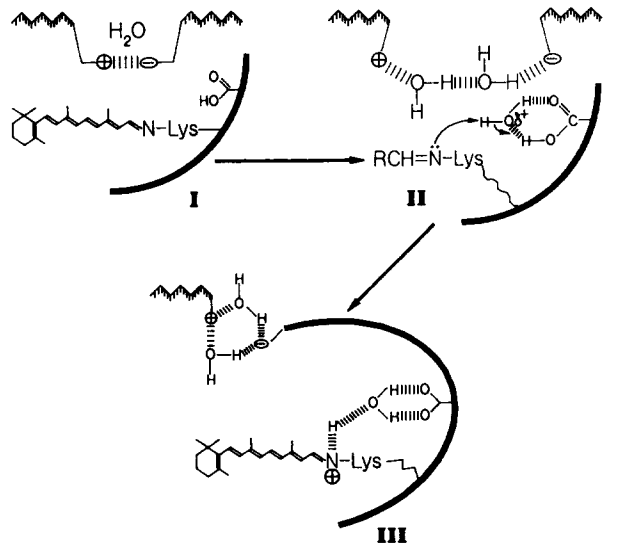


Fig. 7. Schematic representation of a plausible role of ion-pairs near the chromophore of rhodopsin.

Acknowledgment

Support of this work by the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

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