Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 1. Radical Anions[1]

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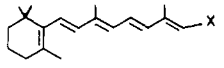
Abstract: The spectra and kinetics of formation and decay of radical anions of a number of retinyl polyenes have been studied in methanol and 2-propanol at room temperature, using pulse radiolysis and kinetic spectrophotometry. The bimolecular rate constants for the attachment of solvated electrons, e^-_{MeOH}, to the retinyl polyenes are in the diffusion-controlled limit (8.6×10^9–1.6×10^{10} M^{-1} s^{-1}). The radical anions of retinol and retinol acetate have their spectral maxima at 370–390 nm, and undergo decay very slowly with second-order kinetics. On the other hand, the radical anions of retinal, retinal *n*-butylamine Schiff base, and retinoic acid/ester have spectral maxima at 430–510 nm, and decay by first-order kinetics in methanol with rate constants in the range 1×10^4–1×10^6 s^{-1}. The decay rates of radical anions of retinal and retinoic acid/ester become considerably longer on going from methanol to less acidic alcohol, 2-propanol, suggesting that protonation by solvent is the major mode of their decay in protic media. In the case of retinal Schiff base, an additional slow process with bimolecular rate constant 9.0×10^7 M^{-1} s^{-1} in methanol is observed for the formation of radical anion and is ascribed to the electron-transfer reaction from the methanol radical, $\cdot CH_2OH$.

Introduction

Retinyl polyenes participate in a variety of biological processes related with vitamin A, retinochromes, visual pigments, photosynthetic pigments, and anticancer agents. Because of their roles as chromophores in visual excitation (e.g., in rhodopsin) and light transduction (e.g., in bacteriorhodopsin), the excited-state properties of retinyl polyenes have been studied[2] quite extensively. From time to time there have been suggestions that charge-transfer interaction[3] between the retinyl moiety and suitable donor groups such as sulfhydryl and indole in the protein (opsin) might be operative in the early stages of vision. Although the involvement of charge transfer in the early steps of visual excitation is yet to be substantiated by definitive evidence, the electron donor–acceptor behaviors of retinyl polyenes and their excited states, and the properties of their radical ions, are of current interest.

Another reason why we have been interested in the charge-transfer properties of retinyl polyenes is that systems[4] with extended π electrons may mediate the transport of charge across a biological membrane either through electron tunnelling or by a "give-and-take" mechanism where an electron (or hole) is accepted on one side and donated on the other. Carotenoids,[5] including retinal[5d,e] and retinoic acid,[5e] have been the subjects of study for such roles in interfacial processes. Although the π-electron system in retinyl polyenes is only half as extended as in carotenes, the fact that some of them can form aggregates (dimers)[6] under certain conditions of solvent and temperature makes them potentially capable of carrying charge and excitation energy along biological membranes through exciton interaction.



X = CHO , 1
= CH_2OH, 2
= CH_2OOCCH_3 , 3
= COOH , 4
= $COOCH_3$, 5
= $CH{=}NCH_2CH_2CH_2CH_3$,6

Figure 1. Structures of retinyl polyenes under study: (1) retinal, (2) retinol, (3) retinol acetate, (4) retinoic acid, (5) methyl retinoate, (6) *n*-butylamine Schiff base of retinal.

There have been only a few studies concerning the generation of ions and radicals from retinyl polyenes. Grady and Borg[3a] have irradiated retinal and retinol at low temperatures and observed ESR signals believed to originate from radical anions formed as a result of charge-transfer phenomena. Rosenfeld et al.[7] observed that the laser flash photolysis of retinol, retinol acetate, and retinylamine in polar solvents at room temperature leads to ionization in appreciable yields. Lang et al.[3c] have reported on the ESR spectra of radical anions of retinal and retinal Schiff base generated electrochemically in tetrahydrofuran at low temperature. More recently, pulse radiolytic studies concerning the radical ions of all-trans retinal,[8] its homologues,[8] and various related carotenoids[9] have appeared in the literature.

We have carried out a detailed pulse radiolysis study of a number of retinyl polyenes ending with various functional groups. These include the all-trans isomers of retinal, retinol, retinol

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(2) For reviews, see: Kliger, D. S. *Int. J. Quantum Chem.* **1979**, *26*, 809-817, Becker, R. S.; Hug, G.; Das, P. K.; Schaffer, A. M.; Takemura, T.; Yamamoto, N.; Waddell, W. *J. Phys. Chem.* **1975**, *80*, 2265-2273, Land, E. J. *Photochem. Photobiol.* **1975**, *22*, 286-288, Honig, B.; Ebrey, T. G. *Annu. Rev. Biophys. Bioeng.* **1974**, *3*, 151-177.

(3) (a) Grady, F.; Borg, D. *Biochemistry* **1968**, *7*, 675-682, (b) Bensasson, R. E.; Land, E. J.; Truscott, T. G. *Nature* (*London*) **1975**, *258*, 768-770, Bensasson, R. E.; Land, E. J.; Truscott, T. G. *Photochem. Photobiol.* **1977**, *26*, 601-605, (c) Lang, C. M.; Harbour, J.; Guzzo, A. V. *J. Phys. Chem.* **1971**, *75*, 2861-2866, (d) Shirane, K. *Nature* (*London*) **1975**, *254*, 722-723.

(4) (a) Kuhn, H. *Chem. Phys. Lipids* **1972**, *8*, 401-404, (b) Jahn, T. L. *J. Theoret. Biol.* **1962**, *2*, 129-138, (c) Platt, J. R. *Science* **1959**, *129*, 372-374, (d) Tien, H. T. *Photochem. Photobiol.* **1972**, *16*, 271-290, *Ibid.* **1976**, *24*, 97-116.

(5) (a) Berns, D. S. *Photochem. Photobiol.* **1976**, *24*, 117-139, (b) Almgren, M.; Thomas, J. K. *Ibid.* **1980**, *31*, 329-335, (c) Hong, F. T. *Ibid.* **1976**, *24*, 155-189, and references therein, (d) Kobamoto N.; Tien, H. T. *Biochem. Biophys. Acta* **1971**, *241*, 129-146; **1972**, *266*, 56-66, (e) Schadt, M. *Ibid.* **1973**, *323*, 351-366.

(6) (a) Takemura, T.; Hug, G.; Das, P. K.; Becker, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 2631-2634, (b) Chihara, K.; Takemura, T.; Yamaoka, T., Yamamoto, N.; Schaffer, A. M.; Becker, R. S. *Photochem. Photobiol.* **1979**, *29*, 1001-1008, (c) Takemura, T.; Chihara, K.; Becker, R. S.; Das, P. K.; Hug, G. L. *J. Am. Chem. Soc.* **1980**, *102*, 2604-2609, (d) Moore, T. A.; Song, P. S. *Nature* (*New Biol.*) **1973**, *243*, 30-32, (e) Chihara, K.; Waddell, W. H. *J. Am. Chem. Soc.* **1980**, *102*, 2963-2968.

(7) (a) Rosenfeld, T.; Alchalel, A.; Ottolenghi, M. *Chem. Phys. Lett.* **1973**, *20*, 291-297, (b) Rosenfeld, T.; Alchalel, A.; Ottolenghi, M. In "Excited States of Biological Molecules"; Birks, J. B., Ed.; Wiley: London, 1976; pp 540-554.

(8) Land, E. J.; Lafferty, J.; Sinclair, R. S.; Truscott, T. G. *J. Chem. Soc., Faraday Trans. 1*, **1978**, *74*, 538-545.

(9) (a) Dawe, E. A.; Land, E. J. *J. Chem. Soc. Faraday Trans. 1*, **1975**, *71*, 2162-2169, (b) Lafferty, J.; Roach, A. C.; Sinclair, R. S.; Truscott, T. G.; Land, E. J. *Ibid.* **1977**, *73*, 416-429.

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acetate, retinoic acid and its methyl ester, and *n*-butylamine Schiff base derivative of retinal. The structure of these compounds are shown in Figure 1. In the present paper, we report the results of our study in methanol and 2-propanol where the transient phenomena are concerned primarily with the formation and decay of radical anions.

Experimental Section

all-trans-Retinal, retinol, retinol acetate, and retinoic acid were purchased from Sigma and stored at 0 °C. Of these, retinal was recrystallized from *n*-hexane; the other compounds were used from freshly opened vials. The presence of cis isomers as impurities in these samples was estimated at less than 5% by thin layer chromatography and from absorption spectral intensity in the regions of cis peaks. Methyl retinoate was prepared by refluxing a mixture of retinoic acid and methyl iodide (in the molar ratio 1:2) over anhydrous potassium carbonate in methyl ethyl ketone. The crude product was chromatographed on silica gel using petroleum ether + ether (3%) as eluent and then crystallized from *n*-hexane. *all-trans*-Retinal Schiff base was made by mixing retinal with an excess of freshly distilled *n*-butylamine in dry methanol and storing the solution over molecular sieve (3A) at 0 °C under an atmosphere of argon for 12 h. The Schiff base was recovered by removing the solvent and excess *n*-butylamine, first by blowing nitrogen over the solution and then by applying high vacuum (10^{-3} torr).

Methanol (Aldrich, Spectrophotometric, Gold Label) and 2-propanol (Baker Analyzed) were used without further purification. Unless otherwise mentioned, the solutions were deaerated before pulse radiolysis by bubbling nitrogen for at least half an hour. The deaeration was continued in the reservior from which the solution was allowed to flow slowly and continuously through the pulse radiolysis cell. All solutions containing retinyl polyenes were protected from room light during experiments.

A description of the computer-controlled spectrophotometric pulse radiolysis set up is available elsewhere.[10] The irradiation was carried out with 5-ns electron pulses from the Notre Dame 7-MeV ARCO LP-7 linear accelerator using dose rates of ~2 × 10^{16} eV/g per pulse. Suitable Corning filters were used in the path of analyzing light to minimize its absorption by the samples during pulse radiolysis experiments.

Results

Most of the experiments described here were performed in nitrogen-saturated methanol and 2-propanol at room temperature. In these protic, polar solvents, the radical anions are formed[11] through the attachment of the solvated electron e^-_{sol} to the solute molecule. The alcohol positive ions disappear fast producing ketyl and alkoxy radicals[12] according to eq 1 and 2.

$$RR'CHOH^+ + RR'CHOH \rightarrow RR'COH + RR'CHOH_2^+ \quad (1)$$

$$\rightarrow RR'CHO\cdot + RR'CHOH_2^+ \quad (2)$$

The ketyl radicals, e.g., $\cdot CH_2OH$ in methanol, can also reduce a solute forming the radical anion. However, the electron-transfer reactions from these species are comparatively slow, and except in the case of retinal Schiff base (see later) these were not generally important. The alkoxy radicals, on the other hand, can take part in addition and hydrogen abstraction reactions with the polyenes under study. We, however, did not observe any significant contribution to transient formation from processes parallel to the radical anion buildup in the time scale commensurate with the lifetimes of alkoxy radicals.[13]

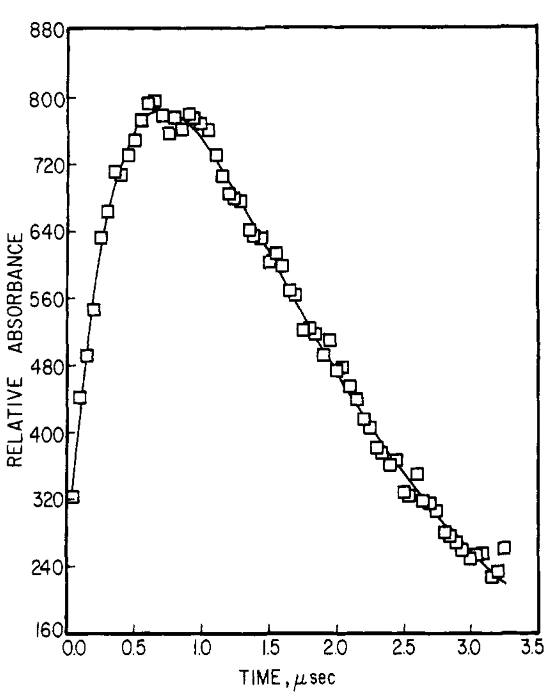

Figure 2. The formation and decay profile for radical anion of methyl retinoate (9.0 × 10^{-5} M in methanol) monitored at 485 nm. The solid line is the least-squares best fit based on two consecutive first-order processes.

The kinetics of the formation of radical anions was studied by following either the formation of the anions in the spectral region 350–550 nm or the decay of the solvated electron in the spectral region 600–700 nm. The decay rate of the solvated electron as a function of the concentration of the substrates was practically the same as that of the formation of the transients, and this was the most important criterion[14] we have used for the assignment of the transients as radical anions formed through electron attachment. In the cases where the radical anions were relatively long-lived, e.g., retinol, retinol acetate, and retinal Schiff base, the formation profile reached a clearly defined plateau and the experimental trace was fitted into a simple integrated growth equation: $A_t = A_\infty(1 - e^{-k_{expt}t})$. However, in the cases of retinal and retinoic acid/ester in methanol, the decay of the radical anions was relatively fast. In these cases, a least-squares best fit to the experimental trace based on consecutive reaction kinetics was obtained and the formation and decay rate constants were extracted from it. A typical example of such a fit is shown in Figure 2.

The rate constant, k_e, for electron attachment was obtained from linear plots based on the equation $k_{expt} = k_0 + k_e[R]$, where k_{expt} is the formation rate constant for polyene anion or the decay rate constant for solvated electron at substrate concentration [R] and k_0 is the decay constant for solvated electron in the absence of a reactant. At least five solutions of different polyene concentrations in the range 1 × 10^{-5}–3 × 10^{-4} M were used for each measurement. The average value for k_0, as obtained from the intercepts of the plots for electron-attachment reactions in methanol, is 0.8 ± 0.2 μs^{-1}. This value is slightly higher than that reported by other workers[11] for the decay of solvated electron in methanol (~0.67 μs^{-1}). The discrepancy is tolerable in view of

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(10) Patterson, L. K.; Lilie, J. *Int. J. Radiat. Phys. Chem.* **1974**, *6*, 129–141, Schuler, R. H.; Buzzard, G. K. *Ibid.* **1976**, *8*, 563–574.

(11) (a) Taub, I. A.; Harter, D. A.; Sauer, Jr., M. C.; Dorfman, L. M. *J. Chem. Phys.* **1964**, *41*, 979–985, (b) Arai, S.; Dorfman, L. M. *Ibid.*, **1964**, *41*, 2190–2194, (c) Dorfman, L. M. *Acc. Chem. Res.* **1970**, *3*, 224–230.

(12) Baxendale, J. H.; Mellows, F. W. *J. Am. Chem. Soc.* **1961**, *83*, 4720–4726, (b) Ellison, D. H.; Salmon, G. A.; Wilkinson, F. *Proc. R. Soc. London, Ser. A* **1972**, *328*, 23–26, (c) Sargent, F. P.; Gardy, E. M. *Can J. Chem.* **1974**, *52*, 3645–3650, (d) Sargent, F. P. *J. Phys. Chem.* **1977**, *81*, 89–90.

(13) Methoxy radical,[12b] for example, has a lifetime of 150 ns in pure methanol. In the concentration range, 1 × 10^{-5}–3 × 10^{-4} M, used for [R], the rate constants for reactions of $CH_3O\cdot$ with retinyl polyenes would have to be >1 × 10^9 M^{-1} s^{-1} for a favorable competition against its decay. Our inability to detect processes parallel to the formation of radical anions strongly suggest that the rates of reactions of $CH_3O\cdot$ with retinyl polyenes are too slow and/or the products of the reactions do not absorb appreciably in the region where the radical anions absorb.

(14) It is important to note that, for each of the polyenes under examination, we have carried out pulse radiolysis experiments in air- and N_2O-saturated methanol solutions. Except for retinal Schiff base (see text), no significant transient phenomena were observed in N_2O-saturated solutions. The yields and lifetimes of the transients assigned as radical anions were found to be very sensitive to the presence of O_2.

Table I. Spectral and Kinetic Data of Radical Anions of Retinyl Polyenes in Methanol at 22 °C

polyene	λ_{max}, nm[a]	$G \cdot \epsilon_{max}$, M^{-1} $cm^{-1} \times 10^{-3}$ [b]	rate constant for formation, M^{-1} s^{-1} [c]	decay rate constant,[c] s^{-1}
retinal	445 (460)	46	1.4×10^{10}	7.0×10^5 (8.1×10^3)
retinol	370 (370)	54	8.8×10^9	3.4[d]
retinol acetate	390 (390)	83	8.6×10^9	0.20[d]
retinoic acid	480 (505)	58	1.1×10^{10}	4.4×10^5 (2.7×10^4)
methyl retinoate	480 (510)	70	1.2×10^{10}	5.4×10^5 (1.5×10^4)
retinyl Schiff base	430 (435)	96	1.6×10^{10}	1.4×10^4

[a] ±5 nm; absorption maxima in 2-propanol are given in the parentheses. [b] Estimated error, ±30%; G is the yield of e^-_{sol}, per 100 eV, in methanol. [c] Estimated error, ±10%; decay rate constants given in the parentheses correspond to 2-propanol (for retinoic acid) and 2-propanol + 0.01 M triethylamine (for retinal and methyl retinoate). [d] Value of $2k\, c_0/\epsilon_{max}$ in M cm s^{-1}, where k is second-order rate constant, c_0, end-of-pulse concentration of transient, and ϵ_{max}, extinction coefficient at spectral maximum. The polyene concentrations used are 2.0×10^{-4} and 1.8×10^{-4} M for retinol and retinol acetate, respectively.

the differences in the procedures for purifying solvents and degassing solutions.

The extinction coefficient data in methanol were obtained by comparing the absorbance due to radical anion with that due to $(SCN)_2^{-}\cdot$ in aqueous solution. To obtain the absorbance corresponding to complete reaction with e^-_{MeOH}, the observed absorbance at the maximum of the composite profile for formation and decay was divided by a factor f, where f is related with the expression[15] for the maximum concentration of the intermediate (radical anion).

The kinetic and spectral data for the various polyene radical anions are given in Table I.

(a) Retinal. Our results concerning the pulse radiolysis of retinal in N_2-saturated methanol were similar to those reported by Land et al.[8] Also, in sufficiently dilute solution (~10^{-5} M) we observed, as Land et al. did, that a transient species with spectral maximum at 385 nm is formed at the same rate as that of the decay of the radical anion. The species at 385 nm has been assigned[8] as the protonated form of the radical anion. In order to throw some light on this aspect, we have carried out pulse radiolysis experiments in 2-propanol. In this solvent, the spectral maximum of retinal anion is red-shifted by 15 nm, and its decay occurs on a time scale ~100 times longer than that in methanol. The absorption spectrum and a decay profile in 2-propanol are shown in Figure 3. While the decay of retinal anion in methanol is strictly of first order, that in 2-propanol fits into neither clean first-order nor clean second-order kinetics. These results suggest that the radical anion decays in methanol predominantly through protonation with solvent (eq 3). Reaction 4, i.e., protonation with solvated proton,

$$R^{-}\cdot + ROH \rightarrow \dot{R}H + RO^{-} \qquad (3)$$

$$R^{-}\cdot + ROH_2^{+} \rightarrow \dot{R}H + ROH \qquad (4)$$

generated pulse radiolytically in micromolar concentrations by reactions 1 and 2, appears to be unimportant in methanol. This is supported by the lack of a second-order component in the decay process in methanol, and explained by the fact that even with large rate constants,[11] 2–5 × 10^{10} M^{-1} s^{-1}, for such reactions with solvated protons, one would expect them to occur on a time scale much longer than that actually observed for retinal anion decay in methanol. In 2-propanol where the anion has a much longer lifetime, both processes 3 and 4 appear to be involved in the decay (as shown by mixed kinetics). This is confirmed by the fact that in the presence of ~0.01 M triethylamine in 2-propanol, the decay takes place predominantly with first-order kinetics, with rate constant 8.1×10^3 s^{-1}.

(b) Retinol and Retinol Acetate. Retinol and retinol acetate on pulse radiolysis in methanol produce transient species with spectral maxima at 370–390 nm. The absorption spectra, shown in Figure 4, are quite sharp in comparison to the spectra of the other retinyl polyene anions. The spectra remain practically unchanged on going from methanol to 2-propanol. The fact that the spectrum of radical anion of retinol differs from that of retinol acetate in relative intensities at 370 and 390 nm (Figure 4) is taken to mean that the observed transient phenomena are probably not due to a polyene radical or ion that could be formed through the loss of OR^- or $\cdot OR$ (R = H, CH_3CO) from radical anions.

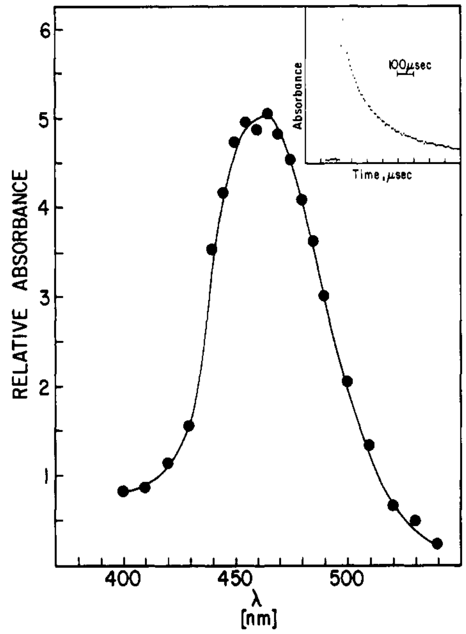

Figure 3. Absorption spectra of radical anion of radical (1.4×10^{-4} M) in 2-propanol, recorded at 2.6 μs following the pulse; inset: experimental trace showing the decay of retinal anion in 2-propanol.

The decay of the radical anions of retinol and retinol acetate follows second-order kinetics and occurs in the millisecond time range. It appears that the protonation by solvent is relatively slow in these cases and the predominant mode of decay is protonation or ion-pair formation with ROH_2^+. Another possible mode of second-order decay is dimerization, eq 5. Our attempt to observe

$$R^{-}\cdot + R^{-}\cdot \rightarrow R\text{–}R^{2-} \qquad (5)$$

the dianion, $R\text{–}R^{2-}$, in the spectral region 350–750 nm, however, proved to be unrewarding.

(c) Retinoic Acid/Ester. The radical anions of both retinoic acid and its methyl ester have spectra maxima at 480 nm in methanol (Figure 5) and at 505–510 nm in 2-propanol. The spectra are relatively broad and the decay of the anions in methanol occurs at rates slower by a factor of ~2 than the rate of decay of retinal anion. Again, the decay lifetimes become significantly longer on going from methanol to 2-propanol (Table I). Addition of sodium hydroxide to the solution of retinoic acid in methanol does not make any significant difference in the spectrum or decay kinetics.

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(15) In our notations, the complete expression for f is as follows:

$$f = \frac{k_e[R]}{k_0 + k_e[R] - k_d}[\exp(-k_d t_{max}) - \exp\{-(k_0 + k_e[R])t_{max}\}]$$

where $t_{max} = (k_0 + k_e[R] - k_d)^{-1}[\ln (k_0 + k_e[R]) - \ln k_d]$.

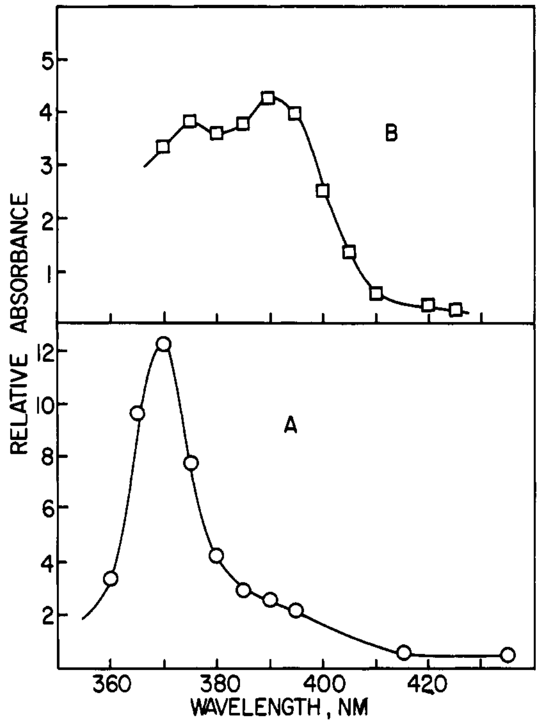


Figure 4. Absorption spectra of radical anion of (A) retinol (2.0×10^{-4} M in methanol) observed at 3 μs after the pulse and (B) retinol acetate (1.8×10^{-4} M in methanol) observed at 0.2 μs after the pulse.

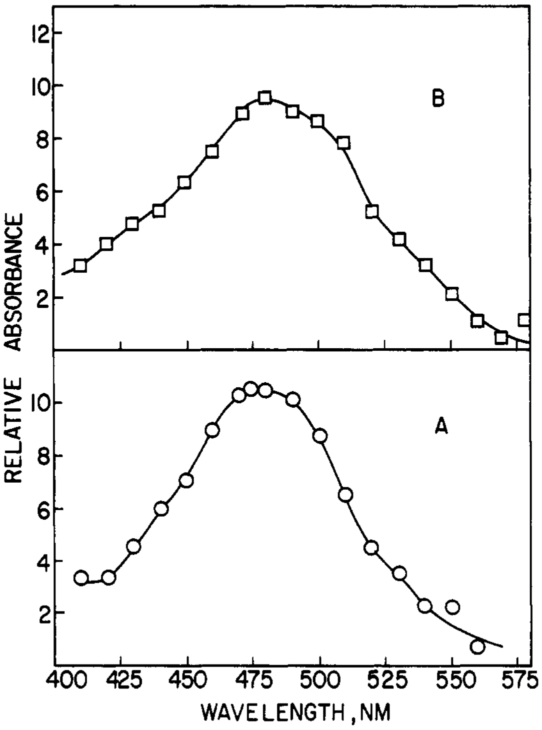


Figure 5. Absorption spectra of radical anion of (A) methyl retinoate (1.3×10^{-4} M in methanol) observed at 1.3 μs after the electron pulse and (B) retinoic acid (2.0×10^{-4} M in methanol) observed at 0.4 μs after the electron pulse.

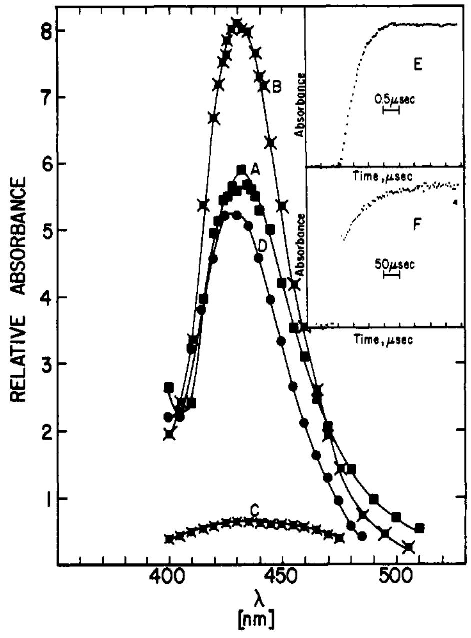


Figure 6. Absorption spectra of transient(s) observed in 2.9×10^{-4} M solution of retinal Schiff base in N_2-saturated methanol at (A) 2.0 μs and (B) 350 μs after the pulse, and in N_2O-saturated methanol at (C) 1.5 μs and (D) 350 μs after the pulse. Insets: experimental traces showing the formation of radical anion in N_2-saturated methanol on (E) short- and (F) long-time scale.

(d) Retinal Schiff Base. The formation kinetics of the anion of retinal Schiff base provides an interesting situation. In addition to the fast process which becomes complete within a few microseconds, a slow belated process is observed. The spectral maximum of the fast-formed transient is found to be the same as that of the transient that is slowly formed, i.e., at 430 nm (Figure 6). In a solution of 5×10^{-5} M retinal Schiff base in methanol, the contribution from the transient that is slowly formed is about 25% of the total optical density observed at the spectral maximum. Similar behavior is also observed in 2-propanol where the spectral maximum of anion is slightly red-shifted (λ_{max} 435 nm). The kinetics of formation of the slowly formed transient is found to be linearly dependent on the substrate concentration and gives a rate constant of 9.0×10^7 M^{-1} s^{-1} for its formation in methanol. Based on the kinetics of formation and spectral position, we have assigned the slowly formed transient also as the radical anion of retinyl Schiff base formed through reaction with ketyl radicals, e.g., $\cdot CH_2OH$ in methanol (reaction 6).

$$R + \cdot CH_2OH + CH_3OH \rightarrow R^{-}\cdot + CH_2O + CH_3OH_2^+ \quad (6)$$

Experiments in N_2O-saturated methanol confirm this assignment. N_2O scavenges solvated electrons forming $\cdot CH_2OH$ (in methanol) through reactions 7 and 8. The pulse radiolysis of

$$N_2O + e^-_{sol} \rightarrow N_2 + O^- \quad (7)$$

$$O^- + CH_3OH \rightarrow OH^- + \cdot CH_2OH \quad (8)$$

retinal Schiff base in N_2O-saturated methanol shows that the relative amount of the transient formed by the slow process is significantly increased at the expense of the fast process (both relative to N_2-saturated methanol). The spectral maxima (Figure 6) remain practically unchanged, although the spectrum (Figure 6C) corresponding to the fast process in N_2O-saturated methanol is broader, and has a relatively high intensity in the long-wavelength region (440–480 nm). As a matter of fact, in N_2O-saturated methanol one would expect very little formation of retinal Schiff base anion by e^-_{MeOH} attachment because the scavenging process represented by reactions 7 and 8 is very fast ($t_{1/2} \sim 1$ ns). On the contrary, the observed rate for the fast component in N_2O-saturated methanol containing 8.1×10^{-5} M retinal Schiff base is 2.2×10^6 s^{-1}. We attribute the fast process in N_2O-saturated methanol primarily to the protonation of the Schiff base (ground state) by solvated protons produced pulse radiolytically, i.e., by reactions 1 and 2 (λ_{max} 445 nm for retinal Schiff base protonated with trifluoroacetic acid in methanol). This conclusion is supported by the fact that the fast component vanishes completely in the presence of triethylamine (~0.01 M) in N_2O-saturated methanol, but remains practically unchanged in yields and kinetics on adding triethylamine in N_2-saturated methanol.

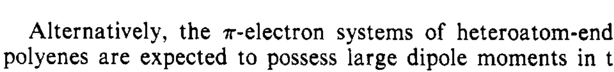
Processes that can be best explained in terms of protonation of retinal Schiff base (ground state), leading to spectral absorption at 440–460 nm, are also observed in air- and oxygen-saturated methanol solutions (but not when triethylamine is present in excess).

Discussion

The present work reports for the first time on the kinetics of formation and decay of radical anions of various retinyl polyenes. In addition, spectral data are presented for the first time regarding the radical anions of retinol, retinoic acid, and their esters. These data are important for understanding the role, if any, of electron transfer interactions involving these polyenes in various biological processes.

It has been shown in recent studies[8,9b] that the wavelength maxima of radical ions of polyenes are red-shifted in a linear manner with increase in the number of conjugated double bonds. This has been supported[9b] by the results of semiempirical calculations based on Hückel and PPP methods. From the spectral data[8] on radical anions of homologues of retinal in methanol, the spectral maximum of radical anion of retinol with chain length shorter than that of retinal by one double bond is expected to be located at ~390 nm in neutral methanol. The observed maxima for radical anions of retinol (λ_{max} 370 nm) and retinol acetate (λ_{max} 390 nm) are in agreement with this expectation. It is noted that for polyenes of identical chain length there is a nonnegligible dependence of the radical-anion spectral maxima on the nature of the functional group present in the polyene. Thus, the radical anions of retinoic acid/ester have spectral maximum at longer wavelengths than that of retinal. The ground-state spectral maxima of these polyenes are, however, in opposite order.[6a,e] Also, it is interesting to note that the absorption maxima of anions of retinal, retinoic acid/ester, and retinal Schiff base move to lower energy on going from methanol to the less polar alcohol, 2-propanol. This trend is supported by the fact that the spectral maximum of retinal anion in hexane is 580 nm.[8] This solvent effect on the absorption maxima of anions is opposite to that observed for ground-state absorption maxima. Clearly, the solvent and substituent effects on the doublet–doublet transitions of the anions of the heteroatom-ended polyenes are quite different from those on the singlet–singlet transitions ($^1B_u \leftarrow {}^1A_g$) of their ground states, and are subject to further studies.

As expected for reactions with solvated electron, the bimolecular rate constants for the formation of polyene radical anions are relatively large (8.6×10^9–1.6×10^{10} M^{-1} s^{-1}) and are in the limit of diffusion control. Even in the diffusion-controlled limit, a difference beyond the range of experimental errors is noticeable in the rate constants for the reactions of various polyenes. Thus, retinol and retinol acetate react relatively slowly in comparison to the other polyenes (Table I). We attribute this to the fact that, of the polyenes under consideration, only retinol and retinol acetate do not have a heteroatom as a part of the polyene moiety (Figure 1). The heteroatom-ended polyenes, i.e., retinal, retinoic acid/ester, and retinal Schiff base, offer a more favorable site of attachment for the solvated electron by virtue of the presence of the more electronegative heteroatom (oxygen/nitrogen); the reaction is represented by the following equation using a trienal as an example:



(9)

Alternatively, the π-electron systems of heteroatom-ended polyenes are expected to possess large dipole moments in the ground state and, hence, offer themselves as better substrates for solvated electron.

Retinal Schiff base appears to be the most reducible among the polyenes under consideration. This is indicated not only by the rate constant for its reaction with e^-_{MeOH} being the highest, but also the fact that this is the only polyene where we have observed the formation of radical anion on a slow time scale through reaction with methanol radical, $\cdot CH_2OH$. Another reason why we have been able to observe this slow reaction for retinal Schiff base is the fact that the radical anion is quite long-lived in this case (see Table I) in comparison to other heteroatom-ended polyenes. The lack of observation of slow formation of long-lived radical anion in the case of retinol and retinol acetate implies that the reaction of these polyene systems with $\cdot CH_2OH$ is very slow ($<10^7$ M^{-1} s^{-1}) and cannot compete favorably with the decay of $\cdot CH_2OH$ by dimerization/disproportionation.

An examination of the decay behaviors of the radical anions of various polyenes presents an interesting situation. The radical anions of retinol and retinol acetate decay very slowly with second-order kinetics. For other polyenes, we observe first-order decay kinetics in methanol with rate constants lying in the range, 1×10^4–1×10^6 s^{-1}. Our observation regarding the large increase in decay times on going from methanol to less acidic alcohol,[16] 2-propanol, clearly shows that protonation with solvent (reaction 3) is the most dominant mode of decay for heteroatom-ended polyenes, particularly in methanol. Decay by protonation by solvent in protic media is quite common to radical anions of other kinds, e.g., aromatic hydrocarbons.[11,17-19] The reason why the protonation is slow or lacking in the case of radical anions of retinol and retinol acetate may be sought in the fact that these radical anions would have smaller dipole moments than the radical anions of the heteroatom-ended polyenes where the negative charge is expected to be localized more on one end of the polyene moiety than on the other. The correlation of radical anion decay rate (by protonation) with dipole moments has also been observed for geometric isomers of stilbene[17] and terphenyl,[19] where the radical anions of bent isomers have been shown to decay faster by protonation.

In a relatively recent study[18] it has been shown that the logarithm of the protonation rate constant for radical anions of polycyclic alternant hydrocarbons is linearly related with their singlet energy separation $\Delta E_{S_1 \rightarrow S_0}$. A theoretical basis for this correlation comes from the fact that $^1/_2\, \Delta E_{S_1 \rightarrow S_0}$ is a measure of exothermicity upon protonation. This is because in the radical anion the unpaired electron occupies the lowest unoccupied molecular orbital while in the protonated radical the unpaired electron occupies the nonbonding orbital. In the present study, however, we find that the order in which the first-order protonation rate constants increase, viz., retinal > retinoic acid/ester > Schiff base, is opposite to the order in which the energies corresponding to the intense, low-energy band maximum ($^1B_u \leftarrow {}^1A_g$ transition) increase, that is, Schiff base ~ retinoic acid/ester > retinal. This difference in behavior may be taken to mean that the site of attachment of the proton in the three polyene systems varies significantly as far as the charge density is concerned.

Lang et al.[3c] have shown that on electrolysis of *n*-butylamine Schiff base of retinal in tetrahydrofuran at −20 °C a transient species with characteristic ESR spectrum is produced. The long-wavelength absorption peak for this species has been shown to be at 610 nm. The transient species we have assigned as radical anion of *n*-butylamine Schiff base of retinal has its absorption maximum at 430 nm in methanol at room temperture and shows no hint of a shoulder or second maximum at long wavelengths. As discussed earlier, the absorption spectral maxima of radical anions of heteroatom-ended polyenes appear to be strongly dependent on environmental conditions. The discrepancy between our spectral data with those of Lang et al. can be resolved, only if a temperature-dependence study is carried out regarding the absorption spectral maxima of polyene radical anions.

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(16) Hine, J.; Hine, M. *J. Am. Chem. Soc.* **1952**, *74*, 5266–5271.

(17) Levanon, H.; Neta, P. *Chem. Phys. Lett.* **1977**, *48*, 345–349.

(18) Levanon, H.; Neta, P.; Trozzolo, A. M. *Chem. Phys. Lett.*, **1978**, *54*, 181–185.

(19) Arai, S.; Tremba, E. L.; Brandon, J. R.; Dorfman, L. M. *Can. J. Chem.* **1967**, *45*, 1119–1123.