Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 1459

www.rsc.org/obc

**PAPER**



# Kinetic studies of retinol addition radicals†‡

**Ali El-Agamey,*[a,b,c] Shunichi Fukuzumi,*[a,d] K. Razi Naqvi[e] and David J. McGarvey[b]**

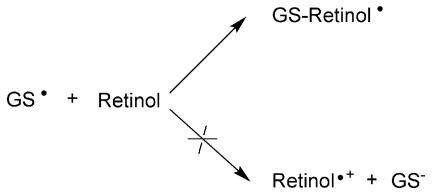
***Received 29th September 2010, Accepted 22nd November 2010***
**DOI: 10.1039/c0ob00799d**

Retinol neutral radicals (RS-retinol•), generated from the reaction of retinol with 4-pyridylthiyl and 2-pyridylthiyl radicals in argon-saturated methanol, undergo β-elimination, which can be monitored *via* the slow secondary absorption rise at 380 nm attributed to the rearrangement of the unstable retinol neutral addition radicals to the more stable addition radicals. Rate constants for the β-elimination reactions ($k_\beta$) of 4-PyrS-retinol• were measured at different temperatures and the Arrhenius equation for the reaction is described by $\log (k_\beta/s^{-1}) = (12.7 \pm 0.2) - (54.3 \pm 1.3)/\theta$, where $\theta = 2.3RT$ kJ mol$^{-1}$. The reactivities of retinol addition radicals (RS-retinol•), generated from the reaction of retinol with various thiyl radicals, towards oxygen have also been investigated in methanol. In the presence of oxygen, the decay of RS-retinol• fits to biexponential kinetics and both observed rate constants for the RS-retinol• decay are oxygen-concentration dependent. This suggests that at least two thiyl addition radicals, formed from the reaction of RS• with retinol, undergo oxygen addition reactions. In light of the estimated rate constants for oxygen addition to RS-retinol• and RS-CAR• (CAR: carotenoid), the antioxidant-prooxidant properties of retinol are discussed.

## Introduction

Vitamin A and various natural retinoids are essential in many biological processes such as vision, reproduction and normal cell differentiation.[1] They have been used in the treatment of some skin diseases and cosmetic formulations.[1–3] In the literature, there are many reports about the antioxidant activities of retinoids.[4–6] However, the prooxidant effects of retinoids have also been observed.[7–9] Moreover, there is considerable debate about whether retinoids combat or promote photocarcinogenesis and heart diseases.[1,10]

Whilst there are many reports on the photochemistry of retinoids,[11–20] there are comparatively few studies of time-resolved free radical reactions with them.[16,19] The reactions of trichloromethylperoxyl radical ($CCl_3O_2$•) with different retinoids, in 2% Triton X-100, lead to the formation of retinoid radical cations (retinoid•+, $\lambda_{max}$ = 590 nm) and another unidentified intermediate absorbing at shorter wavelength.[19] Retinoids can be regenerated from retinoid•+ *via* their efficient reactions ($k$ ~1–7 × 10$^8$ M$^{-1}$ s$^{-1}$) with vitamin C.[19] Moreover, glutathione thiyl radical (GS•) reacts with vitamin A (retinol) in aqueous methanolic (60%) solution to form a transient species that absorbs strongly at 380 nm ($\varepsilon_{max} = 4 \times 10^4$ M$^{-1}$ cm$^{-1}$).[16] This transient was previously attributed to either GS-retinol• (addition radical) or retinol•+. However, since retinol•+ is known to absorb at ~590 nm,[14,19] the transient can be assigned to GS-retinol• (Scheme 1).[21]




**Scheme 1**

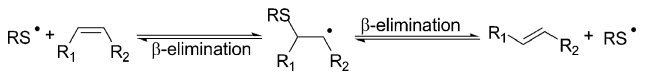
[a]*Department of Material and Life Science, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan. E-mail: a_el_agamey@yahoo.co.uk, fukuzumi@chem.eng.osaka-u.ac.jp; Fax: +81-0668797370; Tel: +81-0668797369*
[b]*School of Physical and Geographical Sciences, Keele University, Keele, Staffordshire, ST5 5BG, UK*
[c]*Chemistry Department, Faculty of Science, Mansoura University, New Damietta, Damietta, Egypt*
[d]*Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Korea*
[e]*Department of Physics, Norwegian University of Science and Technology (NTNU), N-7491, Trondheim, Norway*

† This work is dedicated to the memory of Athel Beckwith, a teacher and scientist from whom we learned how to study chemistry by example. His pioneering advances in radical chemistry laid the foundation for much of the current radical clock methodology.

‡ Electronic supplementary information (ESI) available: Transient profiles (380 nm) for 4-PyrS-retinol•, 4-PyrS•, 2-PyrS-retinol•, 2-PyrS• and PhS-retinol• in argon-saturated methanol at 334 K, transient profiles (380 nm) for RS-retinol• at different retinol concentrations, transient profiles for the decay of PhS• at 450 nm in air- and argon-saturated methanol, transient spectra of 2-PyrS•, PhS•, 2-PyrS-retinol• and PhS-retinol• in methanol and transient spectra of 4-PyrS-retinol• in cyclohexane, dependence of $k_{obs}$ for the formation of RS-retinol• on retinol concentration, dependence of $k_{obs}$ for the decay of 4-PyrS-retinol• and PhS-retinol• on oxygen concentration, data for the influence of the temperature on secondary growth rate constants, Eyring and Arrhenius plots for the slow growth (at 380 nm) generated from the reaction of 4-PyrS• or 2-PyrS• with retinol in argon-saturated methanol, and the influence of temperature on 2-PyrS-retinol• and PhS-retinol• in air-saturated methanol. See DOI: 10.1039/c0ob00799d

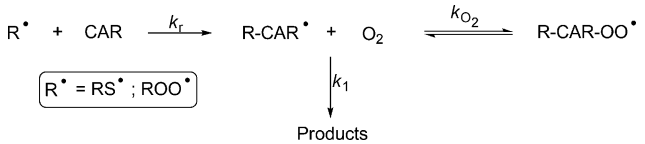
Therefore, retinol$^{\bullet+}$ and vitamin A neutral addition radical are the principal initial intermediates involved in the free radical reactions with retinol. However, formation of retinol$^{\bullet+}$ in biological systems is less likely since lipid peroxyl radicals, involved in lipid peroxidation, are much weaker electron accepting oxidizing agents than $CCl_3O_2^{\bullet}$.[19] On the other hand, formation of vitamin A neutral addition radicals is favored due to the formation of a highly resonance-stabilized neutral radical.[16]

In general, thiyl addition radicals can undergo two important reactions, namely β-elimination and oxygen addition reactions.[22–26] Firstly, β-elimination reactions of thiyl addition radicals are the key steps in the thiyl radical catalyzed *Z*/*E* isomerization reactions of unsaturated fatty acids (Scheme 2).[23,24] Chatgilialoglu *et al.*[23,24,27–29] have investigated these reactions extensively, due to the possible adverse effects on the functions of biological membranes if these reactions occur *in vivo*. In these reactions, thiyl radicals add to the double bond of the unsaturated fatty acid to form thiyl addition radicals. These thiyl addition radicals can undergo β-elimination of the thiyl radical to form either *Z*- or *E*-isomers depending on the conformation of the thiyl addition radicals. *Trans*-isomers are the major products of these reactions because of their greater stability relative to *cis*-isomers.[23,24,27–29] In addition, Ito *et al.*[22,30–34] studied extensively the addition reactions of various arylthiyl radicals with non-conjugated and conjugated olefins using the selective radical trapping flash photolysis method. Using this method they estimated the rate constants for the forward and backward reactions.




**Scheme 2**

Secondly, oxygen addition reactions to various carotenoid thiyl addition radicals have been investigated,[25,26,35] where it has been shown that the oxygen addition rate constants generally decrease as the number of conjugated double bonds increases in the parent carotenoid.[26] In addition, the reversibility of oxygen addition to PhS-77DH$^{\bullet}$, formed from the reaction of PhS$^{\bullet}$ with 7,7′-dihydro-β-carotene (77DH) in benzene, was observed (Scheme 3 and Fig. 1).[25] In 1984, Burton and Ingold proposed a reversible oxygen addition to carotenoid peroxyl addition radicals to explain the prooxidant-antioxidant behavior of carotenoids (Scheme 3).[36]




**Scheme 3**

To the best of our knowledge, the chemistry of retinol thiyl addition radicals is unexplored. We report herein studies on β-elimination reactions of thiyl addition radicals of retinol and the reactivities of these addition radicals towards oxygen. In addition, the influence of the thiyl radical reactivity on β-elimination and oxygen addition reactions has been examined.

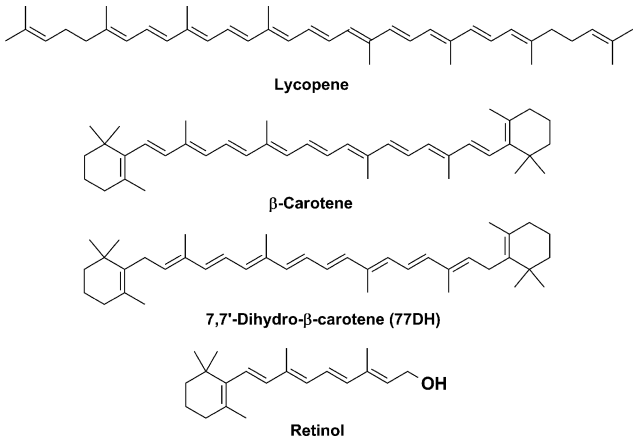
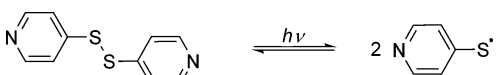



**Fig. 1** Structures of retinol and some carotenoids.

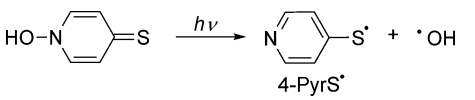
## Results and discussion

### Generation of thiyl radicals

Laser flash photolysis (LFP), with 266 nm excitation, of air-saturated methanolic solutions of 4,4′-dipyridyl disulfide leads to the formation of a transient spectrum with two absorption bands at 420 nm and <350 nm (Fig. 2 and eqn (1)). The 420 nm absorption band was observed previously following LFP of 4,4′-dipyridyl disulfide or *N*-hydroxypyridine-4-thione (eqn (2)) in THF and was attributed to 4-pyridylthiyl radical (4-PyrS$^{\bullet}$);[30] however, the transient spectra in this work do not cover wavelengths shorter than 390 nm. The decay of 4-PyrS$^{\bullet}$ radical has been ascribed to radical recombination, leading to the formation of the parent compound.[30]




(1)




(2)

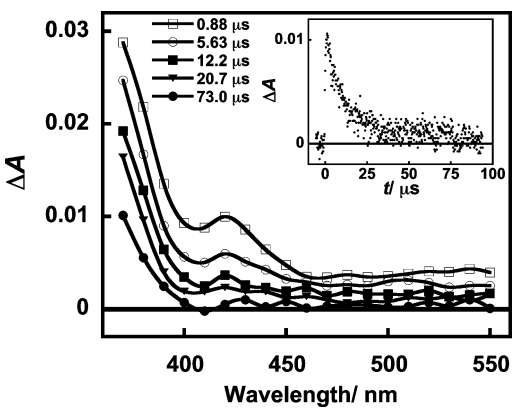
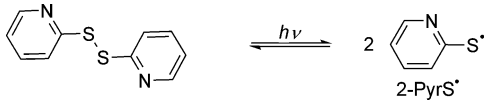



**Fig. 2** Transient spectra of 4-PyrS$^{\bullet}$ obtained following LFP (266 nm) of 4,4′-dipyridyl disulfide (~$2 \times 10^{-4}$ M) in air-saturated methanol (laser energy ~6.0 mJ). The inset shows the transient profile at 420 nm.

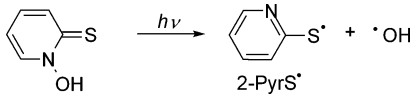
On the other hand, LFP of 2,2′-dipyridyl disulfide in air-saturated methanol gives a transient spectrum with $\lambda_{max}$ at 490 nm.

The transient decay at 490 nm leads to the growth of a transient ($\lambda_{max}$ < 410 nm, see Fig. S1‡).[37] Similar observations have been reported for LFP of 2,2′-dipyridyl disulfide in THF (eqn (3)) or *N*-hydroxypyridine-2-thione in acetonitrile (eqn (4)) and the transient spectrum was ascribed to 2-pyridylthiyl radicals (2-PyrS•).[31,38–41] Both 4-pyridylthiyl and 2-pyridylthiyl radicals are unreactive toward oxygen.[22,30,31,39,40]

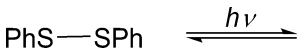



(3)




(4)

LFP of phenyl disulfide in methanol forms phenylthiyl radical (PhS•) (Fig. S2‡ and eqn (5)) with an absorption maximum at 450 nm. A similar transient spectrum has been reported for PhS• in benzene.[25] It is known that PhS• is unreactive toward oxygen (see Fig. S3‡).[22]




(5)

### Generation of retinol thiyl addition radicals

LFP (266 nm) of methanolic solutions of 4,4′-dipyridyl disulfide, 2,2′-dipyridyl disulfide or phenyl disulfide in the presence of retinol leads to the formation of transient absorption features ($\lambda_{max}$ ~ 370 nm; see Fig. 3, S4 and S5,‡ respectively), similar to those observed for addition radicals formed in the reaction of retinol with GS• radicals in aqueous methanolic solution.[16] Therefore, these transients can be attributed to 4-PyrS-retinol•, 2-PyrS-retinol• and PhS-retinol•, respectively. In addition, LFP of 4,4′-dipyridyl disulfide and retinol in cyclohexane gives a similar transient spectrum ($\lambda_{max}$ ~ 370 nm; see Fig. S6‡).

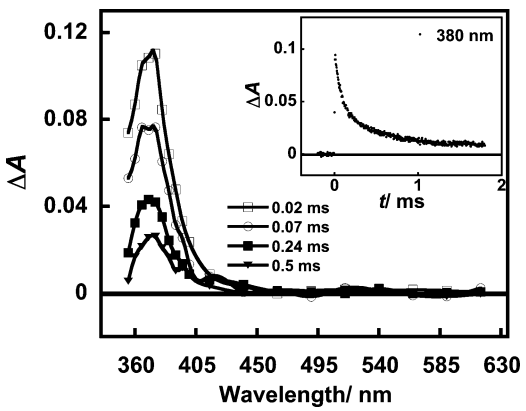


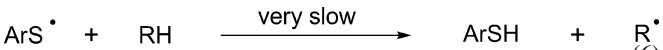

**Fig. 3** Transient spectra of 4-PyrS-retinol• obtained following 266 nm laser photolysis of 4,4′-dipyridyl disulfide (~$2 \times 10^{-4}$ M) in the presence of retinol (~$4.0 \times 10^{-5}$ M)[45,46] in air-saturated methanol at room temperature (laser energy ~4 mJ). The inset shows a kinetic absorption profile for the decay of 4-PyrS-retinol• at 380 nm.

The formation of retinol neutral radical (retinol•), instead of thiyl addition radicals, is ruled out due to the poor H-abstraction ability of arylthiyl radicals (eqn (6)).[22,26,31] In addition, it has been reported that thiyl radical reactions with various carotenoids give rise to thiyl addition radicals.[25,26,42–44]

**Table 1** Rate constants ($k_r$) for the reactions of thiyl radicals (RS•) with retinol in methanol[a]

| RS• | $k_r/10^9$ M$^{-1}$ s$^{-1}$ |
|---|---|
| 4-PyrS• | 8.1 ± 0.5 |
| 2-PyrS• | 5.3 ± 0.4 |
| PhS• | 1.48 ± 0.1 |

[a] The estimated rate constants represent the sum of the addition rate constants leading to the formation of various thiyl addition radicals.




(6)

It should be noted that no transient was detected at 380 nm following 266 nm LFP of retinol alone in methanol, which indicates that the transient observed at 380 nm is due to the reaction of thiyl radicals with retinol.

Moreover, the electron-transfer reaction between RS• and retinol is excluded because of the absence of the absorption band due to retinol•+, ($\lambda_{max}$ ~ 590 nm).[14,19] Similarly, contribution of triplet retinol ($^3$retinol, $\lambda_{max}$ ~ 400 nm) to the observed spectra has been eliminated.[47–49] Furthermore, the assignment of the transient spectra (Fig. 3, S4 and S5‡) to RS-retinol-OO• has been excluded because of the similarity of the transient spectra for the reaction of RS• and retinol in air and argon-saturated solutions (Fig. 3 and S7‡).

By varying the concentration of retinol and maintaining it higher than that of the generated RS•, the rate constants ($k_r$) for the reaction of 4-PyrS•, 2-PyrS• and PhS• radicals with retinol were determined (see Table 1 and Fig. S8‡) from the plot of the observed pseudo-first-order rate constant ($k_{obs}$) for the growth of RS-retinol• at 380 nm *versus* retinol concentration using eqn (7), where $k_0$ (intercept) is the apparent rate constant for the decay of RS• in the absence of retinol (Fig. S8–S11‡).

$$k_{obs} = k_o + k_r\,[\text{retinol}] \quad (7)$$

The rate constants for the reaction of PyrS• with retinol are comparable to that reported for the reaction of 2-PyrS• with β-carotene ($7.3 \times 10^9$ M$^{-1}$ s$^{-1}$).[39] Furthermore, the rate constant for PhS• is close to the reported rate constant for the reaction of GS• with retinol in aqueous methanol ($1.4 \times 10^9$ M$^{-1}$ s$^{-1}$).[16] The lower reactivity of PhS•, compared to 2-PyrS• and 4-PyrS•, can be attributed to the higher electrophilicity of the S atom in pyridylthiyl radicals than in phenylthiyl radical.[22,30,31] Also, this order of reactivity (4-PyrS• ≈ 2-PyrS• > PhS•) is the same as the order of reactivities toward isoprene in THF (1.0, 1.2 and 0.047 × $10^9$ M$^{-1}$ s$^{-1}$, respectively).[22,31]

### β-Elimination of retinol-thiyl addition radical[50]

At room temperature, LFP (266 nm) of argon-saturated methanolic solutions of either 4,4′-dipyridyl disulfide or 2,2′-dipyridyl disulfide in the presence of retinol leads to a slow secondary absorption rise ($k_\beta$ = $1.4 \times 10^3$ and $3.5 \times 10^3$ s$^{-1}$ at 296 K, respectively) after the fast formation of 4-PyrS-retinol• or 2-PyrS-retinol•. As the temperature increases, the rate of secondary growth, which is monitored at 380 nm, increases (Fig. S12, S13,‡ 4 and 5). These observations can be attributed to the rearrangement of the less stable addition radical to the more stable one *via* β-elimination (Scheme 4).

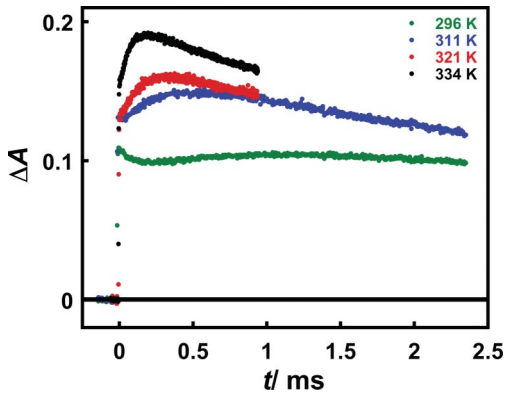



**Fig. 4** The influence of temperature on the transient profiles, at 380 nm, of 4-PyrS-retinol• following 266 nm LFP (laser energy ~4 mJ) of 4,4′-dipyridyl disulfide (~$2 \times 10^{-4}$ M) in the presence of retinol (~$8.0 \times 10^{-5}$ M), in argon-saturated methanol.

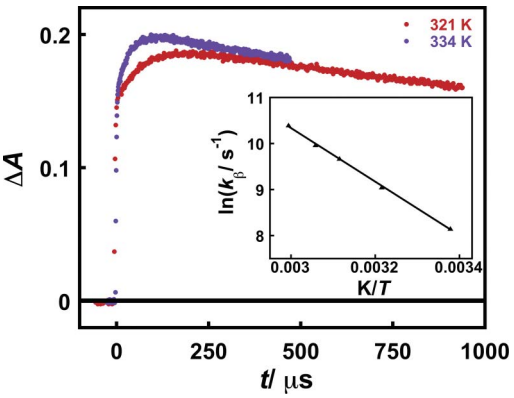
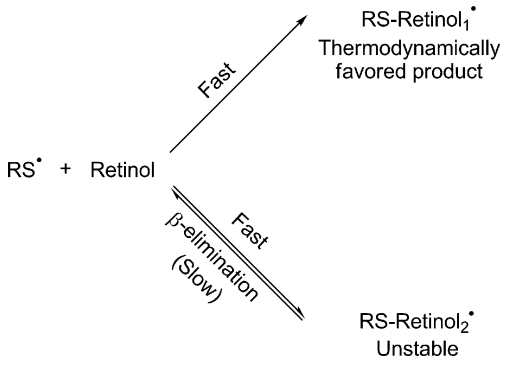



**Fig. 5** The influence of temperature on the transient profiles, at 380 nm, of 2-PyrS-retinol• following 266 nm LFP (laser energy ~4 mJ) of 2,2′-dipyridyl disulfide (~$3.0 \times 10^{-4}$ M) in the presence of retinol (~$8.0 \times 10^{-5}$ M), in argon-saturated methanol. The inset shows an Arrhenius plot for the slow growth (at 380 nm) generated from the reaction of 2-PyrS• with retinol.

Activation enthalpies ($\Delta H^{\ddagger}$) and entropies ($\Delta S^{\ddagger}$) were determined from measurements of the secondary growth rate constants for the different retinol addition radicals (4-PyrS-retinol• and 2-PyrS-retinol•) as a function of temperature (Tables 2, S1, S2 and Fig. S14‡). In Table 2, the small negative activation entropies are consistent with many β-elimination reactions, which require some degree of organization to achieve the transition state. For example, β-elimination of α-cumyloxyl radical and decarboxylation of alkoxycarbonyl radicals have log A values in the range of 12–13.[28e,51–53]

Moreover, previous studies[22] for the reactions of PhS• with 1,3-butadiene and non-conjugated olefins indicate that as the number of conjugated double bonds increases the resonance stabilization of the addition radical formed increases. This rise in the stability of




**Scheme 4**

addition radical will decrease the activation energy of the forward reaction and increase that of the reverse reaction, *i.e.* the rate constant for the forward reaction will increase while that for the reverse reaction will decrease. For example, the rate constants for PhS• addition to 1,3-butadiene and non-conjugated olefins are $3.0 \times 10^7$ and $4.0 \times 10^3$ $M^{-1}$ $s^{-1}$, respectively, while those for the β-elimination reactions of their corresponding addition radicals are $>3.0 \times 10^5$ and $3.3 \times 10^7$ $s^{-1}$, respectively (Scheme 5).[54]

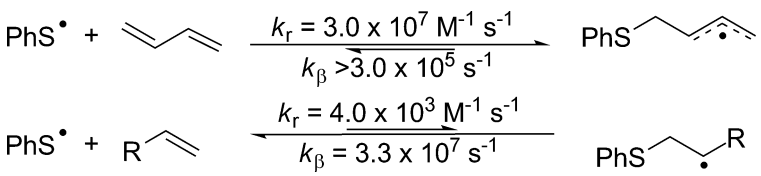
Further elongation of the conjugated chain, as in retinol, is expected to increase the rate constant for the forward reaction and decrease the rate constant for β-elimination reactions. The rate constants for the reactions of 4-PyrS• and 2-PyrS• radicals with retinol ($k_r = (8.1 \pm 0.5) \times 10^9$ and $(5.3 \pm 0.4) \times 10^9$ $M^{-1}$ $s^{-1}$, respectively) and those for β-elimination reactions of 4-PyrS-retinol• and 2-PyrS-retinol• ($k_\beta = 1.4 \times 10^3$ and $3.5 \times 10^3$ $s^{-1}$ at 296 K, respectively) are in agreement with this trend. In addition, for carotenoids with longer conjugated chains (*e.g.* β-carotene and lycopene have 11 conjugated double bonds), the rate constants for β-elimination reactions are expected to be much smaller than that for retinol (5 conjugated double bonds). This could explain the difficulty of observing the β-elimination reactions for these longer carotenoids.[25,26]

It should be noted that LFP of an argon-saturated methanolic solution of retinol shows no transient at 380 nm. Also, following

**Table 2** Activation enthalpies ($\Delta H^{\ddagger}$) and entropies ($\Delta S^{\ddagger}$) for the slow absorption rise following the fast formation of PyrS-retinol• (at 380 nm) from the reaction of retinol (~$8 \times 10^{-5}$ M) with 4-PyrS• or 2-PyrS• in argon-equilibrated methanol (laser energy ~4 mJ)

| Precursor | $\Delta H^{\ddagger}$/kJ mol$^{-1}$ | $\Delta S^{\ddagger}$/J K$^{-1}$ mol$^{-1}$ |
|---|---|---|
| 4,4′-dipyridyl disulfide | 51.7 ± 1.3 | −10.1 ± 4.1 |
| 2,2′-dipyridyl disulfide[a] | 45.8 ± 0.9 | −22.6 ± 2.7 |

[a] Arrhenius equation for the β-elimination reaction of 2-PyrS-retinol• is described by $\log (k_\beta/s^{-1}) = (12.1 \pm 0.14) - (48.4 \pm 0.9)/\theta$, where $\theta = 2.3\ RT$ kJ mol$^{-1}$.




**Scheme 5**

LFP of argon-saturated methanolic solutions of 2,2′-dipyridyl disulfide or 4,4′-dipyridyl disulfide at 334 K, there is no slow absorption rise at 380 nm after the fast formation of 2-PyrS• and 4-PyrS• (Fig. S15 and S16‡). Therefore, the observed slow absorption rise is not due to thiyl radicals (2-PyrS• or 4-PyrS•) or retinol; but it arises from the reaction of 2-PyrS• or 4-PyrS• with retinol.

There is no evidence for the β-elimination reaction following LFP of phenyl disulfide and retinol in argon-saturated methanolic solutions even at higher temperatures (Fig. S17‡). This can be attributed to the lower reactivity of PhS•, which will lead to the formation of either one addition radical or a mixture of addition radicals with much higher stability than those obtained from the reaction of retinol with 2-PyrS• and 4-PyrS•. In the latter case, the extra stability of these addition radicals will slow down the β-elimination reaction.

### Oxygen addition reactions of retinol-thiyl addition radicals

Oxygen addition reactions play an essential role in determining the antioxidant–prooxidant properties of carotenoids and retinoids.[21,55] In Scheme 3, if the equilibrium associated with the oxygen addition reaction is in favor of the formation of R-CAR-OO•, a prooxidant effect may result. On the other hand, if the equilibrium shifts toward the formation of stable R-CAR•, the antioxidant effect may prevail (Scheme 3).[35,36]

The influence of oxygen concentration on the decay of PyrS-retinol• following 266 nm LFP of 4,4′-dipyridyl disulfide or 2,2′-dipyridyl disulfide in the presence of retinol has been investigated in methanol (Fig. S18‡ and 6). The decay of 4-PyrS-retinol• and 2-PyrS-retinol• fits to biexponential kinetics. Both the observed rate constants for PyrS-retinol• decay are oxygen-concentration dependent. This observation can be ascribed to the oxygen addition to various thiyl addition radicals absorbing at 380 nm,[56] which have different thermodynamic stabilities. Moreover, this result supports our earlier observation for β-elimination reactions, which involve at least two addition radicals (*vide supra*).

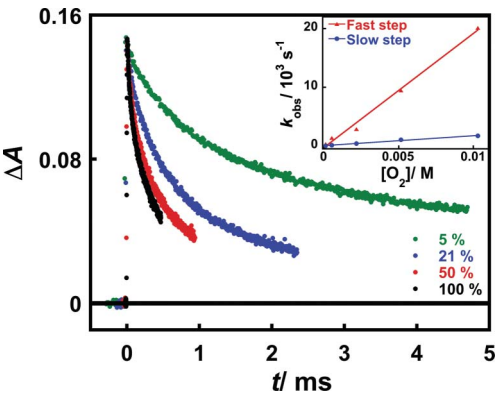



**Fig. 6** Normalized kinetic absorption profiles for the decay of 2-PyrS-retinol• at 380 nm in methanol at various oxygen concentrations (5, 21, 50, 100%) formed following 266 nm LFP (laser energy ~4.0 mJ) of 2,2′-dipyridyl disulfide (~$3.0 \times 10^{-4}$ M) in the presence of retinol (~$8.0 \times 10^{-5}$ M). The inset shows plots of pseudo-first-order rate constants ($k_{obs}$) for the fast and slow decay of 2-PyrS-retinol•, at 380 nm, *versus* the oxygen concentration.

The rate constants for each step (fast and slow) can be calculated by plotting $k_{obs}$ of each step *versus* the oxygen concentration (eqn (8)). The slope equals the rate constant for oxygen addition of each step ($k_{O_2}$) and the intercept equals the apparent first-order rate constant for the decay of the RS-retinol• ($k_1$) in the absence of oxygen.

$$k_{obs} = k_1 + k_{O_2}[O_2] \quad (8)$$

The rate constants for oxygen addition to 4-PyrS-retinol• of the fast and slow steps were estimated as $(20.4 \pm 2.3) \times 10^5$ and $(2.1 \pm 0.3) \times 10^5$ $M^{-1}$ $s^{-1}$, respectively (see inset of Fig. S18‡). Similarly, for 2-PyrS-retinol•, the rate constants were calculated as $(19.5 \pm 1.0) \times 10^5$ and $(1.7 \pm 0.1) \times 10^5$ $M^{-1}$ $s^{-1}$, respectively (see inset of Fig. 6).

In addition, the influence of the temperature on 4-PyrS-retinol• and 2-PyrS-retinol• transients has been investigated in air-saturated methanol (Fig. 7 and S19‡).[57,58] At high temperature, a small residual baseline offset was observed which may be attributed to the β-fragmentation of oxygen from 4-PyrS-retinol-OO• and 2-PyrS-retinol-OO•, *i.e.* the reversibility of oxygen addition to 4-PyrS-retinol• and 2-PyrS-retinol• at high temperature (see Scheme 3).[59] Similar behavior has been observed for the oxygen addition to PhS-77DH•, formed from the reaction of PhS• with 7,7′-dihydro-β-carotene (77DH) in benzene, at high temperature.[25]

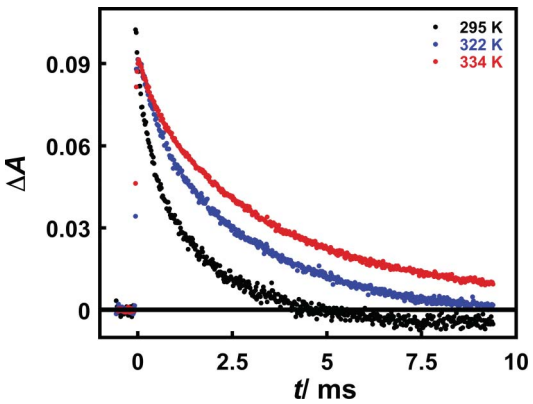



**Fig. 7** The influence of temperature on the normalized transient profiles, at 380 nm, of 4-PyrS-retinol• following 266 nm LFP (laser energy ~4 mJ) of 4,4′-dipyridyl disulfide (~$2 \times 10^{-4}$ M) in the presence of retinol (~$9.0 \times 10^{-5}$ M), in air-saturated methanol.

The influence of oxygen concentration on the decay of PhS-retinol• has also been investigated (Fig. S20‡). The PhS-retinol• decay fits to biexponential kinetics similar to those observed for PyrS-retinol•, and is oxygen-concentration dependent. This supports the assumption for the formation of a mixture of addition radicals (*vide supra*). Using eqn (8), the rate constants for the fast and slow steps were estimated as $(3.5 \pm 0.3) \times 10^5$ and $(1.1 \pm 0.1) \times 10^5$ $M^{-1}$ $s^{-1}$, respectively (Fig. S20‡).

The small difference between the oxygen addition rate constants for the fast and slow steps, compared with those obtained with PyrS-retinol• is consistent with a smaller difference in the stabilities of the PhS-retinol• radicals formed compared to PyrS-retinol• radicals. Also, the rate constants for oxygen addition to PyrS-retinol• and PhS-retinol• radicals indicate lower reactivity of PhS-retinol• radicals compared with PyrS-retinol• radicals.[60] Therefore,

the failure to observe β-elimination can be attributed to the greater stabilities of the different PhS-retinol• radicals formed. This stability, as indicated from their oxygen addition rate constants, will significantly hinder the β-elimination reaction (*vide supra*).

Furthermore, the rate constants for oxygen addition to PhS-retinol• are 1–2 orders of magnitude larger, depending on the carotenoid, than those reported for oxygen addition to thiyl addition radicals (PhS-CAR•) generated from the reaction of PhS• with long chain carotenoids.[25,26] The lower reactivity of PhS-CAR• toward oxygen can be attributed to the greater stability, by resonance, of the longer chain neutral addition radical formed.

## Conclusions

β-Elimination and oxygen addition reactions have been observed for the retinol–thiyl addition radicals, generated from the reaction of various thiyl radicals with retinol. The observation of the β-elimination reaction and of biexponential kinetics observed for the oxygen addition reaction have been interpreted as evidence for the formation of more than one addition radical. Moreover, it was shown that the reactivity of the RS• radicals defines the reactivity of RS-retinol• radicals formed. As the reactivity of the RS• radicals increases, the selectivity decreases *i.e.* more reactive thiyl addition radicals are generated. Therefore the antioxidant–prooxidant properties of retinol are partly determined by the reactivity of the thiyl radical involved. Finally, the rate constants for oxygen addition to RS-retinol• are 1–3 orders of magnitude larger, depending on the reactivity of the thiyl radical, than those reported for carotenoids (CAR). On this basis one may conclude that, if β-fragmentation reactions (*i.e.* loss of oxygen) for RS-retinol-OO• and RS-CAR-OO• are slow, carotenoids are expected to be more potent antioxidants than retinoids under the same conditions (temperature, oxygen concentration, medium and thiyl radical).

## Experimental section

### Materials

Methanol (Aldrich, HPLC grade), retinol (Sigma, ≥99%), 4,4′-dipyridyl disulfide (Aldrich, 98%), 2,2′-dipyridyl disulfide (Aldrich, 98%) and phenyl disulfide (Fluka) were used as received. Oxygen (1, 5, 50 (balance nitrogen) and 100%) and argon were supplied by the British Oxygen Company.

### Laser flash photolysis experiments

The details of the laser flash photolysis system (time resolution is 50 ns) have been described previously.[61] Unless otherwise stated, laser energies were in the range 1–4 mJ pulse$^{-1}$ with a beam diameter of ~4 mm. Quartz sample cells (2 mm excitation pathlength × 10 mm monitoring pathlength) fitted with vacuum taps (where necessary) were employed for the laser flash photolysis measurements. If necessary, (for example, during acquisition of transient absorption spectra) fresh solution was introduced into the sample cell following each exposure to the laser. All the experiments were carried out at ~20 °C. The errors in the measurements are expressed as standard errors.

## Acknowledgements

We thank Dr Thor Bernt Melø (Department of Physics, Norwegian University of Science and Technology (NTNU), N-7491, Trondheim, Norway) for his valuable discussions. Dr El-Agamey is grateful to the Leverhulme Trust and JSPS (Japan Society for the Promotion of Science) for financial support. We are also grateful for financial support provided by Grants-in-Aids (Nos. 20108010 and 21111501) and a Global COE program, "the Global Education and Research Center for Bio-Environmental Chemistry" from the Japan Society of Promotion of Science (JSPS), and by KOSEF/MEST through WCU project (R31-2008-10010-0).

## Notes and references

1 P. P. Fu, S.-H. Cheng, L. Coop, Q. Xia, S. J. Culp, W. H. Tolleson, W. G. Wamer and P. C. Howard, *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.*, 2003, **21**, 165–197.
2 A. M. Young and G. Gregoriadis, *Photochem. Photobiol.*, 1996, **63**, 344–352.
3 M. A. Freyaldenhoven, R. V. Lloyd and V. M. Samokyszyn, *Chem. Res. Toxicol.*, 1996, **9**, 677–681.
4 (*a*) N. P. Das, *J. Neurochem.*, 1989, **52**, 585–588; (*b*) M. Ciaccio, M. Valenza, L. Tesoriere, A. Bongiorno, R. Albiero and M. A. Livrea, *Arch. Biochem. Biophys.*, 1993, **302**, 103–108; (*c*) G. F. Vile and C. C. Winterbourn, *FEBS Lett.*, 1988, **238**, 353–356; (*d*) S. A. Keys and W. F. Zimmerman, *Exp. Eye Res.*, 1999, **68**, 693–702; (*e*) M. A. Livrea, L. Tesoriere, A. Bongiorno, A. M. Pintaudi, M. Ciaccio and A. Riccio, *Free Radical Biol. Med.*, 1995, **18**, 401–409.
5 V. M. Samokyszyn and L. J. Marnett, *Free Radical Biol. Med.*, 1990, **8**, 491–496.
6 L. Tesoriere, M. Ciaccio, A. Bongiorno, A. Riccio, A. M. Pintaudi and M. A. Livrea, *Arch. Biochem. Biophys.*, 1993, **307**, 217–223.
7 F. Dal-Pizzol, F. Klamt, M. S. Benfato, E. A. Bernard and J. C. F. Moreira, *Free Radical Res.*, 2001, **34**, 395–404.
8 M. Murata and S. Kawanishi, *J. Biol. Chem.*, 2000, **275**, 2003–2008.
9 F. Klamt, F. Dal-Pizzol, E. A. Bernard and J. C. F. Moreira, *Photochem. Photobiol. Sci.*, 2003, **2**, 856–860.
10 V. P. Palace, N. Khaper, Q. Qin and P. K. Singal, *Free Radical Biol. Med.*, 1999, **26**, 746–761.
11 K. Bhattacharyya, K. Bobrowski, S. Rajadurai and P. K. Das, *Photochem. Photobiol.*, 1988, **47**, 73–83.
12 S. K. Chattopadhyay, K. Bobrowski and P. K. Das, *Chem. Phys. Lett.*, 1982, **91**, 143–148.
13 G. G. Gurzadyan, J. Reynisson and S. Steenken, *Phys. Chem. Chem. Phys.*, 2007, **9**, 288–298.
14 K. Bobrowski and P. K. Das, *J. Phys. Chem.*, 1985, **89**, 5079–5085.
15 K. Bobrowski and P. K. Das, *J. Phys. Chem.*, 1985, **89**, 5733–5738.
16 M. D'Aquino, C. Dunster and R. L. Willson, *Biochem. Biophys. Res. Commun.*, 1989, **161**, 1199–1203.
17 T. Rosenfeld, A. Alchalal and M. Ottolenghi, *Chem. Phys. Lett.*, 1973, **20**, 291–297.
18 K. K. N. Lo, E. J. Land and T. G. Truscott, *Photochem. Photobiol.*, 1982, **36**, 139–145.
19 M. Rozanowska, A. Cantrell, R. Edge, E. J. Land, T. Sarna and T. G. Truscott, *Free Radical Biol. Med.*, 2005, **39**, 1399–1405.
20 K. Bobrowski and P. K. Das, *J. Am. Chem. Soc.*, 1982, **104**, 1704–1709.
21 A. El-Agamey, G. M. Lowe, D. J. McGarvey, A. Mortensen, D. M. Phillip, T. G. Truscott and A. J. Young, *Arch. Biochem. Biophys.*, 2004, **430**, 37–48.
22 O. Ito, in *S-Centered Radicals*, ed. Z. B. Alfassi, John Wiley & Sons, Chichester, 1999, pp. 193-224.
23 C. Chatgilialoglu, A. Altieri and H. Fischer, *J. Am. Chem. Soc.*, 2002, **124**, 12816–12823.
24 C. Chatgilialoglu, A. Samadi, M. Guerra and H. Fischer, *ChemPhysChem*, 2005, **6**, 286–291.
25 A. El-Agamey and D. J. McGarvey, *Org. Lett.*, 2005, **7**, 3957–3960.
26 A. El-Agamey and D. J. McGarvey, *Free Radical Res.*, 2007, **41**, 295–302.

27 C. Chatgilialoglu, L. Zambonin, A. Altieri, C. Ferreri, Q. G. Mulazzani and L. Landi, *Free Radical Biol. Med.*, 2002, **33**, 1681–1692.

28 (*a*) C. Ferreri, M. Panagiotaki and C. Chatgilialoglu, *Mol. Biotechnol.*, 2007, **37**, 19–25; (*b*) C. Ferreri, C. Costantino, L. Landi, Q. G. Mulazzani and C. Chatgilialoglu, *Chem. Commun.*, 1999, 407–408; (*c*) A. Samadi, I. Andreu, C. Ferreri, S. Dellonte and C. Chatgilialoglu, *J. Am. Oil Chem. Soc.*, 2004, **81**, 753–758; (*d*) I. N. Lykakis, C. Ferreri and C. Chatgilialoglu, *Angew. Chem., Int. Ed.*, 2007, **46**, 1914–1916; (*e*) C. Chatgilialoglu, C. Ferreri, M. Ballestri, Q. G. Mulazzani and L. Landi, *J. Am. Chem. Soc.*, 2000, **122**, 4593–4601.

29 (*a*) C. Ferreri and C. Chatgilialoglu, *Chem. Bio. Chem.*, 2005, **6**, 1722–1734; (*b*) L. Zambonin, C. Ferreri, L. Cabrini, C. Prata, C. Chatgilialoglu and L. Landi, *Free Radical Biol. Med.*, 2006, **40**, 1549–1556; (*c*) C. Ferreri, M. R. Faraone-Mennella, C. Formisano, L. Landi and C. Chatgilialoglu, *Free Radical Biol. Med.*, 2002, **33**, 1516–1526; (*d*) C. Ferreri, C. Costantino, L. Perrotta, L. Landi, Q. G. Mulazzani and C. Chatgilialoglu, *J. Am. Chem. Soc.*, 2001, **123**, 4459–4468; (*e*) C. Chatgilialoglu and C. Ferreri, *Acc. Chem. Res.*, 2005, **38**, 441–448; (*f*) C. Ferreri, A. Samadi, F. Sassatelli, L. Landi and C. Chatgilialoglu, *J. Am. Chem. Soc.*, 2004, **126**, 1063–1072.

30 M. M. Alam, O. Ito, G. N. Grimm and W. Adam, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2471–2476.

31 M. M. Alam, A. Watanabe and O. Ito, *J. Org. Chem.*, 1995, **60**, 3440–3444.

32 O. Ito and M. Matsuda, *Prog. Polym. Sci.*, 1992, **17**, 827–874.

33 (*a*) O. Ito, R. Omori and M. Matsuda, *J. Am. Chem. Soc.*, 1982, **104**, 3934–3937; (*b*) O. Ito and M. Matsuda, *J. Am. Chem. Soc.*, 1979, **101**, 5732–5735.

34 O. Ito, S. Tamura, K. Murakami and M. Matsuda, *J. Org. Chem.*, 1988, **53**, 4758–4762.

35 A. El-Agamey and D. J. McGarvey, in *Carotenoids Volume 4: Natural Functions*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander, Birkhäuser, Basel, 2008, pp. 119-154.

36 G. W. Burton and K. U. Ingold, *Science*, 1984, **224**, 569–573.

37 The transient rise at 380 nm was attributed to the formation of *N*-hydropyridine-2-thione *via* hydrogen abstraction from the solvent by a small fraction of the radical formed.[31,38].

38 M. M. Alam, A. Watanabe and O. Ito, *Photochem. Photobiol.*, 1996, **63**, 53–59.

39 B. M. Aveline, I. E. Kochevar and R. W. Redmond, *J. Am. Chem. Soc.*, 1996, **118**, 10113–10123.

40 B. M. Aveline, I. E. Kochevar and R. W. Redmond, *J. Am. Chem. Soc.*, 1995, **117**, 9699–9708.

41 M. P. DeMatteo, J. S. Poole, X. Shi, R. Sachdeva, P. G. Hatcher, C. M. Hadad and M. S. Platz, *J. Am. Chem. Soc.*, 2005, **127**, 7094–7109.

42 A. Mortensen, L. H. Skibsted, J. Sampson, C. Rice-Evans and S. A. Everett, *FEBS Lett.*, 1997, **418**, 91–97.

43 A. Mortensen, *Asian Chem. Lett.*, 2000, **4**, 135–143.

44 S. A. Everett, M. F. Dennis, K. B. Patel, S. Maddix, S. C. Kundu and R. L. Willson, *J. Biol. Chem.*, 1996, **271**, 3988–3994.

45 $\varepsilon_{max}$ of retinol in ethanol is $5.28 \times 10^4$ $M^{-1}$ $cm^{-1}$ at 325 nm.[46].

46 G. G. Garwin and J. C. Saari, *Methods Enzymol.*, 2000, **316**, 313–324.

47 The reviewer has drawn our attention to the possibility of $^3$retinol contribution to the transient spectrum; however, the very low quantum yield of $^3$retinol formation ($\phi_T \sim 0.003$ in polar solvent) and the efficient oxygen quenching of $^3$retinol efficiently reduces any contribution of $^3$retinol to the absorption transient spectra.[48,49].

48 M. Montalti, A. Credi, L. Prodi and M. T. Gandolfi, *Handbook of Photochemistry*, CRC Press, Boca Raton, 2006.

49 I. Carmichael and G. L. Hug, *J. Phys. Chem. Ref. Data*, 1986, **15**, 1–250.

50 Thiyl-eliminations from retinol-thiyl addition radicals are not strictly β-elimination reactions since the formed highly conjugated radicals are delocalized over the conjugated backbone, *i.e.* the unpaired electron is not localized at the β-position.

51 P. A. Simakov, F. N. Martinez, J. H. Horner and M. Newcomb, *J. Org. Chem.*, 1998, **63**, 1226–1232.

52 A. Baignee, J. A. Howard, J. C. Scaiano and L. C. Stewart, *J. Am. Chem. Soc.*, 1983, **105**, 6120–6123.

53 A. L. J. Beckwith and V. W. Bowry, *J. Am. Chem. Soc.*, 1994, **116**, 2710–2716.

54 In order to estimate the rate constants for β-elimination reactions the authors assumed that the rate constant for oxygen addition to thiyl addition radicals is ~$10^9$ $M^{-1}$ $s^{-1}$.[22].

55 L. Tesoriere, D. D'Arpa, R. Re and M. A. Livrea, *Arch. Biochem. Biophys.*, 1997, **343**, 13–18.

56 For the more stable addition radical, the length of the conjugated double bonds is expected to be longer than that of the less stable radical, therefore $\lambda_{max}$ of the latter radical is blue-shifted with respect to the former one, *i.e.* at 380 nm, $\varepsilon$ of the less stable radical is less than that of the more stable radical. This assumption has been supported by the biexponential kinetics of oxygen addition to RS-retinol$^\bullet$ and by the absorption rise due to the rearrangement of the less stable radical.

57 The reviewer raises the possibility that the temperature change of the 380 nm decays may be related to the lowering oxygen concentration with rising temperature. However, this possibility has very little effect on the observed kinetics since the oxygen concentration change in methanol over the temperature range of our experiments is very small.[58].

58 P. G. T. Fogg and W. Gerrard, *Solubility of Gases in Liquids: A Critical Evaluation of Gas/Liquid Systems in Theory and Practice*, John Wiley & Sons, New York, 1991, pp. 296-297.

59 LFP of phenyl disulfide and retinol in air-saturated methanol shows a small residual baseline offset for the PhS-retinol$^\bullet$ transient at high temperature (Fig. S21‡). This can be ascribed to the β-fragmentation of PhS-retinol-OO$^\bullet$ (*i.e.* the reverse of oxygen addition).

60 Since PyrS$^\bullet$ radicals are more reactive than PhS$^\bullet$, their reactions with retinol will generate highly reactive PyrS-retinol$^\bullet$.

61 A. El-Agamey, *J. Photochem. Photobiol., A*, 2009, **203**, 13–17.