Lycopene is more potent than beta carotene in the neutralization of singlet oxygen: role of energy transfer probed by ultrafast Raman spectroscopy

Kong-Thon Tsen
Arizona State University
Department of Physics and Astronomy
Tempe, Arizona 85287-1504
E-mail: tsen@asu.edu

Shaw-Wei D. Tsen
Johns Hopkins University
Department of Biology
Baltimore, Maryland 21218

Juliann G. Kiang
Uniformed Services University of the Health Sciences
Scientific Research Department
Armed Forces Radiology Research Institute
and
Department of Medicine
and
Department of Pharmacology
Bethesda, Maryland 20814-4799

Abstract. Energy transfer processes between beta carotene, lycopene, and singlet oxygen ($^1O_2$) have been studied by ultrafast Raman spectroscopy. Our experimental results demonstrate that during the neutralization of singlet oxygen by beta carotene the excitation energy of singlet oxygen is transferred directly to the first excited electronic state S_1 of beta carotene. In contrast, the excitation energy of singlet oxygen is transferred directly to the ground excited vibronic state S_0 of lycopene. Our data not only provide the first direct experimental elucidation of energy transfer processes in such important biological systems but also help explain why lycopene is a more potent antioxidant than beta carotene in the neutralization of singlet oxygen. [DOI: 10.1117/1.2398884]

Keywords: energy transfer; beta carotene; lycopene; singlet oxygen; Raman spectroscopy.

Paper 06074R received Mar. 22, 2006; revised manuscript received Jul. 3, 2006; accepted for publication Aug. 18, 2006; published online Dec. 21, 2006.

1 Introduction

Recently, a lot of experimental studies have demonstrated that free radicals play an important role on various diseases in human beings.[1] Free radicals are molecules that contain unpaired electrons.[2] The unpaired electron is highly reactive. It can either cause oxidative damage to the molecules or be passed from one molecule to another so as to turn the recipient into a free radical. In the case of lipid peroxidation, there is a chain reaction that involves damage and passing of radicals.[3] In general, cellular macromolecules, such as proteins, nucleic acids, and lipids, are vulnerable to free radical damage. For example, oxidation of low-density lipoprotein (LDL) cholesterol by free radicals leads to atherosclerosis.[4] Removal or neutralization of the free radicals can, therefore, protect against cardiovascular diseases. It has also been reported that free radicals might underlie the aging process itself because low caloric intake that reduces the generation of free radicals has been shown to increase the life span in *C. elegans*[5] and others.[6]

Most free radicals in biological systems are derivatives of oxygen that are usually referred to as reactive oxygen species (ROSs). They primarily are produced by mitochondria and their damage is mainly to mitochondrial membranes and mitochondrial DNA. Between 1 and 5% of the oxygen used by mitochondrial to generate adenosine triphosphate results in the formation of superoxide radicals. Although mitochondria are the major source of free radicals, there are numerous other sources. For example, free radicals are released from white blood cells (neutrophils) associated with inflammation. Neutrophils use oxidative free radicals (superoxide, hydrogen peroxide, hydroxyl) to kill intruding bacteria. The lysosomal enzyme—myeloperoxidase—catalyzes the production of bacteriocidal hypochlorite from hydrogen peroxide and chloride ions. Free radicals are also generated by eicosanoids from arachidonic acid during ischemia-reperfusion injuries. Air pollution, tobacco smoking, and ultraviolet irradiation can produce free radicals that cause oxidative damage to lungs, blood vessels, and other body tissues.

On the other hand, antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically, this means that the antioxidant molecule becomes a free radical in the process of neutralizing another free radical molecule. However, the antioxidant molecule will be a much less reactive free radical than the free radical neutralized. The antioxidant molecule may be very large, thereby allowing it to dilute the unpaired electron; it may be readily neutralized by another antioxidant molecule; or it may have another mechanism for terminating its free radical condition.

Recent studies have shown that high dietary intake of carotenoids tremendously lowers the risk of various diseases.[1] Carotenoid molecules such as beta carotene and lycopene are powerful antioxidants that usually accumulated in the human body through consumption of fruits and vegetables. These molecules play the role of scavengers for free radicals,[7] singlet oxygen,[8,9] and other harmful ROSs[10] that are formed during the biological and chemical processes in the cell. Carotenoids also have great promise for use as inhibitors of

Address all correspondence to: Dr. Kong-Thon Tsen, Arizona State University, Department of Physics and Astronomy, Tempe, AZ 85287-1504; Tel: 480-965-5206; Fax: 480-965-7954; E-mail: tsen@asu.edu

1083-3668/2006/11(6)/064025/6/$22.00 © 2006 SPIE

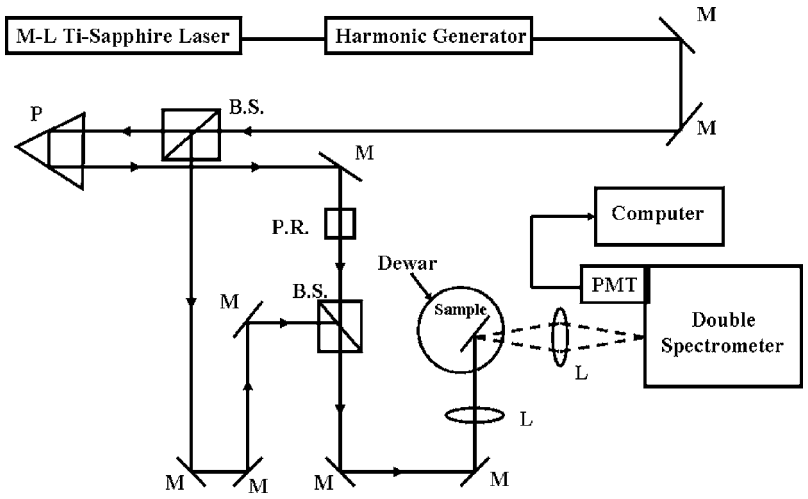

Fig. 1 The experimental setup used to study energy transfer processes in the neutralization of singlet oxygen molecules by beta carotene and lycopene. M: mirror; P: prism; B.S.: beamsplitter; P.R.: polarization rotator; L: lens; PMT: photomultiplier tube.

various cancers and precancers.[11,12] An inverse relationship between beta carotene intake and the incidence of certain type of cancers, such as lung and gastrointestinal tract cancers, has been observed.[13]

Despite evidence of the effects of carotenoids on the health of human beings, no knowledge about their neutralization processes has been available. It becomes, therefore, very essential to unfold the neutralization processes of carotenoids on free radicals. In this paper, we report our spectroscopic findings of the energy transfer process during the neutralization of singlet oxygen by beta carotene and lycopene. Our experimental results demonstrate that during the neutralization of singlet oxygen by beta carotene, the excitation energy of singlet oxygen is transferred directly to the first excited electronic state S_1 of beta carotene; whereas, the excitation energy of singlet oxygen is transferred to the ground excited vibronic state S_0 of lycopene, implying that lycopene is capable of more efficiently neutralizing singlet oxygen than beta carotene. Therefore, our findings not only provide the first direct experimental elucidation of energy transfer processes in such important biological systems but also help explain why lycopene is a more potent antioxidant than beta carotene in the neutralization of singlet oxygen.

2 Methods and Materials

The experimental technique—ultrafast Raman spectroscopy employed in this work has been described in detail elsewhere.[14–17] As shown in Fig. 1, the output of the second harmonic generation of a mode-locked Ti-sapphire laser is used as both the excitation and probing sources in the case of pump-probe experiments. In the single beam (pump only) experiments, our experimental results represent an average over the duration of the pulse width of the laser. The laser can provide a continuous pulse train of 80 MHz in repetition rate, at photon wavelengths ranging from 350 to 450 nm, having a pulse width of about 100 fs and with average power of about 100 mW. One advantage of probing with Raman spectroscopy is that the Raman scattering signal is present only when the probing photons are present; as a result, the time resolution in ultrafast Raman spectroscopy is limited only by the pulse duration of the laser and not by the response time of the detection system. In addition, Stokes processes can provide information on both the ground and excited states; whereas, anti-Stokes processes reflect the very existence of the excited states. The Raman signal is collected and analyzed by a standard computer-controlled Raman system that includes a double spectrometer, a photomultiplier tube, and associated photon counting electronics. All the experimental data were taken at $T=300$ K and with a laser operating at a 390-nm wavelength.

Beta carotene, lycopene, and porphyrin samples used in this study were purchased from Aldrich Co.

3 Results and Discussions

The dynamics of beta carotene have been studied extensively in the literature.[18–21] As depicted in Fig. 2, absorption of a photon having a wavelength around 450 nm brings the beta

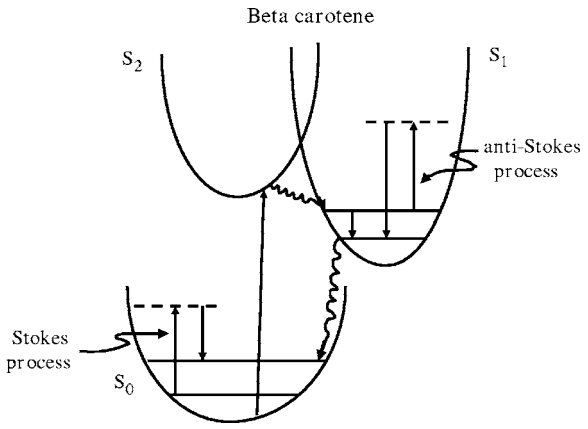

Fig. 2 Schematic energy diagram of a beta carotene molecule excited by a near uv photon. S_2 state relaxes toward S_1 state in about 200 fs; S_1 thermalizes to S_0 state in a few picoseconds. Also shown are the Stokes and anti-Stokes Raman processes associated with S_0, S_1 states, respectively.

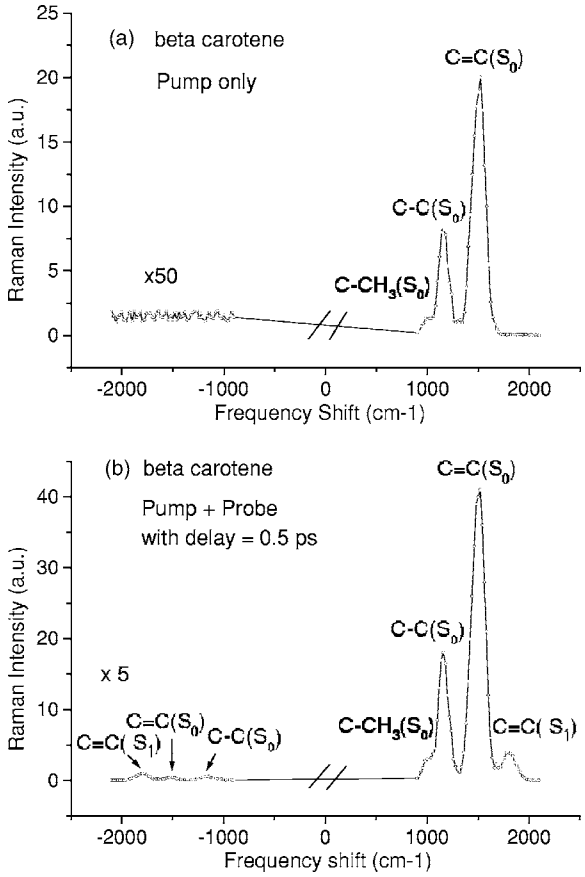

Fig. 3 Typical Raman spectra for beta carotene in toluene solution excited by an ultrashort laser with pulse width about 100 fs in (a) pump pulse only, and (b) pump/probe configuration with time delay about 0.5 ps. See text for discussions.

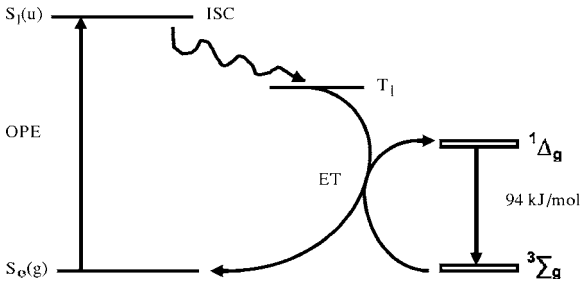

Fig. 4 Energy diagram demonstrating the production of singlet oxygen molecules from porphyrin in air-saturated toluene solution. OPE: one photon excitation; ISC: intersystem crossing; ET: energy transfer. See text for discussions.

carotene from the ground electronic state S_0 to the second excited electronic state S_2 (excitation to the first excited electronic state S_1 from the ground electronic state S_0 by photons is forbidden because of the selection rule). The excited S_2 state relaxes to S_1 state on a timescale of about 200 fs.[22–26] The excited vibronic state associated with S_1 thermalizes to its ground vibronic state within 1 ps.[21] The transition then occurs within a few picoseconds with the C═C stretching motion as the major accepting mode.[22,27,28] The excited beta carotene relaxed to its ground state within about 20 ps.

A typical Raman spectrum for beta carotene taken by the ultrafast laser when only the pump pulse is present is shown in Fig. 3(a) (after a suitable subtraction of the luminescence background on the Stokes side). There are no observable structures found on the anti-Stokes side of the spectrum. This is because the first excited electronic state S_1 is populated by the pump pulse about 200 fs after the pump excitation, but the pulse duration of the excitation laser is about 100 fs. On the other hand, the three profound Raman lines on the Stokes side come from scattering of light by the rocking motion of $C-CH_3$ ($\cong 1005\ cm^{-1}$), the stretching motion of C–C ($1157\ cm^{-1}$), and the stretching motion of C═C ($1524\ cm^{-1}$), respectively, all of which are associated with the ground electronic state S_0 of beta carotene.[20]

Figure 3(b) shows a typical Raman spectrum for beta carotene taken by the ultrafast laser in the pump-probe configuration in which the time delay between the pump and probe is about 0.5 ps. We have found that on the Stokes side of the spectrum, in addition to those appearing in the single beam excitation of Fig. 3(a), there is an additional feature at around $1800\ cm^{-1}$ that is attributed to the scattering of light by the stretching motion of C═C associated with the first excited electronic state S_1 of beta carotene.[21,29] This unique feature will be used later to justify unambiguously the direct involvement of S_1 electronic state in the neutralization of singlet oxygen molecules by beta carotene. The small structures observed on the anti-Stokes side indicate that there are sizable excited vibronic states associated with both the S_1 and S_0 present during the probing.

Figure 4 shows a schematic diagram of how the free radicals—singlet oxygen molecules—are produced in our experiment. Absorption of a near uv photon brings the photosensitizer molecule—porphyrin—into the excited state S_1.[30–32] The excited porphyrin then undergoes an intersystem crossing to a triplet state T_1 with energy of 110 to 130 kJ/mol on the timescale of nanoseconds. From the triplet state, the energy is transferred to the nearby oxygen molecules by turning them from a triplet ground state $^3\Sigma_g$ into an excited singlet state $^1\Delta_g$ with excitation energy of 94 kJ/mol, which happens on the timescale of a few milliseconds. The presence of excited singlet oxygen molecules in solution is usually detected by their $^1\Delta_g \rightarrow {}^3\Sigma_g$ luminescence at around 1270 nm. Figure 5 shows a typical luminescence spectrum detected when the porphyrin in air-saturated toluene solution is excited by our laser pulses.

Figure 6(a) shows a Raman spectrum for a mixture of beta carotene with porphyrin in toluene and under about 10^{-4} Torr air pressure, that is, without the presence of oxygen molecules, taken with the pump pulse present only. We notice that the spectrum is strikingly similar to Fig. 3(a) in which only beta carotene is present. However, as the solution in Fig. 6(a) is air-saturated, the Raman spectrum, which is shown in Fig. 6(b), mimics that of Fig. 3(b) in which a delayed probe pulse is also present. Apparently, the addition of oxygen molecules in the mixture causes the presence of Stokes as well as anti-Stokes signal at around $1800\ cm^{-1}$ in Fig. 6(b), which provides[21,29] unambiguous evidence that the first excited vibronic state S_1 of beta carotene is populated during the energy transfer processes.

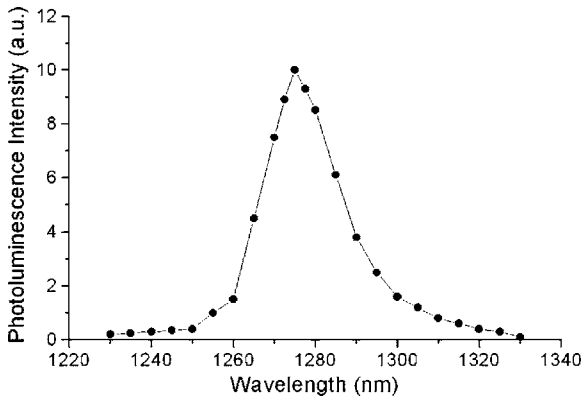

Fig. 5 A typical luminescence spectrum detected in the mixture of porphyrin in air-saturated toluene after excitation of an ultrafast laser having a 390-nm wavelength. The luminescence comes from the radiative relaxation of the singlet oxygen toward its triplet ground state.

Comparison of the relative intensities of C═C (S_0) versus C–C (S_0) on the anti-Stokes side between Figs. 3(b) and 6(b) indicates that intramolecular vibrational redistributions do depend on the mechanism of $2A_g^-$ generation.

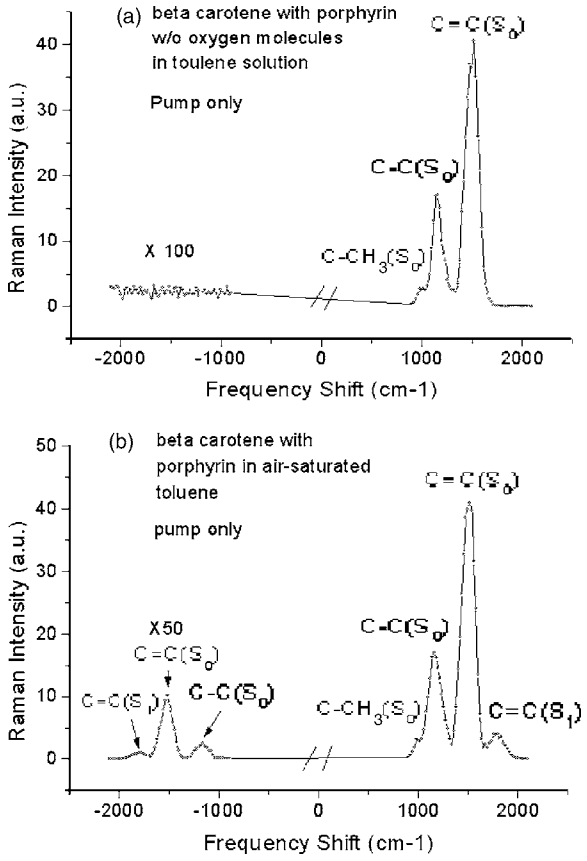

Fig. 6 Typical Raman spectra for beta carotene with porphyrin in toluene solution (a) without oxygen molecules, (b) with oxygen molecules, excited by a 390-nm pump laser only. The presence of an additional Raman line at around 1800 cm^{-1} on the Stokes side in (b) as compared with (a) is indicative of the direct involvement of the excited vibronic state S_1 of beta carotene in the neutralization of singlet oxygen molecules.

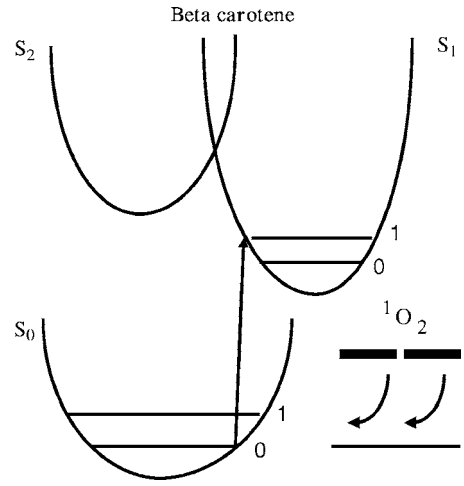

Fig. 7 A proposed energy transfer process suggested by our experimental results in the neutralization of singlet oxygen molecules by beta carotene. In the process, two singlet oxygen molecules simultaneously lose their excitation energy to beta carotene, which is excited to the first excited vibronic state S_1.

We note that because the energy transfer between the triplet state T_1 of porphyrin and the nearby oxygen molecule occurs on the timescale of milliseconds,[32] while the repetition rate of our excitation or probing laser is 80 MHz, we are exciting or probing under a quasi-steady-state condition in our current experimental configuration. It is this quasi-steady-state condition that enables us to detect the transient and therefore the intermediate states of energy transfer processes with a single–laser beam experiment.

We notice that the first excited vibronic state S_1 of beta carotene can be populated through the relaxation of higher excited states in beta carotene. However, this possible scenario is unlikely, because it involves simultaneous relaxation of three or more singlet oxygen molecules. Another possible energy transfer pathway is that the excitation energy of singlet oxygen transfers partly to the vibronic states associated with the ground electronic state S_0 of beta carotene and partly to that associated with the first excited state S_1. This is not possible considering the fact that in Fig. 6(b) nonequilibrium C═C (S_1) stretching mode is observed and its occupation number ($\Delta n \cong 0.0054$) has been found to be about the same as that of C═C (S_0) ($\Delta n \cong 0.0058$) and much larger than that of C–C (S_0) ($\Delta n \cong 0.0012$) stretching modes. On the other hand, these experimental observations are consistent with what one would expect under the quasi-steady-state conditions when the excitation energy of singlet oxygen is transferred directly to the S_1 state. Therefore, the presence of Stokes as well as anti-Stokes Raman lines coming from the scattering of light by the stretching motion of C═C at around 1800 and −1800 cm^{-1} seen in Fig. 6(b), provides concrete evidence that during the neutralization of singlet oxygen by beta carotene, the excitation energy of singlet oxygen molecules transfers primarily to the excited vibronic states associated with the first excited electronic state S_1 of beta carotene. As shown in Fig. 7, because the energy difference between the S_1 and S_0 states of beta carotene is about 14 500 cm^{-1},[33] in order for such an energy transfer to occur

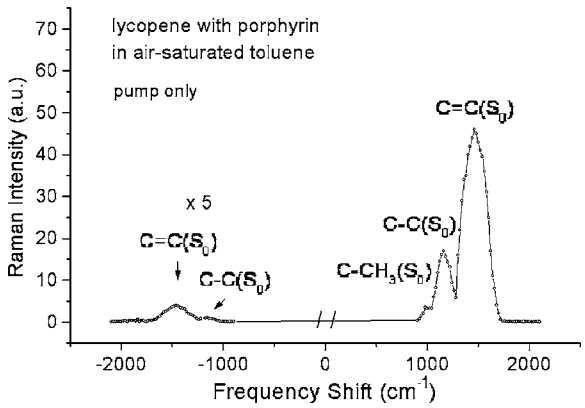

Fig. 8 A typical Raman spectrum for lycopene with porphyrin in air-saturated toluene solution, excited by 390-nm pump laser only. The absence of Raman lines at around 1800 cm^{-1} on the Stokes side and anti-Stokes side as compared with Fig. 6(b) suggests that the excitation energy of singlet oxygen is transferred primarily to the excited vibronic state S_0 of lycopene in the neutralization of singlet oxygen molecules.

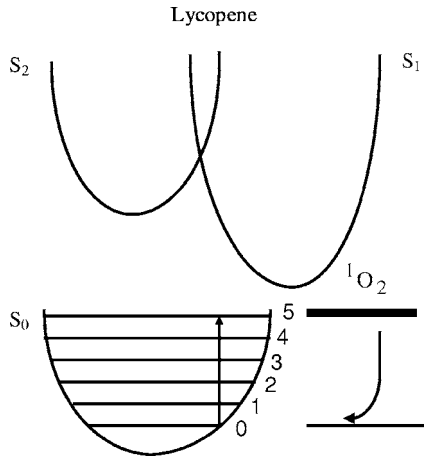

Fig. 9 A proposed energy transfer process suggested by our experimental results in the neutralization of singlet oxygen molecules by lycopene. In the process, one singlet oxygen molecule loses its excitation energy to lycopene, which is excited to the ground excited vibronic state S_0.

while conserving the energy, two singlet oxygen molecules (each of them has excess energy of about 7875 cm^{-1}) must be simultaneously losing their energy by relaxing to their ground triplet states with the excitation of a beta carotene molecule to its first excited vibronic state associated with S_1.

Figure 8 shows a Raman spectrum for a mixture of lycopene with porphyrin in air-saturated toluene solution, taken with the pump pulse present only. All the experimental parameters are kept the same except that beta carotene has been replaced by lycopene. The intriguing feature of this spectrum, when compared with Fig. 6(b) in which beta carotene is used, is the disappearance of 1800 cm^{-1} structures on both Stokes and anti-Stokes sides. This observation provides direct evidence that the first excited state S_1 in lycopene is not involved in the energy transfer processes. On the other hand, a relatively large occupation number ($\Delta n \cong 0.017$) is detected for the C═C stretching mode associated with the ground electronic state S_0, suggesting that the excitation energy of singlet oxygen is transferred primarily to the ground excited vibronic state S_0 of lycopene. We notice that the full width at half maximum (FWHM) for Raman signals associated with C═C (S_0) is significantly wider for lycopene (Fig. 8) than for beta carotene [Fig. 6(b)]. This is because under the quasi-steady-state condition, significantly more higher vibronic modes are populated in lycopene (up to $\upsilon \approx 5$) than in beta carotene ($\upsilon \approx 1$). In addition, the peak has been found to be redshifted, as a result of the effect of anharmonicity of the C═C vibrational mode. A proposed energy transfer process for a lycopene–single oxygen system based on our experimental results is depicted in Fig. 9. Because an energy transfer process involving simultaneous deexcitation of two singlet oxygen molecules is much less likely than a process involving a single one, our findings provide a possible answer to the following question: Why is lycopene a more potent antioxidant than beta carotene in the neutralization of singlet oxygen?[34,35]

We now address why beta carotene can be excited by a pair of singlet oxygen molecules, whereas lycopene can be excited by a singlet oxygen molecule. One likely explanation is that the pair of beta-ionone rings attached to both ends of beta carotene may be relevant, because it is known that their double bonds can be oxidized to form epoxides, see for example the case of the violaxanthin cycle. Another possible explanation is that when a pair of 1 $^3B_u(T_1)$ fragments is formed in a beta carotene molecule by energy transfer from two $^1O_2$, the 2 $^1A_g^-$ (S_1) state of the beta carotene can be formed by fusion, because 1 $^3B_u \times 1$ $^3B_u = 2$ ^1A_g in symmetry.[36]

4 Conclusion

We have studied energy transfer processes in the beta carotene–singlet oxygen and lycopene–singlet oxygen systems by using ultrafast Raman spectroscopy. Our experimental results provide concrete evidence that excitation energy of singlet oxygen is transferred primarily to the first excited electronic vibronic state S_1 of beta carotene, suggesting that the neutralization process involves simultaneous relaxation of two singlet oxygen molecules. On the other hand, the excitation energy of singlet oxygen is transferred primarily to the ground excited vibronic state S_0 of lycopene. These experimental results indicate that lycopene is capable of more efficiently neutralizing singlet oxygen than that of beta carotene. Therefore, our findings not only provide the first direct experimental elucidation of energy transfer processes in such important biological systems but also help explain why lycopene is a more potent antioxidant than beta carotene in the neutralization of singlet oxygen.

Acknowledgments

This work is supported in part by the National Science Foundation under Grant No. DMR-0305147 and by Department of Defense Research Area Directorate II Science and Technology Objective C. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the U.S. Department of the Army, the Uniformed Services University of the Health Sci-

ences, or the U.S. Department of Defense. The authors would also like to thank Y. F. Chen and S. H. Lin for useful discussions.

References

1. T. W. M. Boileau, A. D. Moore, and J. W. Erdman, Jr., "Carotene and vitamin A," in *Antioxidant Status, Diet, Nutrition, and Health*, A. M. Papas, Ed., pp. 133–158, CRC Press, Boca Raton, FL (1999).
2. B. Halliwell and J. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford (1999).
3. W.-Y. Lee and S.-M. Lee, "Ischemic preconditioning protects post-ischemic oxidative damage to mitochondria in rat liver," *Shock* **24**, 370–375 (2005).
4. R. Stocker and J. F. Keaney, Jr., "New insights on oxidative stress in the artery wall," *J. Thromb. Haemost.* **3**, 1825–1834 (2005).
5. Y. Luo, "Long-lived worms and ageing," *Redox. Rep.* **9**, 65–69 (2004).
6. L. Kappeler and J. Epelbaum, "Biological aspects of longevity and ageing," *Rev. Epidemiol. Sante Publique* **53**, 235–241 (2005).
7. F. Bohm, J. H. Tinkler, and T. G. Truscott, "Carotenoids protect against cell membrane damage by the nitrogen dioxide radical," *Nat. Med.* **1**, 98–99 (1995).
8. C. S. Foote and R. W. Denny, "Chemistry of singlet oxygen. Quenching by β-carotene," *J. Am. Chem. Soc.* **90**, 6233–6235 (1968).
9. A. Farmillo and F. Wilkinson, "On the mechanism of quenching of singlet oxygen in solution," *J. Photochem. Photobiol.* **18**, 447–450 (1973).
10. P. F. Conn, W. Schalch, and T. G. Truscott, "The singlet oxygen and carotenoid interaction," *J. Photochem. Photobiol.* **11**, 41–47 (1991); "Erratum," **17**, 89 (1993).
11. L. C. Chen, L. Sly, C. S. Jones, R. De Tarone, and L. M. Luca, "Differential effects of dietary beta-carotene on papilloma and carcinoma formation induced by an initiation-promotion protocol in SENCAR mouse skin," *Carcinogenesis* **14**, 713–717 (1993).
12. H. S. Garewal, R. Katz, F. Meyskens, J. Pitcock, D. Morse, S. Friedman, Y. Peng, D. Pendrys, S. Mayne, D. Alberts, T. Kiersch, and E. Graver, "Beta-carotene produces sustained remissions in patients with oral leukoplakia: Results of a multicenter prospective trial," *Arch. Otolaryngol. Head Neck Surg.* **125**, 1305–1310 (1999).
13. R. Peto, R. Doll, J. D. Buckley, and M. B. Sporn, "Can dietary beta-carotene materially reduce human cancer rates?," *Nature (London)* **290**, 201–208 (1981).
14. K. T. Tsen, "Electron velocity overshoot, ballistic electron transport and non-equilibrium phonon dynamics in nanostructure semiconductors," in *Ultrafast Phenomena in Semiconductors*, K. T. Tsen, Ed., pp. 191–259, Springer-Verlag, New York (2001).
15. K. T. Tsen, "Ultrafast dynamics in wide bandgap wurtzite GaN," in *Ultrafast Physical Processes in Semiconductors*, K. T. Tsen, Ed., pp. 109–149, Semiconduct. Semimetals, Vol. **69**, Academic Press, New York (2001).
16. K. T. Tsen, "Optical studies of electric-field induced electron and hole transient transports and optical phonon instability in semiconductor nanostructures," in *Ultrafast Dynamical Processes in Semiconductors*, K. T. Tsen, Ed., pp. 193–258, Topics in Applied Physics, Vol. **92**, Springer-Verlag, Heidelberg (2004).
17. K. T. Tsen, "Optical studies of carrier dynamics and non-equilibrium optical phonons in nitride-based semiconductors," in *Non-equilibrium Dynamics of Semiconductors and Nanostructures*," K. T. Tsen, Ed., CRC Press, Inc., New York (2005).
18. T. Polivka, D. Zigmantas, H. A. Frank, J. A. Bautista, J. L. Herek, Y. Koyama, R. Fujii, and V. Sundstrom, "Near-infrared time-resolved study of the S_1 state dynamics of the carotenoid Spheroidene," *J. Phys. Chem. B* **105**, 1072–1080 (2001).
19. G. Cerullo, G. Lanzani, M. Zavelani-Rossi, and S. De Silvestri, "Early events of energy relaxation in all-trans-beta carotene following sub-10 fs optical-pulse excitation," *Phys. Rev. B* **63**, 241104-1–241104-4 (2001).
20. D. W. McCamant, J. E. Kim, and R. A. Mathies, "Vibrational relaxation in beta carotene probed by picosecond Stokes and anti-Stokes resonance Raman spectroscopy," *J. Phys. Chem. A* **106**, 6030–6038 (2002).
21. D. W. McCamant, P. Kukura, and R. A. Mathies, "Femtosecond time-resolved stimulated Raman spectroscopy: Application to the ultrafast internal conversion in beta carotene," *J. Phys. Chem. A* **107**, 8208–8214 (2003).
22. A. P. Shreve, J. K. Trautman, T. G. Owens, and A. C. Albrecht, "Determination of the S_2 lifetime of β-carotene," *Chem. Phys. Lett.* **178**, 89–96 (1991).
23. J. P. Zhang, T. Inaba, Y. Watanabe, and Y. Koyama, "Excited-state dynamics among the $1B_u^+$, $1B_u^-$ and $2A_g^-$ states of all-*trans*-neurosporene as revealed by near-infrared time-resolved absorption spectroscopy," *Chem. Phys. Lett.* **332**, 351–358 (2000).
24. H. Kandori, H. Sasabe, and M. Mimuro, "Direct determination of a lifetime of the S_2 state of beta-carotene by femtosecond time-resolved fluorescence spectroscopy," *J. Am. Chem. Soc.* **116**, 2671–2672 (1994).
25. S. Akimoto, I. Yamazaki, S. Takaichi, and M. Mimuro, "Excitation relaxation of carotenoids within the S_2 state probed by the femtosecond fluorescence up-conversion method," *Chem. Phys. Lett.* **313**, 63–68 (1999).
26. A. N. Macoherson and T. Gillbro, "Solvent dependence of the ultrafast S_2-S_1 internal conversion rate of beta-carotene," *J. Phys. Chem. A* **102**, 5049–5058 (1998).
27. H. Nagae, M. Kuki, J. P. Zhang, T. Sashima, Y. Mukai, and Y. Koyama, "Vibronic coupling through the in-phase, C═C stretching mode plays a major role in the $2A_g^-$ to $1A_g^-$ internal conversion of all-trans-beta-carotene," *J. Phys. Chem. A* **104**, 4155–4166 (2000).
28. M. R. Wasielewski, D. G. Johnson, E. G. Bradford, and L. D. Kispert, "Temperature dependence of the lowest excited singlet-state lifetime of all-trans-beta-carotene and fully deuterated all-trans-beta-carotene," *J. Chem. Phys.* **91**, 6691–6697 (1989).
29. H. Hashimoto and Y. Koyama, "The C═C stretching Raman lines of β-carotene isomers in the S_1 state as detected by pump-probe resonance Raman spectroscopy," *Chem. Phys. Lett.* **154**, 321 (1989).
30. B. W. Henderson and T. J. Dougherty, "How does photodynamic therapy work?," *Photochem. Photobiol.* **55**(1), 147–157 (1992).
31. R. Bonnett, "Photosensitizers of the porphyrin and phtalocyanine series for photodynamic therapy," *Chem. Soc. Rev.* **24**(1), 19–33 (1995).
32. A. Karotki, M. Kruk, M. Drobizhev, A. Rebane, E. Nickel, and C. W. Spangler, "Efficient singlet oxygen generation upon two-photon excitation of new porphyrin with enhanced nonlinear absorption," *IEEE J. Sel. Top. Quantum Electron.* **7**(6), 971–975 (2001).
33. K. Onaka, R. Fujii, H. Nagae, M. Kuki, Y. Koyama, and Y. Watanabe, "The state energy and the displacements of the potential minima of the $2A_g^-$ state in all-*trans*-β-carotene as determined by fluorescence spectroscopy," *Chem. Phys. Lett.* **315**, 75–81 (1999).
34. P. Di Mascio, S. Kaiser, and H. Sies, "Lycopene as the most efficient biological carotenoid singlet oxygen quencher," *Arch. Biochem. Biophys.* **274**(2), 532–538 (1989).
35. V. Bhuvaneswari and S. Nagini, "Lycopene: A review of its potential as an anticancer agent," *Curr. Med. Chem.* **5**, 627–635 (2005).
36. P. Tavan and K. Schulten, "Electronic excitations in finite and infinite polyenes," *Phys. Rev. B* **36**, 4337 (1987).