acids, and cell membranes are also damaged in photodynamic action, carotenoids also protect against these effects,[3] presumably by the same mechanism.

The behavior of these carotenoids in triplet MB quenching also serves to explain the fact that shorter carotenoid chains are effective in protecting against *anaerobic* chlorophyll photoreduction, since triplet chlorophyll a is probably intermediate in this reaction, and quenching of this triplet is effective even with carotenoids of only 5 conjugated double bonds.[8]

The implications of these observations for the mechanism of singlet oxygen quenching by carotenoids are discussed in the accompanying communication.[6]

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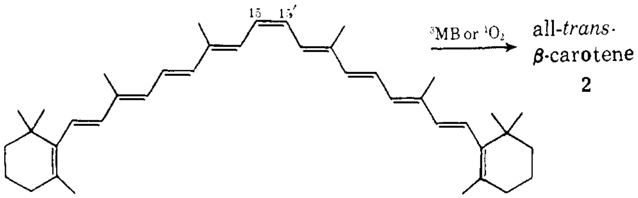
Chemistry of Singlet Oxygen. XI. Cis–Trans Isomerization of Carotenoids by Singlet Oxygen and a Probable Quenching Mechanism[1]

Sir:

Numerous reports of cis–trans isomerization of carotenoids by direct or sensitized irradiation have appeared.[2] The isomerization of carotenoids sensitized by chlorophyll a appears to occur only in the direction cis → trans.[2,3] We now report that isomerization of 15,15′-*cis*-β-carotene (**1**) is sensitized by singlet oxygen ($^1O_2$), as well as by triplet methylene blue (^{3}MB) with high efficiency. The product is, at least largely, all-*trans*-β-carotene (**2**). Under the same conditions, **2** does not appear to undergo appreciable isomerization.



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The apparatus and conditions used are similar to those used for the quenching experiments,[1,4] except that 2-methyl-2-pentene was omitted from most experiments (having been found not to affect the results), and that changes in carotene composition were monitored by absorption spectroscopy.[5] All-*trans*-β-carotene has strong (ε 114,000) absorption at 464 nm and only weak (ε 7500) absorption at 345 nm; 15,15′-*cis*-β-carotene has weaker absorption at 456 nm (ε 85,000) but much stronger absorption at 345 nm (ε 49,400); this latter absorption is referred to as the "cis peak" and is much stronger for the symmetrical 15,15′-cis isomer than for other *cis*-carotenes.[2a]

Solutions of **1** and methylene blue (MB) were irradiated under O_2 through a filter[1] which ensured that light was absorbed only by MB. The results are summarized in Table I, and show a rapid decrease in the "cis peak" and a simultaneous increase in absorbance at 464 nm. The results are consistent with a clean isomerization **1** → **2**; the absorbance at 464 nm calculated from the decrease at 345 nm on the assumption that no other reaction occurs is in excellent agreement with the observed values. Reaction under N_2 was equally rapid, but no reaction whatever occurred under O_2 without MB.

Table I. Absorbance of a Solution of 15,15′-*cis*-β-Carotene[a] and Methylene Blue[b,c]

Irradn time, sec	Absorbance 345 nm	Absorbance 464 nm	Isomerization, %[d]	A_{464}, calcd[e]
0	0.68	1.39	14	1.40
3	0.49	1.54	43	1.53
6	0.39	1.66	58	1.60
9	0.28	1.70	76	1.68
12	0.19	1.77	89	1.74

[a] **1**, 1.57×10^{-4} *M*. [b] 4×10^{-5} *M*. [c] In C_6H_6:CH_3OH (80:20); irradiated through $K_2Cr_2O_7$ filter; see ref 1. [d] Calculated from decrease in absorbance at 345 nm. [e] Calculated on assumption that reaction **1** → **2** is quantitative.

The carotene was isolated (in 80% yield) from an isomerization run under the conditions of the quenching experiments[1] (containing 2-methyl-2-pentene) by $Ca(OH)_2$ chromatography, and was found to be pure all-*trans*-β-carotene by melting point and mixture melting point, R_f, uv–visible spectrum, and ir spectrum (absence of cis band[6] at 772 cm^{-1}). Other cis isomers were shown to be absent by tlc (isomerization of **2** with I_2 produces a mixture which contains several other cis isomers which are readily separable by chromatography[7]). Similar experiments using all-*trans*-β-carotene gave no isomerization. From the amount of 2-methyl-2-pentene peroxide produced in photooxygenation experiments[1,4] and the rate of isomerization, it can be calculated that each singlet oxygen molecule produced causes isomerization of slightly less than one molecule of **1** to **2**.

A similar series of experiments was carried out using $^1O_2$ generated from NaOCl-H_2O_2[8] in C_6H_6:CH_3OH:diglyme (1:1:1);[4] all experiments were carried out in darkness to avoid photoisomerization of carotenes. As in the photochemical experiments, **1** underwent smooth isomerization to the all-trans isomer (identified as above), but **2** was not isomerized detectably under identical conditions. No isomerization of **1** occurred on addition of spent reagents nor of H_2O_2 alone. The initial efficiency of isomerization

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(1) Paper X: C. S. Foote, Y. C. Chang, and R. W. Denny, *J. Amer. Chem. Soc.*, **92**, 5216 (1970). Contribution No. 2570; supported by a grant from the USPHS-NAPCA (No. AP-00681).

(2) (a) L. Zechmeister, "*Cis-trans*-Isomeric Carotenoids, Vitamins A and Arylpolyenes," Academic Press, New York, N. Y., 1962, pp 46–57; (b) H. Claes and T. O. M. Nakayama, *Nature (London)*, **183**, 1053 (1959); (c) H. Claes, *Biochem. Biophys. Res. Commun.*, **3**, 585 (1960); (d) H. H. Inhoffen, F. Bohlmann, K. Bartram, G. Rummert, and H. Pommer, *Justus Liebigs Ann. Chem.*, **570**, 54 (1950).

(3) L. Zechmeister, A. LeRosen, W. A. Schroeder, A. Polgár, and L. Pauling, *J. Amer. Chem. Soc.*, **65**, 1940 (1943).

(4) C. S. Foote and R. W. Denny, *ibid.*, **90**, 6233 (1968).

(5) β-Carotene (Eastman Organic Chemicals) was recrystallized from benzene-methanol (mp 180–181°). 15,15′-*cis*-β-Carotene was kindly supplied by Dr. U. Schwieter, Hoffmann-LaRoche, Basel, and had mp 145°, with resolidification and melting at 167–170°. (The material as used subsequently had undergone some (~10%) isomerization to all-*trans*-β-carotene.)

(6) O. Isler, *Helv. Chim. Acta*, **39**, 249 (1956).

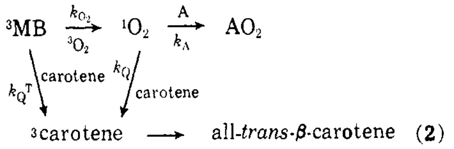
(7) A. Polgár and L. Zechmeister, *J. Amer. Chem. Soc.*, **64**, 1865 (1942).

(8) C. S. Foote, S. Wexler, W. Ando, and R. Higgins, *ibid.*, **90**, 975 (1968).

of **1** (moles of **1** isomerized/moles of $^1O_2$ generated[9]) was 36%.

An attempt to measure the singlet O_2 quenching rate of **1** failed because complete isomerization to **2** occurred before sufficient product was formed from 2-methyl-2-pentene to be detected accurately by the usual techniques. The overall quenching rate measured in these runs agreed well with that previously determined for all-*trans*-β-carotene.

These results and those in the accompanying communication[1] support the mechanism suggested[4] for carotene quenching of singlet oxygen, in which energy transfer from $^1O_2$ to the carotene occurs, as shown below.



In this scheme, all terms have the previous meaning;[1] carotene is either *cis*- or *trans*-β-carotene, and ³carotene is a carotene triplet. Radiationless decay processes for ³MB and $^1O_2$ do not compete under these conditions and are omitted for clarity. ³Carotene must collapse largely to all *trans*-β-carotene, whether formed from **1** or **2** initially.

The presence or absence of O_2 was found not to affect the rate of photochemical isomerization, which is consistent with the above scheme since $k_Q[\text{car}] \gg k_A[A]$.[1] On the other hand, if isomerization were caused only by ³MB, O_2 would inhibit the reaction whereas if $^1O_2$ were the sole cause, isomerization would not occur under N_2.[10] In O_2 saturated solutions, isomerization occurs entirely by way of $^1O_2$, since $k_Q{}^T[\text{car}] \ll k_{O_2}[O_2]$.

The dependence of quenching rate on the length of the conjugated chain[1] is consistent with the above scheme if the compounds with 5 and 7 double bonds have triplet energies above 22 kcal (the energy of $^1\Delta_gO_2$) but below that of methylene blue. The triplet energy of the 9-double bond carotenoid would be near 22 kcal, and that of β-carotene (11 C=C) would be lower. No reliable energy data for any of these compounds are available.[11]

Alternative mechanisms are not ruled out. For example, reversible electron transfer from carotene to $^1O_2$ could accommodate both the isomerization results and the chain-length dependence. Such mechanisms are well established for quenching,[13] and the ionization potential of carotenoids should increase with decreasing conjugation, so that the rate should drop off, though perhaps not so sharply; furthermore, the carotene radical cation should isomerize relatively easily (although one might expect it to undergo other reactions as well). However, the energy-transfer mechanism seems more satisfactory.

The quenching of $^1O_2$ by carotenes not only explains at least part of the protective action of carotenes in natural systems, but also implicates singlet oxygen as a causative agent in photodynamic action, though probably not the sole one.

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[9] Calculated by oxidizing 2-methyl-2-pentene under identical conditions, determining the amount of product produced,[1,4] correcting for the trapping efficiency by this olefin at the concentration used, and back-calculating to give the yield of $^1O_2$ based on NaOCl (50% in this solvent, 0°, 0.76 *M* 2-methyl-2-pentene, 0.11 *M* H_2O_2).

[10] Isomerization of two poly-*cis*-lycopenes is reportedly inhibited by O_2.[2b]

[11] The emission from β-carotene which was reported[12] is due to an impurity: P.-S. Song, private communication.

[12] R. J. Cherry, D. Chapman, and J. Langelaar, *Trans. Faraday Soc.*, **69**, 2309 (1968).

[13] C. S. Foote, *Science*, **162**, 963 (1968); N. J. Turro, *Photochem. Photobiol.*, **9**, 555 (1969).

Conformational Aspects of Polypeptide Structure. XXXI. Helical Poly[(*S*)-thiazolidine-4-carboxylic acid] and Poly[(*S*)-oxazolidine-4-carboxylic acid]. Theoretical Results

Sir:

Conformational analysis of polypeptides has proven to be extremely useful in confirming and predicting ordered structures for isolated polymer chains.[1-3] We have calculated the structure for poly[(*S*)-thiazolidine-4-carboxylic acid], and believe that this polypeptide assumes a helical conformation. The structural relationship of this peptide to poly-L-proline is clear (Figure 1).

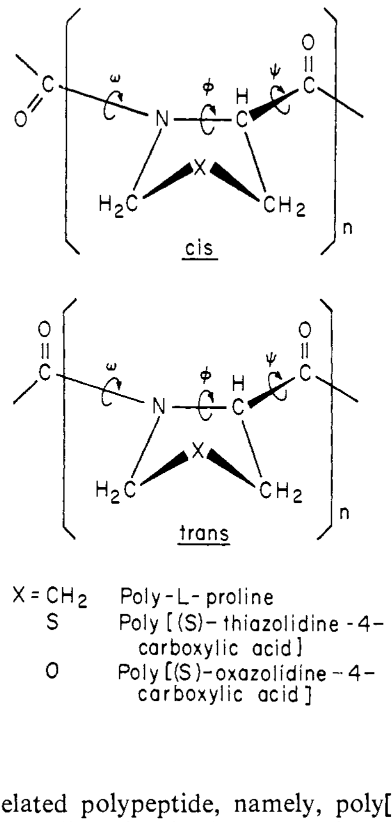

Figure 1.

Another related polypeptide, namely, poly[(*S*)-oxazolidine-4-carboxylic acid], is also under investigation in our laboratory. Although we have not succeeded in

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[1] (a) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966); (b) *ibid.*, **46**, 4410 (1967).

[2] G. N. Ramachandran and C. M. Venkatachalam, *Biopolymers*, **6**, 1255 (1968).

[3] (a) J. E. Mark and M. Goodman, *J. Amer. Chem. Soc.*, **89**, 1267 (1967); (b) J. E. Mark and M. Goodman, *Biopolymers*, **5**, 809 (1967); (c) A. M. Liquori and P. DeSantis, *ibid.*, **5**, 815 (1967); (d) P. R. Schimmel and P. J. Flory, *Proc. Natl. Acad. Sci. U. S.*, **58**, 52 (1967).