ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Vol. 49 N° 1-S, 1999

Lycopene entrapped in human albumin protects 2'- deoxyguanosine against singlet oxygen damage

Lydia F. Yamaguchi, Glaucia R. Martinez, Luiz H. Catalani, Marisa H.G. Medeiros, Paolo Di Mascio

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brasil.

SUMMARY. The generation of electronically excited molecular oxygen $^1O_2$ has been shown to occur in several biological systems, such as photooxidation of a variety of biological compounds and xenobiotics ("photodynamic action") and also enzymatic reactions. The high reactivity of $^1O_2$ with unsaturated compounds, sulfides and amino groups arises from its electrophilicity and relatively long lifetime. Thus, biological targets for $^1O_2$ having the above functional groups include unsaturated fatty acids, proteins, enzymes and DNA. There is interest in the role of nutrition in the prevention and pathogenesis of cancer. Epidemiological studies in humans have suggested that carotenoids aid in cancer prevention. Lycopene and oxycarotenoids are present at significant levels in cells and plasma. Extensively conjugated biomolecules such as carotenoids act largely on physical quenching of $^1O_2$ and in much lesser extent on chemical reaction. In this study we observed the protective effect of ß-carotene and lycopene entrapped in human albumin (HSA) against the oxidative $^1O_2$ attack of 2'-deoxyguanosine (dGuo). Photosensitization with methylene blue associated with Chelex® resine or Polymer-Rose bengal (Sensitox®) and thermodecomposition of water-soluble endoperoxide 3,3'-(1,4-naphthylidene) dipropionate were employed to generate $^1O_2$. The detection of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) and 4-hydroxy-8-oxo-7,8-dihydro-2'-deoxyguanosine (4-OH-8-oxodGuo) were performed using reversed phase HPLC with UV, electrochemical detection and by electrospray ionization mass spectrometry. Results showed a significant decrease in the amount of 8-oxodGuo in the presence of lycopene. The percentages of 4-OH-8-oxodGuo and 8-oxodGuo measured were 50% and 70% lower than the control, respectively. These data indicate that carotenoids entrapped in albumin can be an efficient quencher of $^1O_2$ and may be of interest in protecting against the deleterious effect of this excited state molecule.

Key words: Singlet oxygen, carotenoids, lycopene, 2'-deoxyguanosine, DNA, mass spectrometry.

RESUMEM. Lycopeno "atrapado" en la albúmina humana protege el 2'- deoxyguanosina contra el daño de singletes de oxígeno. La generación de singletes de oxígeno molecular ($^1O_2$) ha sido mostrada en sistemas biológicos, tales como en la fotooxidación de una variedad de compuestos biológicos y xenobióticos ("acción fotodinámica") y también en reacciones enzimáticas. La alta reactividad que presenta el $^1O_2$ con compuestos no saturados, sulfuros y grupos aminos es debido a su electrofilicidad y tiempos de vida relativamente altos. Así, compuestos biológicos que contengan estos grupos funcionales que incluyan ácidos grasos, proteínas, enzimas y DNA interactuan con singletes de oxígeno. Es interesante hacer notar el rol de la nutrición en la prevención y patogénesis del cáncer. Estudios epidemiológicos en humanos han sugerido que hay un importante rol de los carotenoides en la prevención del cáncer. Compuestos como lipoproteínas y oxicarotenoides están presentes en niveles significantes en las células y el plasma. Biomoléculas que presentan dobles enlaces conjugados extensos como los carotenoides, actúan eficientemente como inhibidores físicos del $^1O_2$ y son mucho menos eficientes químicamente. En este estudio hemos observado el efecto protector del ß-caroteno y licopeno, "atrapados" en albúmina humana (HSA), al ataque oxidativo de $^1O_2$ a 2'-deoxiguanosina (dGuo). Para generar singletes de oxígeno hemos usado la fotosensibilización con azul de metileno asociado con resina Chelex®, o bien, rosa de bengala-polímero (Sensitox®) y la termodescomposición del endoperóxido soluble en agua 3,3'-(1,4-naftilideno) dipropionato. Se registró la detección de 8-oxo-7,8-dihidro-2'-deoxiguanosina (8-oxodGuo) y 4-hidroxi-8-oxo-7,8-dihidro-2'-deoxiguanosine (4-OH-8-oxodGuo) por medio de cromatografía HPLC en fase reversa, con detección UV electroquímica y "electrospray ionization mass spectrometry". Los resultados muestran una significante reducción de la cantidad de 8-oxodGuo en presencia de licopeno. Los porcentajes de 4-OH-8-oxodGuo y 8-oxodGuo fueron 50% y 70% menores que los controles, respectivamente. Estos datos indican que los carotenoides "atrapados" en albúmina pueden ser eficientes inhibidores del $^1O_2$, y de esta manera ejercer un efecto de protección frente al deterioro capaz de producir esta molécula en estado excitado.

Palabras clave: Singletes de oxígeno, carotenoides, licopeno, 2'-deoxiguanosina, DNA, espectrometria de masa.

INTRODUCTION

The higher occupied electronic level of molecular oxygen is constituted of two π^* orbital of the same energy, so-called degenerated, occupied by only two electrons. In ground state, each of these electrons lies in one π^* orbital and their spins are parallel; hence it is a triplet state $^3\Sigma^-_g$, noted $^3O_2$ (Figure 1). As a consequence, the direct oxygen reduction by two electrons

is spin-forbidden. The activation of oxygen to electronically excited states with antiparallel spin requires overcoming of spin restriction. The first excited singlet state, $^1\Delta_g$, has two electrons with opposite spins in the same π^* orbital. The next higher excited singlet state, $^1\Sigma^+_g$, has one electron in each degenerate π^* orbital with opposite spins. The energies of the first ($^1\Delta_g$) and the second ($^1\Sigma^+_g$) singlet state of oxygen are 22.5 and 37.5 kcal/mol above the ground state, respectively (1). The second singlet state is extremely short lived (10^{-11}s), being rapidly deactivated to the $^1\Delta_g$ state and does not seem to play an important role in the oxidation processes operating in condensed phase. Thus, the term $^1O_2$ usually stands for $^1\Delta_g$ O_2.

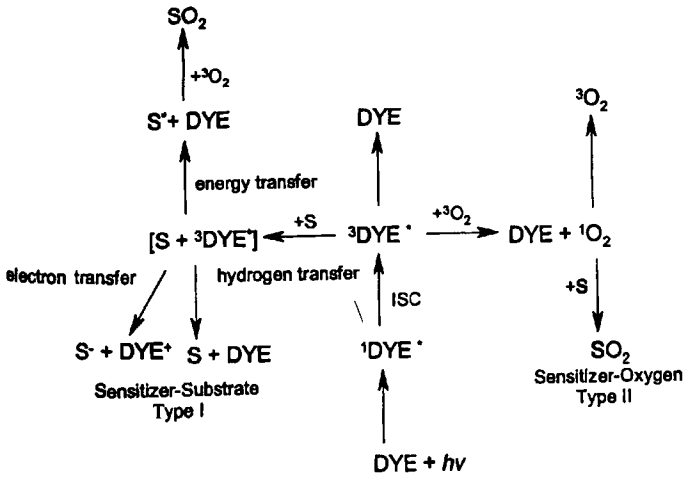
FIGURE 1

State	Orbital Occupancy	Energy (kcal/mol)	Lifetime (s)
Ground $^3\Sigma^-_g$	⟦↑ ↑⟧		
First $^1\Delta_g$	⟦↕ ○⟧	22.5	10^{-6}
Second $^1\Sigma^+_g$	⟦↑ ↓⟧	37.5	10^{-11}

Chemical and photochemical generation of singlet oxygen

Photosensitized generation of singlet oxygen: There are two fundamental types of sensitized photooxygenation (2,3). They differ in that the triplet sensitizer (DYE) reacts directly with the substrate in the Type I photoreaction, while in the second (Type II), it interacts first with oxygen to produce $^1O_2$ (Figure 2).

FIGURE 2



Type I chemistry usually involves the production of free radicals or radical-ions by interaction of the triplet sensitizer with a reducing substrate (SH or S). The produced radicals can undergo a wide variety of possible reactions such as insertion of oxygen or electron transfer to oxygen, electron or hydrogen abstraction from other substrates, initiation of free radical autoxidations, or back electron transfer reactions (3).

Gaseous discharge: The generation of $^1O_2$ by microwave discharge is well suited for studying gas phase reaction (4). The processes occurring in this system can be summarized in Scheme 1.

Scheme 1

$$^3O_2 \xrightarrow{\text{discharge}} {}^1O_2$$

$$^3O_2 \xrightarrow{\text{discharge}} 2O$$

The least desirable contaminants in this system are oxygen atoms and ozone. The main advantage of the microwave discharge method is the absence of the undesirable interactions of the substrate with an excited sensitizer or other chemical compounds, including solvent if the reaction is performed in the gas phase.

Thermal decomposition of organic ozonides: In 1969, Murray and Kaplan (5), using the triphenyl phosphite ozonide, provided experimental evidence for $^1O_2$ generation during the thermal decomposition of ozone-phosphite adducts (reaction 1).

$$(C_6H_5O)_3P\begin{smallmatrix}O\\ \diagup\ \diagdown\\ O\quad O\\ \diagdown\ \diagup\\ O\end{smallmatrix} \longrightarrow (C_6H_5O)_3P{=}O + {}^1O_2 \qquad \text{(reaction 1)}$$

The ozonide seems not to serve as a clean thermal source for $^1O_2$ at temperatures below -25°C.

Oxidation and disproportionation of superoxide anion: The possible generation of $^1O_2$ through oxidation or disproportionation of superoxide anion radical, $O_2^{\cdot-}$, led to contradictory results (6-8). Corey et al. provide evidence that $^1O_2$ *is produced through the oxidation of* $O_2^{\cdot-}$ *by various* oxidizers including Ce(IV), Pb(IV), iodobenzene and tetranitromethane in acetonitrile since the characteristic emission at 1270 nm could be recorded (9). These questions are important in biological systems since it had been recognized for a long time by Fridovich that $O_2^{\cdot-}$ is generated *in vivo* (10). The mechanism and kinetics for the oxidation of hydrogen peroxide by hypochlorite have been studied in detail (11) (reaction 2).

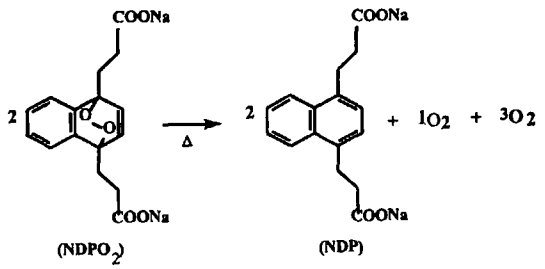
$$H_2O_2 + OCl^- \xrightarrow{HO^-} H_2O + Cl^- + {}^1O_2 \qquad \text{(reaction 2)}$$

Reaction of peroxynitrite with hydrogen peroxide: Peroxynitrite ($ONOO^-$) is a biologically active species produced

by reaction of the O_2·- with nitric oxide (NO·) (12). Nitric oxide, identified as the endothelium-derived relaxing factor (13), is formed by conversion of L-arginine to L-citrulline by NO· synthase. Endothelial cells, macrophages, neutrophils and neuronal cells have been shown to produce NO· and $ONOO^-$. Di Mascio et al. (14) propose that the peroxynitrite anion reacts with hydrogen peroxide releasing excited oxygen, once this reaction is accompanied by the characteristic chemiluminescence emission of $^1O_2$. An electronically excited intermediate of $ONOO^-$ may also be considered, *via* the reaction of the $ONOO^-$ with CO_2 (15).

Thermal decomposition of endoperoxides: The thermolysis of many endoperoxides of polycyclic aromatic compounds was suggested to occur *via* the generation of molecular oxygen and the parent aromatic species (16-19). Experimental proof for this reaction has been provided by Wasserman and Larsen (19) using 9,10-diphenylanthracene-9,10-endoperoxide. The endoperoxide is stable when stored in the solid state at 0 to 5°C, but dissociates at 80°C. The use of endoperoxide decomposition is advantageous as compared with other methods of singlet oxygen-driven oxygenations. The undesirable side-photoreactions associated with dye-photosensitization are avoided and there is no need to work at reduced pressure as in the microwave discharge methods or with low-temperature techniques as the triphenyl phosphite ozonide method. As a reproducible and clean source of $^1O_2$ for the investigation of the role of $^1O_2$ in biological systems, the use of the thermodissociation of the water-soluble endoperoxide of 3,3'-(1,4-naphthylidene) dipropionate ($NDPO_2$) is suitable. With this method, $^1O_2$ can be obtained in an easy and simple way without reactive intermediates or byproducts (reaction 3) (20). For example, this endoperoxide was used as a chemical source of $^1O_2$ to study oxidative damage to DNA (21-23).

(reaction 3)



Detection and identification of singlet oxygen

An important method for detection and characterization of $^1O_2$ is to measure the chemiluminescence arising from the radioactive transition of $^1O_2$ to the ground state. There are two types of chemiluminescence derived from $^1O_2$ (24).

Dimol emission: The bimolecular transition can be monitored by means of a red sensitive, thermoelectrically cooled photomultiplier tube connected to a discriminator, amplifier and recording system as developed by Boveris et al. (25) (reaction 4).

$^1O_2 + {}^1O_2 \rightarrow 2\ {}^3O_2 + h\nu$ (λ = 634 and 703 nm) (reaction 4)

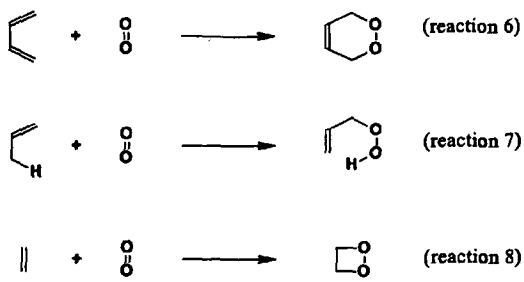
Dimol emission has often been used in complex systems such as enzymatic model reactions, suspensions of subcellular fractions and cells, perfused organ or *in situ* for an exposed organ (26).

Monomol emission: In 1979, Khan and Kasha (27) developed a spectroscopic instrumentation capable of direct solution spectral studies of $^1O_2$ emission, using a thermoelectrically cooled lead sulfite detector (reaction 5).

$^1O_2 \rightarrow {}^3O_2 + h\nu$ (λ = 1270 nm) (reaction 5)

The further development of a more sensitive spectrometer by Khan (28) based on a germanium diode photodetector and augmented the capability for examination of many reactions generating $^1O_2$. The intensity of this emission is directly proportional to the concentration of $^1O_2$, for example using the endoperoxide $NDPO_2$ (reaction 3) as a source of $^1O_2$ (20), and provides a measure of the amount produced.

Chemical traps: Trapping techniques are based on detection of the chemical product resulting from $^1O_2$ added to an appropriate substrate. The reactions of various types of substrates with $^1O_2$ are quite well established. They include the Diels-Alder reaction of dienes to form endoperoxides ([2+4] cycloaddition) (reaction 6) (29) and the "ene" reaction of alkenes to give allylic hydroperoxides (30) (reaction 7). In the $^1O_2$ "ene" reaction, olefins containing allylic hydrogens are oxidized to the corresponding allylic hydroperoxides in which the double bond is shifted to the adjacent position. In addition, $^1O_2$ reacts with electron-rich alkenes without allylic hydrogens or sterically hindered to form 1,2-dioxethanes ([2+2] cycloaddition) (reaction 8) (31).



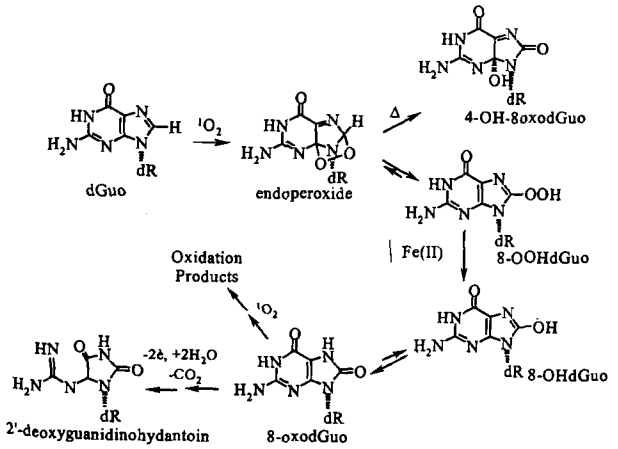
Use of deuterated solvent: The use of deuterated solvent as a tool to characterize the presence of $^1O_2$ has become universal. It is based on the remarkable fact that the lifetime of $^1O_2$ (τ) in D_2O is approximately 15-18 times longer than in water, and also longer in deuterated organic solvents (32). In cases where either $^1O_2$ and $O_2^{-\cdot}$ might be involved and the reaction is accompanied by product formation, the technique based on deuterated solvents can not be used because both $^1O_2$ and $O_2^{-\cdot}$ lifetimes are longer in those solvents (33).

Singlet oxygen in biological systems

Singlet oxygen is of substantial importance in chemical and biological systems due to its high reactivity and involvement in physiological and pathological processes. It has been shown to be generated in biological systems and implicated in (a) defense mechanisms of living organisms such as in phagocytosis, (b) hormonal activity of prostaglandins, (c) photochemotherapy utilizing the photodynamic action of synthetic dyes, (d) clinical manifestations of toxic agents like psoralens, and (e) inborn errors of metabolism exemplified by erythropoietic porphyria (34,35). The reactivity of $^1O_2$ with unsaturated compounds, sulfides and amino groups arises from its electrophilicity. Thus, biological targets for $^1O_2$ having the above functional groups include unsaturated fatty acids, proteins, enzymes and DNA. These lesions have been suggested to play an important role in aging, mutagenesis and carcinogenesis (35).

Singlet oxygen and 2'- deoxy guanosine: In DNA, $^1O_2$ reacts preferentially with 2'- deoxyguanosine (dGuo) residues, leading to the formation of at least four different reaction products: two 4R* and 4S* diasteromers of 4-hydroxy-8-oxo-7,8-dihydro-2'-deoxyguanosine (4-OH-8-oxodGuo), the main product, and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) (Scheme 2) (36,37).

Scheme 2



The formation of 4-OH-8-oxodGuo is prevented within double-stranded DNA. The reactivity of 8-oxodGuo toward $^1O_2$ is close to two orders of magnitude higher than dGuo yielding oxidation products as cyanuric acid (38). The oxidation of 8-oxodGuo via the Type I mechanism yielding 2'-deoxyguanidinohydantoin was also proposed (39).

Quenching process: Quenchers have also been employed as a means to identify $^1O_2$. Two types of quenching process may occur: chemical and/or physical (reactions 9 and 10, respectively). Chemical quenching has been already discussed as chemical trapping. Excited molecular oxygen can be physically quenched by two types of mechanism: electron transfer and energy transfer (40).

$$Q + {}^1O_2 \xrightarrow{kr} QO_2 \quad \text{(reaction 9)}$$

$$Q + {}^1O_2 \xrightarrow{kq} Q + {}^3O_2 + \text{heat} \quad \text{(reaction 10)}$$

Two classes of compounds, which apparently quench $^1O_2$ by energy transfer, are carotenoids (e.g. ß-carotene, lycopene, etc.) (41,42) and nickel complexes; in these cases the quenching rate constants ($k_t = k_r + k_q$) are *ca.* 10^{10} $M^{-1}s^{-1}$, that is slightly above those of diffusion controlled processes (43).

1,4-Diazabicyclo[2.2.2]octane, phenols, sulfides and azides are known to quench $^1O_2$ by a charge-transfer mechanism. For example, polyamines like spermine and spermidine, well known constituents of the eukaryotic chromatin, may also protect DNA against damage by $^1O_2$ (44, 45).

Carotenoids as singlet oxygen quencher

Numerous studies indicate that dietary carotenoids help reduce the risk of cancer, cardiovascular diseases, macular degeneration and cataracts (46). These are important plant pigments found in the photosynthetic pigment - protein complex of plants, photosynthetic bacteria, fungi and algae and are responsible for the bright colors of various fruits and vegetables. Several biological effects have been attributed to carotenoids. Lycopene is one of the major carotenoids in Mediterranean diets, found mostly in tomatoes and tomato products (47), and attracted attention due to its biological and physicochemical properties, especially those related to its effect as antioxidant. Color and antioxidant activities of carotenoids are a consequence of their structure, an extended system of conjugated double bonds. Carotenoids are tetraterpenes formed by tail-to tail linkage of two C-20 units, and in many carotenoids the end-groups are modified into five- or six-membered rings giving monocyclic or dicyclic compounds. Singlet oxygen quenching by carotenoids occurs via physical or chemical quenching. The efficacy of physical quenching greatly exceeds that of chemical quenching and involves the transfer of excitation energy from $^1O_2$ to the carotenoid, resulting in ground-state oxygen and excited triplet-state carotenoid (reaction 11). The energy is dissipated through rotational and

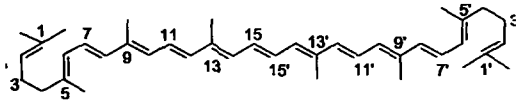
vibrational interaction between the excited carotenoid and surrounding solvent to yield the ground state carotenoid and thermal energy (reaction 12).

$^1O_2$ + Carotenoids $\rightarrow$ $^3O_2$ + ^{3} Carotenoids (reaction 11)

^{3} Carotenoids $\rightarrow$ Carotenoids + heat (reaction 12)

The quenching ability of carotenoid mainly depends on the number of conjugated double bonds and is influenced to a lesser extent by carotenoid end groups (cyclic or acyclic) or the nature of substituents in carotenoids containing cyclic groups. Lycopene (11 conjugated and two nonconjugated double bonds) is among the most efficient $^1O_2$ quenchers of the natural carotenoids (Figure 3) (42).

FIGURE 3



In the following experiments two different sources of $^1O_2$ were employed: (a) photosensitization with polymer-bound Rose bengal (Sensitox®) or with Chelex® resine-associated methylene blue (MB-Chelex®) through a type II photoreaction (Figure 2) (48); (b) thermal decomposition of the water-soluble endoperoxide $NDPO_2$ excluding the type I photoreaction (reaction 3) (20).

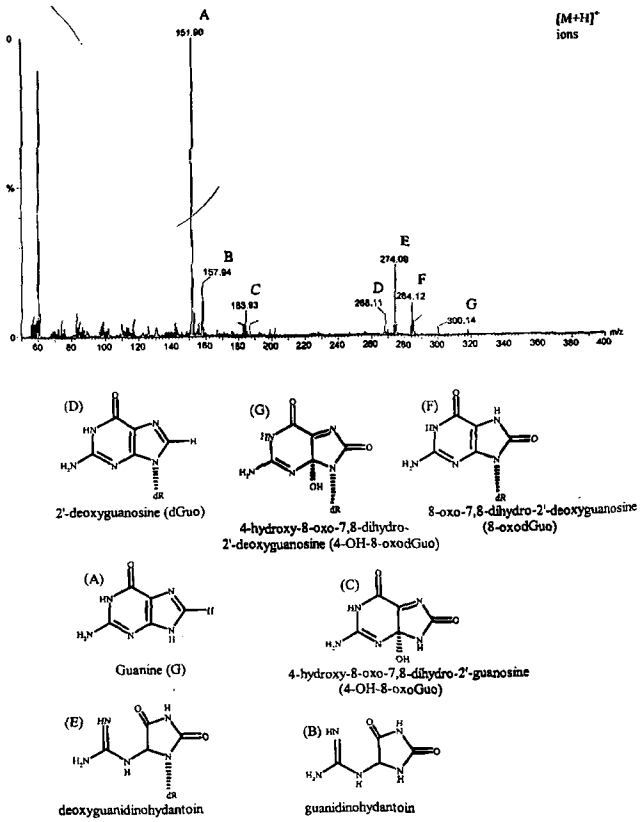
Experimental procedures

Carotenoids entrapped in human albumin: Carotenoids were dissolved in 100 µL of destilled THF/0.025% BHT. Five µL of this mixture was added to a solution of human albumin (HSA) (1 mg/mL) every 5 min for 20 min. The amount of carotenoid associated with albumin was determinated spectrophotometrically after extraction in chloroform (ε_{456} ß-carotene = 128,800 $M^{-1}cm^{-1}$ and ε_{473} lycopene = 199,526 $M^{-1}cm^{-1}$).

Detection of 8-oxodGuo and 4-OH-8-oxodGuo: The amount of 8-oxodGuo present in the solution was analyzed in a HPLC, Shimadzu (Kyoto, Japan) system, connected to a UV, set at 285 nm, and an electrochemical detectors at a potential of + 650 mV. A reversed phase column C-18 (Spherex, 250 x 4.6 mm, 5 µm) was used and the mobile phase was KH_2PO_4, 50 mM, pH 5.5 with 10% methanol and 2.5 mM EDTA (34). The 4-OH-8-oxodGuo was measured by HPLC and UV detector using a normal phase amino substituted silica gel Hypersil NH_2 column (250 x 4.6 mm, 5 µm) and a mobile phase consisting of a mixture of 25 mM ammonium formate and acetonitrile (40:60). Electrospray ionization mass spectrometry was also used to identify the oxidation products of dGuo after reaction with $^1O_2$. Samples were analyzed with a Quattro II (Micromass, Manchester, U.K.) mass spectrometer with an electrospray ion source.

A 10 µl sample of the mixture of 1mM dGuo and 1 O.D. (optical density) MB in H_2O, pH 7.0 irradiated for 30 minutes, was injected. Positive ion electrospray (LC-ESI-MS) were recorded at a capillary voltage of 3.5 kV, a cone voltage of 50 V and a source temperature of 80°C. Data were processed with MassLynx software. Positive-ion electrospray spectra exhibited $[M+H]^+$ ions. The mass spectrum obtained (Figure 4) exhibited peaks at m/z = 268.11, 284.12 and 300.14 attributed to $[M+H]^+$ of dGuo, 8-oxodGuo (49) and 4-OH-8-oxodGuo, respectively. The peak at m/z =274.09 is attributed to 2'-deoxyguanidinohydantoin (39, 50). The loss of the sugar ring produced peaks at m/z = 151.90, 157.94 and 183.93 corresponding to guanine, guanidinohydantoin, 4-OH-8-oxoGuo, respectively.

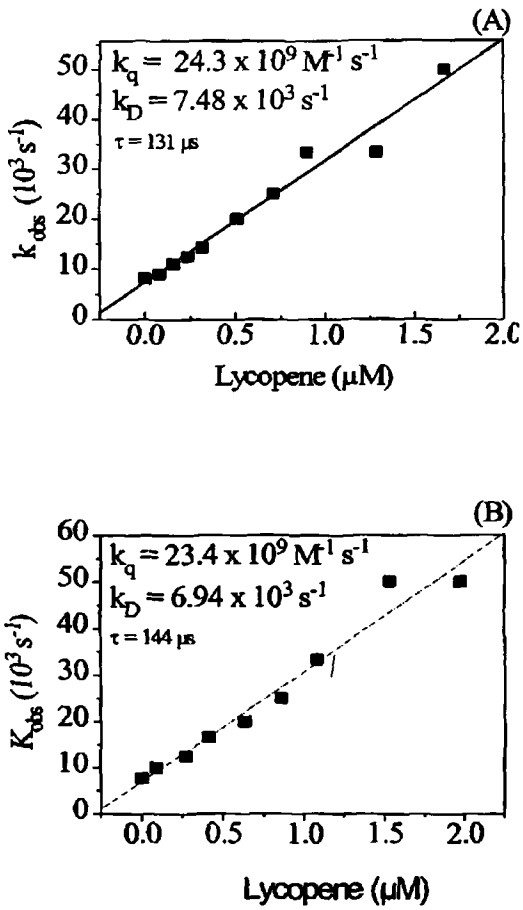
FIGURE 4



Reaction systems: System 1: Time-resolved infrared luminescence technique. Since $^1O_2$ can be generated by light excitation of a specific sensitizer in an oxygenated media, pulsed light sources have been used in order to generate a burst of such species. Then, its decay to ground state or products can be followed by any monitoring system that senses directly its

presence. By the end of the 70's, it became widely recognized that $^1O_2$ elicits phosphorescence at 1270 nm region due to its $^1\Delta g \rightarrow {}^3\Sigma_g^-$ transition (27,51) (reaction 5), with the use of fast response Germanium-based photodiodes coupled to amplifiers of appropriate bandwidth and gain, after a laser pulse excitation of a sensitizer. The most used excitation systems are Q-switched, frequency doubled or tripled Nd:YAG lasers (λ excitation = 532 and 355 nm, respectively). The signal from the diode can be fed into a box-car or transient digitizer oscilloscope. Precise determination of lifetimes ($k_D = 1/\tau$ $^1O_2$) provides an easy and accurate method to calculate quantum yields and quenching constants (kq) using Stern-Volmer plots. The kq is the slope of a plot of k_{obs} *vs* the concentration compound (K_{obs} is the observed rate constant) (Figure 5) (52). Time resolved near-infrared luminescence of $^1O_2$ was obtained by collecting the 1270 nm light emitted at right angle using a liquid nitrogen cooled germanium photodiode (EG&G Judson model J16D-M204-R05M-60), after excitation at 532 nm with a frequency doubled Q-switched Nd-YAG laser (1500 W) (Spectron Laser System). The $^1O_2$ was generated using tetraphenylporphyrin (7 ng/ml) in chloroform (53).

FIGURE 5



We investigated the quenching ability of lycopene (98% purity) (Figure 5A) and industrial tomato extracts containing 10% of lycopene in oleoresins (Figure 5B), applying the time-resolved infrared luminescence technique (52,53). Using the same concentrations of lycopene, the physical quenching rate constant (k_q) of the extracts was similar ($k_q = 24.3 \times 10^9$, $23.4 \times 10^9\ M^{-1}\ s^{-1}$, respectively), indicating that oleoresins present in the solution did not affect the quenching ability of lycopene (Figure 5). A singlet oxygen quenching constant of $31 \times 10^9\ M^{-1}\ s^{-1}$ was measured using the technique of steady state $^1O_2$ production by $NDPO_2$ and the monomol photoemission by Di Mascio et al. (41). Significant variations can arise when comparing rate constants determined by different methods. The value obtained with this technique is higher, probably due to the high lycopene quality (99.9 %).

System 2: Photosensitization with 2 mg of Sensitox® or MB-Chelex® was employed to generate $^1O_2$. One mM of dGuo was incubated in the presence of 1 mM of $FeSO_4$ in water at 37°C and irradiated during 30 minutes with a 50 W tungsten lamp placed 10 cm from the solution.

2'- Deoxyguanosine is the main target in DNA constituent that can be oxidized by $^1O_2$ through the type II photoreaction. So, this provides a good model system to evaluate the photoprotection of lycopene. Photosensitization of an aqueous solution of dGuo containing MB-Chelex® (Figures 6A, and B) or Sensitox® (Figure 7A) gave rise to the formation of 8-oxodGuo (Figure 6A, 7A and B) and 4-OH-8-oxodGuo (Figure 6B). In the presence of 300 μM lycopene associated to 0.5 mg/ml albumin, the yield of these products decreased as a function of time under irradiation due to the $^1O_2$ quenching effect of lycopene. Using MB-Chelex® and light as a source of $^1O_2$ and Calf Thymus DNA (CT-DNA) as a target, lycopene was also able to protect DNA against dGuo oxidation measured after CT-DNA hydrolysis (Figure 7B). A clear protection was observed over 10 min irradiation time.

System 3: Using the thermodissociation of 5mM $NDPO_2$ as a source of $^1O_2$, the incubation was performed in the presence of 1 mM of dGuo and different concentrations of ß-carotene or lycopene in phosphate buffer 0.1 M, pH 7.4 at 37°C (Figure 8).

In conclusion, lycopene and ß-carotene were entrapped in albumin (reactions 13 and 14) to make possible the interaction of these carotenoids with $^1O_2$ in aqueous solution. Lycopene was a better quencher of $^1O_2$ than ß-carotene in protecting dGuo from $^1O_2$ generated by $NDPO_2$, a chemical generator, or MB-Chelex®, a photosensitizer.

$^1O_2$ + Carotenoids-HSA→ $^3O_2$ + *Carotenoids-HSA (reaction 13)

*Carotenoids-HSA → Carotenoids-HSA + heat (reaction 14)

FIGURE 6

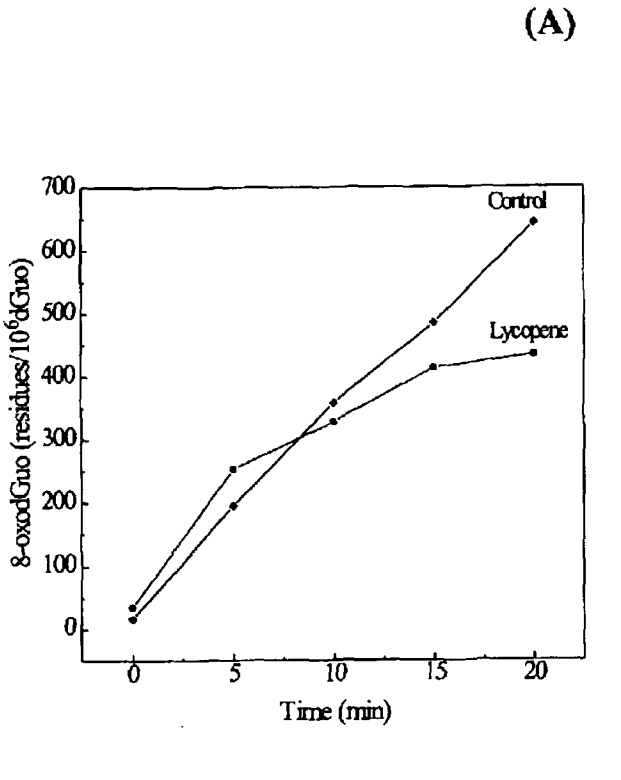


FIGURE 7

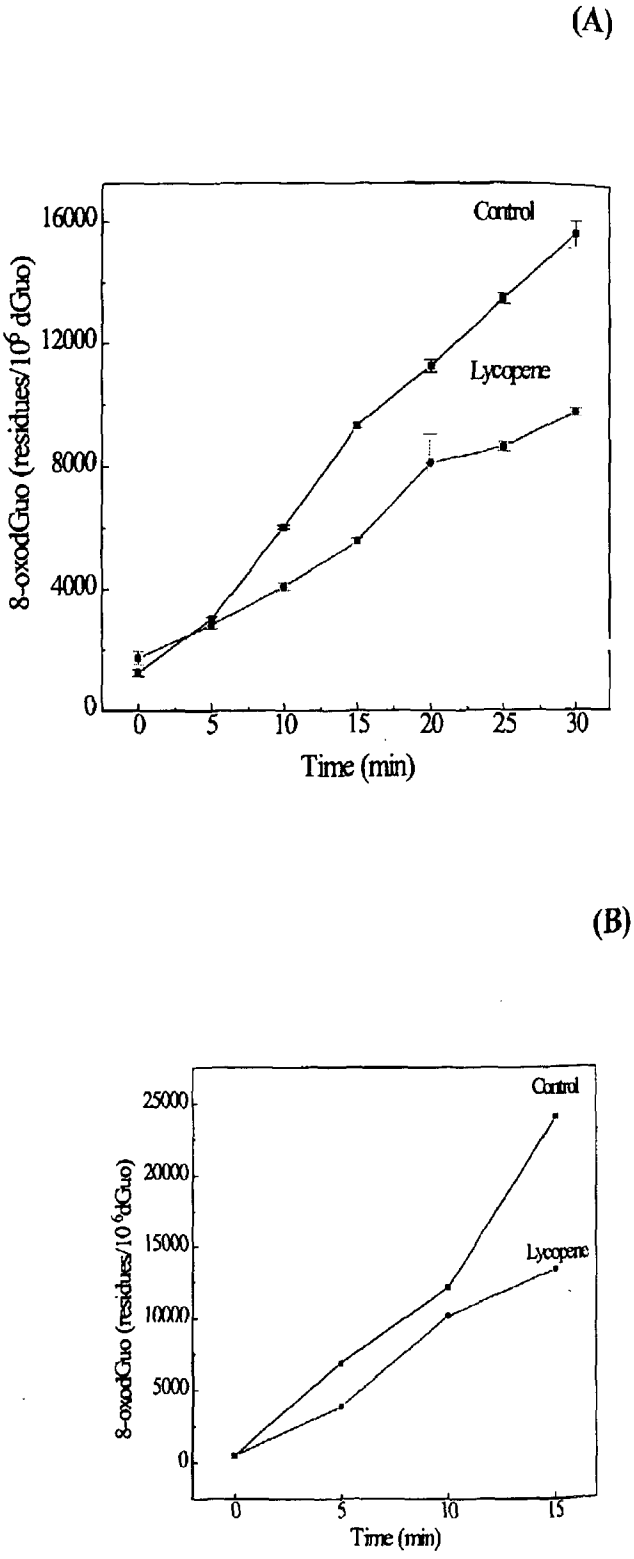
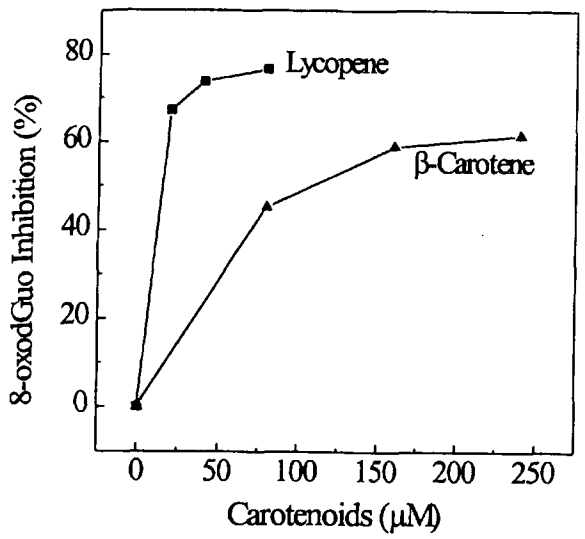


FIGURE 8



The quenching efficiency of lycopene may be of interest in protecting against $^1O_2$-induced damage of biological macromolecules like DNA, as may occur in lung oxidant injuries, skin photosensitivity, erythropoietic porphyria and toxicity of certain photosensitizers used in photochemotherapy.

ACKNOWLEDGMENTS

This work was supported by the "Fundação de Amparo à Pesquisa do Estado de São Paulo", FAPESP (Brazil), the "Conselho Nacional para o Desenvolvimento Científico e Tecnológico", CNPq (Brazil), and the "Programa de Apoio aos Núcleos de Excelência", PRONEX/FINEP, (Brazil). We thank Dr. Zohar Nir (Lycored Ltd; Israel) for kindly providing lycopene. L. F. Y. and G. R. M. are recipients of FAPESP and CNPq fellowships respectively. We are grateful to Dr. S. Bertolotti for the Summary translation.

REFERENCES

1. Kasha M. Singlet oxygen electronic structure and energy transfer. In: Frimer AA, editor. Singlet O_2 Vol I. Boca Raton: CRC Press, 1985:1-14.
2. Gollnick K. Photoxygenation reactions in solution. Adv Photochem 1968; 6:1-122.
3. Foote CS. Photosensitized oxidation and singlet oxygen: consequences in biological systems. In: Pryor WA, editor. Free radicals in biology Vol 2. NY: Academic Press, 1986: 85-133.
4. Ogryzlo EA. Gaseous singlet oxygen. In: Wasserman HH, Murray RW, editors. Singlet oxygen. NY: Academic Press, 1979:35-56.
5. Murray RW, Kaplan ML. Singlet oxygen sources in ozone chemistry. Chemical oxygenations using the adducts between phosphite esters and ozone. J Am Chem Soc 1969; 91: 5358-64.
6. Khan AU. Singlet molecular oxygen from superoxide anion and sensitized fluorescence of organic molecules. Science 1970; 168: 476-7.
7. Aubry JM, Rigaudy J, Ferradini C, Pucheault J. A search for singlet oxygen in the disproportionation of superoxide anion. J Am Chem Soc 1981; 103: 4965-6.
8. Corey EJ, Mehrotra MM, Khan AU. Water induced dismutation of superoxide anion generates singlet molecular oxygen. Biochem Biophys Res Commum 1987; 145: 842-6.
9. Corey EJ, Mehrotra MM, Khan AU. Antiarthritic gold compounds effectively quench electronically excited singlet oxygen. Science 1987; 236: 68-9.
10. Fridovich I, Handler P. Xanthine oxidase. IV. Participation of iron in internal electron transport. J Biol Chem 1958; 233: 1581-5.
11. Held AM, Halko DJ, Hurst JK. Mechanisms of chlorine oxidation of hydrogen peroxide. J Am Chem Soc 1978; 100: 5732-40.
12. Moro MA, Darley-Usmar VM, Goodwin DA, Read NG, Zamora-Pinto R, Feelisch M, et al. Paradoxical fate and biological action of peroxynitrite on human platelets. Proc Natl Acad Sci USA 1994; 91: 6702-6.
13. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-6.
14. Di Mascio P, Bechara EJH, Medeiros MHG, Briviba K, Sies H. Singlet molecular oxygen production in the reaction of peroxynitrite with hydrogen peroxide. FEBS Letters 1994; 355: 287-9.
15. Sharov VS, Driomina ES, Briviba K, Sies H. Sensitization of peroxynitrite chemiluminescence by the triplet carbonyl sensitizer coumarin-525. Effect of CO2. Photochem Photobiol 1998; 68: 797-801.
16. Moureu C, Dufraisse C, Dean PM. Sur un hydrocarbure coloré: le rubrène. C R Acad Sci 1926; 182: 1440-3.
17. Dufraisse C, Velluz L. L'union labile de l'oxygène au carbone: un peroxyde spontanément dissociable à froid. C R Acad Sci 1939; 208: 1822-4.
18. Rigaudy J, Guillaume J, Maurette D. Effet des substituants sur la photoxygénation des dérives aromatiques. Formation simultanée de photoxydes isomères en 1-4 et 9-10 à partir de diméthyl-1,4 -anthracènes. Bull Soc Chim Fr 1971; 144-52.
19. Wasserman HH, Larsen DL. Formation of 1,4-endoperoxides from the dye-sensitized photo-oxygenation of alkyl-naphthalenes. J C S Chem Comm 1972: 253-4.
20. Di Mascio P, Sies H. Quantification of singlet oxygen generated by thermolysis of 3,3'-(1,4-naphthylidene)dipropionate. Monomol and dimol photoemission and the effects of 1,4-Diazabicyclo[2.2.2]octane. J Am Chem Soc 1989; 111: 2909-14.
21. Sies H, Menck CFM. Singlet oxygen induced DNA damage. Mutation Res 1992; 275: 367-75.
22. Sies H. Damage to plasmid DNA by singlet oxygen and its protection. Mutation Res 1993; 299: 183-91.
23. Menck CFM, Di Mascio P, Agnez LF, Ribeiro DT, Costa de Oliveira R. Genetic deleterious effects of singlet oxygen. Quím Nova 1993; 16: 328-36.
24. Kasha M, Khan AU. The physics, chemistry, and biology of singlet molecular oxygen. Ann NY Acad Sci 1970; 171: 5-9.

25. Boveris A, Cadenas E, Chance B. Ultraweak chemiluminescence: a sensitive assay for oxidative radical reactions. Fed Proc 1981; 40: 195-8.
26. Cadenas E, Sies H. Low-level chemiluminescence as an indicator of singlet molecular oxygen in biological systems. Meth Enzymol 1984; 105: 221-31.
27. Khan AU, Kasha M. Direct spectroscopic observation of singlet oxygen emission at 1268 nm excited by sensitizing dyes of biological interest in liquid solution. Proc Natl Acad Sci USA 1979; 76: 6047-9.
28. Khan AU. Direct spectral evidence of the generation of singlet molecular oxygen ($^1\Delta_g$) in the reaction of potassium superoxide with water. J Am Chem Soc 1981; 103: 6516-7.
29. Bloodworth AJ, Eggelte HJ. Endoperoxides. In: Frimer AA, editor. Singlet O_2 Vol II. Boca Raton: CRC Press, 1985: 93-204.
30. Alder K, Pascher F, Schmitz A. Substituting additions I. Addition of maleic anhydride and azodicarboxylic esters to singly unsaturated hydrocarbons. Substitution processes in the allyl position. Ber Dtch Chem Ges 1943; 76: 27-8.
31. Baumstark AL. The 1,2-dioxetane ring system: preparation, thermolysis, and insertion reactions. In: Frimer AA, editor. Singlet O_2 Vol II. Boca Raton: CRC Press, 1985: 1-38.
32. Kajiwara T, Kearns DR. Direct spectroscopic evidence for deuterium solvent effect on the lifetime of singlet oxygen in water. J Am Chem Soc 1973; 95: 5886-90.
33. Bielski BHJ, Saito E. Deuterium isotope effects on the decay kinetics of perhydroxyl radical. J Phys Chem 1971; 75: 2263-6.
34. Sies H. Biochemistry of oxidative stress. Angew. Chem. (Inst. Ed. Engl.) 1986; 25: 1058-71.
35. Di Mascio P, Medeiros MHG, Bechara EJH, Catalani LH. Singlet molecular oxygen: Generation, reactivity, identification and biological effects. Ciênc C 1995; 47: 297-311.
36. Cadet J, Berger M, Douki T, Ravanat J-L. Oxidative damage to DNA: formation, measurement, and biological significance. Rev Physiol Biochem Pharmacol 1997; 131: 1-87.
37. Ravanat J-L, Cadet J. Reaction of singlet oxygen with 2'-deoxyguanosine and DNA. Isolation and characterization of the main oxidation products. J Chem Res Toxicol 1995; 8(3): 379-88.
38. Cadet J, Berger M, Douki T, Morin B, Raoul S, Ravanat J-L, Spinelli S. Effects of UV and visible radiation on DNA-final base damage. Biol Chem 1997; 378: 1275-86.
39. Goyal RN, Jain N, Garg K. Electrochemical and enzymatic oxidation of guanosine and 8-hydroxyguanosine and the effect of oxidation products in mice. Bioelectrochem Bioeng 1997; 43: 105-14.
40. Foote CS. Quenching of singlet oxygen. In: Wasserman HH, Murray RW, editors. Singlet oxygen. NY: Academic Press, 1979: 139-71.
41. Di Mascio P, Sundquist A, Devasagayam TA, Sies H. Assay of lycopene and other carotenoids as singlet oxygen quenchers. Meth Enzymol 1992; 213: 429-38.
42. Di Mascio P, Kaiser S,. Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989; 274: 532-8.
43. Rabek JF. $^1O_2$ oxidation of polymers and their stabilization. In: Frimer AA, editor. Singlet O_2 Vol IV. Boca Raton: CRC Press, 1985: 1-90.
44. Khan AU, Mei YH, Wilson T. A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen. Proc Natl Acad Sci USA 1992; 89: 11426-7.
45. Khan AU, Di Mascio P, Medeiros MHG. Wilson T. Spermine and spermidine protection of plasmid DNA against single-strand breaks induced by singlet oxygen. Proc Natl Acad Sci USA 1992; 89:11428-30.
46. Sies H, Stahl W. Lycopene: Antioxidant and biological effects and its bioavailability in the human. Proc Soc Exp Biol Med 1998; 218: 121-4.
47. Gerster HMA. The potential role of lycopene for human health J Am Coll Nutr 1997; 16, 109-26.
48. Greenwald RA. Handbook of methods for oxygen radical research. Boca Raton: CRC Press, 1985.
49. Ravanat J-L, Duretz A, Guiller T, Douki T, Cadet J. Isotope dilution high-performance liquid chromatography-electrospray tandem mass spectrometry assay for the measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine in biological samples. J Chromatogr B 1998; 715: 349-56.
50. Burrows CJ, Muller JG. Oxidative nucleobase modifications leading to strand scissions. Chem Rev 1998; 98: 1109-51.
51. Krasnovsky Jr AA. Photoluminescence of singlet oxygen in pigment solutions. Photochem Photobiol 1979; 29: 29-36.
52. Wilkinson F, Helman WP, Ross AB. Quantum yields for the photosensitized formation of the lowest electronically excited singlet state of molecular oxygen in solution. J Phys Chem Ref Data 1993; 22: 113-262.
53. Di Mascio P, Medeiros MHG, Sies H, Bertolotti S, Braslavsky SE, Veloso DP, et al. Quenching of singlet molecular oxygen by natural furan diterpenes. Photochem Photobiol 1997; 38: 169-73.