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Mechanism for the enhanced peroxidation of linoleic acid by a titanium dioxide/hypochlorite system

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ABSTRACT

Nanosized titanium dioxide (TiO_2) is a common component of sunscreen preparations and cosmetics as it reflects UV and visible light in accordance to Rayleigh's law. However, in aqueous environments, TiO_2 is an efficient photocatalyst, producing superoxide ($O_2^{\cdot-}$) and hydroxyl ($HO^{\cdot}$) radicals, which are highly damaging to biomolecules. We investigated the role of TiO_2 in promoting the peroxidation of linoleic acid (LA) alone and in the presence of hypochlorous acid (HOCl). TiO_2 significantly enhanced peroxidation of LA, which was further enhanced in the presence of HOCl. This latter finding involved the formation of singlet molecular oxygen in a Russell-type mechanism appearing to involve preformed lipid hydroperoxides (LOOH). In addition to lipid peroxidation, HOCl also mediated formation of 18:1 monochlorohydrins, which in the presence of TiO_2 appeared to decompose to kinetic products which supplemented peroxidation of linoleic acid. We present a theoretical mechanism which fits the available experimental data and may partially explain the dichotomy associated with HOCls role in lipid modification.

1. Introduction

Nanosized titanium dioxide (TiO_2) is a key component of many sunscreen formulations as it efficiently scatters UVB (290–320 nm) and UVA (320–400 nm) light in accordance to Rayleigh's law [1]. In addition to this, TiO_2 also absorbs UV light, which results in the promotion of electrons from the valence band to the conduction band, liberating an electron and leaving a positively-charged gap (the electron–hole) [2]. In an aqueous environment, liberated electrons can react with oxygen to form superoxide radicals ($O_2^{\cdot-}$) and the electron-hole interacts with water to form hydroxyl radicals ($HO^{\cdot}$), both of which damage macromolecular structures [3]. The formation of free radicals on the surface of nanosized TiO_2 is ostensibly circumvented in sunscreen preparations by coating with a photochemically inert layer of zinc or aluminium oxide. Yet, despite these precautions, there is evidence to suggest continued free radical formation [4].

The issue of radical formation on TiO_2 nanoparticles may be further complicated when one considers the interaction of waterproof sunscreens with hypochlorite anions OCl^-, formed when chlorate(I) salts are used to sanitise water in swimming pools. On reaction with water, chlorates liberate molecular chlorine (1), forming hypochlorous acid (2), in equilibrium with its conjugate base, hypochlorite (3). Hypochlorite ($E^{\ominus} = +1.49\ V$) is sufficiently reactive to reduce the superoxide anion to a more damaging hydroxyl radical (4) [5]. Since superoxide is formed on TiO_2, it follows that significant levels of hydroxyl radicals could be formed in a TiO_2/HOCl system, especially since halides have also been shown to accelerate the photocatalytic process [6]

$$4NaClO + 2H_2O \rightarrow 4NaOH + 2Cl_2 + O_2 \quad (1)$$

$$Cl_2 + H_2O \rightleftharpoons HOCl + HCl \quad (2)$$

$$HOCl \rightleftharpoons OCl^- + H^+ \quad (3)$$

$$HOCl + O_2^{\cdot-} \rightarrow HO^{\cdot} + O_2 + Cl^- \quad (4)$$

Hydroxyl radicals are believed to be the most toxic reactive oxygen species identified to date [7,8]; they are highly reactive and participate in hydrogen atom abstraction, addition reactions and one-electron transfers ($E^{\ominus} = +2.31\ V$) [9]. Of particular relevance to this investigation is the reaction of hydroxyl radicals with polyunsaturated fatty acids (PUFAs) such as linoleic acid (LA) during lipid peroxidation. Free radicals, such as hydroxyl radical, initiate lipid peroxidation by abstracting allylic hydrogen atoms from *cis–cis* pentadiene centres [10] forming a carbon-centred pentadienyl radical. This rapidly undergoes oxygen addition to produce a lipid peroxyl radical which serves as the chain carrying species in lipid peroxidation. This process produces a variety of conjugated diene hydroperoxides, including *E,E*-9-hydroperoxy-10,12-octadienoic

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acid (9-HPODE) and *E,E*-13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE). The lipid peroxidation chain reaction only ends through a termination reaction involving chain-breaking antioxidants or biradical quenching, or when the substrate has been exhausted [11].

Overall, lipid peroxidation is thermodynamically and kinetically favourable under physiological conditions (for linoleate, $\Delta E^{\ominus} = +1.71\ V$; $k = 6 \times 10^1\ M^{-1}\ s^{-1}$) [12]. Given the central role of oxygen for life and the wide distribution of lipids in cell membranes, lipid peroxidation is of significant biological importance [13]. In this work, we investigated the potential for TiO_2-catalysed formation of hydroxyl radicals to initiate lipid peroxidation and assessed the role of hypochlorite, which may be of relevance to research in premature ageing and cancer, as both these processes may involve TiO_2-mediated free radical damage.

2. Materials and methods

2.1. Preparation of reagents

All reagents were obtained from the Sigma–Aldrich Chemical Co. (Dorset, UK) unless otherwise stated. All aqueous solutions were prepared using Milli-Q double-deionised water (resistance > 18 mΩ/cm^2) (Millipore, MA, USA) stored over Chelex-100 resin to eliminate adventitial transition metal ions. A high SPF sunscreen was obtained from a local pharmacy and the TiO_2 content recovered using solvent extraction (acetonitrile/acetone/chloroform). The TiO_2 concentration was determined by complex formation with disodium 1,2-dihydroxybenzene-3,5-disulfonate (λ_{max} 404 nm; ε_{max} 827 $M^{-1}\ cm^{-1}$) using a Shimadzu UV–visible 240 spectrophotometer (Antwerp, BE) [14]. Sodium hypochlorite solutions were prepared fresh daily by appropriately diluting a stock solution (*ca.* 0.1 M in 0.1 M NaOH) in 50 mM phosphate buffer (pH 7.4), verifying its concentration through chloramine adduct formation with taurine [15]. Linoleate liposomes were prepared as previously described [16].

2.2. UV irradiation of TiO_2

TiO_2 (0–25 μg/mL) was dispersed in aqueous 4-nitrophenol (200 μM, pH 5.5), positioned 10 cm below a VL-15 M UVB source (Vilber Lourmat, Torcy, Fr) and irradiated at room temperature for 60 min. The UVB source had peak irradiance at 312 nm and the incident fluence rate was measured as 15–17 J $m^{-2}\ s^{-1}$ (UVX 31 Sensor; UltraViolet Products, Upland, CA). At appropriate time points, aliquots (1 mL) were removed, the hydroxylation reaction quenched by addition of 100 μL HCl (10 M) and the TiO_2 removed by filtration (0.1 μm). The product (1,2-dihydroxy-4-nitrobenzene) was extracted using 2 × 1 mL portions of diethyl ether, which were combined with 500 μL NaOH (0.1 M) and the absorption (λ_{max} 507 nm; ε_{max} $1.13 \times 10^4\ M^{-1}\ cm^{-1}$) assessed using diethyl ether as a blank.

2.3. Peroxidation of linoleate liposomes

Samples containing TiO_2 (0–25 μg/mL) and linoleate (0.16 mM) were irradiated as before in the presence or absence of HOCl (2.5 mM). Control samples were incubated in the dark but under otherwise identical conditions. Aliquots were removed at 5 min intervals and lipid peroxidation was terminated by addition of 20 μL of butylated hydroxytoluene (5 mM in MeOH). Levels of 9-/13-HPODE were assessed by reversed-phase HPLC (Perkin–Elmer Series 200 HPLC) on a C18 column (Phenomenex) using MeOH/ammonium acetate (10 mM, pH 5) (95/5% v/v) as mobile phase with post-column chemiluminescent detection [17].

2.4. Formation of 18:1 monochlorohydrin

Aliquots were removed as above and the fatty acid content transmethylated with BF_3/MeOH and derivatized by a standard method [18]. One microlitre of the *O*-methylester-*O*-TMS-ether derivative was resolved by GC (HP 5790 A Series Chromatograph) on a 25 μm fused silica column (Phenomenex) with helium carrier gas at a flow rate of 1 mL/min. The temperature of the oven was maintained at 60° C (2 min), followed by a linear increase of 40° C/min to 200° C. A second temperature ramp of 0.5° C/min was applied with an isothermal hold at 230° C for 15 min. The GC was linked to a mass spectrometer (HP 5970 A MSD), operating at 70 eV with an emission current of 200 μA and an ion source temperature of 150° C.

2.5. Formation of singlet oxygen

Formation of $O_2(^1\Delta_g)$ from preformed lipid hydroperoxides was assessed in samples containing linoleate (0.16 mM) and HOCl (2.5 mM) through monomolecular photoemission at 1270 nm using a photodiode detector (Edinburgh Instruments, Livingstone, UK) [19]. To verify the role of preformed lipid hydroperoxides, linoleate liposomes were prepared in the presence of triphenylphosphine (10 mM in MeOH), a reductant of lipid hydroperoxides [20].

3. Results and discussion

3.1. Characterisation of TiO_2 photocatalysis

TiO_2 was recovered from a homogeneous sample of commercially available sunscreen using mixed solvent extraction to remove all organic components. The recovered TiO_2 was standardised to six concentrations (0–25 μg/mL) and hydroxyl radical efflux for each TiO_2 concentration (irradiated at 312 nm for 60 min) assessed by following the formation of 1,2-dihydroxy-4-nitrobenzene from 4-nitrophenol (5).

$$TiO_2 + H_2O - (h\nu) \rightarrow HO^{\cdot} + C_6H_4NO_2OH \rightarrow C_6H_3NO_2(OH)_2 \qquad (5)$$

Under the experimental conditions employed $[C_6H_4NO_2OH] \ll [TiO_2]$ and therefore the concentration of $C_6H_3NO_2(OH)_2$ had a linear dependence on $[HO^{\cdot}]$ until all the substrate was consumed. Thus, the gradient (m) of a plot of absorbance *vs.* time (Fig. 1A) will equal the rate of reaction. Substituting dilution factor = 10, $\varepsilon = 1.13 \times 10^4\ M^{-1}\ cm^{-1}$ and $l = 1.0$ in (6) and differentiating both sides gives us an expression for the rate of reaction (7).

$$[C_6H_3NO_2(OH)_2] = \frac{A}{\varepsilon l} \qquad (6)$$

$$\frac{10A}{\varepsilon l}\frac{dA}{dt} = (8.85 \times 10^{-4})m \qquad (7)$$

From the stoichiometry of (5) the rate of formation of $C_6H_3NO_2(OH)_2$ is related to $[HO^{\cdot}]$ and m by (8). Thus, the rate constant (k_1) governing the formation of $[HO^{\cdot}]$ may be readily evaluated (9).

$$\frac{d[HO^{\cdot}]}{dt} = \frac{d[C_6H_3NO_2(OH)_2]}{dt} = (4.43 \times 10^{-4})m \qquad (8)$$

$$\frac{d[C_6H_3NO_2(OH)_2]}{dt} = k_1[C_6H_3NO_2(OH)_2]^a = (4.43 \times 10^{-4})m \qquad (9)$$

Results from kinetic experiments in which the concentrations of reactants were systematically varied demonstrated that m had a first order dependence on $[C_6H_3NO_2(OH)_2]$ (Fig. 1B). This gives a first order rate constant (k_1) of $5.21 \pm 0.14 \times 10^{-5}\ s^{-1}$, which is consistent with that available in the literature [21]. The significance of this value is that represents the rate constant for chain initiation

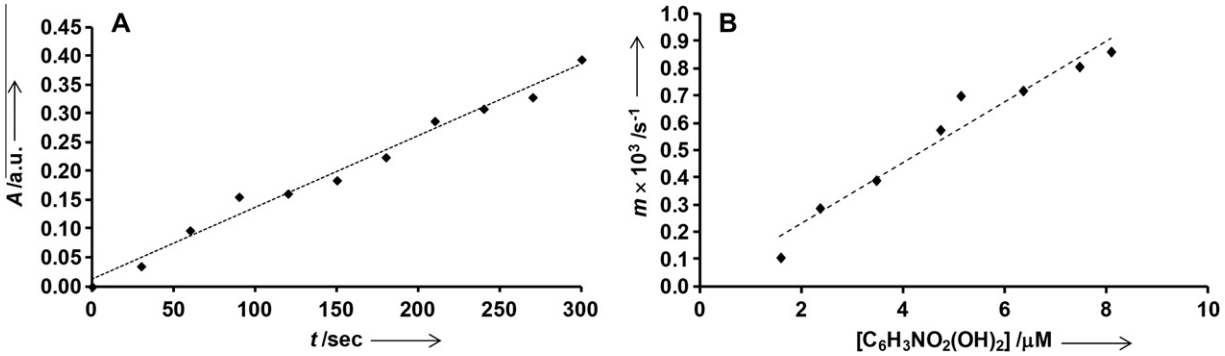


Fig. 1. A. Absorbance *vs.* time plot for production of 1,2-dihydroxy-4-nitrobenzene. B. First order dependence of rate on concentration of 1,2-dihydroxy-4-nitrobenzene.

(formation of lipid pentadienyl radicals) in the aqueous phase. Interestingly, this value is similar to that that reported for the more commonly used azo initiators, such as 2,2′azobis(2-amidoinopropane) dihydrochloride which has $k_1 = 1.36 \times 10^{-6}\ s^{-1}$ [22].

The ability of TiO_2 to photocatalytically degrade organic compounds was originally believed to proceed exclusively *via* $HO^{\cdot}$ formation. However, when rate constants for these process are compared, the rate constant for $HO^{\cdot}$ production on TiO_2 is much lower than that for the degradation of organic compounds, *e.g.* oxidation of propan-2-ol, $k = 2 \times 10^1$. This discrepancy may be due to formation of an oxygen anion radical ($O^{\cdot -}$) which is covalently bound to titanium atoms (e.g. $Ti^{IV}OTi^{IV}O^{\cdot}$ [23]. Taking this into consideration, it is possible that a portion of the 4-nitrophenol in our experiments was oxidised at this site, rather than undergoing 2-hydroxylation by liberated $HO^{\cdot}$. However, given that oxidation of 4-nitrophenol in this way is more likely to proceed *via* an *ipso*-attack to produce a 1,4-benzoquinone [24], the formation of 1,2-dihydroxy-4-nitrobenzene as a metric of $HO^{\cdot}$ formation is sufficient for our purposes and produced a value for k_1 consistent with that expected for aqueous-phase $HO^{\cdot}$ radical generating reactions.

3.2. Involvement of TiO_2 in lipid peroxidation

Sodium linoleate liposomes were prepared by quantitatively deprotonating linoleic acid with NaOH in borate buffer and storing under argon at 4 °C. Despite all reasonable precautions, a small degree of autooxidation occurred (<5%), verified by conjugated diene formation (λ_{max} 234 nm; ε_{max} $2.95 \times 10^4\ M^{-1}cm^{-1}$) and IR absorption [=3400, 1750 cm^{-1} (–OOH, C = O)]. However, such autooxidation is unavoidable under normal laboratory conditions and represents only a small portion of the total lipid substrate.

To determine the relationship between [TiO_2], HOCl and lipid peroxidation, the concentrations of 9-HPODE and 13-HPODE were measured by HPLC in the following experiments:

1. Control: TiO_2 (0–25 μg mL^{-1}), linoleate (0.16 mM) incubated at 37 °C in the dark ± HOCl (2.5 mM).
2. UVB irradiated: TiO_2 (0–25 μg mL^{-1}), linoleate (0.16 mM) irradiated at 312 nm ± HOCl (2.5 mM).
3. Kinetic control: TiO_2 (0–25 μg mL^{-1}), linoleate (0.16 mM), α-tocopherol (5 μM) incubated at 37 °C in the dark ± HOCl (2.5 mM).
4. Kinetic UVB irradiated: TiO_2 (0–25 μg mL^{-1}), linoleate (0.16 mM), α-tocopherol (5 μM) irradiated at 312 nm ± HOCl (2.5 mM).

The latter two experiments were necessary to evaluate k_5, the rate constant for the termination reaction, which is used in the steady state evaluation of the rate constant of propagation, k_3 (10) [25].

$$R_5 = k_3[LA]\sqrt{\frac{R_1}{2k_5}} \tag{10}$$

Results (Table 1, column A) clearly demonstrate a dose-dependent relationship between the rate of propagation and [TiO_2]. In control samples (incubated in the dark) the level of $HO^{\cdot}$ formation by photocatalytic breakdown of water should be minimal. However, peroxidation was still observed in these samples (Table 1, column B), suggesting either production of radical species independently of UV irradiation [26] or decomposition of preformed lipid hydroperoxides on the TiO_2 surface, which could form chain-carrying lipid peroxyl radicals. Given that the redox potential for TiO_2 is in the region of −0.20 V [27] and that of lipid hydroperoxides is +0.6 V, it is unlikely that TiO_2 could oxidise lipid hydroperoxides directly. However, it is also possible that lipid hydroperoxides could be absorbed onto surface sites on TiO_2, which could lower the activation energy and make the oxidation thermodynamically feasible.The rate of propagation in UVB irradiated samples was further enhanced by the presence of HOCl (Table 1, column C). This is consistent with the notion that HOCl produces $HO^{\cdot}$ in the presence of superoxide (4), a further product of TiO_2 photocatalysis. However, linoleate and HOCl incubated in the dark also exhibited marked peroxidation (Table 1, column D). This may be attributable to the phenomena discussed above, though the magnitude of peroxidation in these samples is higher, suggesting an alternative mechanism in which HOCl supplements the peroxidation process. The potential of HOCl to initiate lipid peroxidation is contentious. Nevertheless, it is well-established that in the presence of peroxides, HOCl can form singlet molecular oxygen $O_2(^1\Delta_g)$ (11) [28].

$$HOCl + H_2O_2 \rightarrow O_2(^1\Delta_g) + H_2O + H^+ + Cl^- \tag{11}$$

In linoleate liposomes, a potential source of peroxide is preformed lipid hydroperoxide (LOOH), which could react with HOCl in a Russell-type mechanism to form $O_2(^1\Delta_g)$ (12) [29]. Since we detected preformed lipid hydroperoxides in liposomes due to autooxidation, we explored formation of $O_2(^1\Delta_g)$ by this mechanism.

Table 1
Variation of the rate constant for propagation (k_3) with concentration of TiO_2 in the absence or presence of HOCl.[a]

TiO_2/μg μL^{-1}	$k_3/\times 10^3\ M^{-1}\ s^{-1}$			
	A (UV)	B (control)	C (UV)[b]	D (control)[b]
0	0.2 ± 0.1	0.00 ± 0.00	0.4 ± 0.1	0.4 ± 0.1
5.0	0.2 ± 0.0	0.04 ± 0.02	0.7 ± 0.2	0.6 ± 0.1
10.0	1.5 ± 0.1	0.04 ± 0.01	3.8 ± 0.4	1.7 ± 0.3
15.0	1.7 ± 0.2	0.06 ± 0.01	5.3 ± 0.3	2.4 ± 0.2
20.0	2.2 ± 0.1	0.09 ± 0.02	6.9 ± 0.2	3.1 ± 0.1
25.0	2.7 ± 0.3	0.09 ± 0.01	8.7 ± 0.2	3.9 ± 0.1

[a] Results are mean ± s.d. ($n = 6$).
[b] In the presence of 2.5 mM HOCl.

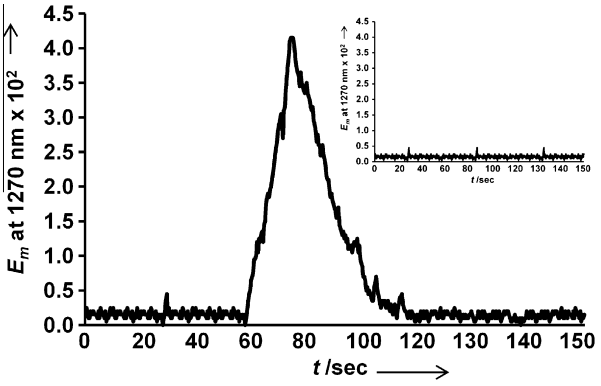


Fig. 2. Photoemission from reaction of HOCl and LOOH. HOCl was added to LA liposomes at 60 s, which was followed by rapid monomolecular emission at 1270 nm, corresponding to the decay $O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-) + h\nu$. However, when liposomes contained TPP, a reductant of LOOH, no photoemission was observed (inset).

$$2\mathrm{LOOH} \rightarrow [\mathrm{LOOOOL}] \rightarrow \mathrm{LO} + \mathrm{LOH} + \mathrm{O_2}(^1\Delta_g) \quad (12)$$

The monomolecular decay of $O_2(^1\Delta_g)$ is accompanied by emission of light in the IR region (13), which is readily quantified by photoemission counting at 1270 nm. Results (Fig. 2) demonstrate modest formation of $O_2(^1\Delta_g)$ in linoleate liposomes on addition of HOCl. However, when liposomes were prepared in the presence of triphenylphosphine (TPP), which reduces lipid hydroperoxides to the corresponding alcohol, formation of $O_2(^1\Delta_g)$ was abolished (Fig. 2, inset). This suggests that the level of peroxidation observed in control samples incubated in the dark was more than likely due to $O_2(^1\Delta_g)$.

$$2\mathrm{O_2}(^1\Delta_g) \rightarrow 2\mathrm{O_2}(^3\Sigma_g^-) + h\nu(1270\ \mathrm{nm}) \quad (13)$$

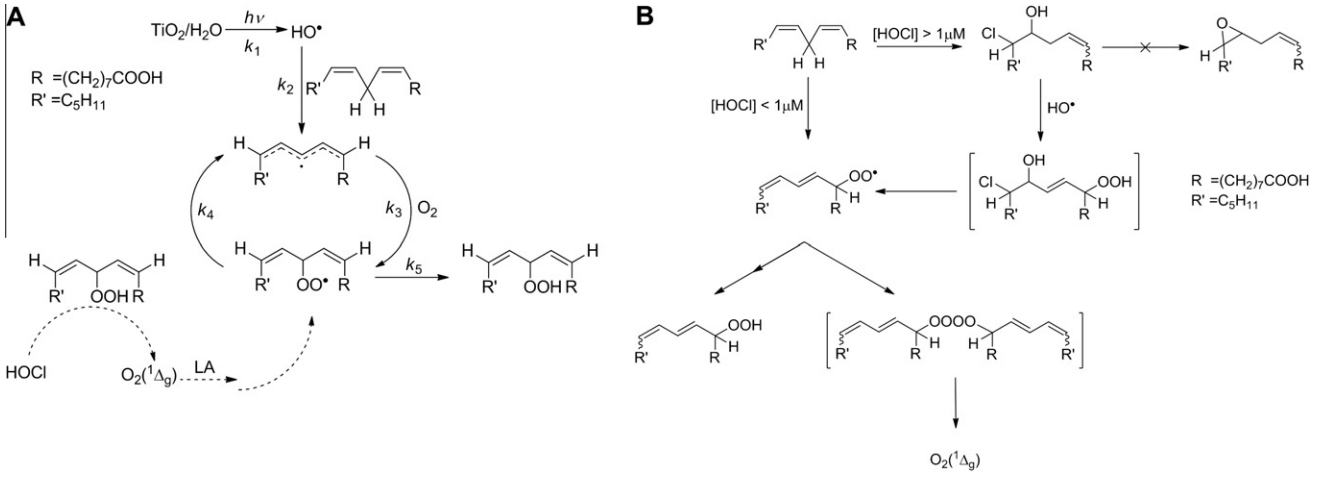
Peroxidation by $O_2(^1\Delta_g)$ occurs by a different mechanism from that of other radical species; it is not a free radical and the mechanism of peroxidation is based on electrophilic addition to double bonds. When PUFAs are oxidised by $O_2(^1\Delta_g)$, 9-HPODE and 13-HPODE are formed, and thus $O_2(^1\Delta_g)$ formation contributes to the overall value of k_3 (Scheme 1A).

3.3. Formation of 18:1 monochlorohydrins

Aside from its role in lipid peroxidation, HOCl is known to promote formation of lipid chlorohydrins in an electrophilic addition reaction [30]. This reaction is thermodynamically favourable under physiological conditions and thus raises the question of whether HOCl preferentially oxidises or chlorinates lipids. This has been addressed by a number of laboratories [31] and is investigated here as it is a significant reaction of HOCl. TiO_2 (0–25 μg mL^{-1}), linoleate (0.16 mM) and HOCl (2.5 mM) were irradiated as before for 30, 60 or 90 min and the concentration of 18:1 monochlorohydrin assessed by GC–MS of the *O*-methylester-*O*-TMS-ether derivative. For comparison, the levels of 9-HPODE and 13-HPODE were measured in the same samples using HPLC as before.

At all combinations of reactants assessed, the major GC peak eluted at 8.4 min and had a mass spectrum that contained several peaks characteristic of the fragmentation pattern of an 18:1 monochlorohydrin. Therefore, this GC peak was selected for measurements of the concentration of 18:1 monochlorohydrin using a linoleate monochlorohydrin standard. Results (Fig. 3A) demonstrated that control samples, incubated in the dark and in the presence of HOCl, maintained a fairly constant concentration of 18:1 monochlorohydrin, which is consistent with a radicalindependent mechanism. The levels of 9-HPODE and 13-HPODE in the same samples increased as before (Fig. 3B). These results imply that at a [HOCl] $\geqslant$ 2.5 mM, chlorohydrin formation proceeds independently of [TiO_2], suggesting that these compounds are not decomposed on the surface of TiO_2. Even though we did not assess the formation of *bis*chlorohydrins, the percent change displayed in Fig. 3A is lower than expected given that HOCl was in a 15-M excess to lipid. This may suggest some interaction between chlorohydrins and the TiO_2 surface. However, the relatively steady concentration of 18:1 monochlorohydrin does not fit with classical Langmuir absorption kinetics.

Samples which were irradiated at 312 nm displayed a decrease in 18:1 monochlorohydrins with increasing concentrations of TiO_2



Scheme 1. A. Peroxidation of linoleic acid (LA). Hydroxyl radicals (HO·) are initially formed on the surface of TiO_2 which abstract allylic hydrogen atoms from LA to form a resonance-stabilised pentadienyl radical. This undergoes O_2 addition, forming a peroxyl radical (LOO·), the chain-carrying species in lipid peroxidation. LOO· is sufficiently reactive to abstract a hydrogen atom from LA, forming a lipid hydroperoxide (LOOH) and a further pentadienyl radical. Alternatively, LOO· may undergo a termination reaction through biradical quenching, forming a hydroperoxide. The formation of $O_2(^1\Delta_g)$ (dotted lines) may arise from reaction of HOCl with LOOH; singlet oxygen so-formed may then undergo electrophilic addition to LA, forming LOO· and thus accelerating the rate of peroxidation. B. Proposed pathway for lipid hydroperoxide formation from fatty acid chlorohydrins. At high [HOCl], mono- and *bis*-chlorohydrins preferentially form *via* a chloronium ion intermediate. In the presence of an additional radical source (e.g. the TiO_2 system), the chlorohydrin may undergo electrophilic addition of *HO*·, forming a lipid chlorohydrin peroxide intermediate. This may then form a lipid hydroperoxide or participate in a Russell-like mechanism to produce singlet oxygen.

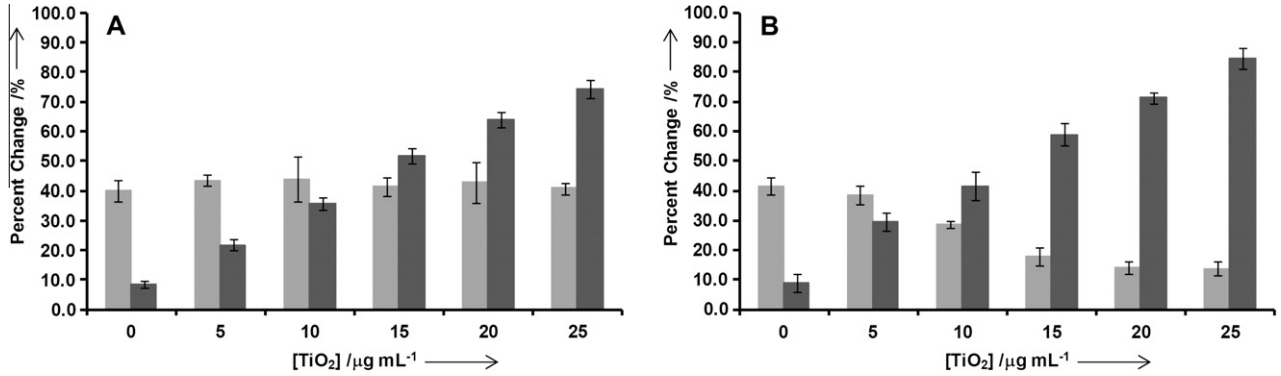


Fig. 3. A. Percent change in 18:1 monochlorohydrin (light grey) and lipid hydroperoxides (dark grey) for various concentrations of TiO_2 incubated in the dark. B. Percent change in 18:1 monochlorohydrin (light grey) and lipid hydroperoxides (dark grey) for various concentrations of TiO_2 irradiated at 312 nm. Results presented are the mean of the three time points examined (three replicates of each).

(Fig. 3B) while the levels of 9-HPODE and 13-HPODE increased more than observed in control samples (Fig. 3B). These latter findings may support the decomposition of chlorohydrins on the surface of TiO_2, or alternatively, they may imply either consumption of chlorohydrins or conversion to epoxides. We assessed the latter by FT-IR, which revealed no significant absorption at 915 cm^{-1} (the region characteristic of epoxides). Furthermore, the generation of an epoxide would not account for the observed formation of $O_2(^1\Delta_g)$, as we were unable to detect $O_2(^1\Delta_g)$ release on reaction of 9,10-epoxy-12-octadecenoic acid with HOCl. Therefore, we assume (*a priori*) that in the presence of an additional radical source, chlorohydrins are consumed in a peroxidation reaction ultimately producing $O_2(^1\Delta_g)$.

Given that both peroxidation and chlorination are thermodynamically feasible under these conditions, it would appear that kinetic factors ultimately dictate HOCls preferred pathway. As a potential model, the reaction of HOCl with pentenoic acid proceeds in a simple pseudo first order reaction with $k = 8.7 \pm 1.9\ M^{-1}\ s^{-1}$ [32]. Given that the rate of peroxidation is slower, it seems likely that chlorohydrins are the initial species formed. For linoleic acid, the likely structure of the 18:1 monochlorohydrin is shown in Scheme 1B. This is based on the understanding that the chloronium ion forms at the least substituted carbon of the double bond, while the hydroxyl group adds to the most substituted carbon of the same bond. We speculate that in the presence of $HO^{\cdot}$, a chlorohydrin hydroperoxide intermediate could form, which decomposes to a peroxyl radical. Although a peroxyl radical could feed directly into the classic propagation cycle of lipid peroxidation, this does not account for formation of $O_2(^1\Delta_g)$. Therefore, we suggest that at least a portion of the peroxyl radicals combine in a Russell mechanism to produce $O_2(^1\Delta_g)$.

We have demonstrated that commercial grade titanium dioxide can produce hydroxyl radicals when exposed to UV light, despite being coated with a layer of ZnO or Al_2O_3. Furthermore, in the presence of aqueous hypochlorite anions, peroxidation of linoleate liposomes was supplemented by electrophilic oxidations mediated by singlet oxygen. This, in combination with the formation of 18:1 monochlorohydrins, suggests that current sunscreen formulations may not fully protect against premature ageing or skin cancer. These findings may go some way to explain the dichotomy associated with lipid modification by HOCl as it would seem that in the presence of an additional radical source, fatty acid chlorohydrins supplement lipid peroxidation.

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