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PERSPECTIVE

UVA and endogenous photosensitizers – the detection of singlet oxygen by its luminescence†

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UVA irradiation (320–400 nm) comprises about 95 percent of incident midday solar ultraviolet irradiation. It penetrates skin much deeper than UVB irradiation. The absorption of UVA irradiation in endogenous chromophores frequently leads to the generation of reactive oxygen species such as singlet oxygen ($^1O_2$). $^1O_2$ is an important biochemical intermediate in multiple biological processes. Beside other procedures, the direct detection of $^1O_2$ by its luminescence is a powerful tool that helps to understand the generation of $^1O_2$ during UVA exposure in solution, *in vitro* and *in vivo*. This article describes the endogenous photosensitizers, their ability to generate $^1O_2$ under UVA irradiation, and the detection technology to visualize the action of $^1O_2$.

1. Introduction

Solar UV radiation causes many adverse effects in tissue such as skin, which can be attributed to DNA damage. Consequently, skin cancer has accounted for about 40 percent of all cancers in the United States and their frequency has been increasing.[1] UVB (280–320 nm) is directly absorbed by cellular DNA, resulting in dipyrimidine lesions that include cyclobutane pyrimidine dimers (CPD), especially thymine dimers or pyrimidine photoproducts.[2]

The major component of solar radiation (~95%) is UVA (320–400 nm), which has been shown to produce likewise deleterious biological effects.[3] Exposure to UVA irradiation has been recognized as a source of aging of eye lens proteins and as a risk factor for cataract formation.[4] Revisiting the photochemistry of solar UVA in human skin, it was stated that the importance of UVA in skin cancer is undeniable.[5,6] Cyclobutane pyrimidine dimers were detected in significant yield in whole human skin exposed to UVA radiation.[7] UVA irradiation is also responsible for the most frequent photo-dermatosis of the skin.[8] Recent epidemiological data showed that UVA radiation is even involved in the genesis of cutaneous melanoma.[9]

UVA radiation can penetrate deeper into tissue than UVB, which may lead to interaction with more tissue constituents as compared to UVB. However, UVA radiation is not sufficiently absorbed in proteins or DNA, which leads to different mechanisms of action in tissue for UVA and UVB. It is known that also UVA radiation leads to oxidative damage, single- and doublestrand breaks, produces secondary photoreactions, damages DNA by indirect photosensitizing reactions, and induces the photoproduct 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8oxoG) in human skin.[10] Thus, UVA radiation acts indirectly by producing reactive oxygen species (ROS), in which the highly reactive singlet oxygen ($^1O_2$) plays a major role.[3]

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† Contribution to the themed issue on the biology of UVA.

2. Photodynamic generation of singlet oxygen

The first prerequisite of photodynamic generation of $^1O_2$ is the absorption of radiation in molecules (Fig. 1). These could be either endogenous photosensitizers or exogenous molecules, which are accidentally present in UVA exposed skin.[11,12] Many molecules convert the absorbed energy simply to heat. However, after light absorption, some molecules can effectively cross over to a long-lived triplet T_1 state. This is known as intersystem crossing (ISC). In contrast to the short-lived singlet S_1 state, which shows a lifetime in the order of nanoseconds, the triplet T_1 state is long-lived with lifetimes in the order of microseconds to milliseconds. This allows an efficient transfer of energy or charge to substrate or molecular oxygen generating radicals or $^1O_2$, respectively (Fig. 2).

These specific generation mechanisms of ROS comprise the same mechanisms as known in photodynamic therapy of tumors (PDT)[13] or in antimicrobial PDT (aPDT).[14] Those molecules in tissue that convert the energy to reactive oxygen species (ROS) are called endogenous photosensitizers. Some of the endogenous photosensitizers in cells or tissue are identified such as flavins,[15] NADH/NADPH,[16] urocanic acid,[15,17] sterols,[18] and anthraquinones.[19]

3. Detection of singlet oxygen

The detection of $^1O_2$, in particular when excited with UVA, is performed by using various methods that can be assigned to indirect and direct procedures. The main methods applied are the use of quenchers, measurement of $^1O_2$ luminescence, and electronic paramagnetic spin resonance (Table 1).

Table 1 Singlet oxygen generation and detection under UVA exposure

Authors	Photosensitizer	Environment	Detection
Zhang *et al.*[52]	thiopurine DNA bases	solution	luminescence
Musson *et al.*[50]	protein phosphatase calcineurin	*in vitro*	quencher
Thomas *et al.*[53]	pterins	solution	luminescence
Baier *et al.*[15]	urocanic acid, flavins	solution	luminescence
Avalle *et al.*[49]	lipofuscin	solution	luminescence
Lamore[51]	protein epitope dihydropyridine (DHP)-lysine	solution	RNO bleaching[b]
Yin *et al.*[54]	anhydroretinol	solution	EPR[a]
Agrawal *et al.*[25]	Ciprofloxacin	*in vitro*	RNO bleaching
Ray *et al.*[57]	fluoroquinolones	solution	RNO bleaching
Jantova *et al.*[56]	quinolones	*in vitro*	EPR
Barbierikova *et al.*[58]	Selenadiazoloquinolones	solution	EPR
Martinez *et al.*[60]	NSAID	solution	luminescence
Vargas *et al.*[61]	levomepromazine	solution	quencher
Bilski *et al.*[62]	antiepileptic drug Lamotrigine	solution	luminescence
Wolnicka *et al.*[63]	neuroleptic drug Chlorpromazine	solution	luminescence
Onoue *et al.*[64]	imidazopyridine derivatives	solution	RNO bleaching
Bao *et al.*[68]	polycyclic aromatic hydrocarbons (PAH)	*in vitro*	quencher
Regensburger[12]	polycyclic aromatic hydrocarbons	solution	luminescence
Zhao *et al.*[67]	fullerene	solution, *in vitro*	EPR, quencher
Baier *et al.*[36]	skin constituents	*in vivo*	luminescence

[a] EPR: electron paramagnetic resonance spectroscopy. [b] Absorbance decrease of *N*,*N*-dimethyl-4-nitrosoaniline at 440 nm.

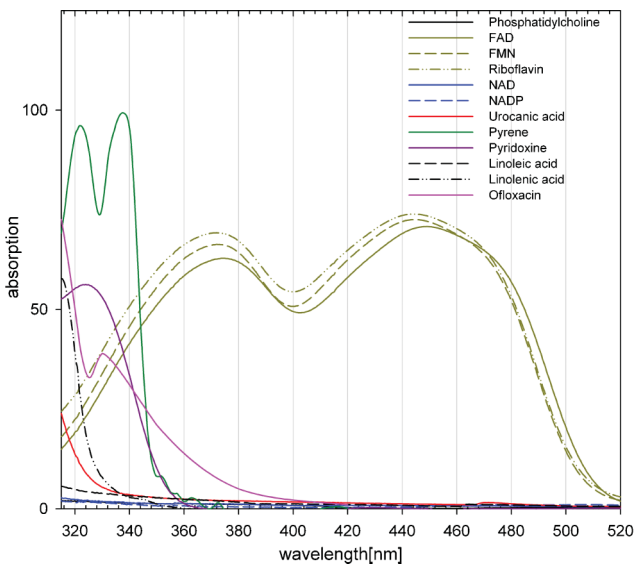

Fig. 1 The absorption of some molecules that may act as endogenous photosensitizers to generate singlet oxygen. For a better illustration, absorption is given in percent (absorption = 100% – transmission). The values are measured with spectrophotometer (Beckman, DU 640) in polar or nonpolar solvents at a concentration of 50 µmol L^{-1}, except for phosphatidylcholine (2.2 mmol L^{-1}), fatty acids (10 mM), ofloxacin (10 µg ml^{-1}) and pyridoxine (200 µM). The fatty acids (purity: 99%) contain traces of oxidized products that enables absorption of UVA radiation. Two substances were exemplarily added to the figure that may be present in skin accidentally and can act as UVA photosensitizer: pyrene (in black tattoo inks), ofloxacin (systemic treatment with antibiotics).

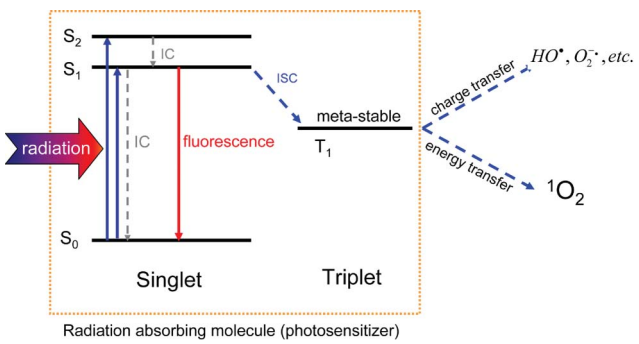

Fig. 2 The scheme shows the generation of reactive oxygen species that is initiated by radiation absorption in a photosensitizer molecule. The absorption leads to population of excited singlet states in the molecule, which rapidly (picoseconds) yields S_1 population *via* internal conversion (IC). Within nanoseconds, the absorbed energy is either converted to heat (IC) or to fluorescence. A third pathway is the intersystem crossing (ISC) in the molecule to the metastable triplet T_1 state (lifetime: µs to ms), which allows energy or charge transfer to other molecules such as oxygen.

Indirect detection

An important indirect proof of $^1O_2$ is the chemical analysis of its reaction products,[20–22] although this method frequently yields no unequivocal results. Specific quenchers such as sodium azide shorten the lifetime of $^1O_2$, whereas the use of the solvent deuterium oxide (D_2O) extends the lifetime of $^1O_2$. By shortening or extending the $^1O_2$ lifetime, the related damage of cellular structures is reduced or pronounced, which can be detected with different cellular parameters (*e.g.* mitochondrial activity or cell survival).[23] When adding spin traps, ESR signals show the presence of $^1O_2$.[24] In addition, $^1O_2$ can be detected by exploiting its chemical reactivity in different settings such as RNO-bleaching[25,26] or chemoluminescence assays.[27] The chemoluminescence assays are frequently based on switch-on fluorescence of rhodamines or fluoresceins such as '$^1O_2$ Sensor Green (SOSG)'.

Except for chemical analysis, the disadvantages of these indirect methods are obvious since they require the application of reporter molecules, which may show limited access to living cells *in vitro*. It is hence not warranted that the quencher reach the site of $^1O_2$ concentration with an appropriate concentration. The application *in vivo* is also limited because some of these substances are toxic or do not penetrate tissue to a sufficient extent.

Direct detection

The non-radiative deactivation of $^1O_2$ is accompanied by radiative deactivation yielding infrared luminescence at about 1270 nm. The luminescence detection is a great tool to directly detect $^1O_2$ without adding any reporter molecules, in particular for experiments with UVA radiation (Fig. 3). This technology can be applied for experiments in solution, *in vitro* and *in vivo*.

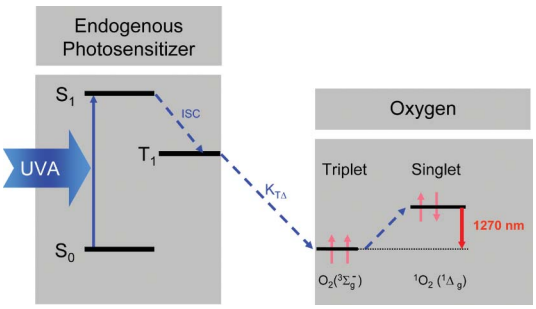

Fig. 3 The rate constant $K_{T\Delta}$ describes the formation of $^1O_2$, which corresponds to the transition from the molecular oxygen ground state (triplet state) to its first singlet state. $^1O_2$ can be detected by its weak luminescence that occurs at 1270 nm.

The major disadvantage of luminescence detection is the very low quantum yield of the radiative decay in the order of 10^{-7}, which requires a very sensitive detection system. For more than 10 years, however, special photomultipliers have been available that show a high sensitivity in the infrared spectrum up to 1400 nm. Now, these photomultipliers are successfully and frequently used for $^1O_2$ detection.[28–34]

Several groups world-wide have undertaken much effort to optimize the detection technology and to gain more detailed information from such $^1O_2$ luminescence signals.[35] The detection of $^1O_2$ luminescence in living cells or tissue has proven to be a technically challenging problem for the following reasons. Due to low oxygen concentrations,[29,36] short $^1O_2$ lifetime,[37] and changing oxygen diffusion coefficients in cells or tissue,[28] the signal intensity may be substantially weaker than in solution, showing diverse rates. Scattering in turbid media, *e.g.* cell suspensions, may lead to an increased superposition of the signals with other radiation sources, which was recently investigated by the groups of Wilson[29] and Röder.[28] In addition, flash photolysis experiments can be applied for triplet spectroscopy and the results can be compared to singlet oxygen luminescence at 1270 nm.[28] Spatially resolved singlet oxygen luminescence were detected either on a microscopic scale, in particular in living cells,[38] or *in vivo* using a scanning laser system.[39]

Time-resolved luminescence detection

Using excitation lasers with short pulse durations and kHz repetition rates, along with the highly sensitive IR photomultipliers, the single photon counting allows very sensitive luminescence detection with a high time resolution. This technique, meanwhile rather standardized, shows advantages over the conventional analogue detection mode that was used in the past.[40]

To generate $^1O_2$ by UVA radiation, the emission of a frequency tripled Nd:YAG laser (355 nm) or an appropriate OPO laser can be applied to excite endogenous photosensitizers. The laser pulse energy for luminescence experiments should be kept low (a few μJ) to minimize the damage of photosensitizer and cellular structures.[28] The $^1O_2$ luminescence at 1270 nm can be detected in either perpendicular or near-backward direction with respect to the excitation beam using infrared sensitive photomultipliers (*e.g.* Hamamatsu).[15,28,29,33,38] To avoid detection of non-$^1O_2$ photons, appropriate interference filters and cut-off-filters are usually placed in front of the photomultiplier (Fig. 4).

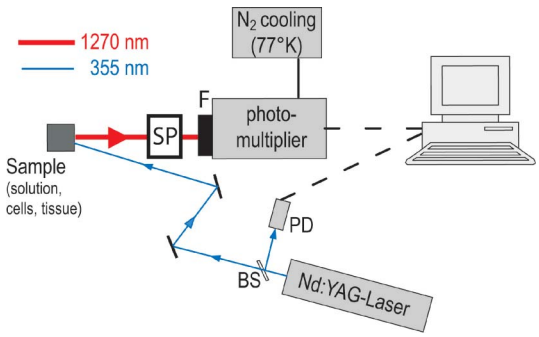

Fig. 4 A schematic setting for detection of singlet oxygen luminescence. The sample is excited with a frequency tripled Nd:YAG laser that emits at 355 nm. The luminescence is collected and transferred to the infrared sensitive photomultiplier, which is equipped with a 1270 nm interference filter. The single photon counting setting enables the time-resolved detection, whereas the stop signal is provided by a photodiode (PD): *via* beam splitter (BS). The spectral resolution is either achieved with different interference filters from 1100 to 1400 nm or by using a spectrometer (SP).

Spectrally resolved luminescence detection

Due to the very low luminescence signals, which is potentially superposed by other near infrared signal such as fluorescence or phosphorescence, it is important to detect the luminescence signal spectrally resolved in order to confirm that the signal can be attributed to $^1O_2$.[29,41,42] When combining the time- and spectrally resolved luminescence, the resulting 3D-image provides evidence for clear luminescence signal of $^1O_2$, an example is displayed in Fig. 5.

Mathematical fit of time-resolved singlet oxygen luminescence

After detection of such time resolved signals, it is a first and practical step to fit the gained signal curve using a constant C, the rise (τ_R) and decay (τ_D) times as well as different fit routines such as least square fit.[43]

$$I(t) = \frac{C}{\tau_R^{-1} - \tau_D^{-1}}\left[\exp(-\frac{t}{\tau_D}) - \exp(-\frac{t}{\tau_R})\right] \quad (1)$$

When applying different experiments, the values can be attributed to the respective rates and rate constants in the respective experiment.[42] The counted luminescence photons can be summed to calculate the total amount of $^1O_2$ detected. The spectrally resolved luminescence signal, either using a monochromator[34] or different interference filters,[36] is fitted with a Lorentzian function.

The time resolved luminescence signal provides information about the generation and deactivation of $^1O_2$ in its present environment. The in-depth evaluation of time-resolved luminescence signals can be performed along with mathematical equations, which describe the generation and decay of $^1O_2$.[29,42] Briefly, the coupling of a photosensitizer molecule with molecular oxygen *via*

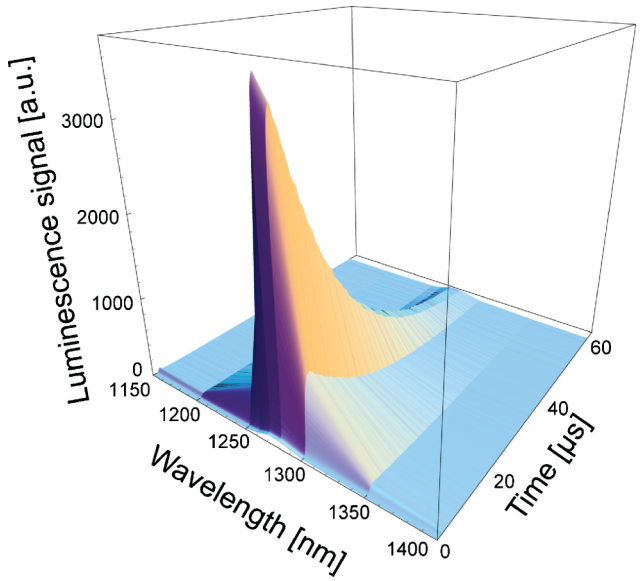

Fig. 5 The time- and spectrally resolved signal can be combined to have a clear confirmation of $^1O_2$ signal. This is important in case of weak luminescence signals, in particular from cells and tissue.

energy transfer from its triplet T_1 state to molecular oxygen yields a coupled system of differential equations, which can be reduced to the rate constants of the triplet T_1 state or first $^1O_2$ state:

$$\frac{d[T_1]}{dt} = -K_T[T_1] \quad (2)$$

$$\frac{d[^1O_2]}{dt} = -K_\Delta[^1O_2] \quad (3)$$

The signal will rise with the largest of the two rates and decay with the lowest. The assignment of the measured rise (τ_R) and decay (τ_D) times to the rates of $^1O_2$ or photosensitizer T_1 state remains challenging.

In non-viscous media (*e.g.* solvents), for high oxygen concentrations, the rise time is equivalent to K_T and the decay time to K_Δ, whereas for small oxygen concentrations it is the other way around. After this classification, the rates can be assigned to the respective lifetimes. In highly viscous media (*e.g.* in cells or tissue), the diffusion of oxygen molecules plays an additional role that complicates the evaluation of time resolved measurements and the assignment of the lifetimes.

The rates are intrinsically tied to the concentration and diffusion of oxygen in the environment of $^1O_2$ production. When evaluating such luminescence signals, it is therefore important to know, to measure or to estimate the oxygen concentration at the site of $^1O_2$ production during luminescence detection. In solution or cell suspensions, small needle sensors can be applied to monitor the oxygen concentration.[44]

Quantification of singlet oxygen

To quantify the $^1O_2$ generation of endogenous photosensitizers, the quantum yield Φ_Δ can be determined by using the Wilkinson definition[45] or by comparing the luminescence signals of the photosensitizer with unknown Φ_Δ to photosensitizer with known Φ_Δ.[15] Values are listed for a selection of photosensitizers in Table 2. It should be noted that the quantum yields are predominantly determined for photosensitizers in aerated solvents. In case of low oxygen concentrations (*e.g.* inside cells or tissue), Φ_Δ values may clearly differ from those values as shown in Table 2. For example, Φ_Δ of Riboflavin in solution decreases from 0.54 (oxygen concentration: 280 µM) to about 0.20 (oxygen concentration: 2 µM).[15] This decrease can be different for different photosensitizers.

Table 2 UVA-induced Φ_Δ measured by means of luminescence detection

Photosensitizer	Category	Φ_Δ
11-*cis* retinal[93]	vitamin A	0.55
Riboflavin[15]	vitamin B_2	0.54
Riboflavin[94b]	vitamin B_2	0.49
FMN[15]	vitamin B_2	0.51
FAD[15]	vitamin B_2	0.07
Pyridoxal[95]	vitamin B_6	0.44
Pyridoxine[95]	vitamin B_6	0.28
Pyridoxamine[95]	vitamin B_6	0,06
Pyrocobester[96]	vitamin B_{12}	0.21
Vitamin E [97a]	vitamin E	0.10
Ergosterol[98]	provitamin D_2	0.85
Lipofuscin[99]	aging pigment	0.08
Naproxen[60]	NSAID	0.28
Nabumetone[60]	NSAID	0.19
Ciprofloxacin[100]	antibiotics	0.09
Ofloxacin[100]	antibiotics	0.08
Anthracene[12]	PAH	0.81
Chlorpromazine[101]	neuroleptics	0.27
Lamotrigine[62]	antiepileptics	0.22
Hypericin[102]	antidepressants	0.43
6-thioguanine[52]	immunosuppression	0.56
Pterin[53c]	UV receptors	0.30

[a] 308 nm excitation. [b] indirect (EPR, RNO-bleaching). [c] pD = 10.5.

4. Photosensitized generation of singlet oxygen

In contrast to UVB, UVA radiation mainly provokes photosensitized reactions.[46] The major prerequisite of photosensitized reactions is UVA absorption in such molecules. The list of molecules has been fairly extended during recent years. It starts with endogenous porphyrins of heme synthesis, flavins, and the cellular pyrimidine nicotinamide cofactors (NADH and NADPH).[47] Meanwhile, the list also contains exogenous molecules, which are frequently administered to skin along with medical treatments. The papers cited below represent a non-exhausting selection of endogenous photosensitizers.

Endogenous photosensitizers

Among others, endogenous porphyrin molecules such as Protoporphyrin IX, the precursor of heme, efficiently generate $^1O_2$ under UVA irradiation leading to activation of heme oxygenase-1 (HO-1).[48]

Urocanic acid produces $^1O_2$ with UVA irradiation (355 nm) that was directly proven by luminescence signal.[15] In the same work, excitation with UVA of Riboflavin, FMN and FAD yielded strong luminescence signals and the respective quantum yields could be determined with $\Phi_\Delta = 0.54$ (riboflavin), $\Phi_\Delta = 0.51$ (FMN), and $\Phi_\Delta = 0.07$ (FAD). Depending on their concentration in the skin, the flavins are potential generators of $^1O_2$, even more effective than exogenous porphyrins used for cell killing in photodynamic therapy. In view of these high values, it seems to be reasonable that

these substances, even though at low concentrations, can provide sufficient amount of $^1O_2$ during UVA exposure that leads to gene regulation, photoaging, and possibly carcinogenesis.

The human retinal pigment epithelial (RPE) layer contains a complex mixture of components called lipofuscin; this mixture forms with age and with various genetic disorders such as Stargardt's disease. It is well accepted that lipofucsin generates $^1O_2$ when excited with UVA, which contributes to retinal maculopathies.[49]

The protein phosphatase calcineurin has been gradually revealing itself as the central controller of our immune response. UVA1 radiation suppresses calcineurin activity. Evidence was provided that this activity loss is partly due to $^1O_2$ generated by photosensitization.[50] Recently, experiments showed that the malondialdehyde-derived protein epitope dihydropyridine (DHP)-lysine is a potent endogenous UVA-photosensitizer of human skin cells.[51]

The photophysics and photochemistry of thiopurine DNA bases are far less understood than those of normal DNA bases, although some of them, such as azathioprine, 6-mercaptopurine, and 6-thioguanine, have been used as cancer therapeutic and immunosuppressive agents for five decades. The incorporation of 6-thioguanine into DNA increases the risk of $^1O_2$-initiated skin cancer. It was very recently shown that UVA irradiation of 6-thioguanines in solution produced $^1O_2$ with quantum yields Φ_Δ from 0.49 to 0.58.[52] 6-Thioguanines absorb UVA radiation in a broad range from 320–370 nm with a maximum at around 340 nm.

Pterins (2-amino-4-hydroxypteridin derivatives) are a family of heterocyclic compounds present in a wide variety of biological systems. Pteroyl-L-glutamic acid (folic acid) is a precursor of coenzymes involved in the metabolism of nucleotides and amino acids. All investigated pterins produced significantly amounts of $^1O_2$ with Φ_Δ in the range from 0.30 to 0.47 (pD Value 10.5). Only folic acid showed a very small quantum yield of less than 0.02.[53]

Anhydroretinol is a metabolite of vitamin A (retinol) and a major photodecomposition product of retinyl palmitate and retinyl acetate. There is sufficient evidence that irradiation of anhydroretinol with UVA light generates reactive oxygen species, *e.g.* $^1O_2$, which mediate the induction of lipid peroxidation.[54]

Fluorescent proteins are increasingly applied in different sections of experimental biology. One of those techniques is chromophore-assisted laser inactivation, which is employed to specifically inactivate the function of target proteins or organelles by producing photochemical damage.[55] Using time resolved luminescence detection, singlet oxygen was proven to be generated by the red fluorescent protein TagRFP with an estimated quantum yield of 0.004.

Antibiotics

Many substances, which are used in antibiotics such as quinoline derivatives,[56] are known to generate $^1O_2$ under UVA exposure. Ciprofloxacin produced ROS by Type I and Type II photodynamic reactions, interacted with nucleic acid moiety and inhibited cell viability.[25] The production of $^1O_2$ by various antibiotics was found to be concentration dependent. In the fluoroquinolone group, enoxacin generated most $^1O_2$ under UVA irradiation followed by lomefloxacin, norfloxacin, and ofloxacin.[57] In light of the undesirable photosensitized reactions of fluoroquinolones, new selenium-containing heterocyclic compounds were investigated. However, also these substances produced $^1O_2$.[58]

Other medical drugs

Several classes of drugs including thiazide diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), and tricyclic antidepressants, even when not toxic by themselves, may become reactive under exposure to environmental radiation, inducing undesired side effects. Providing a few examples, the following section should highlight the problems with $^1O_2$ that is generated by medical drugs exposed to UVA.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a chemically heterogeneous group of drugs mainly used as anti-inflammatory particularly in the treatment of rheumatic diseases. Some of the NSAID are potent photosensitizers when exposed to UVA radiation.[59] Due to the presence of the naphthalene chromophore, substances like Nabumetone or Naproxen generate $^1O_2$ with quantum yields of 0.19 or 0.28 in solution.[60]

The neuroleptic drug levomepromazine (known as methotrimeprazine) is photolabile under UVA and UVB radiation in aerobic conditions. Irradiation of a methanol solution of this drug produces one photoproduct, resulting from oxidation. It is demonstrated that photodegradation occurs *via* type II mechanism involving irreversible trapping of self-photogenerated singlet molecular oxygen.[61] Lamotrigine (LTG) is an anticonvulsant (antiepileptic) drug specifically blocking voltage-gated sodium channels. LTG occasionally causes cutaneous problems including exaggerated sunburn or skin photo-irritation. Depending on the solvent, the quantum yield Φ_Δ of LTG shows values up to 0.22.[62] Chlorpromazine (CPZ), a phenothiazine derivative, is a neuroleptic drug widely used in medicine because of its tranquilizing and antipsychotic properties. Experimental data indicate that in hydrophobic environment CPZ is a relatively efficient generator of $^1O_2$.[63]

Imidazopyridine derivatives are designed as 5-HT_4 receptor agonist for the clinical treatment of gastroesophageal reflux disease. Also these substances produce $^1O_2$ under UVA exposure.[64] Dihydropyridine-type calcium channel antagonists such as nifedipine and nitrendipine are important drugs for the treatment of hypertension and coronary heart disease. A novel substance and some of the derivates produced significant amounts of $^1O_2$ under simulated solar radiation.[65]

Nano-materials such as water-soluble fullerenes have shown potential uses as drug carriers to bypass the brain and ocular barriers. However, photoexcitation of fullerene derivatives may produce $^1O_2$.[66] The water-soluble fullerene derivative γ-cyclodextrin bicapped C_{60} efficiently generates $^1O_2$ under UVA exposure. Since this compound has an absorbance maximum of 349 nm, it has the potential to cause lenticular damage when in the human eye.[67]

Other exogenous compounds

Polycyclic aromatic hydrocarbons (PAHs) are widely spread substances in environment that have been identified as essential risk factors for various benign or malignant human diseases, either alone or in combination with UVA. Specific sources are cigarette smoke and diesel particles extracts (DPE). *In vitro* experiments

showed that sodium azide significantly inhibited both cellular and DNA damage induced by DPE + UVA treatment, which provide evidence of $^1O_2$ generation by PAHs [68]

PAHs are injected into the skin along with black tattoo inks and may stay intradermally for years. After incubation of skin cells with extracts of black tattoo inks, the cells were exposed to UVA and cell viability decreased in a dose-dependent manner. The quantum yield Φ_Δ of different PAHs were determined yielding high values of up to 0.85 for benz[a]anthracene.[12]

Nanoparticles such as silicon nanoparticles can generate $^1O_2$ on its surface *via* energy transfer from an exciton singlet state to oxygen.[69] This might be of particular interest when using nanoparticles in ointments and medical drugs.

5. UVA-mediated singlet oxygen generation

Besides the photosensitized generation triggered by UVA, $^1O_2$ can be produced by various chemical reactions involving different radicals and other reactive species.[70] In the presence of oxidizable biomolecules like lipids, proteins or DNA, photosensitization and chemical reactions (*e.g.* lipid peroxidation) may occur at the same time yielding various products.[71,72]

Usually lipids and fatty acids are the target of $^1O_2$ that has been generated by any photosensitizer. However, we have shown that $^1O_2$ is generated in suspensions of egg yolk phosphatidylcholine during irradiation with UVA that included the action of oxygen radicals.[36] Under 355 nm irradiation, different fatty acids (oleic acid 18 : 1, linoleic acid 18 : 2, linolenic acid 18 : 3) in aerated ethanol solution (50 mmol L^{-1} each) showed clear $^1O_2$ luminescence signals, without any photosensitizer (Fig. 6), which was confirmed by the spectrally resolved detection of the signals (Fig. 7). The decay time of the signal $\tau_\Delta = (K_\Delta)^{-1}$ was in the range from 13 to 14 µs, which is the life time of $^1O_2$ in ethanol.[42]

Oxidized products of such fatty acids must be present to enable initial absorption of UVA radiation.[73] Once singlet oxygen is generated, the amount of oxidized products increases, which in turn enhances radiation absorption. Usually, luminescence signals of photosensitizer induced $^1O_2$ show a rise time and a decay time according to the eqn (1)–(3). However, time-resolved luminescence signals of $^1O_2$ in fatty acid solutions showed a decay time but no rise time. That is, the luminescence signal was maximal within a time span of less than 200 ns. The difference of such signals shapes, with and without rise time, is illustrated in Fig. 8. A photosensitized generation of $^1O_2$ by a porphyrin photosensitizer in water shows a clear rise time, which is $\tau_T = (K_T)^{-1} = 1.9$ µs, whereas the decay time was $\tau_\Delta = (K_\Delta)^{-1} = 3.6$ µs. We suggested that due to the lack of rise time, the light absorbing molecules are not able to form such a triplet state (*e.g.* linear-shaped molecules like fatty acids). Thus, we assumed that $^1O_2$ is generated with the assistance of chemical reactions, but initiated by the applied UVA radiation.[74]

In addition, hydroperoxides of fatty acids can be further decomposed to acid-peroxyl radicals and/or alkoxyl radicals, which are responsible for the propagation of peroxidation.[75] The decomposition of lipid hydroperoxides into peroxyl radicals has been shown to be a potential source of $^1O_2$ in biological systems.[3,76] In 1957, Russell proposed a self-reaction mechanism of such peroxyl radicals involving the formation of a cyclic mechanism from a linear tetraoxide intermediate that decomposes to give different products as well as $^1O_2$.[77] It could be demonstrated that

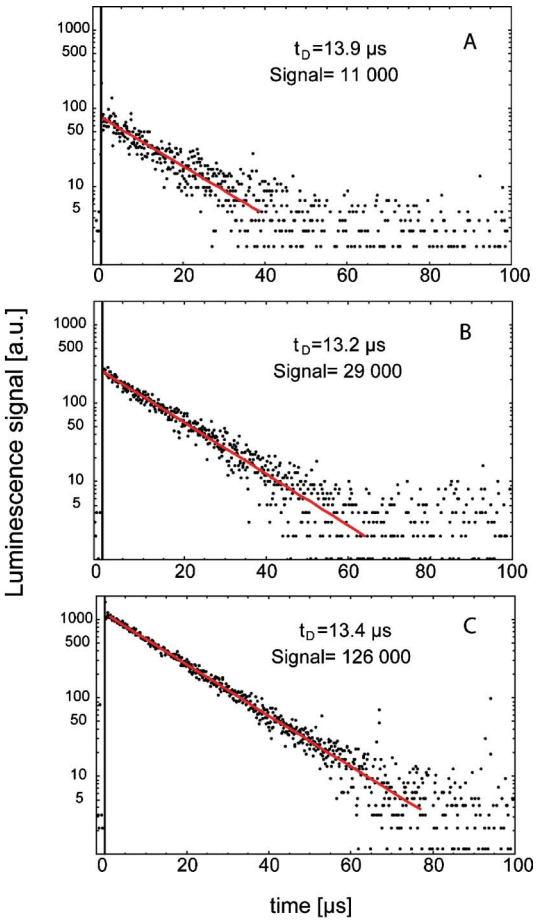

Fig. 6 The time-resolved $^1O_2$ luminescence signals of different fatty acids, oleic acid 18 : 1 (A), linoleic acid 18 : 2 (B), linolenic acid 18 : 3 (C) in aerated ethanol solution at 50 mmol L^{-1}. The decay time t_D and the values of integrated signal are shown (Signal, arbitrary units).

such a self-reaction formation of peroxyl radicals deriving from fatty acids generates predominantly $^1O_2$.[78] Fig. 9 shows a scheme of UVA-mediated generation of $^1O_2$ generation in fatty acids, which is probably not yet complete.

Such fatty acids are major constituents of many cellular membranes that should underline their potential role in UVA mediated activation of cellular signaling. Skin contains sufficient amount of oxygen ($pO_2 \sim 20$ Torr).[79] When exposed to UVA radiation, initial concentrations of oxidized fatty acids are present in skin[80,81] to initiate the generation of $^1O_2$ (see Fig. 9). In cells, a molecule such as ceramide is a key component of stress responses. UVA radiation and $^1O_2$ both generated ceramide in protein-free, sphingomyelin-containing liposomes.[82] Furthermore, human skin, especially the stratum corneum, contains free saturated and unsaturated fatty acids with mostly chain lengths of C_{16} to C_{18} atoms.[83]

Fatty acids are also constituents of creams and ointments, in particular used in sunscreens that protect skin from solar UV radiation. When exposing different creams or ointments in ethanol solution to UVA laser radiation at 355 nm, we detected a clear and impressive signal of $^1O_2$ generation in many of the samples investigated. As for experiments with fatty acids, the decay time of $^1O_2$ was about 14 µs according to the used solvent ethanol. The maximum signal intensity among the investigated creams yielded

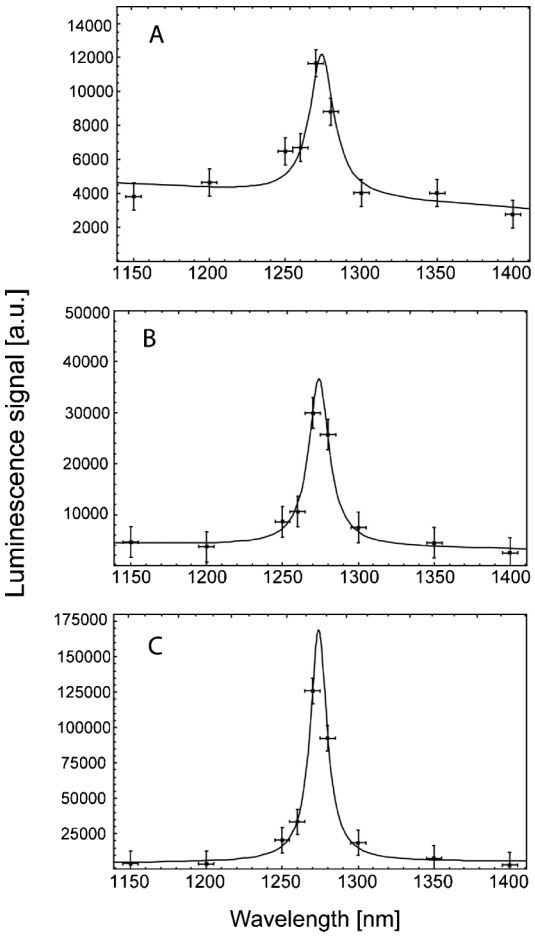

Fig. 7 The spectrally resolved $^1O_2$ luminescence signals of different fatty acids, oleic acid 18 : 1 (A), linoleic acid 18 : 2 (B), linolenic acid 18 : 3 (C) in aerated ethanol solution at 50 mmol L^{-1}. All signals show the transition of $^1O_2$ at 1270 nm.

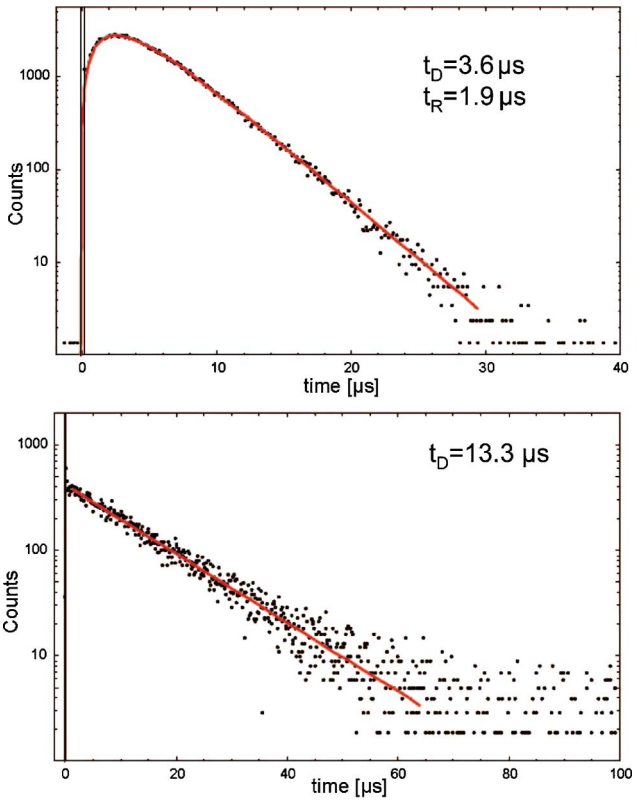

Fig. 8 The time-resolved $^1O_2$ luminescence signals of a porphyrin photosensitizer (5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)-21*H*,23*H*-porphine, TMPyP) in water and linoleic acid in ethanol. The signal of fatty acids shows no rise time.

the sample shown in Fig. 10. The impact of such photosensitizing compounds, which are topically administered to skin and exposed to solar radiation, should be elucidated.

6. Detection of singlet oxygen generation in skin

Solar ultraviolet A (UVA; 320–400 nm) radiation is a well-known trigger of signaling responses in dermal fibroblasts in human skin *in vivo*.[48,84] Investigations have been performed to assess the contribution of $^1O_2$ to lipid peroxidation under in *vivo* conditions. It is known that the generation of $^1O_2$ in turn activates interstitial collagenase like matrix-metalloproteinases (MMPs)[80,85] such as MMP-1, which causes extracellular protein degradation and thereby contributes to photoaging of human skin.[86] The activation of MMP-9 is an essential step in the skin photoaging on exposure to ultraviolet A (UVA). MMP-9 activity is clearly related to the presence of cholesterol-hydroperoxides such as cholesterol 5α-hydroperoxide, which is specifically occurs with $^1O_2$.[87] Gene expression in keratinocytes, which is induced by solar UVA radiation, is initiated at the level of the cell membrane *via* generation of $^1O_2$ and subsequent formation of ceramide from sphingomyelin.[88]

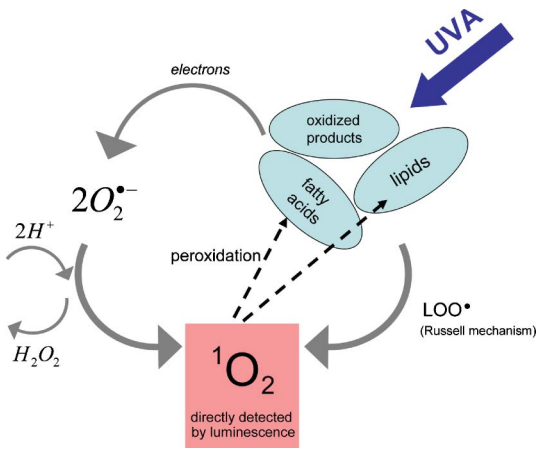

Fig. 9 Proposed scheme of UVA-initiated singlet oxygen generation with the assistance of radicals (taken from reference Baier *et al.*[74]).

Additionally, UVA radiation is now recognized as a class I carcinogen[89] and is suspected to play a significant role in the induction of melanoma,[90,91] although the latter is still a controversial issue.[92] $^1O_2$ is clearly induced in living skin by UVA radiation, but one has to consider that the quantum yield Φ_Δ depends critically on the respective oxygen concentration, which is in skin substantially smaller as compared to aerated solutions (Fig. 11).[15]

In light of these facts, there is a need for a detection system that monitors the generation of $^1O_2$ in tissue *in vivo* for different

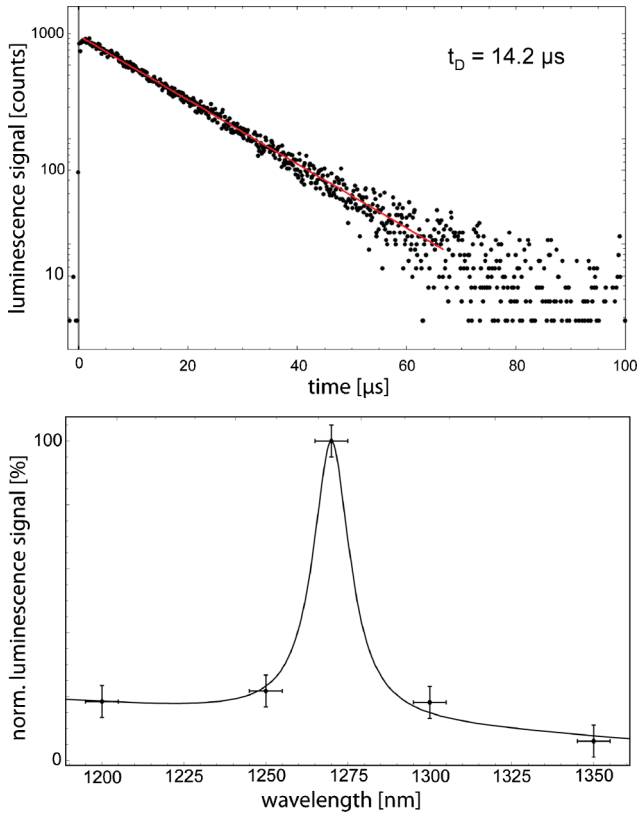

Fig. 10 The clear and impressive time- and spectrally resolved $^1O_2$ luminescence signals of skin moisture.

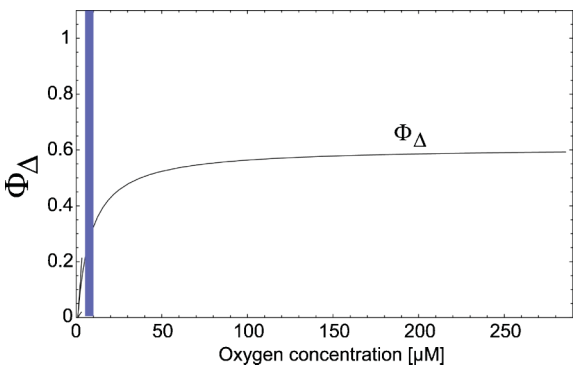

Fig. 11 The quantum yield of $^1O_2$ generation for different oxygen concentrations. The vertical bar indicates the oxygen concentration in a living cell (taken from Schenkman *et al.*[103]).

experimental conditions. The results should be correlated to the biological findings. By means of the highly sensitive luminescence detection, we succeeded in detecting $^1O_2$ in living cells and even in skin *in vivo*, without any exogenous photosensitizer.[36] At present, the luminescence technology seems to be the only method to detect $^1O_2$ *in vivo* since it is difficult or impossible to apply quencher or other reporter substances.

Conclusion

The direct detection of $^1O_2$ by its luminescence is a fascinating tool, which has been used in photodynamic therapy and UVA-mediated photoreactions. The luminescence may report on the generation and the lifetime of $^1O_2$ in various environments. Due to the weak signal intensity, the luminescence detection was limited to experiments in solution at the beginning. For more than ten years, the development of new infrared sensitive photomultipliers has enabled many researchers to extend such luminescence detection to living cells *in vitro* and to tissue *in vivo*. However, there is still need to improve this technology, *e.g.* by looking for detectors that are even more sensitive.

- The high sensitivity of new detection technology should be used to reduce the excitation energy, which still affects the cell integrity during such experiments.
- The luminescence curve appears as a simple signal but contains a lot of information about $^1O_2$ dynamics, which requires a careful analysis and interpretation.
- In the case of living cells, it would be of great importance to develop a CCD camera-like detector of $^1O_2$ that allows the luminescence detection with spatial resolution.
- Computer-assisted modeling may help to simulate the diffusion of excitation and luminescence photons as well as the movement of $^1O_2$ in cells.

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