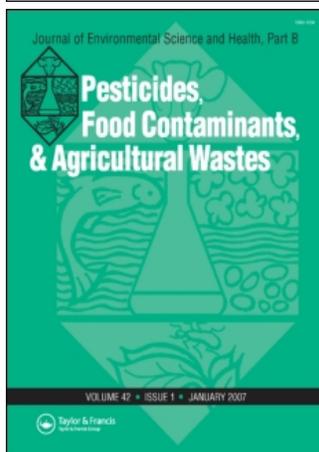


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Photocatalytic mechanisms of indoleamine destruction by the quinalphos metabolite 2-hydroxyquinoxaline: a study on melatonin and its precursors serotonin and *N*-acetylserotonin

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The redox-active quinalphos main metabolite, 2-hydroxyquinoxaline, is particularly effective under excitation by light. We have studied the photocatalytic destruction of melatonin and its precursors, because the cytoprotective indoleamine has been detected in high quantities in mammalian skin. In photooxidation reactions, in which melatonin, *N*-acetylserotonin and serotonin are destroyed by 2-hydroxyquinoxaline, the photocatalyst is virtually not consumed. Rates of melatonin and serotonin destruction are not changed by the singlet oxygen quencher 1,4-diazabicyclo-(2,2,2)-octane, indicating that this oxygen species is not involved in the primary reactions, so that the persistence of 2-hydroxyquinoxaline has to be explained by redox cycling. This should imply formation of an organic radical, presumably the quinoxaline-2-oxyl radical, from which 2-hydroxyquinoxaline is regenerated by electron abstraction from indolic radical scavengers. Electron donation by 2-hydroxyquinoxaline is demonstrated by reduction of the 2,2'-azino-bis-(3-ethylbenzthiazolyl-6-sulfonic acid) cation radical under ultrasound excitation. The compound 2-hydroxyquinoxaline interacts with the specific superoxide anion scavenger Tiron. Formation of oligomeric products from melatonin and serotonin is strongly inhibited by sodium dithionite. Products from photocatalytic indoleamine conversion are predominantly dimers and oligomers. No kynuramines were detected in the case of serotonin oxidation, and melatonin's otherwise prevailing oxidation product *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine, another cytoprotective metabolite, is only formed in relatively small quantities. The proportion between products from melatonin is changed by 1,4-diazabicyclo-(2,2,2)-octane: singlet oxygen, also formed under the influence of excited 2-hydroxyquinoxaline, only affects secondary reactions.

Keywords: *N*-acetylserotonin; 2-hydroxyquinoxaline; melatonin; quinalphos; redox cycling; serotonin; singlet oxygen.

Introduction

Quinalphos [*O,O*-diethyl-*O*-(2-quinoxaliny)-phosphorothioate; Ekalux; Bayrusil; Fig. 1], a widely used insecticide and acaricide, is a source of toxicity to humans and vertebrate animals. A three-year evaluation (2000–2002) of forensic pesticide and herbicide intoxications in Portugal attributed about 29% of total cases to quinalphos.^[1] Primary toxicity by inhibition of acetylcholinesterase is terminated by hydrolysis of the pesticide. Phosphorus-containing hydrolytic products were detected in human serum and urine,^[2] so that an organic product should have been formed

in the intoxicated persons, too. Hydrolysis of the ester bond to the aromatic moiety leads to 2-hydroxyquinoxaline (HQO), which has been identified as the main metabolite in soil, in water and on crops.^[3–5] HQO was recently shown to be cytotoxic to ciliates, dinoflagellates and rotifers, to increase hydroperoxides and protein carbonyl, and to be genotoxic in an Ames test.^[6]

Many toxicological effects have been ascribed to quinalphos, which cannot be easily explained by its primary toxicity, including estrogenic/antiandrogenic actions, oxidative stress, decrease of the radical scavenger melatonin, impairment of immunological parameters, chromosomal aberrations and micronuclei formation.^[7] Therefore, a secondary toxicity by a quinalphos metabolite seemed highly likely. With regard to its chemical structure and properties, 2-hydroxyquinoxaline should be capable of exerting the effects mentioned. As a hydroxylated aromate, it might interact with steroid receptors, what is highly unlikely for the organophosphate. Owing to the free electron

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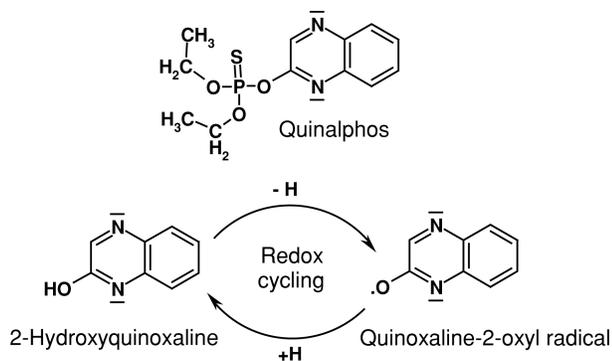


Fig. 1. Chemical structures of quinalphos, 2-hydroxyquinoxaline (HQO) and its redox cycling with the presumed quinoxaline-2-oxyl radical.

pairs of two nitrogen atoms and the presence of a reactive hydroxyl residue, the electron-rich molecule should easily donate an electron to suitable acceptors, thereby forming an organic free radical, most likely the *O*-centered quinoxaline-2-oxyl radical (Fig. 1), which in turn could oxidize other compounds. Additional hints for such a secondary toxicity by a quinalphos metabolite came from studies on phototoxicity.^[8] When directly applied to the depilated guinea pig skin, quinalphos did not elicit any photosensitization, but phototoxicity became apparent when the pesticide was administered orally to mice, so that the effect should have resulted from a metabolite formed in the organism. In fact, we were able to demonstrate photooxidation of various compounds, such as vitamins C and E, catecholamines and other biogenic amines, and anthranilate derivatives, by 2-hydroxyquinoxaline *in vitro*.^[7] Experiments on photocatalytic destruction of ascorbate, in ethanolic solution, indicated that 2-hydroxyquinoxaline was not consumed in these reactions. Such a result might be explained in two different ways, either by energy transfer leading to the formation of reactive singlet oxygen, or by electron transfer reactions, in which HQO acts first as a reductant, and is subsequently regenerated by oxidizing a suitable radical scavenger. In the present study on melatonin and its precursors, we have conducted experiments to decide between these alternatives of energy transfer vs. redox cycling. These indoleamines are potent radical scavengers, differing, however, in details of their oxidation chemistry.^[9] For several reasons, melatonin seemed to be particularly important in this regard. (i) Its levels were found to be decreased in quinalphos-intoxicated animals, along with a deterioration of immunological parameters (data by Haldar et al., as cited by Riediger et al.)^[6], in accordance with the immunomodulatory role of the hormone.^[10,11] (ii) Melatonin and its metabolites of the kynuramine pathway are potent cytoprotective and, at least melatonin, also photoprotective agents.^[12–16] (iii) Melatonin has been found to be synthesized and to be converted into *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK) in mammalian skin^[17,18]

and to attain unexpectedly high concentrations. Even HaCaT keratinocytes *in vitro* produced levels in the range of 30 μM , which is by about 5 orders of magnitude higher than in the circulation.^[19] (iv) Melatonin protected HaCaT cells from UV-induced apoptosis.^[20] Photocatalytic destruction of melatonin by the quinalphos metabolite HQO would, therefore, be highly relevant in terms of an impairment of photoprotection of the skin.

Materials and methods

Chemicals

Substances of highest purity available were obtained from the following sources. Sigma-Aldrich, Taufkirchen (Germany): 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS); 1,4-diazabicyclo-(2,2,2)-octane (DABCO), 4,5-dihydroxy-1,3-benzene-disulfonic acid (Tiron), 2-hydroxyquinoxaline (HQO), melatonin, *N*-acetylserotonin, serotonin hydrochloride. *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK) was synthesized^[21] according to Kennaway et al.,^[22] with slight modifications. All other chemicals were purchased from Merck, Darmstadt (Germany).

Reaction systems

Photooxidation of melatonin, *N*-acetylserotonin and serotonin was followed in either ethanol or potassium phosphate buffer 50 mM, pH 7.4. In the case of buffer, melatonin was predissolved in ethanol, 2.5 mg/0.1 mL, whereas serotonin was directly dissolved. The indoleamines were incubated, in the presence or absence of HQO, DABCO or sodium dithionite (for final concentrations see figures). Mixtures were exposed to light in thin-walled glass tubes (Fiolax, outer diameter 12 mm; wall 0.4–0.5 mm: Schott, Mainz, Germany) using a slide projector (FA 150 Liesegang, Düsseldorf, Germany, with projector bulb Philips type 7158), at a photon fluence rate of 1400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Tubes containing ethanolic solutions were closed by Parafilm, to avoid losses of volume. Throughout the incubation, mixtures were continually shaken (GFL laboratory shaker, type 3016, Burgwedel, Germany). In order to avoid heating, the equipment was kept under the steady air stream of a ventilator. The entire laboratory was kept at 25°C. Changes in optical density, due to formation of photoproducts (400 nm for melatonin, 500 nm for serotonin and *N*-acetylserotonin), were followed directly in the reaction vials, using a Spectronic 88 photometer (Bausch & Lomb, Rochester, NY, USA). In one series with melatonin, reaction mixtures were irradiated with UV light in cultures dishes (Cellstar, 35 mm diameter, Greiner, Frickenhausen, Germany), using a lamp with preferential emission at 366 nm (DESAGA, Heidelberg, Germany), at an intensity of 1926 $\mu\text{W/cm}^2$, at 25°C (in a temperature-controlled room).

Electron donation by HQO was investigated in a system containing ABTS cation radicals ($\text{ABTS}^{\cdot+}$) prepared according to Re et al.^[23] Excitation of HQO by light was avoided because of light sensitivity of ABTS; instead, activation energy was provided by sonication, using an ultrasound generator 281/101 with sonotrode TU 175/1 (KLN, Heppenheim, Germany), operated at intensity 7 for 6 min. The reaction system contained 0.25 mM HQO (controls without HQO) and 0.1 mM $\text{ABTS}^{\cdot+}$, 1.25 mM potassium phosphate buffer pH 7.5, 0.095 mM EDTA, 2.5% ethanol. $\text{ABTS}^{\cdot+}$ reduction was measured at 420 nm (photometer PM 7, Zeiss, Oberkochen, Germany). Photocatalytic electron transfer was studied, in potassium phosphate buffer 50 mM, pH 7.4, using the specific superoxide anion scavenger Tiron (final concentration: 2 mM; HQO: 1 mM; photon fluence rate and other conditions as with indoleamines).

Analytic procedures

Educts and products from ethanolic mixtures were concentrated by evaporation; those from potassium phosphate buffer were extracted with the 2- to 10-fold volume of ethylacetate, which was subsequently concentrated. Initial product analyses were carried out by thin-layer chromatography on silica gel, using various mobile phases, e.g. ethylacetate/methanol 9:1 or 7:3, propanol/HCl 7:3 pH 2.6, or butanol/glacial acetic acid/water 16:1:2, depending on the products of interest.^[7] Fluorescent compounds were detected under UV light at 366 and/or 254 nm (UV lamp DESAGA, Heidelberg, Germany). Some products were re-eluted; absorbance and, where appropriate, fluorescence spectra were measured.

Quantitative determinations of melatonin, *N*-acetylserotonin, serotonin, HQO and the melatonin metabolite AFMK were carried out by reversed-phase high performance liquid chromatography (HPLC) with electrochemical detection (HPLC/ECD; Gynkotec HPLC pump M 480 HD, precolumn Perisorb RP-18, separation column Spherisorb ODS 1, column thermostat STH 585 operated at 40°C, pulsation reducer PD-1, from Gynkotec, Germering, Germany; syringe loading sample injector model 9125 from Rheodyne, Cotati, CA, USA; electrochemical detector EP 30 from Biometra, Goettingen, Germany). The equipment was operated at a flow rate of 800 $\mu\text{L}/\text{min}$, at oxidation potentials of 1050 mV (for melatonin, *N*-acetylserotonin, serotonin, and HQO) or 1200 mV (for AFMK), using an eluent containing 20% methanol, 3.75 g/L NaH_2PO_4 , 11.25 g/L citric acid, 320 mg/L octanesulfonic acid, and 32 mg/L EDTA. Reaction mixtures consisted of 2 mM of the respective indoleamine, 2 mM HQO, and, if present, 10 mM DABCO, in ethanol, and were exposed to light as described above, and diluted before injection with eluent to concentrations suitable for HPLC analyses. Chromatograms were quantitatively evaluated using the Gynkosoftware (Gynkotec, Germering, Germany).

Because of a minor, unavoidable coelution of HQO with a product from *N*-acetylserotonin, reaction mixtures were, in additional experiments, concentrated by evaporation, separated by thin-layer chromatography (mobile phase: ethyl acetate/methanol 9:1). Thereafter, HQO was re-eluted from the respective band in 20 mL HPLC eluent, remaining silica gel removed by centrifugation (30 min 4,500 rpm), and the supernatant directly injected into the HPLC.

Results

Prior to this study, we had already reported that HQO can photocatalytically destroy numerous oxidizable compounds, such as antioxidant vitamins and several biogenic amines, including melatonin and serotonin.^[7] Reactions with the amphiphilic molecule melatonin took place in both aqueous (buffer) and ethanolic reaction systems, a finding which may be relevant with regard to the distribution of melatonin within an organism and between aqueous and lipid phases of cells. In order to avoid a contribution of H_2O_2 -derived free radicals, in particular, hydroxyl and hydroperoxyl radicals to the photocatalytic processes studied, we investigated photocatalytic melatonin destruction and HQO concentrations in ethanolic solution.

Figure 2a shows the formation of colored products from melatonin under the influence of UV or visible light, as followed at 400 nm, a wavelength at which HQO does not

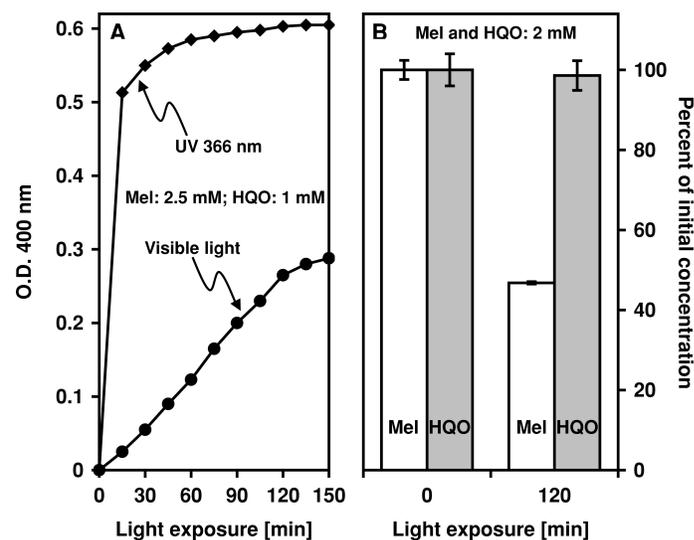


Fig. 2. Photocatalytic destruction of melatonin (Mel) by 2-hydroxyquinoxaline (HQO), in the absence of a substantial decrease in HQO. (a) Conversion of melatonin by UV and visible light, as indicated by rises in optical density (O.D.) at 400 nm, a wavelength at which oligomers formed from melatonin absorb. (b) Quantitative changes determined by high performance liquid chromatography with electrochemical detection (HPLC/ECD). Concentrations refer to initial molarity in the ethanolic reaction mixture. Vertical lines: standard error of the mean (s.e.m.)

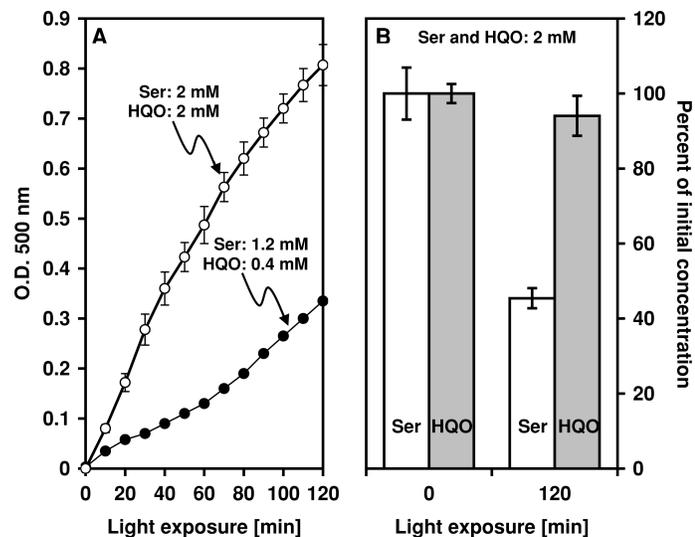


Fig. 3. Photocatalytic destruction of serotonin (Ser) by 2-hydroxyquinoxaline (HQO), in the absence of a substantial decrease in HQO. (a) Conversion of serotonin by visible light, as indicated by rises in O.D. at 500 nm, a wavelength at which di- and oligomers formed from serotonin absorb. (b) Quantitative changes determined by high performance liquid chromatography with electrochemical detection (HPLC/ECD). Concentrations refer to initial molarity in the ethanolic reaction mixture. Vertical lines: s.e.m.

absorb. The products detected are composed of a complex mixture of more than 20, mainly yellowish and brownish oligomers and a certain, minor amount of AFMK.^[7] When melatonin and HQO were jointly exposed to light

at equimolar concentrations and subsequently analyzed by HPLC, melatonin was consumed to a considerable extent, whereas HQO concentration showed virtually no change (Fig. 2b). In the absence of HQO, melatonin was poorly photooxidized during light exposure: at 400 nm, a wavelength at which the oligomers absorb, the change in optical density was nil; at 340 nm, at which kynuramines such as AFMK absorb, an O.D. increase of only 0.005 was measured within 2.5 h of irradiation, in both ethanol and buffer (details not shown).

Very similar results were obtained with serotonin. Rises in optical density could be studied at 500 nm (Fig. 3a), a wavelength at which dimers and subsequently formed oligomers of serotonin absorb.^[7] No kynuramines, which might have been produced by pyrrole ring cleavage, were detected in chromatographic analyses (details not shown). Again, HQO concentration remained almost stable, as judged from HPLC data, whereas serotonin was strongly consumed (Fig. 3b).

Corresponding data were obtained with *N*-acetylserotonin. Homologous di- and oligomers as known from serotonin were formed and could be followed at 500 nm (Fig. 4a). Changes in absorbancy measured with serotonin and *N*-acetylserotonin were very similar. HPLC analyses revealed a strong decline in *N*-acetylserotonin, whereas no consumption of HQO was evident (Fig. 4b). The small increase in apparent HQO concentration was, however, due to the minor coelution mentioned. Therefore, HQO concentration was re-investigated after previous separation by thin-layer chromatography. In this case, a comparison of HQO exposed to light in the presence or absence of *N*-acetylserotonin revealed virtually no difference (Fig. 4c).

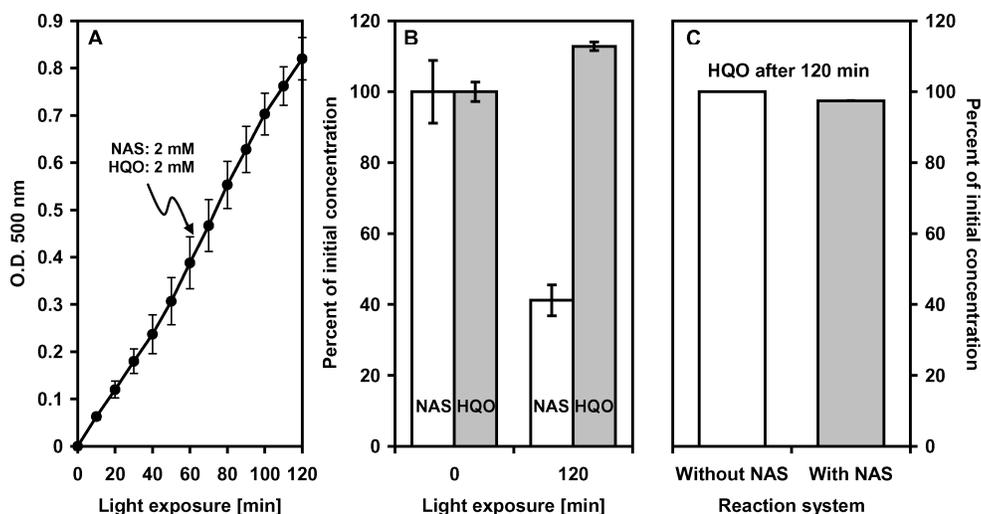


Fig. 4. Photocatalytic destruction of *N*-acetylserotonin (NAS) by 2-hydroxyquinoxaline (HQO), in the absence of a substantial decrease in HQO. (a) Conversion of NAS by visible light, as indicated by rises in O.D. at 500 nm, a wavelength at which di- and oligomers formed from NAS absorb. (b) Quantitative changes determined by high performance liquid chromatography with electrochemical detection (HPLC/ECD). (c) Determinations of HQO after light exposure, separation by thin-layer chromatography, reelution and determination by HPLC/ECD. This procedure was applied because of a minor coelution of HQO in B. Other details (s.e.m.) as in Fig. 2. In 4C, s.e.m. values were too small for well-discernable graphic representation.

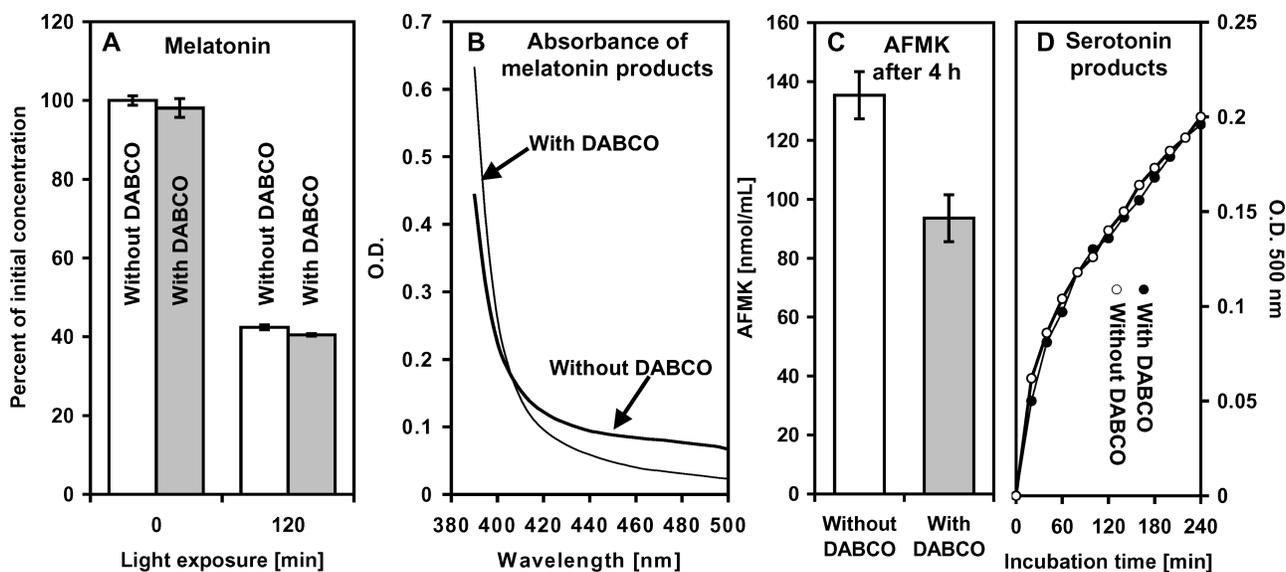


Fig. 5. Effects of the oxygen quencher 1,4-diazabicyclo-(2,2,2)-octane (DABCO) on photocatalytic conversion of melatonin and serotonin. (a) DABCO does not substantially change the extent of melatonin destruction. Determinations by high performance liquid chromatography with electrochemical detection (HPLC/ECD). (b) DABCO changes the composition of secondary products from melatonin, as indicated by lower absorbance at longer wavelengths, at which higher oligomers absorb. Light exposure 2 h. (c) DABCO moderately diminishes the formation of the melatonin metabolite *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK). Determinations by HPLC/ECD. (d) DABCO has no detectable effect on di- and oligomerization of serotonin. A-C in ethanol, D in potassium phosphate buffer, pH 7.4. Other details (s.e.m.) as in Figure 2.

When melatonin destruction was followed in the presence and absence of the singlet oxygen quencher DABCO, no substantial difference was detected by HPLC/ECD (Fig. 5a). However, a difference was observed in the composition of products. Higher oligomers mainly absorbing at wavelengths above 400 nm were diminished by DABCO (Fig. 5b), and also amounts of AFMK measured after a long exposure of 4 h were somewhat reduced (Fig. 5c). Product formation from serotonin was obviously not affected by DABCO (Fig. 5d).

To further test for the involvement of redox processes and oxygen requirement, photocatalytic destruction of indoleamines by HQO was investigated in the presence of sodium dithionite, for reasons of solubility in potassium phosphate buffer. This reducing and oxygen-consuming compound strongly inhibited the conversion of both melatonin (Fig. 6a) and serotonin (Fig. 6b). Almost no AFMK was detected in product analyses from these experiments (not shown). The specific superoxide anion scavenger Tiron^[24] was photocatalytically converted by HQO (Fig. 6c), indicating the capability of the pesticide metabolite of donating electrons. This was confirmed by incubating HQO with ABTS^{•+}. In this case, ultrasonication was used as an energy source instead of light, because of light-induced cation radical formation from ABTS, which would diminish the conversion rate. In the absence of HQO, only 1/4 of the ABTS^{•+} was reduced within 6 min of sonication, whereas

more than 90% were reduced in the presence of HQO (Fig. 6d).

Discussion

All results presented here demonstrate unanimously that melatonin as well as its precursors *N*-acetylserotonin and serotonin are photocatalytically converted by HQO, whereas this pesticide metabolite is practically not consumed within 2 h of light exposure. These findings are in agreement with similar observations of HQO stability during ascorbate destruction in ethanolic solution.^[7] In that earlier study, melatonin had been shown to be dose-dependently destroyed by HQO, and light-exposed serotonin was stable in the absence of HQO for hours.

Without further analysis, HQO stability during indoleamine oxidation might have been explained by two different mechanisms, either by energy transfer from the photoexcited HQO molecule to oxygen, causing the formation of singlet oxygen, or by redox cycling involving single-electron transfer reactions. Singlet oxygen can, in fact, interact with melatonin, however, only with relatively low affinity.^[25–27] The principal product formed from melatonin by this non-radical species is AFMK.^[27,28] The alternate possibility of electron transfer reactions within an organic redox cycle would imply that the excited, any-way electron-rich HQO molecule donates an electron to a

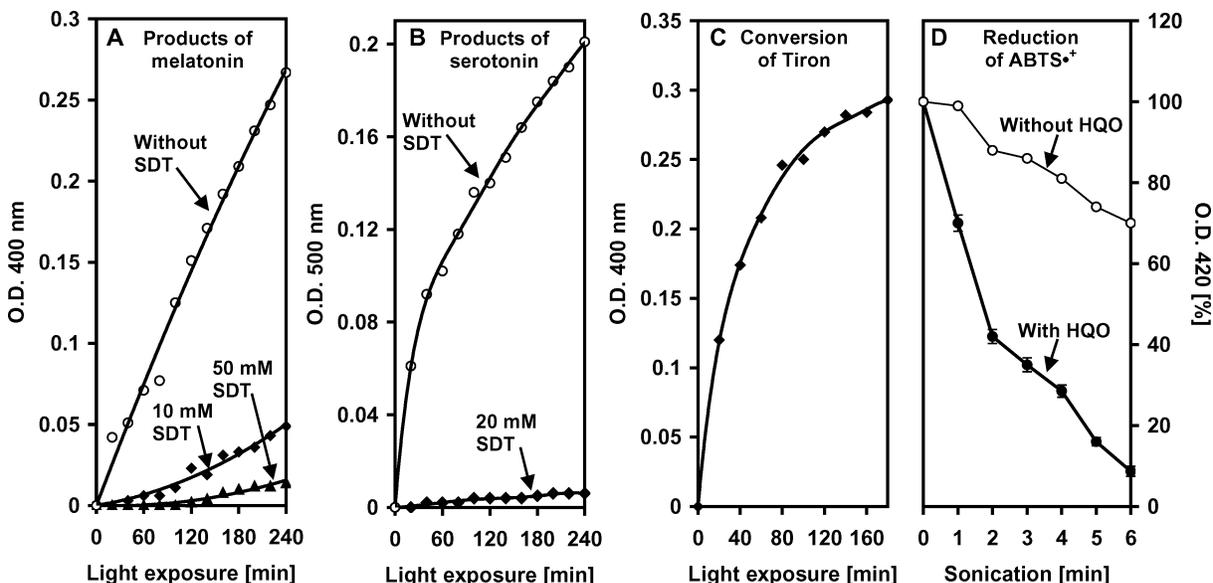


Fig. 6. The involvement of oxygen and electron exchange reactions in photocatalytic actions of 2-hydroxyquinoxaline (HQO). (a) Oligomer formation from melatonin is strongly inhibited by sodium dithionite (SDT). (b) Di- and oligomerization of serotonin is strongly inhibited by SDT. (c) Photocatalytic reaction of the superoxide anion scavenger Tiron with HQO. (d) HQO strongly enhances electron donation to 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) cation radicals (ABTS^{•+}) under energy transfer by ultrasonication. Experiments in potassium phosphate buffer, pH 7.4 (A–C) or 7.5 (D).

suitable acceptor molecule, e.g. O₂, to form a superoxide anion, O₂^{•-}. The resulting organic radical, which should presumably be the *O*-centered quinoxaline-2-oxyl radical, can be expected to abstract, in turn, an electron from an easily oxidizable compound such as melatonin, *N*-acetylserotonin or serotonin.

To distinguish between these alternate modes of reaction, we conducted experiments on photooxidation of melatonin and serotonin by HQO, in the presence of an excess of the singlet oxygen quencher DABCO. Evaluation by HPLC clearly demonstrated that DABCO did not diminish the destruction rates of the indoleamines (Fig. 5). Therefore, interactions with singlet oxygen can only contribute to a very small fraction of photooxidized indoleamines, again a finding which is fully in accordance with corresponding data on singlet oxygen-independent ascorbate destruction by HQO.^[7]

However, with regard to an involvement of singlet oxygen, primary destruction has to be distinguished from secondary reactions. A difference seems to exist between the 5-hydroxylated precursors, on the one hand, and the methoxylated indoleamine melatonin, on the other hand. The only photoproducts formed under catalysis by HQO from serotonin^[7] or *N*-acetylserotonin (unpubl. data) were dimers which progressively oligomerized, whereas kynuramines were virtually absent. The di- and oligomers are not uncommon as oxidation products of the hydroxylated indoleamines, especially by free radicals.^[29–31] In our experiments, the singlet oxygen quencher DABCO did not alter the HQO-dependent oligomerization of serotonin (Fig. 5d),

indicating that the non-radical oxidant did not substantially influence secondary reactions of intermediates deriving from hydroxylated indoleamines. Product analyses by thin-layer chromatography did not reveal any other difference (details not shown). However, in the case of melatonin, the product spectrum was altered by the presence of DABCO. Again, the vast majority of products formed under the influence of HQO were di- and oligomers, according to mass spectrometry,^[7] whereas only about 8.5% of oxidized melatonin (as calculated from data of Figs. 2b and 5c) led to AFMK, which is otherwise a prevailing metabolite in numerous oxidation systems.^[14,15,32] DABCO decreased the fraction leading to AFMK to about 6%, and it strongly diminished the proportion of higher oligomers absorbing at wavelengths above 420 nm. While the small difference in AFMK may be attributed to direct oxidation of melatonin by singlet oxygen, the shift in oligomer composition indicates a more prominent effect of this oxidant on secondary reactions of intermediates deriving from melatonin.

Taken together, these findings indicate that HQO can generate singlet oxygen, but that primary oxidation of the indoleamines involves redox cycling. Photooxidation of melatonin and serotonin was strongly inhibited by sodium dithionite. Since this reductant preferentially donates 2 electrons, this effect may be largely explained by elimination of oxygen rather than single-electron donation to organic free radicals. Since oligomerization of indoleamines does not involve primary oxygen addition, but rather combination of indolyl radicals formed by electron abstraction, the role of oxygen had to be sought in its capability of forming

superoxide anions by electron uptake. In fact, the superoxide scavenger Tiron was photocatalytically converted by HQO. The capacity of HQO to donate electrons was directly demonstrated by ABTS^{•+} reduction (Fig. 6d). In summary, the conclusion has to be that the excited HQO is capable of undergoing organic redox cycling, starting with electron donation from the electron-rich HQO to oxygen, thereby forming an organic free radical, most likely the quinoxaline-2-oxyl radical (deprotonated because of low pK_a of *O*-centered aryl radicals), which regenerates HQO by electron abstraction from the respective indoleamine.

The conclusion on indolyl radical intermediates is strongly supported by the prevalence of oligomer formation, which does not require oxygen addition. Moreover, the difference between hydroxylated and methoxylated indoleamines is well in accordance with this assumption. The 5-hydroxylated indoleamines preferentially produce *C*-centered radicals^[30,31], whereas the 5-methoxylated analogs, in particular, melatonin lead to *N*-centered radicals.^[33] Therefore, oligomers from *N*-acetylserotonin and serotonin differ from those of melatonin in composition and spectral properties.

The photocatalytic properties of HQO have gained new relevance by the discovery of the high amounts of melatonin in mammalian and, in particular, human skin.^[17–20] The complete system of melatonin biosynthesis is present in keratinocytes, so that also the 5-hydroxylated precursors occur in relevant quantities. With regard to the cyto- and photoprotective properties of melatonin,^[12,14–15,20,25,26,34,35] its continual destruction under the influence of HQO may represent a major problem to a light-exposed, intoxicated skin, especially as HQO is not consumed but recycled in these processes, and as other antioxidants such as tocopherols and ascorbate are likewise destroyed.^[7] An additional complication may result from the amphiphilicity of HQO, which is somewhat more lipophilic than melatonin and should, therefore easily cross membranes like this indoleamine, so that the protective action may be strongly impaired in any cell compartment. It will be a future necessity to study the pharmacokinetics and persistence of HQO in the light-exposed skin of quinalphos-intoxicated animals.

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