Water Research 115 (2017) 339–346

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Photo-induced oxidative damage to dissolved free amino acids by the photosensitizer polycyclic musk tonalide: Transformation kinetics and mechanisms

Hansun Fang [a, 1], Yanpeng Gao [b, 1], Honghong Wang [a, c], Hongliang Yin [b], Guiying Li [b], Taicheng An [a, b, *]

[a] State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Resources Utilization and Protection, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, 510640, China
[b] Guangzhou Key Laboratory of Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou, 510006, China
[c] University of Chinese Academy of Sciences, Beijing, 100049, China

ARTICLE INFO

Article history:
Received 6 December 2016
Received in revised form
3 March 2017
Accepted 4 March 2017
Available online 6 March 2017

Keywords:
Polycyclic musks
Amino acids
Oxidative damage
Health effects
Transformation mechanism
Photosensitization damage

ABSTRACT

Residue from the polycyclic musks (PCMs) in household and personal care products may harm human beings through skin exposure. To understand the health effects of PCMs when exposed to sunlight at molecular level, both experimental and computational methods were employed to investigate the photosensitized oxidation performance of 19 natural amino acids, the most basic unit of life. Results showed that a typical PCM, tonalide, acts as a photosensitizer to significantly increase photo-induced oxidative damage to amino acids. Both common and exceptional transformation pathways occurred during the photosensitization damage of amino acids. Experimental tests further identified the different mechanisms involved. The common transformation pathway occurred through the electron transfer from α amino-group of amino acids, accompanying with the formation of $O_2^{\bullet-}$. This pathway was controlled by the electronic density of N atom in α amino-group. The exceptional transformation pathway was identified only for five amino acids, mainly due to the reactions with reactive oxygen species, e.g. $^1O_2$ and excited triplet state molecules. Additionally, tonalide photo-induced transformation products could further accelerate the photosensitization of all amino acids with the common pathway. This study may support the protection of human health, and suggests the possible need to further restrict polycyclic musks use.

1. Introduction

Synthetic musks are ingredients used widely in household products, including perfumes, body lotion, shower gels, deodorants, hair conditioners, and sanitation products (Martinez-Giron et al., 2010; Reiner and Kannan, 2006; Struppe et al., 1997). Nitro-musks and polycyclic musks are two groups of the most widely used synthetic musks. Nitro-musks were used in the early 20th century, but have recently been shown to have active photosensitization properties (Karschuk et al., 2010; Lovell and Sanders, 1988; Parker et al., 1986; Vanhenegouwen, 1991); acute toxic and cancerigenicity (Carlsson et al., 2000; Kafferlein et al., 1998; Neamtu et al., 2000; Schnell et al., 2009; Schramm et al., 1996). As such, many countries and regions have prohibited their use. For instance, musk ambrette was withdrawn from the market due to its phototoxicity during use. In contrast, polycyclic musks are generally thought to be safe and has become alternative fragrances (Ford, 1998; Heberer et al., 1999; Regueiro et al., 2008; Santiago-Morales et al., 2012; Struppe et al., 1997). As a result, their production and use have increased rapidly with a worldwide production of approximately 6000 tons per year. Tonalide and galaxolide are two of the most dominant products, representing approximately 95% and 90% of the EU and U.S. polycyclic musk markets, respectively (Santiago-Morales et al., 2012).

* Corresponding author. Guangzhou Key Laboratory of Environmental Catalysis and Pollution Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou, 510006, China.
E-mail address: antc99@gdut.edu.cn (T. An).
[1] Both authors contributed equally to this work and were considered as co-first authors.

http://dx.doi.org/10.1016/j.watres.2017.03.006
0043-1354/

Unfortunately, polycyclic musks have been associated with ecotoxicological effects for specific organisms (Breitholtz et al., 2003; Chen et al., 2010; Skladanowski et al., 2005); endocrine disruption in humans has also been reported (Dodson et al., 2012). Ingredients containing polycyclic musks in household products, such as skin protectants, shampoo, and perfume, are easily left over skin surfaces, and may cause harm to humans (Wormuth et al., 2005). However, the potential risks risen by residual polycyclic musks on the skin of organisms and humans, especially the photosensitization properties of polycyclic musks are still currently lacking.

Solar light is a natural environmental factor. As such, it is important to validate the impact of the photo-induced transformation of polycyclic musks on human health as a result of using photosensitive ingredients in personal care products. The absorption of solar photons can induce the formation of photoexcited states in skin photosensitizers, and subsequently generate reactive oxygen species (ROSs) and other toxic photoproducts that mediate skin photooxidative stress (Wondrak et al., 2006). Several detrimental effects have been observed during the photosensitization process. For example, the photo-transformation of biomolecules can lead to apoptotic or necrotic signaling pathways and cell death (de Lucas et al., 2014). In addition, photosensitizers can accelerate the injury of cells and other organisms, and even may be associated with the development of human skin cancer (Robinson et al., 2013). All these negative effects could result from the sensitized photoalteration of critical biomolecules, as the macroscopic effects are believed to be consequences of changes at the molecular level. Some of these changes are likely due to photo-induced damages to structural proteins and amino acids.

Proteins are a significant target for oxidative cell damage. Amino acids are essential building blocks of protein, and are found in virtually every cell in human and other mammalian bodies. Since 20 amino acids are assembled in different combinations to create the tens of thousands of different proteins needed to sustain life, these natural amino acids are considered to be the basic units of life. Thus, investigating photo-induced damages to these basic units of life will help us fundamentally understand the photosensitization effects and the mechanisms of polycyclic musks on human health.

In this study, an important polycyclic musk, tonalide, was selected to study its damages to amino acids through photosensitization. Firstly, the study examined the photosensitized damage kinetics of 19 amino acids (all of the 20 natural amino acids except proline due to the inability to detect secondary amines) in the absence and presence of tonalide. Secondly, both experimental and computational methods were used to study the photosensitization mechanisms of amino acids at a molecular level. Finally, the solutions of tonalide irradiated for different period of time were collected to explore the photosensitization properties of tonalide's products. The goal of the study was to assess the risks and understand the potential pathopoiesia mechanisms of this potential organic pollutant under sunlight irradiation. In addition, the study was intended to provide a scientific basis for the early prevention and treatment of skin diseases.

2. Experimental section

2.1. Materials

Tonalide and *p*-Nitroanisole (PNA) were purchased from Adamas Reagent, Ltd. (Shanghai, China, purity > 95%). *O*-Phthalaldehyde (OPA, purity 97%) and nitro blue tetrazolium (NBT, purity 99%) were from Sengon Biotech Co., Ltd. (Shanghai, China). *N*,*N*-dimethyl-4-nitrosoaniline (RNO, analytic grade) was from AOPLLO Scientific Ltd. (Bredbury, England). N-acetylglycine (N-Gly), N(α)-acetyllysine (N-Lys), 2,4,6-trimethylphenol (TMP), imidazole and 19 amino acids were all analytical grade reagents used without further purification. The structural information and the abbreviation of 19 amino acids as well as acetyl amino acids are summarized in Table S1 in Supporting Information (SI).

Tonalide was primarily prepared in acetonitrile (HPLC grade) with a concentration of 20 mM, forming the storage solution, which was then diluted to required concentration just before the irradiation experiments. All other solutions were prepared using high purity deionized water (Millipore Corp., 18 MΩ cm); high-purity oxygen (O_2) or nitrogen (N_2) was used in some specific experiments to change the atmospheric environment of the reaction systems.

2.2. Photo-induced oxidation kinetics of amino acids

The photo-induced oxidative damage of each specific amino acid was assessed through experiments conducted in a 60 mL Pyrex glass tube reactor (diameter: 2.4 cm). The reactor was placed in a Pyrex glass cup with a double-walled cooling water jacket to keep the solution at a constant temperature throughout the experiments (Fig. S1). The reaction solution was prepared with acetonitrile and water at a 50:50 ratio, with a 30 mL volume. Photo-oxidative damage kinetics studies were performed in both acidic and alkaline solutions, with initial tonalide of 500 μM and amino acid concentrations of 50 μM, respectively. The acidic solution was maintained using a 2.5 mM phosphate ($C_{KH_2PO_4} : C_{K_2HPO_4} = 98:2$), and the alkaline solution was maintained using a 2.5 mM carbonate solution ($C_{Na_2CO_3} : C_{NaHCO_3} = 60:40$). Then, the actual pH values of the acetonitrile/water mixtures were calculated as 6.06 and 10.36, which were a little higher than the pH in pure water with the same buffer reagents (Gagliardi et al., 2007). The solution was stirred in the dark for 15 min to achieve equilibrium with respect to gas and temperature before being exposed to irradiation (dissolved oxygen was measured to be 15.6 mg L^{-1} with Winkler's method). Then, the light was turned on, and the 0.75 mL solution was sampled at different time intervals to determine amino acid concentrations. To assess photo-induced oxidative damage to amino acids under O_2 or N_2 saturated condition, the solution was sealed and bubbled with O_2 or N_2 for 20 min before the light was turned on. Samples were collected with a syringe under the positive pressure of specific gas. All experiments were repeated twice, and the average values were obtained for all studies.

A 300 W xenon lamp coupled with a sunlight simulated filter (Perfectlight, Inc., Beijing, China) was housed in one side of the reactor, and was used as the light source. The irradiance spectrum of actual and stimulated sunlight was measured with a spectrometer (USB 2000+, Ocean Optics Inc., USA). As Fig. S2 shows, the filtered light emission spectrum of xenon was similar to actual sunlight.

2.3. Quantitative and qualitative analysis methods

The apparatus and detailed methods associated with the use of high performance liquid chromatography (HPLC) and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) are included in SI.

The apparent quantum yield (ϕ_{aa}) of specific amino acid was calculated using the equation as follows (Kelly and Arnold, 2012):

$$\phi_{aa} = \frac{k_{aa}}{k_{PNA}} \sum_{\lambda} \frac{\varepsilon_{PNA}\lambda_{range}L_{\lambda}}{\varepsilon_{S}\lambda_{range}L_{\lambda}}\phi_{PNA}$$

where ϕ_{PNA} is the quantum yield of the chemical actinometer *p*-nitroanisole (PNA, $\phi_{PNA} = 0.00028$) (Dulin and Mill, 1982), k_{aa} and