

Ultraviolet Irradiation-Dependent Fluorescence Enhancement of Hemoglobin Catalyzed by Reactive Oxygen Species

Leiting Pan^{1*}, Xiaoxu Wang¹, Shuying Yang¹, Xian Wu¹, Imshik Lee¹, Xinzheng Zhang¹, Romano A. Rupp^{1,2}, Jingjun Xu^{1*}

¹ The Key Laboratory of Weak-Light Nonlinear Photonics, Ministry of Education, TEDA Applied Physics School and School of Physics, Nankai University, Tianjin, China,

² Faculty of Physics, Vienna University, Vienna, Austria

Abstract

Ultraviolet (UV) light has a potent effect on biological organisms. Hemoglobin, an oxygen-transport protein, plays an irreplaceable role in sustaining life of all vertebrates. In this study we scrutinize the effects of ultraviolet irradiation (UVI) as well as visible irradiation on the fluorescence characteristics of bovine hemoglobin (Bhb) *in vitro*. Data show that UVI results in fluorescence enhancement of Bhb in a dose-dependant manner. Furthermore, UVI-induced fluorescence enhancement is significantly increased when Bhb is pretreated with hydrogen peroxide (H₂O₂), a type of reactive oxygen species (ROS). Meanwhile, The water-soluble antioxidant vitamin C suppresses this UVI-induced fluorescence enhancement. In contrast, green light irradiation does not lead to fluorescence enhancement of Bhb no matter whether H₂O₂ is acting on the Bhb solution or not. Taken together, these results indicate that catalysis of ROS and UVI-dependent irradiation play two key roles in the process of UVI-induced fluorescence enhancement of Bhb.

Citation: Pan L, Wang X, Yang S, Wu X, Lee I, et al. (2012) Ultraviolet Irradiation-Dependent Fluorescence Enhancement of Hemoglobin Catalyzed by Reactive Oxygen Species. PLoS ONE 7(8): e44142. doi:10.1371/journal.pone.0044142

Editor: Pedro Lagerblad Oliveira, Universidade Federal do Rio de Janeiro, Brazil

Received: March 15, 2012; **Accepted:** August 1, 2012; **Published:** August 30, 2012

Copyright: © 2012 Pan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the Specialized Research Fund for the Doctoral Program of Higher Education (New Teachers) (No. 20110031120004), the Fundamental Research Funds for the Central Universities, the National Natural Science Foundation of China (No. 11074133), the National Basic Research Program of China (Nos. 2007CB307002, 2010CB934101), 111 Project (No. B07013) and International S&T cooperation program of China (2011DFAS2870). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: plt@nankai.edu.cn (LP); jjxu@nankai.edu.cn (JX)

Introduction

There would scarcely be life on earth without light. Main source of visible and ultraviolet (UV) light is the nuclear fusion reactor in the middle of our solar system. Although certain skin diseases can be healed by a proper dose of UV radiation, UV light is in general noxious [1]: For instance, it may cause sunburn, photoaging [2], skin cancers [3], or immune suppression [4]. Since increasing stratospheric ozone depletion of anthropogenic origin results in higher UV intensity to reach the ground, there is a growing concern about possible consequences and thus strong interest in research that might lead to a better understanding of the effects of enhanced UV irradiation (UVI) [5]. Several factors play an crucial role in UVI-induced different cellular physiological and pathological responses. One of them is the production of reactive oxygen species (ROS) through a photosensitized mechanism involving energy transfer by chromophores to oxygen molecules (photodynamic effect). There is a wealth of evidence that ROS trigger, among other physiological responses, also pathological ones in biological organisms [6–8].

As for animals and humans, UV light penetrating into the dermis of the skin can affect erythrocytes inside the capillary [9]. Recently an enhancement of autofluorescence was observed in UVI-treated erythrocytes *in vitro* and attributed to the photodecomposition of hemoglobin (Hb) [10,11], the major component of erythrocytes. Hb contains a benzene-ring-like structure that can exhibit high

photosensitive behaviors. Therefore, as an analog of photosensitizer, Hb was used as a model molecule in the study of photodynamic therapy, a prospective treatment for certain types of cancer and tumor [12]. Since Hb is vital to oxygen transport and plays an essential role in physiological and pathological processes [13], it is necessary to fully understand the interaction between UVI and Hb. To do this, we used bovine hemoglobin (Bhb) as a model in the present work to investigate the relationship among UVI, formation of ROS, photodecomposition by spectroscopy *in vitro*.

Methods

Bovine hemoglobin (Bhb), hydrogen peroxide (H₂O₂), and vitamin C that obtained from Sigma (St. Louis, MO, USA) were dissolved in phosphate buffered saline (PBS) solution. For all experiments, the working solutions were placed in quartz cuvettes with 3 mL. UV treatment was achieved by illumination with a quartz UV sterilizing lamp (electrical power of 20 W) at a distance of 2 cm from the samples emitting essentially at 254 nm. The illumination intensity was approximately 7 mW/cm² (calculated from irradiation geometry with conversion factor of 24% for UV light/electrical power). A comparison group was illuminated by an expanded beam from a 532 nm diode laser (as one representation of visible light) at 60 mW/cm². Fluorescence spectra of the samples were monitored with a spectrofluorometer (FLS920, Edinburgh Instruments, U.K.) at an excitation wavelength of

365 nm. Absorption spectra were acquired with a spectrophotometer (U-4100, Hitachi, Japan).

Results

UVI-induced Fluorescence Enhancement of BHb

A solution containing 10 μM BHb was irradiated with a UV sterilizing lamp for 0 min, 10 min, 20 min, and 30 min, respectively. Fluorescence emission at 365 nm excitation was recorded immediately afterwards by a spectrofluorometer (apart from the 0 min case, excitation irradiation during measurement being negligible in comparison to the prior UVI dose). For the spectra shown in Fig. 1a, the intensities of 3 scans were summed up. We could find that UVI significantly resulted in fluorescence enhancement of BHb solution in a dose-dependent manner. Besides, throughout the irradiation range investigated, we observed no saturation due to consumption of the irradiated molecules: there is a strictly linear increase of fluorescence with UVI dose (Fig. 1b). So the consumption of BHb by UVI-induced processes thus remains negligible in comparison to the total number of BHb.

ROS-catalyzed Fluorescence Enhancement of BHb Induced by UVI

It is well known that ROS plays a crucial role in various UVI-induced different biological effects [6–8]. Therefore, we needed to figure out whether ROS was involved in this process of UVI-induced fluorescence enhancement. First, 1 mM H_2O_2 , one kind of ROS, was selected to preincubated the BHb solution (10 μM) for 10 minutes in the dark. Then, this H_2O_2 -pretreated BHb solution was immediately irradiated by UV light for another 3 min. It was clearly that BHb solution pretreated with H_2O_2 showed much higher fluorescence intensity at the same UVI irradiation time (Fig. 2a), implying that ROS could catalyze UVI-induced fluorescence enhancement of BHb. To further determine the catalytic effect of ROS, low-dose UV excitation light (365 nm) from spectrofluorometer was used to irradiate the BHb solution with or without pretreatment with H_2O_2 . Under this condition, the effect of ROS made by UV excitation itself can be neglected. Therefore, this UV excitation light had two roles in the process of

fluorescence change: to serve as excitation light for fluorescence detection and to provide low-dose UVI for treating samples. We monitored the dynamic variation of the samples fluorescence intensity with spectrofluorometer in a real-time manner with 300 s. As summarized in Fig. 2b, this low-dose UV excitation light resulted in a linear increase of fluorescence of H_2O_2 -pretreated BHb solution, whereas it did not have this increase effect without the assistance of H_2O_2 . These data suggested that ROS revealed a catalytic role in this process of fluorescence enhancement.

The Protection Role of Vitamin C for UVI-induced Phototoxic Effect

To further confirm whether ROS was necessary, the potent antioxidant vitamin C was used in this work. Vitamin C (10 mM) was added to BHb (10 μM) solution for study. As shown in Fig. 3, the fluorescence spectra of pure BHb solution and BHb solution with vitamin C are identical (black line and red line). Then, we irradiated this mixture solution for 10 min using UV sterilizing lamp. Data showed that, after treatment with UVI for the same time, the fluorescence intensity of BHb solution mixed with vitamin C (green line) was markedly lower than that of pure BHb solution (blue line), indicating the obvious protection role of vitamin C.

UVI-dependent Fluorescence Enhancement of BHb

It was reported that visible light could also result in ROS generation and stimulate biological effects in various cell types [14,15]. For that reason we were also searching for fluorescence responses to irradiation by visible light. A 60 mW/cm^2 green laser (532 nm) was selected to be a representative visible light source for the irradiation of 10 μM BHb solution. As shown in Fig. 4a, no fluorescence enhancement is found by green irradiation of BHb solution. Monitoring real-time fluorescence intensity of H_2O_2 -pretreated BHb solution at 595/2 nm for low-dose excitation at 532 nm did not have any effect on fluorescence enhancement (green line in Fig. 4b) despite of a pretreatment with H_2O_2 while dim UV excitation stimulates increase of fluorescence under the same conditions (purple line in Fig. 4b). Likewise, there is fluorescence enhancement upon low-dose UV excitation light that is increased by action of ROS, as already discussed, however

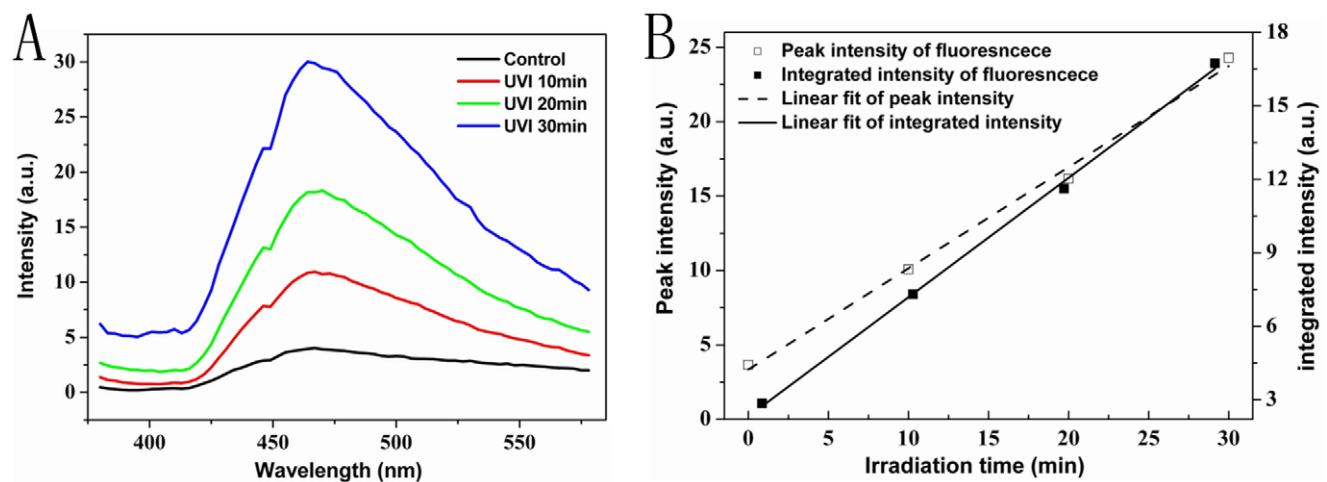


Figure 1. UVI induces fluorescence enhancement of BHb in a dose-dependent manner. (A) Spectra detection of a 10 μM BHb solution (at 365 nm excitation wavelength) after irradiation for 10 min (red line), 20 min (green line), and 30 min (blue line) with 7 mW/cm^2 UV light. Black line is the fluorescence spectrum without prior UVI. (B) Intensity at the peak and integrated intensity (baseline corrected and integrated from 450 nm to 550 nm) plotted versus irradiation time.

doi:10.1371/journal.pone.0044142.g001

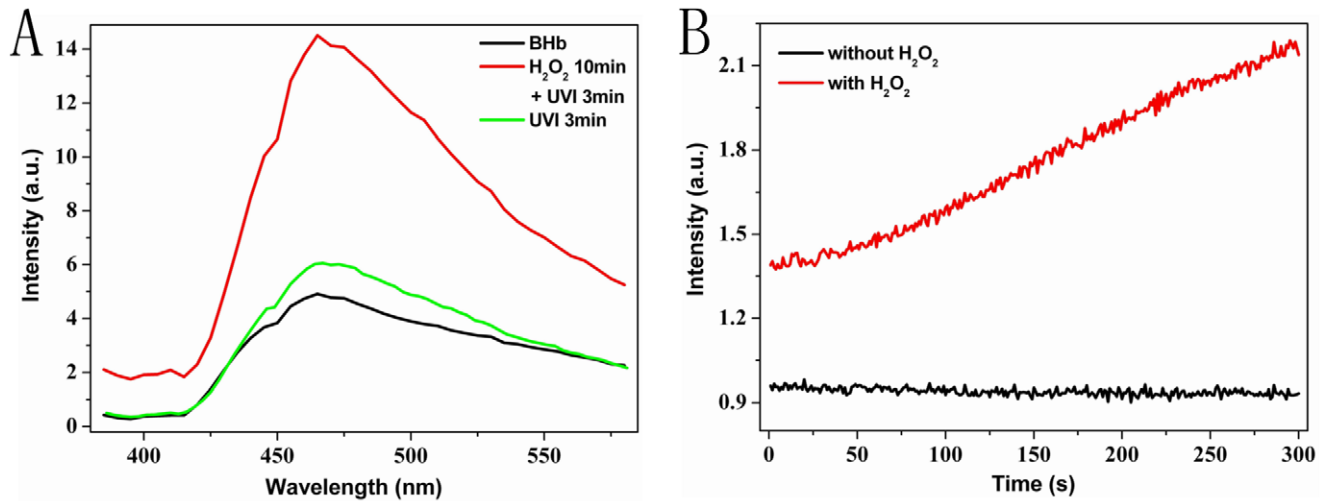


Figure 2. H₂O₂ catalyzes UVI-induced fluorescence enhancement of BHB. (A) Fluorescence spectra of different samples at 365 nm excitation: black line represents a BHB solution (10 μ M) without any treatment; green line indicates the UVI-pretreated (3 min) BHB solution; red line corresponds to H₂O₂-preincubated (1 mM for 10 min) BHB solution with subsequent UVI treatment (7 mW/cm², 3 min). (B) Real-time monitoring of fluorescence at 464/2 nm for a sample with or without 1 mM H₂O₂ treatment at 365 nm excitation. Black line corresponds to the pure BHB solution, red line indicates BHB solution pretreated with 1 mM H₂O₂ for 10 min. doi:10.1371/journal.pone.0044142.g002

no such effect can be found for low-dose green excitation light (Fig. 4c,d). These results clearly demonstrated that fluorescence enhancement of BHB solution depended on UVI.

UV/Vis Absorption Measurement of Samples

Further studies were needed to confirm whether the UVI-induced fluorescence enhancement of BHB was due to the higher fluorescence efficiency of photodecomposition products of BHB because increasing absorption of excitation light maybe also result in strong fluorescence. Therefore, we measured the UV/Vis absorption spectra of different samples. Figure 5 showed the UV/Vis absorption spectra of pure BHB solution (black line), UVI-

pretreated BHB solution (green line), and H₂O₂-pretreated BHB solution (red line), respectively. It was obvious that the effect of UVI or H₂O₂ resulted in a decrease of absorption rate around 365 nm, supporting the production of BHB photodecomposition products with higher fluorescence efficiency.

Discussion

Data clearly showed that UVI could result in fluorescence enhancement of BHB solution in a dose-dependent manner by spectroscopy method (Fig. 1). Kaestner et al. [10] observed an enhancement of autofluorescence in UVA-treated (380 nm) erythrocytes and attributed the increase of fluorescence to fluorescent bilirubin generated by UV-induced photodecomposition of Hb with the argument that the form as well as the wavelength of the maximum of the fluorescence spectrum agrees reasonably with the one reported for isolated bilirubin [16]. It was found that apart from a small indentation at about 450 nm the form of the spectra with a steeper slope on the shorter wavelength side than on the long wavelength side of the maximum is similar to the one shown in [10] for human erythrocytes, however the maximum is at a significantly shorter wavelength, namely 470 nm instead of 508 nm, indicating that fluorescent molecules generated by UV-induced photodecomposition of BHB solution may be different from that of erythrocytes.

UVI can generate large amounts of ROS through photodynamic effect. Meanwhile, it is well known that ROS plays an important role in UVI-induced many biological effects [6–8]. The question remains, whether the fluorescent molecules are generated directly or whether they result indirectly from photo-generated ROS. In the latter case photo-induced fluorescence is a secondary consequence of primary ROS photo-production. This second pathway is the so-called photodynamic effect. In particular, ROS induces structural changes of hemoglobin [17,18] and participates in the degradation process [19,20]. In a further series of experiments we therefore targeted to test for involvement of ROS in the observed UVI-induced fluorescence enhancement. To that aim, we have chosen H₂O₂, a frequently occurring ROS. As illuminated in Fig. 2a, H₂O₂-preincubated (1 mM for 10 min)

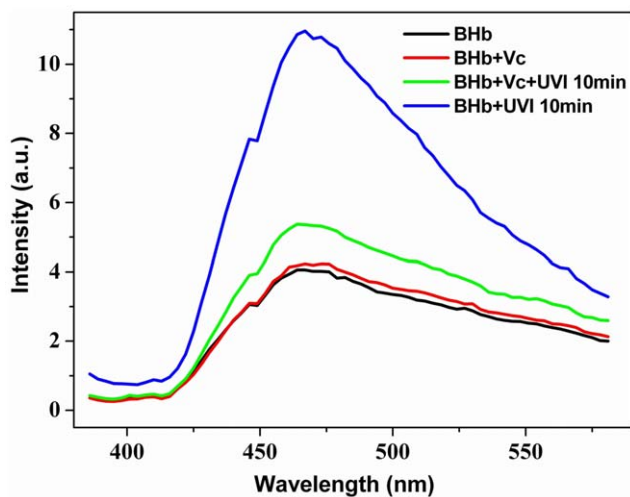


Figure 3. Vitamin C suppresses UVI-induced fluorescence enhancement of BHB. Black line indicates a pure BHB solution (10 μ M); red line represents the mixture of BHB (10 μ M) and vitamin C (10 mM); green line corresponds to the mixture of BHB (10 μ M) and vitamin C (10 mM) after UVI for 10 min; blue line indicates UVI-pretreated (10 min) BHB solution. doi:10.1371/journal.pone.0044142.g003

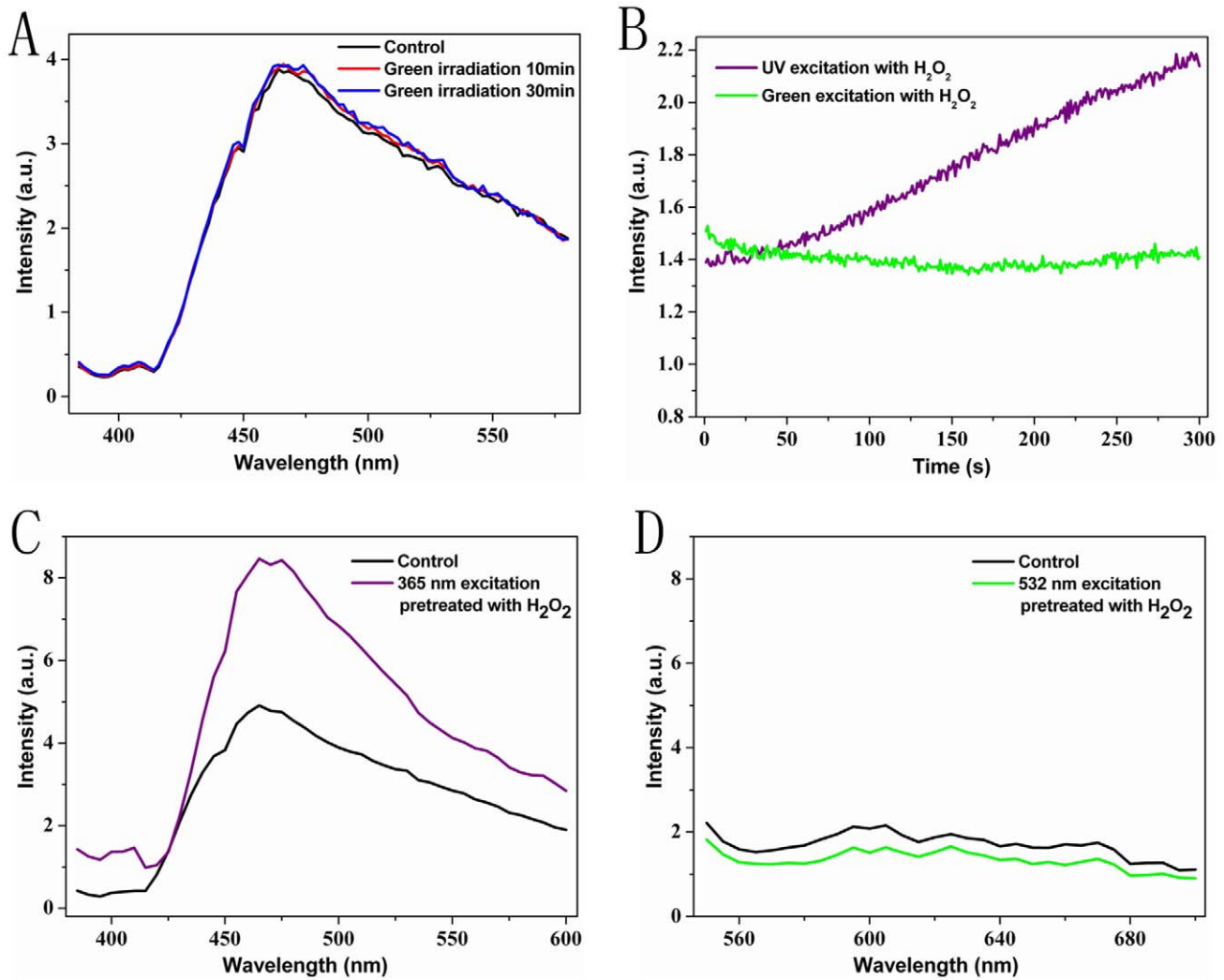


Figure 4. Fluorescence enhancement of BHB depends on UVI. (A) Fluorescence spectra of 10 μM BHB solution (365 nm excitation) after irradiation with 532 nm laser at an intensity of 60 mW/cm^2 for 0 min (black line), 10 min (red line), and 30 min (blue line), respectively. (B) Real-time detection of fluorescence of 1 mM H_2O_2 -pretreated (10 min) BHB solution at 595/2 nm with 532 nm excitation (green line) and at 464/2 nm with 365 nm excitation (purple line). (C) Fluorescence spectra of BHB solution with 365 nm excitation: black line means the pure BHB solution, purple line indicates BHB solution pretreated with 1 mM H_2O_2 for 10 min. (D) Fluorescence spectra of BHB solution with 532 nm excitation: black line corresponds to a BHB solution without any treatment, green line represents the BHB solution pretreated with 1 mM H_2O_2 for 10 min. doi:10.1371/journal.pone.0044142.g004

BHB solution with subsequent UVI treatment (red line) show much higher fluorescence than that of only UVI-preincubated sample (green line). We propose to explain this result by the hypothesis that UVI-induced enhancement of fluorescence is catalyzed by ROS. To further investigate the catalytic effect of ROS, we consider the effect of UV excitation at 365 nm. Besides its role as excitation source during the fluorescence measurement run, it acts in the same time as a low-dose irradiation source. This is to be expected, since ROS production by this low-dose irradiation source at 365 nm is negligible. In a real-time fluorescence measurement we followed the fluorescence without and with H_2O_2 pretreatment at 465 nm as a function of time over 300 s. As can be seen from Fig. 2b for the sample without H_2O_2 , there is no increase of fluorescence. However, one can see a steady increase of fluorescence for the ROS-pretreated solution. In this result we see a confirmation of our view that ROS plays a catalytic role in this process of UV-induced fluorescence enhancement.

Nagababu et al [20] identified two fluorescence bands to appear upon chemical attack by ROS in Hb solution, one having emission centered at 465 nm with 321 nm excitation and one having emission centered at 525 nm with excitation maximum at 460 nm. From the differing time course of the bands it was concluded that they belong to two different compounds. Comparing the ROS-induced fluorescence bands with our UVI-induced bands, one can see that the ROS-induced band with emission at 525 nm can be identified with the UVI-induced fluorescence band in human erythrocytes observed by Kaestner et al. and thus can be attributed to bilirubin, while the band with emission at 465 nm is identical with the UVI-induced band in BHB solution observed in our study and belongs to a hitherto unidentified compound. The most plausible conclusion that can be drawn from these facts is that the same fluorescent molecules are generated by ROS and by UVI. This suggests that the UVI-generated fluorescent species is due to a photodynamic mechanism involving UV generation of ROS in

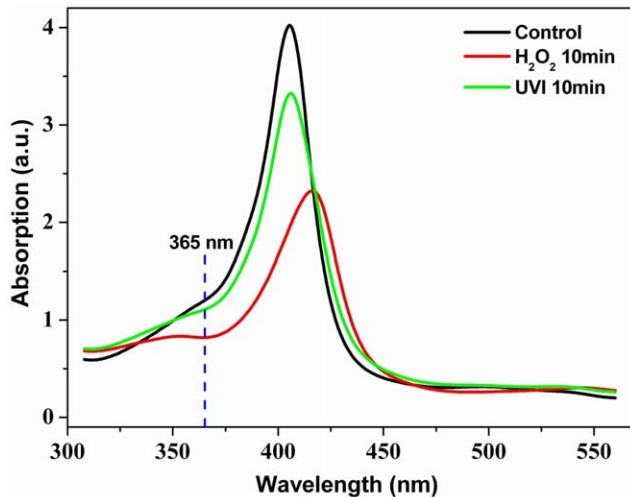


Figure 5. UV/Vis absorption spectra of the samples. Black line indicates the pure BHB solution (10 μ M); green line represents UVI-pretreated (10 min) BHB solution; red line corresponds to BHB solution pretreated with 1 mM H_2O_2 -pretreated for 10 min. Dashed line indicates the position of the excitation wavelength of the fluorescence measurements.

doi:10.1371/journal.pone.0044142.g005

the first step followed by a secondary generation of the fluorescent species by the action of ROS.

Vitamin C reduces UVI-induced damage in all animals and protects skin from photoaging and cancer [21,22] because it is a potent antioxidant [23]. Specially, vitamin C contribute to the decrease in the oxidative stress of erythrocytes [24]. In order to further submit the hypothesis of ROS involvement in the effect of UVI, we added vitamin C (10 mM) to BHB solution. The fluorescence spectra of pure BHB solution and BHB solution with vitamin C are identical (black line and red line in Fig. 3). If we compare now the effect of UVI by the UV sterilizing lamp for 10 min on these two samples, we see that the effect of fluorescence enhancement is significantly suppressed by vitamin C (Fig. 3, green line in comparison with the blue line). We see in these findings a confirmation of our assumption that ROS plays a catalytic effect in the process of UVI-induced fluorescence enhancement. In addition, we have used other ROS scavengers, such as superoxide dismutase, glutathione and catalase. It was found that these scavengers did not block fluorescence enhancement of BHB induced by UVI *in vitro* (data not shown). It seems that vitamin C play a special role in protection against UVI-induced fluorescence enhancement of Hb. Further study is still needed to elucidate the reason for different effects of these ROS scavengers on UVI-induced fluorescence enhancement of Hb.

Furthermore, some studies reported on ROS generation by visible light and its stimulation of biological effects in various cell types [14,15]. For that reason we were also searching for fluorescence responses to irradiation by visible light. A 60 mW/cm² green laser (532 nm) was selected to be a representative visible light source for the irradiation of 10 μ M BHB solution. As shown in Fig. 4a, no fluorescence enhancement is found by green irradiation of BHB solution. Monitoring real-time fluorescence intensity of H_2O_2 -pretreated BHB solution at 595/2 nm for low-dose excitation at 532 nm did not have any effect on fluorescence

enhancement (green line in Fig. 4b) despite of a pretreatment with H_2O_2 while dim UV light stimulates increase of fluorescence under the same conditions (purple line in Fig. 4b). Likewise, there is fluorescence enhancement upon low-dose UV excitation light that is increased by action of ROS, as already discussed, however no such effect can be found for low-dose green excitation light (Fig. 4c,d). Taken together, These results clearly demonstrated that fluorescence enhancement of BHB solution depended on UVI.

Besides, Figure 5 compares the UV/VIS absorption spectra of native BHB solution (black line) with UVI-pretreated BHB solution (green line) and H_2O_2 -pretreated BHB solution (red line), respectively. There are two similarities: Both, UVI as well as chemical treatment with H_2O_2 lead to a decrease of two absorption bands, one around 410 nm and one around 360 nm (only seen as a shoulder of the former band). However, there is also a remarkable difference: while a new band appears at about 425 nm for ROS treatment, this does not happen for UV treatment. We may then roughly sketch the situation at the excitation wavelength 365 nm as follows: The absorption at 365 nm can be thought to consist of two contributions, one proportional to the number density N_1 of molecules with high fluorescence efficiency for 460 nm emission and one proportional to the number density N_2 of non-fluorescent molecules (or molecules of very low fluorescence efficiency). Above we established that fluorescence at 365 nm excitation is increased by UVI as well as by chemical ROS attack. If N_2 were constant, one would therefore expect an increase in absorption at 365 nm – which is not the case. It is therefore beyond doubt that N_2 decreases due to UVI or chemical ROS attack, subsequently resulting in the generation of N_1 . therefore, when N_1 increases, the fluorescence intensity simply increases because more photons are available for fluorescence-effective absorption due to elimination of concurrent non-fluorescent processes.

In conclusion, data show that UVI induces fluorescence enhancement of BHB solution with increasing irradiation dose while irradiation with visible light does not. We suggested that the higher single photon energy of UV light results in photodecomposition of Hb, which is not the case for photons of green light. Furthermore, ROS generated by UVI plays an obviously catalytic role in the process of fluorescence enhancement. Fluorescence enhancement of BHB is due to the catalysis of ROS and UVI-dependent photodecomposition. As a physical therapy, ultraviolet blood irradiation and oxygenation (UBIO) was used to treat many nonspecific diseases in clinics [25,26]. Especially, UBIO could improve the oxygen transport function of erythrocytes [25,27]. We can see that Hb may play an important role in this therapy because it is the main target in UVI-treated erythrocytes. Therefore, We believe that this study of UVI-induced enhancement of BHB might fill an important gap in the research of UBIO.

Acknowledgments

We thank Dr. Jiwei Qi (Nankai University, Tianjin, China) for excellent technical assistance.

Author Contributions

Conceived and designed the experiments: LP JX. Performed the experiments: XW SY XW. Analyzed the data: XW SY XW RAR. Contributed reagents/materials/analysis tools: XZ IL JX. Wrote the paper: LP RAR.

References

- Gallaghera RP, Lee TK (2006) Adverse effects of ultraviolet radiation: a brief review. *Prog Biophys Mol Biol* 92: 119–131.
- Longstreth J, Gruij FR de, Kripke ML, Abseck S, Arnold F, et al. (1998) Health Risks. *J Photoch Photobio B* 46: 20–39.
- Hussein MR (2005) Ultraviolet radiation and skin cancer: molecular mechanisms. *J Cutan Pathol* 32: 191–205.
- Nghiem DX, Kazimi N, Clydesdale G, Ananthaswamy HN, Kripke ML, et al. (2001) Ultraviolet A Radiation Suppresses an Established Immune Response: Implications for Sunscreen Design. *J Invest Dermatol* 117: 1193–1199.
- Madronich S, McKenzie RL, Björn LO, Caldwell MM (1998) Changes in Biologically Active Ultraviolet Radiation Reaching the Earth's Surface. *J Photoch Photobio B* 46: 5–19.
- Yaar M, Gilchrist BA (2007) Photoageing: Mechanism, Prevention and Therapy. *Brit J Dermatol* 157: 874–887.
- Valencia A, Kochevar IE (2006) Ultraviolet A Induces Apoptosis via Reactive Oxygen Species in a Model for Smith-Lemli-Opitz Syndrome. *Free Radical Bio Med* 40: 641–650.
- Scharfetter-Kochanek K, Wlaschek M, Brenneisen P, Schauen M, Blandschun R, et al. (1997) UV-induced reactive oxygen species in photocarcinogenesis and photoaging. *Biol Chem* 378: 1247–1257.
- Svobodová AR, Galandaková A, Šianská J, Doležal D, Ulrichová J, et al. (2011) Acute Exposure to Solar Simulated Ultraviolet Radiation Affects Oxidative Stress-Related Biomarkers in Skin, Liver and Blood of Hairless Mice. *Biol Pharm Bull* 34: 471–479.
- Kaestner L, Juzeniene A, Moan J (2004) Erythrocytes – the house elves of photodynamic therapy. *Photochem Photobiol Sci* 3: 981–989.
- Kaestner L, Tabellion W, Weiss E, Bernhardt I, Lipp (2006) Calcium imaging of individual erythrocytes: problems and approaches. *Cell Calcium* 39: 13–19.
- Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, et al. (1998) Photodynamic Therapy. *J Natl Cancer I* 90: 889–905.
- Reeder BJ, Svistunenko DA, Cooper CE, Wilson MT (2004) The Radical and Redox Chemistry of Myoglobin and Hemoglobin: From In Vitro Studies to Human Pathology. *Antioxid Redox Sign* 6: 954–966.
- Lavi R, Shainberg A, Friedmann H, Shneyvays V, Rickover O, et al. (2003) Low Energy Visible Light Induces Reactive Oxygen Species Generation and Stimulates an Increase of Intracellular Calcium Concentration in Cardiac Cells. *J Biol Chem* 278: 40917–40922.
- Lavi R, Shainberg A, Shneyvays V, Hochauer E, Isaac A, et al. (2010) Detailed Analysis of Reactive Oxygen Species Induced by Visible Light in Various Cell Types. *Laser Surg Med* 42: 473–480.
- Cu A, Bellah GG, Lightner DA (1975) On the Fluorescence of Bilirubin. *J Am Chem Soc* 97: 2579–2580.
- Jia Y, Buehler PW, Boykins RA, Venable RM, Alayash AI (2007) Structural Basis of Peroxide-mediated Changes in Human Hemoglobin. *J Biol Chem* 282: 4894–4907.
- Zhao J, Meng W, Miao P, Yu Z, Li G (2008) Photodynamic effect of hypericin on the conformation and catalytic activity of hemoglobin. *Int J Mol Sci* 9: 145–153.
- Nagababu E, Rifkind JM (2004) Heme Degradation by Reactive Oxygen Species. *Antioxid Redox Sign* 6: 967–978.
- Nagababu E, Rifkind JM (1998) Formation of fluorescent heme degradation products during the oxidation of hemoglobin by hydrogen peroxide. *Biochem Biophys Res Commun* 247: 592–596.
- Lin J, Selim MA, Shea CR, Grichnik JM, Omar MM, et al. (2003) UV photoprotection by combination topical antioxidants vitamin C and vitamin E. *J Am Acad Dermatol* 48: 866–874.
- Farris PK (2005) Topical Vitamin C: A Useful Agent for Treating Photoaging and Other Dermatologic Conditions. *Dermatol Surg* 31: 814–818.
- Heitzer T, Just H, Munzel T (1996) Antioxidant Vitamin C Improves Endothelial Dysfunction in Chronic Smokers. *Circulation* 94: 6–9.
- Krukoski DW, Comar SR, Claro LM, Leonart MSS, do Nascimento AJ (2009) Effect of vitamin C, deferoxamine, quercetin and rutin against *tert*-butyl hydroperoxide oxidative damage in human erythrocytes. *Hematology* 14: 168–172.
- Pamphilon DH (1998) The treatment of blood components with ultraviolet-B irradiation. *Vox Sanguinis* 74: 15–19.
- Rowen RJ (1996) Ultraviolet Blood Irradiation Therapy (Photo-Oxidation). *Int J Biosocial Med Research* 14: 115–132.
- Yu L, Lu X, Zhang J, Song E, Cui X (1997) The effects of UBIO on morphology of red blood cells and characteristics of blood rheology. *Chinese J Med Phys* 14: 136–137.