Photocages for protection and controlled release of bioactive compounds

I. Aparici-Espert, M. C. Cuquerella, C. Paris, V. Lhiaubet-Vallet*, M. A. Miranda*

Instituto Universitario Mixto de Tecnología Química (UPV-CSIC), Universitat Politécnica de Valéncia, Consejo Superior de Investigaciones Científicas, Avda de los Naranjos s/n, 46022 Valencia, Spain. E-mail: lvirgini@itq.upv.es, mmiranda@qim.upv.es

Abstract

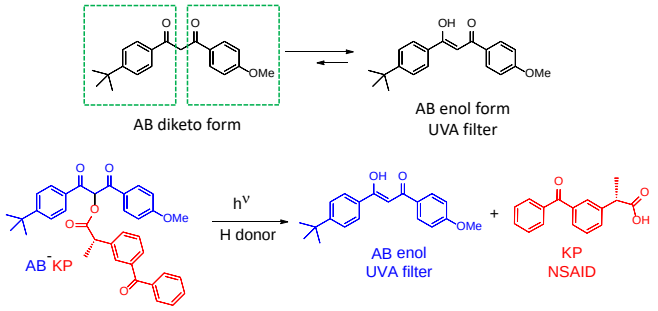
Using a sunscreen-based photocage, we have demonstrated that it is possible to prevent photodegradation of a bioactive compound and to achieve its controlled photorelease. The concept has been proven linking avobenzone, one of the most important UVA blockers, to ketoprofen, which is a representative example of a photosensitive drug.

Results and Discussion

A number of bioactive compounds are used under conditions that involve their exposure to sunlight. This may result in progressive photodegradation, leading to loss of activity and/or the appearance of undesired photoproducts.[1,2] In this context, topical drugs are now in widespread use to treat illnesses as diverse as bacterial or fungal infections, allergic rash, musculoskeletal trauma, etc. The fact that the drug is applied directly to the affected area allows concentrating its effects where needed. As a consequence, for an almost comparable effectiveness, local application shows a better safety profile than oral medication as it minimizes the spread of the active ingredients through the bloodstream to other parts of the body, thus reducing the risk of side effects such as gastric disturbances, generally associated with systemic delivery.[3] However, the use of topical drugs is not innocuous and also presents drawbacks, the most important being related to the instability of some active constituents toward solar radiation. Sunlight exposure leads not only to degradation of the drug, thus decreasing its pharmacological effect, but also to the occurrence of chemical photosensitivity.[4,5] This originates cutaneous side reactions such as phototoxicity or photoallergy, which typically appear as an exaggerated sunburn but they can also provoke severe and/or persistent reactions.[4,5]

In this context, topical pain relievers such as non-steroidal anti-inflammatory drugs (NSAIDs) are available in most of the example of this family is ketoprofen (KP, Scheme 1) that is responsible for pronounced cutaneous photosensitization.[6-10] Nowadays, the

occurrence of severe side effects of drugs is a central public health problem that needs for innovative therapeutic approaches. Hence, specific measures have been taken for establishing their conditions of use, and explicit warnings on sun exposure and persistent photosensitivity are now given in the medication leaflets recommending, for instance, sun protection.[11]



Scheme 1. Tautomeric equilibrium of AB. Structure of AB-KP and photorelease of the latent AB and KP.

It is well established that the in vivo photosensitizing properties of KP are linked to its reactivity in the UVA region of sunlight. Actually, the UV-Vis absorption spectrum of this drug exhibits two peaks, ie. an intense $\pi\pi^*$ absorption centered in the UVC at 254 nm and a weaker UVA band of $n\pi^*$ nature at ca. 330 nm (Figure 1 and S1).[6, 8, 12] In this context, the development of sunscreen-based photocages (equivalent to covalently linked pro-drug/pro-filter systems) could be considered a clever solution. This concept makes use of light-sensitive chemical moieties (photoremovable protecting groups, PPGs) to allow controlled and simultaneous release of the masked drug and the solar filter upon irradiation. This would be clearly advantageous over the mere mixture because it allows a controlled release of the two components.[13] Photocages have become very popular because they provide spatial and temporal control over the activation of molecules triggered by light[14-17] and have previously been employed for biological applications,[15-19] such as photocaged nucleotides,[20, 21 20, 21] proteins,[22-25] ions,[26, 27] neurotransmitters,[28] pharmaceuticals,[29-32] fluorescent dyes,[32-34] or small molecules[34-37]. Interestingly, avobenzone (AB, 4-*tert*-butyl-4´-methoxydibenzoylmethane), which is one of the most important and representative UVA blockers present in commercial sunscreens and cosmetic formulations, contains two phenacyl moieties that could in principle work as PPGs to release carboxylic acids (in green, Scheme 1).[14-17] Being a dibenzoylmethane derivative, AB suffers a keto-enol equilibrium, and the main enol tautomer is responsible for the large UVA absorption.[38, 39] Therefore, the phenacyl structure of AB diketo form could be used as a PPG of the KP carboxylic acid function as shown for compound AB-KP

in Scheme 1. This design should result in a remarkable combination capable to provide a phototriggered slow delivery of the drug together with that of its UVA protective shield. Thus, KP photoreactivity should be inhibited and the risk of adverse skin reactions minimized, as the AB absorption at ca. 350 nm is more than 200 times higher than that of KP (Figure S1).

Here, we report on the synthesis and photochemical evaluation of this photoactivatable dyad (AB-KP, Scheme 1). The synthesis of AB-KP was performed straightforward. First, AB was brominated at the α position of the carbonyl groups by using *N*-bromosuccinimide under solvent free conditions; then, the resulting intermediate was reacted with the (*S*)-ketoprofen cesium salt to afford a diastereoisomeric mixture of the desired AB-KP, which was found to be almost exclusively in the diketo form. Full NMR and HRMS characterization is given in the Supplementary Information. The uncaging process was performed without separating the AB-KP diastereoisomers, because the new chiral carbon located on the AB moiety should be lost during the photochemical release, while the pharmacologically active (*S*) configuration of KP would be preserved.

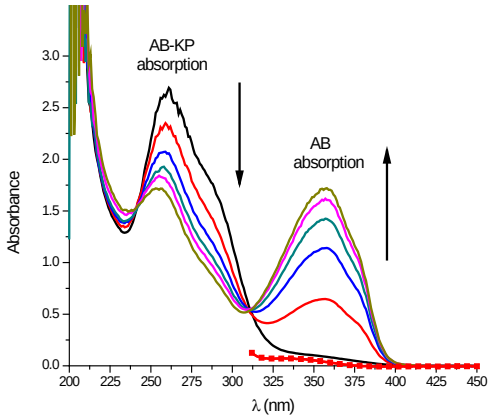


Figure 1. SSL-irradiation (from 0 to 30 min) of 9 x 10^{-5} M AB-KP in deaerated ethanol followed by UV absorption. Red dots shows the absorption of 7 x 10^{-4} M KP in EtOH.

The pro-drug/pro-filter concept was easily checked by UV-Vis absorption spectroscopy monitoring the appearance of the characteristic UVA band of the enolic AB, centered at 355 nm, upon irradiation as diagnostic for the release process (Figure 1).[38, 39] A nitrogen flushed solution of AB-KP (9 x 10^{-5} M) in ethanol, selected as solvent for its hydrogen donor capability, was irradiated with simulated sunlight (SSL) provided by the filtered emission of a Xenon arc lamp.

As observed in Figure 1, the diketonic AB-KP band decreased with irradiation time concomitantly with the increase of the AB band, supporting formation of the enol tautomer of the filter.

Due to the close absorption maxima of KP[12] and AB-KP and the comparatively low molar absorption coefficient of KP at this wavelength, an accurate determination of the released drug required HPLC analysis, which was also achieved in a deaerated ethanol solution of AB-KP at higher concentration (1.1 x 10^{-3} M). Quantitation of the photoproducts was done by comparison with authentic samples of KP and AB. The HPLC traces revealed that the starting AB-KP peak disappeared over time giving actually rise to KP and AB (see Figure S2 of Supplementary Information). The time course of the process is shown in Figure 2. After 15 min, 30% of the initial AB-KP had reacted, while after 2h AB-KP was almost totally consumed. Interestingly, under the same experimental conditions KP was completely photolyzed in less than 30 min, clearly demonstrating the protecting role of the released AB filter (Figure S3, Supplementary Information). Irradiation of an aerated ethanol solution under the same experimental conditions did not lead to formation of AB (Figure S4, Supplementary Information), in agreement with the involvement of a triplet excited state as intermediate.[40]

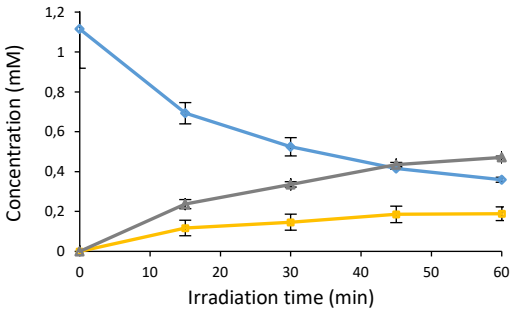


Figure 2. Time-course of the photorelease of KP (orange line) and AB (grey line) through simulated sunlight irradiation of a nitrogen flushed solution of AB-KP (blue line, 1.1 x 10^{-3} M) in ethanol.

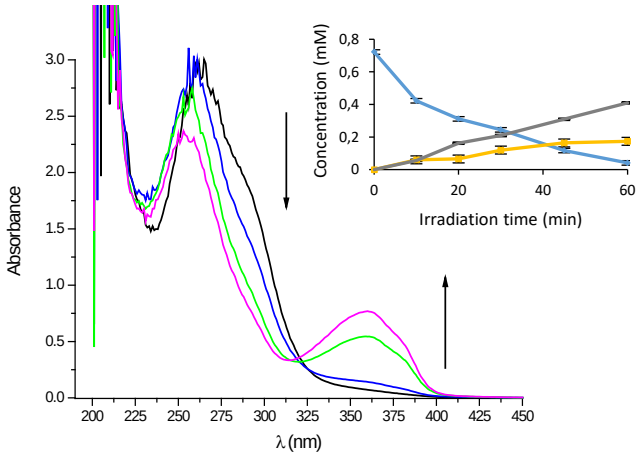


Figure 3. UV absorption changes for AB-KP (9 x 10^{-5} M) in aerated propylene glycol under SSL (from 0 to 35 min). Inset: HPLC analysis for AB-KP (7 x 10^{-4} M).

To go a step further and simulate the more viscous formulation compositions of topical creams, the photorelease was studied under air using propylene glycol as matrix. This way, the medium still presents hydrogen donor capability, but its lower diffusion-controlled rate constant should disfavor the deactivation of excited states by oxygen. As shown in Figure 3, the AB band appeared as a function of SSL irradiation time. This was confirmed by HPLC analysis were both AB and KP peaks were detected.

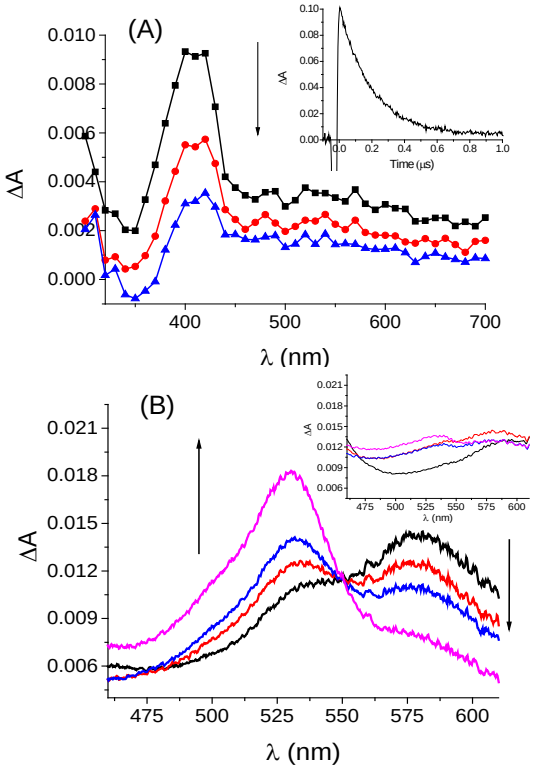


Figure 4. Transient absorption spectra of (A) AB-KP in ethanol under N_2, from 0.05 to 0.3 μs after the 355 nm laser pulse, and (B) ketoprofen and AB-KP (inset) from 3.1 to 15.8 ps after pump excitation.

Finally, transient absorption spectroscopy was run to obtain direct information on the excited states involved in the photochemical process. Nanosecond laser flash photolysis experiments (Nd:YAG, 355 nm) were performed on a nitrogen bubbled ethanol solution of AB-KP (6.4×10^{-4} M). A transient absorption band centered at 400 nm appeared immediately after the pulse (Figure 4A) and decayed with a short lifetime of 0.2 μs without leading to further detectable species. According to the literature data, this band was assigned to the triplet-triplet transition of the avobenzone-like diketo form.[38] At this time window, the signal of the KP-like triplet excited state at ca. 525 nm[12, 41] was not observed. This could mean that it is not formed during the process or that it is indeed formed, but it disappears at a shorter timescale. Hence, ultrafast transient absorption spectroscopy was used to analyze the sub-nanosecond processes. Under these conditions, for an ethanolic solution of KP alone, the characteristic singlet-singlet transition at 580 nm was observed; after few picoseconds intersystem crossing with formation of the triplet excited state absorbing at 525 nm was also noticed (Figure 4B)[42]. By contrast, the 525 nm species was hardly detected in the case of the AB-KP (Figure 4B, inset). This is in agreement with the accepted mechanism involved in the uncaging of compounds using phenacyl as PPG.[40] After light absorption, the triplet excited state of the phenacyl chromophore abstracts hydrogen from the solvent, and subsequently releases KP. Once formed, KP is protected by the AB enolic form that absorbs much more efficiently UVA light, thus avoiding KP excitation.

Conclusion

In summary, the present work has demonstrated that it is possible to develop photocages for protection and controlled release of bioactive compounds. The concept has been proven using sunscreen-based photocages for topical drugs, which are associated with a double beneficial effect: controlled release of the photosensitive active principle upon light exposure coupled with its protection from photodegradation and photoreactivity by the solar filter effect. As both ingredients are registered compounds already in use, the pro-drug/pro-filter concept could in principle be brought to practical application in a time- and cost- efficient way.

Acknowledgements

Spanish Government (CTQ2015-70164-P, RIRAAF RETICS RD12/0013/0009, Severo Ochoa program/SEV-2012-0267 and BES-2013-066566), Generalitat Valenciana (Prometeo

II/2013/005) and VLC/Campus Microcluster “Interacciones Luz-Fármaco en Sistemas Biológicos y Reacciones Adversas” are gratefully acknowledged.

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