Photoinduced oxidation of lipid membrane in the presence of nonsteroidal anti-inflammatory drug ketoprofen

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The damage of cell membranes induced by photosensitive drugs attracts significant attention of researchers in various fields of medicine. Ketoprofen (KP) belongs to the group of non-selective COX-1 and COX-2 inhibitors and is known to be the most photosensitive among the NSAID. The phototoxic side effects of KP and other non-steroidal anti-inflammatory drugs are associated with the action of free radicals, but there is insufficient information about the nature of these radicals. In the present study, free radicals formed upon KP irradiation in lipid membrane were studied using NMR and CIDNP methods, as well as molecular dynamics simulation.

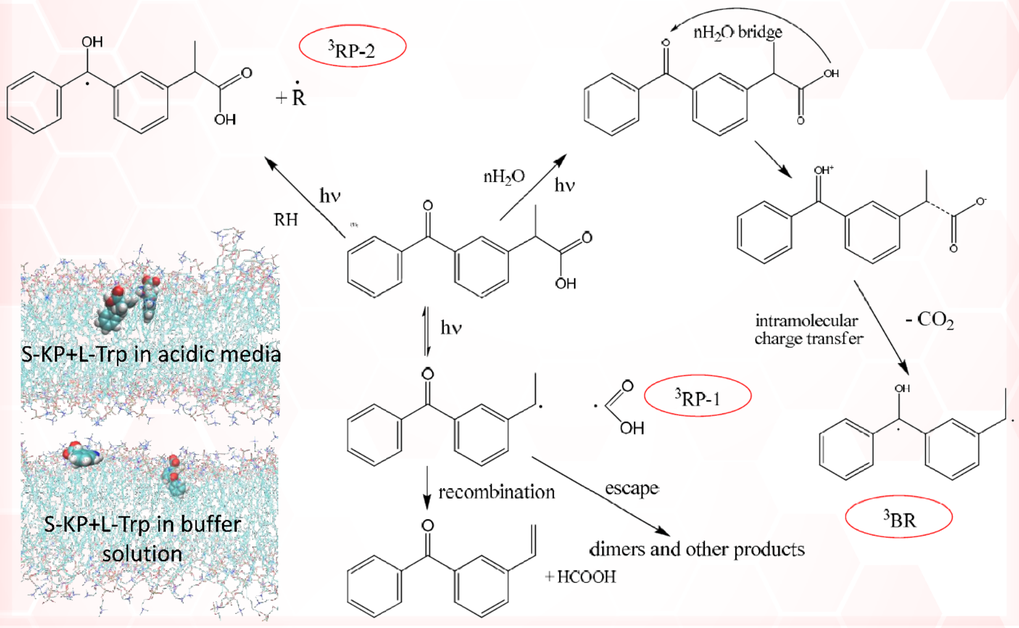
Motivation:

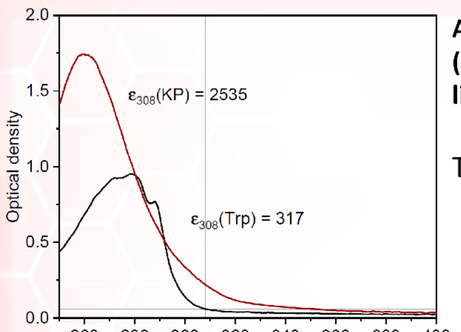
- membranes carry a number of vital functions, including protective and transport.
- unknown mechanism of photodegradation of ketoprofen and other nonsteroidal anti-inflammatory drugs inside the cell under the influence of ultraviolet light.

Methods:

In this work, the methods of NMR (1D, selective gradient NOESY, T_1 relaxation), CIDNP and molecular dynamic were used. The irradiation was carried out at a wavelength of 308 nm.

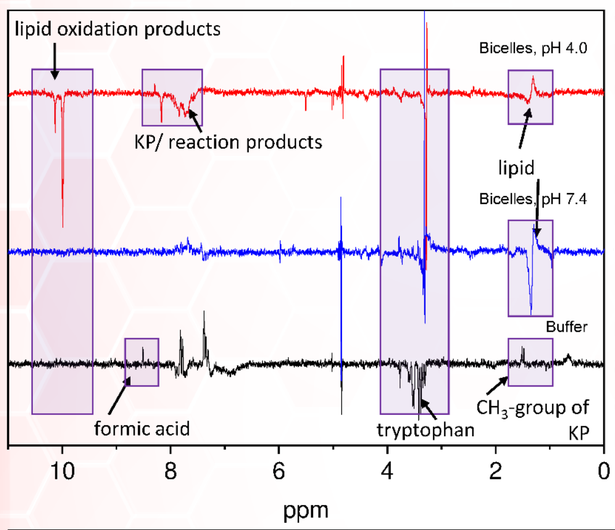
Suggested reaction channels of KP photodegradation:





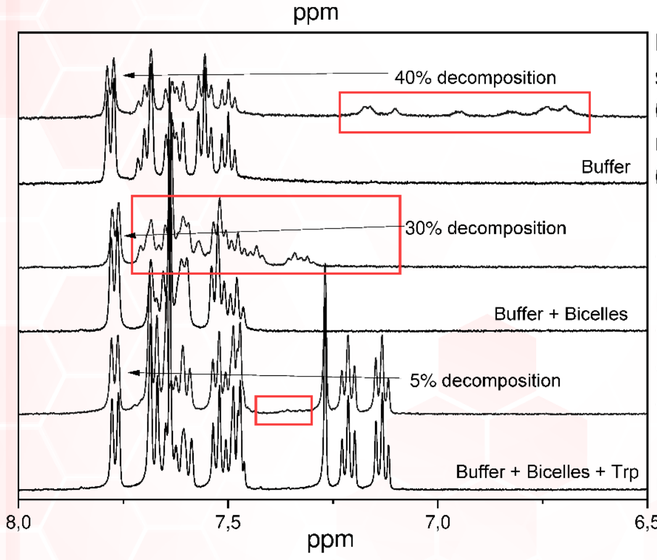
Absorption spectra of KP ($1*10^{-4}$ mM) in bicells (red line) and Trp ($2*10^{-4}$ mM) in bicells (black line) and their extinction coefficients ($M^{-1}\cdot cm^{-1}$).

Thus, light is mainly absorbed by ketoprofen



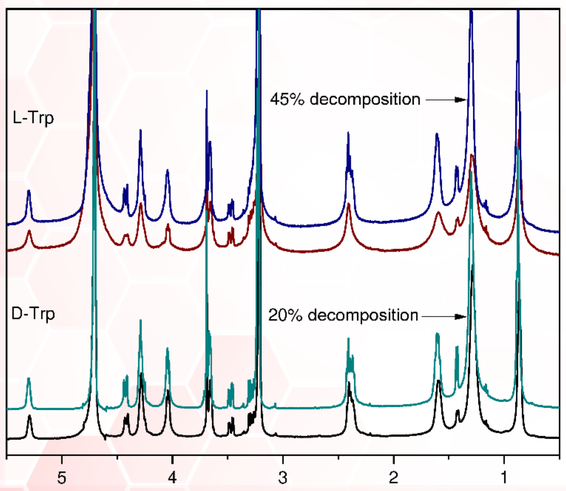
CIDNP spectra recorded during the photolysis of the mixture of KP (2 mM) with Trp (4 mM) in phosphate buffer and DMPC/DHPC bicells at different pH values.

Polarized monomolecular decay products are visible in the buffer solution but in bicells, this mechanism is insignificant. In an acidic environment, reaction products at 8-10 ppm appear in the bicells, there is also polarization on the KP or reaction products at 7.5-7.8 ppm.



Fragments of the ^{1}H NMR spectrum of buffer solution KP (2 mM)/ KP (2 mM) + Trp (4 mM) before and after photolysis (64 laser pulses).

In the presence of tryptophan, there is practically no decrease in the intensity of KP signals, which allows us to suggest that a reversible reaction of KP with tryptophan is the main reaction channel.

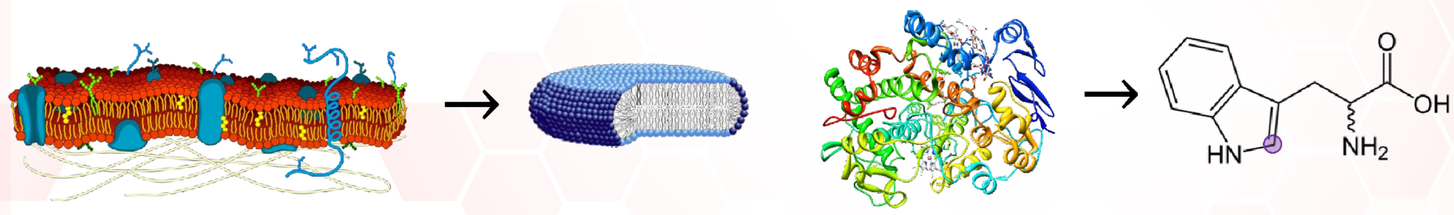


^{1}H NMR spectra obtained before and after irradiation, showing the degree of decomposition of lipids at pH = 7.4

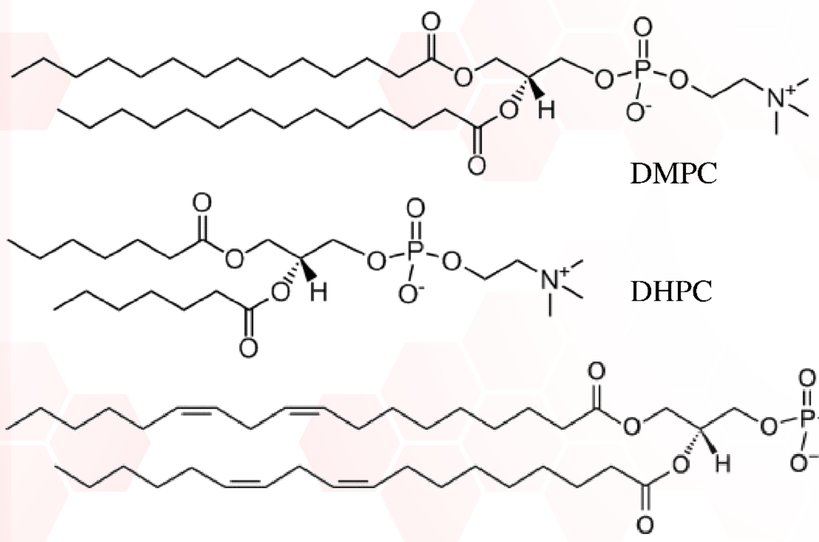
It is important that there is also difference in the degree of decomposition of lipids and KP molecules after irradiation: in the oxygenated system with L-tryptophan, the integral intensity of the peak corresponding to the CH_2 groups of lipids decreased by 45%, but in the system with D-tryptophan by 20% .

Model system:

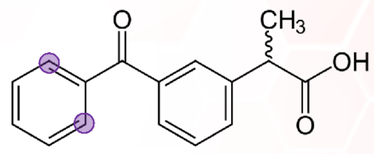
It is difficult to describe a complex system, so let's move on to a simpler one:



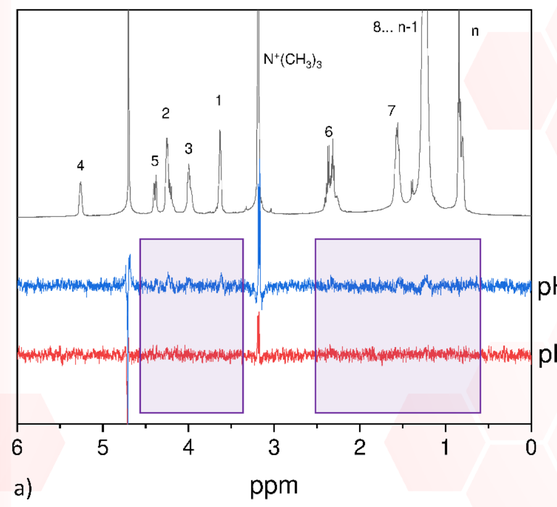
To accelerate the formation of bicelles from DMPC and DHPC, three freeze–thaw cycles were performed. The DMPC (DLPC): DHPC ratio was 1:2, with the total lipid concentration being 24 mM.

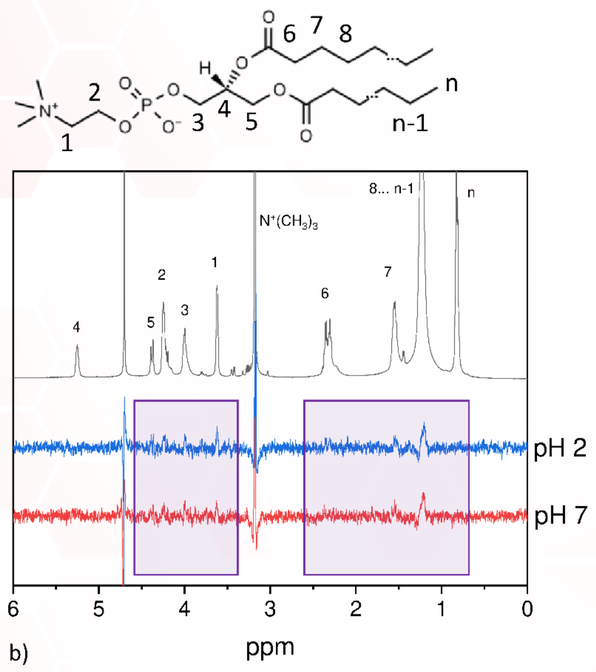


The S-KP : L/D-Trp ratio was 1:2.



1D NOESY spectra (red and blue lines) and ^{1}H NMR (gray line) of a) 2 mM KP; b) 2 mM KP + 4 mM Trp ; in DHPC/DMPC bicells at different pH values.





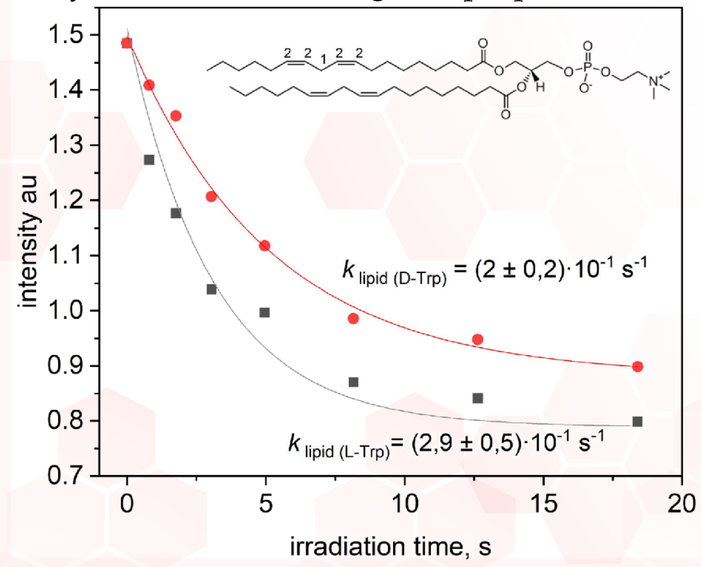
Cross-peaks at NOESY spectra were observed when the distance between the nuclei was less than 0.5 nm. Obtained results mean that, firstly, the KP molecule is able to penetrate into the lipid bilayer, and secondly, the KP–bilayer interaction is sensitive to the presence of Trp.

Spin-lattice relaxation time T_1 of KP and Trp

	KP (7.8 ppm) without Trp	KP (7.8 ppm) with Trp	Trp (7.1 ppm)
Water solution	2.5±0.2s	3.1±0.1s	2.4±0.2s
Bicelles, pH=7	1.2±0.1s	3.0±0.1s	2.6±0.1s
Bicelles, pH=3.5	1.1±0.1s	1.0±0.2s	1.9±0.2s

It is notable that the spin–lattice relaxation times of ketoprofen protons decreases by approximately half in bicelles in comparison to water solution. NMR relaxation times are very sensitive to molecular motion. Limitation of the mobility of the molecule leads to a decrease in the relaxation time.

Decay kinetics of ^{1}H NMR signals lipid protons (1) at 2.7 ppm on irradiation time in DHPC/DLPC bicelles at pH=3.3.



Time dependence of the decay of NMR signal of bis-allylic protons of lipid (1-H) was measured. Since the initiation stage of unsaturated lipids oxidation is the abstraction of a hydrogen atom at this position, and reaction products (lipid radicals and conjugated dienes) do not contain such protons in the structure, the initiation stage leads to a decrease in the intensity of this signal as a function of time. Similar decay kinetics have been observed for NMR signals of protons 2-H (non-conjugated double bonds).

Rate constants of the decay of 1-H and 2-H lipid signals under irradiation in DHPC/DLPC bicelles with KP and L/D-Trp, pH=3.3.

	Rate constant (*10^{-1} s^{-1})
1-H decay, KP+L-Trp	2.9±0.5
1-H decay, KP+D-Trp	2±0.2
2-H decay, KP+L-Trp	2.5±0.3
2-H decay, KP+D-Trp	1.4±0.1

Conclusions:

- The main channels of ketoprofen photolysis in lipid membranes are interaction by radical mechanism with lipid molecules and with tryptophan, in contrast to monomolecular decomposition in homogeneous solutions.
- Stereoselectivity of oxidation of lipid molecules during photolysis of ketoprofen with tryptophan enantiomers in the lipid bilayer was found.

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