Copper(II), in the parts per million range, modulates photochemical and photosensitizing properties of tolmetin *via* electron transfer with a triplet carbanion

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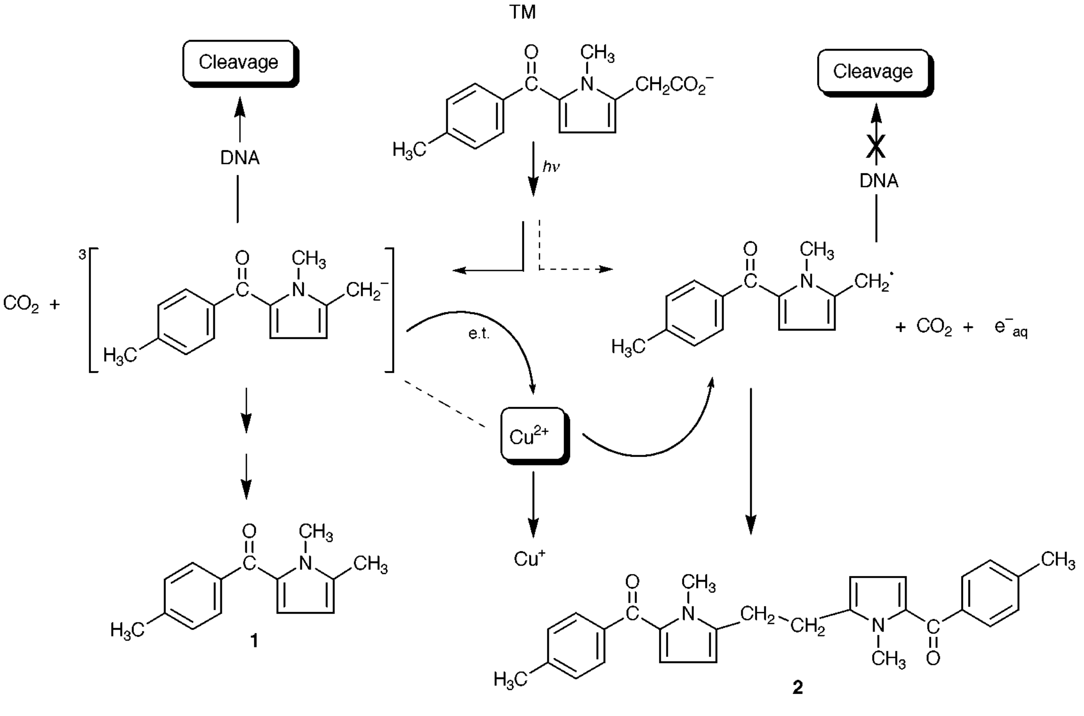
Copper(II), at very low levels of concentration, modulates the distribution of tolmetin stable photoproducts as well as inhibits the DNA cleavage photoinduced by the drug, *via* a highly efficient electron transfer process with the main transient species generated in the tolmetin photolysis.

Tolmetin, 2-[5-(*p*-toluoyl)-1-methylpyrrol-2-yl]acetic acid (TM), is a non-steroidal anti-inflammatory drug (NSAID) and is known to induce toxic effects on biosubstrates which can be associated with light exposure.[1–3] Like other NSAIDs,[4,5] a photodecarboxylation reaction is believed to be responsible for the TM photoinduced damage.[3] In light of this, the problem of finding suitable bio-compatible devices which may control drug photodegradation and, as a consequence, exert a photoprotective action towards the photoinduced damage, is receiving growing attention. Our previous work pointed out that the use of metal ions at very low concentrations may represent a valid tool in inhibiting toxic effects photoinduced by drugs,[6–9] as well as a potential strategy for modulating the photochemical pathways involved in drug photodegradation. Furthemore, patents concerning copper(II) compounds with antioxidant activity have been registered.[10–12] Despite encouraging experimental results, the role played by the metal ions in this field is still unclear and complex. On the basis of these considerations we proposed to elucidate the role of copper(II) in the photochemistry of TM and on its photosensitizing activity towards DNA by combining steady-state and time-resolved techniques. Recent studies on the transient photochemistry of this drug have pointed out that TM photodecarboxylation is triggered by a short-lived excited triplet state involving a triplet carbanion as the key intermediate in the photodecomposition.[13,14] Stable photoproduct **1** is formed. A minor pathway, consisting of a photoionization process followed by fast decarboxylation and formation of a dimeric species such as stable photoproduct **2**, was also noticed (Scheme 1).

The results of the HPLC analysis of a nitrogen saturated, irradiated TM solution at pH 7.4 led to the photoproduct distribution shown in Fig. 1. As shown, addition of copper(II) in the micromolar range does not influence the photodegradation efficiency of the starting compound but provokes remarkable changes in the distribution of the two photoproducts. It can be seen that the reduction of the photoproduct **1** is accompanied by a corresponding increase in the amount of photoproduct **2**.

Since the photodegradation quantum yield is unaffected by the metal ion, the behavior observed accounts for the reaction between copper(II) and the transient intermediates formed in the TM photolysis. Inasmuch as the increase of the percentage of the photoproduct **2** has to involve an increase in the pyrrolyl radical concentration, we can hypothesize that an electron transfer from the triplet carbanion to copper(II), leading to the formation of the pyrrolyl radical and copper(I), may explain the observed behavior. This hypothesis is confirmed by monitoring



Scheme 1

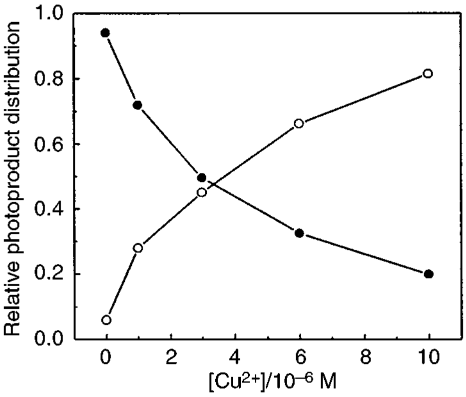


Fig. 1 Relative distribution of TM photoproducts at various copper(II) concentrations: (●) **1** and (○) **2**.

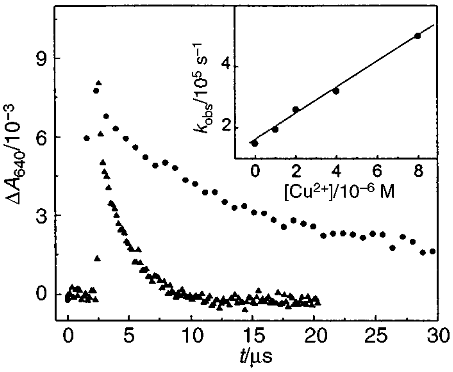


Fig. 2 Kinetic decay of the TM triplet carbanion (●) in the absence and (▲) in the presence of Cu^{2+} (7×10^{-6} M). Inset: plot for the quenching of the triplet carbanion by Cu^{2+}.

the triplet carbanion decay at 640 nm using nanosecond laser flash photolysis techniques. As showed in Fig. 2, this transient species is quenched by addition of copper(II) and a bimolecular quenching constant of $4.5–10^{10}$ M^{-1} s^{-1} (inset in Fig. 2) is obtained.

This value is higher than the diffusion-controlled rate and indicates a strong electrostatic interaction between the two reactants, due to their opposite charges.

These data are in good agreement to those obtained from steady state experiments. Indeed, the fraction of carbanion quenched by a certain concentration of copper(II) fits well both the percentage decrease of the photoproduct **1** and the increase of the photoproduct **2**. Further confirmation that the quenching mechanism occurs through an electron transfer reaction is provided by the high amount of copper(I) detected after TM irradiation in N_2O saturated solution.† Addition of sodium bathocuproinedisulfonate (BCDS) under N_2O atmosphere turns the irradiated solution red, which shows the characteristic absorption of the copper(I)–BCDS complex at 484 nm.[15]

Low concentrations of copper(II) were also used in DNA photocleavage experiments. As shown in Table 1, the metal ion inhibits markedly the amount of DNA damage photoinduced by TM.

Table 1 TM photoinduced DNA cleavage in anaerobic conditions in the presence of different amounts of Cu^{2+}

Cleavage (%)	$[Cu^{2+}]/10^{-7}$ M
67 ± 7	0.0
55 ± 6	1.0
42 ± 5	5.0
30 ± 4	10.0

Since the main transient intermediate produced in these experimental conditions is always the triplet carbanion, we believe that an electron transfer between this species and DNA nucleobases can trigger the photoinduced damage. The hypothesis is supported by formation of reduction products of DNA nucleobases observed in experiments performed using single nucleosides. These results can be rationalized on the basis of the influence of the metal ion on the TM photoproduct distribution. Due to the intermolecular electron transfer with cupric ions, the active DNA photocleaver triplet carbanion is converted into a pyrrolyl radical. Given its chemical structure, this species is highly stable, is inefficient for abstraction of hydrogen from the DNA backbone, and the only method of decay is a self-reaction to generate the photoproduct **2**.

The overall results, summarized in Scheme 1, provide a clear example in which an oligoelement such as copper(II) interacts efficiently at very low concentration with the intermediate of a photochemical reaction. This process modulates remarkably the distribution of the stable photoproducts and inhibits considerably DNA drug photoinduced damage by turning a species very highly reactive towards the biosubstrate into an unreactive one.

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Notes and references

† N_2O was used as an electron scavenger in order to avoid formation of copper(I) by direct capture of the hydrated electron photoejected by TM.

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