Photochemical Inhibition of the Reactions of Copper(II) Peptide Complexes with Molecular Oxygen

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Summary Copper(II) accelerates the oxidation of tetrapeptides by O_2 in neutral solution, but light ($\lambda < 420$ nm) has the novel effect of inhibiting the reaction.

MOLECULAR oxygen reacts spontaneously with tetra- and penta-peptide complexes of nickel(II) to oxidize the peptides under very mild conditions.[1] The corresponding reactions do not occur under normal laboratory light when copper(II) replaces nickel(II). However, the activation of O_2 in the presence of copper(II)-peptides does take place in the dark. Although many reactions are known to be photochemically initiated[2] including the reaction of amides with O_2,[3] the essentially complete photochemical inhibition observed in the present system is most unusual if not unique. Specifically, radiation in the 340—420 nm wavelength range inhibits the O_2 reaction with copper(II)–peptides while radiation of longer wavelength has no effect.

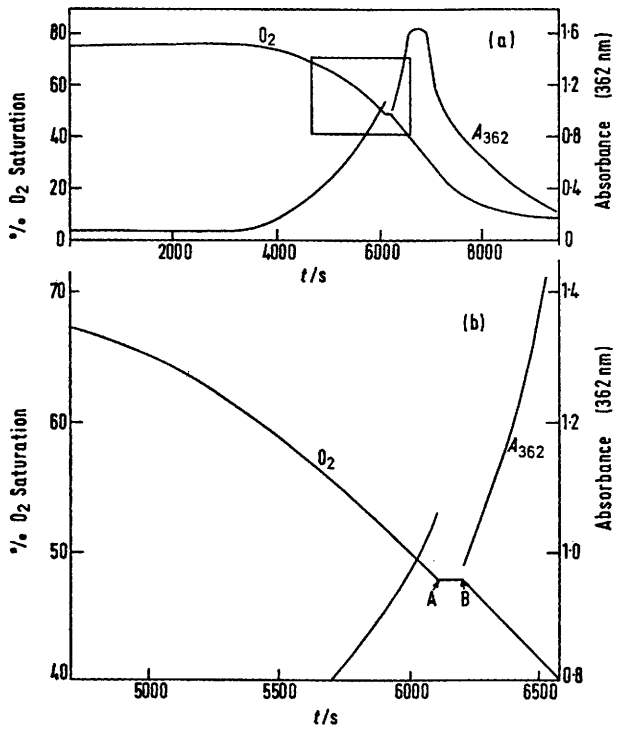

FIGURE. (a) Molecular oxygen reaction with copper(II)–tetraglycine (5×10^{-3} M) at pH 7·4, 37 °C, initially in the absence of light. The absorbance at 362 nm (1 cm cell) is attributed to a Cu^{III}–peptide intermediate which forms and decays as the O_2 is consumed. (b) The reaction system is exposed to white light at point A causing the O_2 uptake to cease. During the interval that the light is on there is only a small loss in the amount of Cu^{III}–peptide. When the light is removed at point B the reaction resumes without delay, indicating that the autocatalytic species has not been destroyed.

On the other hand, similar levels of radiation have virtually no effect on the O_2 reaction with nickel(II)–peptides.

Table 1 compares the reactivity of Cu^{II} and Ni^{II} in their induced autoxidation of oligopeptides in the absence of light. The metal ions are essential to activate O_2, but the Cu^{II}-catalysed reactions are *ca.* 20 times slower than those of Ni^{II}. The reactive Cu^{II} species have (i) a glycyl residue in the third peptide position, (ii) three Cu–N bonds [one amine and two N(peptide) groups], and (iii) an open equatorial position (*i.e.* one without carboxylate or nitrogen bonding).[4] Tripeptides such as GGG^- and GGH^-,† which co-ordinate all four equatorial groups around Cu^{II} are inactive. Substituting an alanyl residue for a glycyl residue has little effect except when this is done in the third position. (The O_2 activation requirements with Ni^{II}–peptides are significantly different because the peptides which co-ordinate all four equatorial positions of Ni^{II} are the most reactive.[5]) The third peptide residue of $Cu(H_{-2}GGGG)^-$ is attacked by O_2 yielding glycylglycylamide (GGa) and glyoxylglycine as products, whereas with Ni^{II} the O_2 attack is at the fourth residue of $GGGG^-$.[1]

TABLE 1

O_2 Activation by metal–peptides

	Ni^{IIa}	Cu^{II}
Reactive peptides†	GGGG, AGGG, AAAA, GGAG, GGH	GGGG, GGGGG, GGGa
Less reactive peptides	GGGGG	AAAA, GGAG
Non-reactive peptides	GGG, GGa, GGGa	GGG, GGa, GGH
Reaction with GGGG		
Main products (pH 8)	GGGa, CO_2, H_2CO	GGa, glyoxylglycine
Effect of light	negligible	inhibition
pH of max. reactivity	8·0	7·4
Intermediate	λ_{max} 380 nm	λ_{max} 362 nm, ϵ 7200 l mol^{-1} cm^{-1} oxidant
Induction period[b]	*ca.* 200 s	*ca.* 4000 s
Max. O_2 uptake rate[b]	$8{\cdot}5 \times 10^{-6}$ l mol^{-1} s^{-1}	$0{\cdot}4 \times 10^{-6}$ l mol^{-1} s^{-1}

[a] The results for nickel are taken in part from unpublished work of F. P. Bossu, E. T. Gray, Jr., and D. W. Margerum. [b] For the reaction of 5×10^{-3} M metal–GGGG with 1×10^{-3} M O_2 at 37 °C at the pH of max. reactivity.

The Figure (a) shows the results of mixing a limited amount of oxygen with an excess of $Cu(H_{-2}GGGG)^-$, initially in the absence of light. After a long induction period there is an autocatalytic uptake of O_2 (monitored with an oxygen electrode) and the formation of an intermediate which absorbs strongly at 362 nm (ϵ 7200 l mol^{-1} cm^{-1}). The intermediate reaches a maximum concentration when the rate of oxygen uptake is a maximum. It has the spectral and redox properties of Cu^{III}-complexes,[6] being able to oxidize iodide and thiocyanate ions but not

† Abbreviations: G: glycyl; A: L-alanyl; H: L-histinyl; a: amide; DPPH: 1,1-diphenyl-2-picrylhydrazyl.

bromide ion. It persists in solution after removing Cu^{II} which is in accord with the more sluggish substitution reactions expected for a metal(III) d^8 complex. It undergoes a first-order decomposition, k *ca.* 10^{-4} s^{-1} at pH 7, but it decomposes more rapidly in acid or in base to give Cu^{II} species.

The rate dependence of the autocatalytic portion of the reaction [equation (1)] was determined by independently varying the concentrations of $Cu(H_{-2}GGGG)^-$, O_2, and the autocatalyst. Although the autocatalyst was not specifically identified, its concentration was varied by dilution of

$$\text{autocatalytic rate: } -dO_2/dt = k[Cu(H_{-2}GGGG)^-][O_2][\text{autocatalyst}] \quad (1)$$

the reaction systems. The autocatalyst can be destroyed and the reaction stopped by the addition of reducing agents or free-radical scavengers. The maximum concentration of autocatalyst appears to be *ca.* 10^{-4} M as this level of But^t_2NO is needed to halt completely the reaction in the autocatalytic stage. The concentration of the Cu^{III}-peptide intermediate parallels the concentration of the autocatalyst. The addition of the much less soluble free-radical scavenger, DPPH, at 10^{-7}—10^{-8} M levels has no effect once the autocatalytic stage of the reaction has been reached. On the other hand, this level of DPPH greatly lengthens the induction period.

Table 2 shows that, even for exhaustive oxygenation, light in the 340—420 nm region stops the reaction completely whereas light above 450 nm affects the reaction only slightly and permits the GGGG to be oxidized. Furthermore, when a solution undergoing autoxidation is exposed to light [point A, Figure (b)] the autoxidation stops immediately. When the solution is again protected from light (point B) the reaction resumes at approximately the same rate. During the 100 s exposure (A to B) the Cu^{III} intermediate decays by only 8%. Clearly, it is not the destruction of the Cu^{III}-peptide which stops the reaction. Also, the lack of a new induction period indicates that the autocatalyst is still present. Therefore a short exposure to light reversibly blocks the reaction without destroying significant amounts of the autocatalyst.

TABLE 2

Wavelength dependence of the light inhibition of the Cu^{II}–GGGG reaction with O_2

λ/nm[a]	%GGGG reacted[b]
330—700	0
330—520	0
340—420	0
450—700	75
540—700	79
550—610	84
No light	90

[a] 75 W W-lamp with band-pass and cut-off filters. [b] $Cu(H_{-2}GGGG)_1^- = 5 \times 10^{-3}$ M, pH 7·4, 25·0 °C, continuous O_2 (1 atm.)-flow for 22 h.

The exact manner in which light inhibits the reaction is still under investigation and any proposed mechanism must be very tentative. One possible explanation is that a very weak copper-peptide-O_2 complex or an activated form of this complex, which is needed for the reaction, is broken up very efficiently by light. A dark-light-reversible reaction of a cobalt(III)–bis(glycylglycine) chelate has been reported[7] in the reaction of O_2 with the $Co^{II}(H_{-1}GG)_2^{2-}$ complex. U.v. irradiation apparently causes a photochemical reduction of $Co^{III}(H_{-1}GG)_2^-$ to the Co^{II} complex and the effect of light is not related to the oxygenation itself which is believed to go through a binuclear peroxo-intermediate. Thus, the effect of light appears to be not the same as in the present system.

Some remarkable properties of the Cu^{II}–GGGG–O_2 system are (i) the light inhibition itself, (ii) the low levels of light which are sufficient to cause inhibition, (iii) the 'off-and-on' response of the autocatalytic rate to light, (iv) the formation of Cu^{III}–peptide intermediates, and (v) the spontaneity of the oxidation under conditions similar to that found physiologically.

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