The Photolytic Release of Copper from the Copper Penicillamine Complex $[Cu(II)_6Cu(I)_8(D\text{-Penicillamine})_{12}Cl]^{5-}$

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Abstract

The light sensitivity of CuPen ($[Cu(II)_6Cu(I)_8(D\text{-penicillamine})_{12}Cl]^{5-}$) was examined. The wavelength range of the photolytic activity was determined in the visible and near-ultraviolet region of the electromagnetic spectrum. No photolytic activity was observed above 450 nm. The quantum yield of copper release was measured between 450 nm and 250 nm and was found to increase from 0 to 0.1. A shoulder around 400 nm corresponding to electronic absorption and CD features was observed in the photo-action spectrum. Inhibition of formazan formation from nitroblue tetrazolium mediated by xanthine oxidase-generated superoxide was used to quantify the copper release as a result of the photolytic decomposition of CuPen. The influence of oxygen on the photolytic reaction was investigated by EPR and electronic absorption spectroscopy. In the absence of oxygen, visible light induces almost total bleaching. However, EPR reveals only slight changes in the spin concentration. Upon introduction of aerobic EDTA all of the copper is immediately oxidised to Cu(II).

Introduction

The photochemistry of copper compounds has been broadly investigated [1, 2]. Due to the easily attainable oxidation states of copper, its complexes tend strongly to undergo photo-redox reactions attributable to excitation of charge-transfer transitions. Very often ultraviolet light is necessary to induce these. If these transitions are shifted to lower energies, e.g. by introducing soft ligands like sulfur, photochemical reactions may be expected in the visible region. In this context, the earlier observed light sensitivity of CuPen* [3] ($[Cu(II)_6Cu(I)_8(D\text{-penicillamine})_{12}Cl]^{5-}$), a copper complex of the cysteine analogue penicillamine (3,3-dimethylcysteine) [4], deserved a closer look.

Of special importance in this regard was the elucidation of the wavelength region capable of degrading CuPen, the quantum yield of this process, and the assignment to an electronic transition. The effect of oxygen during this photochemical reaction was examined. Formation of unspecifically coordinated copper was measured using the inhibition of formazan development from nitroblue tetrazolium (NBT). Electron paramagnetic resonance (EPR) measurements were performed to monitor the oxidation state of copper.

It has been suggested that CuPen [5] takes part in the antirheumatic effect [6] of D-penicillamine, which is known to be a potent ligand to chelate many metal ions including copper. As a mode of action for the antiinflammatory activity of CuPen, its property as a mobile form of copper has been proposed [5]. A beneficial role of copper in the inflammatory process has been proposed [7]. The thermal release of copper from CuPen in biological systems has been proven [5]. As to the photolytic release of copper from CuPen, a mechanism in terms of 'photobiochemistry in the dark' triggered by chemical excitation, as suggested by Cilento [8], might be considered. This is especially the case in those inflammatory regions where chemically excited species, like oxygen radicals, are involved [7].

Experimental

CuPen was prepared as described by Birker and Freeman [4] by mixing aqueous solutions of $CuCl_2$ and D-penicillamine and precipitating CuPen with 50% ethanol. Higher purity was obtained upon gel filtration on Superose 12 (Pharmacia, Uppsala, cross-linked agarose, separation range M_r 1000–30000, which removed extraneously bound copper. The elemental analyses were in accordance with earlier work [9].

Spurious copper formed during the photolysis of CuPen was quantified in the presence of unreacted

*Abbreviations: CuPen = $[Cu(II)_6Cu(I)_8(D\text{-penicillamine})_{12}Cl]^{5-}$; NBT = nitroblue tetrazolium dication.

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CuPen, using its inhibition of formazan formation from NBT mediated by superoxide [3]. Superoxide was generated enzymatically in the course of the xanthine–xanthine oxidase reaction. This assay was calibrated with $CuSO_4$ to allow the determination of copper in the micromolar range. An excess of EDTA over copper abolished the inhibition. CuPen was less inhibitory than $CuSO_4$ by two orders of magnitude. Every photolytic experiment was paralleled by a temperature-controlled sample stored in the dark.

The range of photolytically active wavelengths was detected as follows. Gel-filtered aqueous CuPen was illuminated with a cold light source (Schott KL 105 B equipped with a 150 W Osram Xenophot HLX tungsten lamp and an IR-reflecting Schott KG 37 filter) in a 1-cm quartz cuvette for 2 h at 4 °C. Different absorption edge filters (Schott BG26, GG400–GG495, edges between 350 and 495 nm) were used to eliminate the short wavelength part of the spectrum. Spurious copper released as a result of the photolysis was assayed in the NBT test.

The quantum yield of the photolysis of CuPen was determined between 250 nm and 450 nm. Freshly gel-filtrated aqueous CuPen (800 µl) was illuminated with monochromatic light of 8 nm bandwidth for 2.5 h at 23 °C in a 1-cm quartz cell. The optical system of a JASCO J 20 A spectropolarimeter (xenon lamp, prism monochromator, variable slit) was used as a light source. Photolytically generated copper was assayed in the NBT test. The photon flux was obtained by ferrioxalate actinometry [10] under the same conditions.

Photolysis of CuPen in the absence of oxygen was studied using the cold light source described above without filters. Gel-filtrated aqueous CuPen was purged of oxygen by repeatedly evacuating and flushing with nitrogen. The solution was irradiated under nitrogen. Before, during and after illumination, X-band EPR measurements at 77 K and electronic absorption spectra were recorded. EPR, CD and electronic absorption spectra were taken on a Varian E-109 EPR spectrometer, a JASCO J 20 A spectropolarimeter and a Beckman DU-40 spectrophotometer, respectively.

Results

It has been shown earlier that CuPen slowly releases Cu(II) when irradiated with visible light in the presence of oxygen [3]. After total bleaching, only Cu(II) is left, part of which could be attributed to Cu(II) penicillamine disulfide.

When aqueous CuPen was irradiated anaerobically with a tungsten lamp, the colour of the deeply red–violet solution developed within 120 h to faintly brown (see Fig. 3c). The absorption coefficient at 518 nm dropped from 1840 to approximately 150

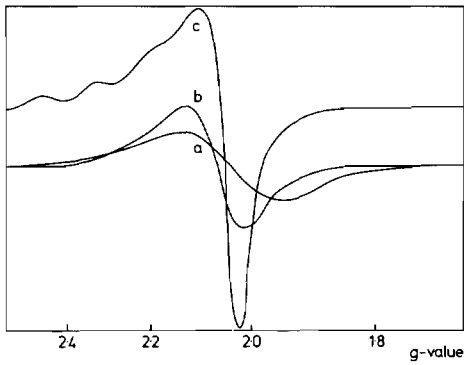

Fig. 1. EPR spectra of CuPen before and after anaerobic bleaching. Aqueous CuPen (1.15 mM Cu) under N_2 was irradiated with a tungsten light source at 4 °C for 120 h. Aliquots were passed into EPR tubes anaerobically. EPR spectra of 300 µl samples were taken: (a) before irradiation, (b) after irradiation, and (c) as (b) after addition of 20 µl of air-equilibrated aqueous EDTA (2 mM). Recording conditions: microwave frequency 9.24 GHz, microwave power 20 mW, modulation frequency 100 kHz, modulation amplitude 10 G, temperature 77 K.

$M^{-1}\,cm^{-1}$ per Cu atom. The broad symmetric EPR spectrum of CuPen gradually changed into an almost symmetric but narrower line shape (Fig. 1); at the same time, the g value drifted from 2.038 to 2.063. No anisotropic contribution could be seen. After irradiation, the amount of EPR-detectable copper was approximately 10% higher, as judged from the area confined by the integrated spectra. However, if air-equilibrated EDTA was added to irradiated samples in a two-fold molar excess, a dramatic rise in EPR-detectable copper was observed attributable to the total oxidation of copper to Cu(II). Conversely, EDTA did not affect the EPR spectrum of native CuPen. Thus, during anaerobic irradiation of CuPen solution, an EDTA- and/or oxygen-sensitive copper complex is generated having the same spin multiplicity as CuPen. When aqueous CuPen was decomposed by heating to 100 °C for 2 h, the resulting products – a brown precipitate in an uncoloured solution – did not show any EPR signal at 77 K (data not shown).

In order to learn more about the energy of the photolytically active light, samples of purified aqueous CuPen were irradiated in the presence of oxygen with a tungsten light source. Various edge filters opaque on the short wavelength side were used to constrict the wavelength range. Photolytic activity was determined by measuring the copper release using the NBT test. The active wavelength range could thus be confined to the high energy side of the visible light spectrum (Fig. 2). In order to exclude the possibility that traces of UV light passing the filters might be assigned to the observed effects, the short wavelength pass Schott UG5 filter (low energy edge at 386 nm) was used in the same experiment. However, only negligible copper release was observed.

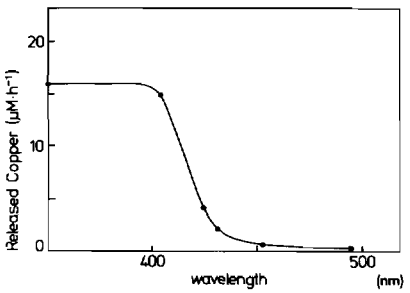

Fig. 2. Copper release from CuPen during irradiation with visible light. Aqueous CuPen (1 mM Cu) was irradiated with a tungsten light source at 4 °C for 2 h. The blue part of the spectrum was excluded with different edge filters. Their edge (point of 50% transmission) was taken for the positioning of the values on the wavelength scale. The filters used were from left to right: Schott BG 26, GG 400, GG 420, GG 435, GG 455 and GG 495. After irradiation, the inhibitory activity of a 50-μl aliquot was determined in the NBT test. The assay system had been calibrated with $CuSO_4$. The copper release was deduced from the inhibition. The experiment was paralleled by controls stored in the dark.

Thus, the UV light from the high energy end of the emission spectrum of the tungsten lamp did not suffice to induce the breakdown of CuPen. As a consequence, the curve shown in Fig. 2 might be seen as integrated photo-action spectrum for the release of copper from CuPen in the visible region. Subsequently, the quantum yield of the photolysis of CuPen in terms of copper release was determined between 250 nm and 450 nm. Copper atoms liberated per photon are plotted *versus* wavelength in Fig. 3. Below 350 nm the quantum yield steadily rises to reach 0.1 at 250 nm. There is a distinct shoulder around 400 nm that correlates well with a negative Cotton band in the circular dichroism spectrum at 405 nm and an ill-defined shoulder in the visible electronic spectrum around 420 nm (Fig. 3).

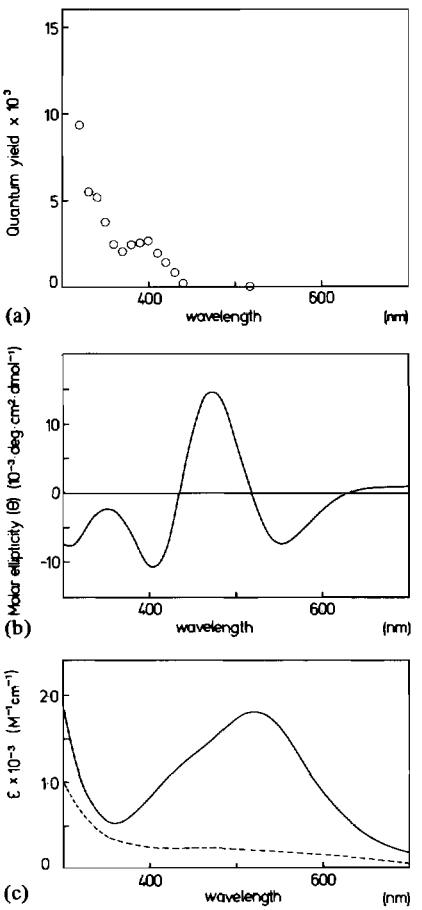

Fig. 3. Wavelength dependency of the quantum yield of photolytic copper release from CuPen (a) and the correlation with circular dichroism (b) and electronic absorption (c) spectra. The quantum yield was determined by irradiating aqueous CuPen for 2 h at 23 °C with monochromic light (bandwidth 8 nm). Photon flux was measured using ferrioxalate actinometry. Copper release was assayed as described in the legend to Fig. 2. The averages of the results from two experiments are shown. Standard deviation was less than 7%. Circular dichroism of CuPen (a) and electronic absorption spectra (c) are given relative to copper; (c) shows the UV–Vis spectra of CuPen (——) and of CuPen irradiated anaerobically, as described in the legend to Fig. 1 for 120 h (- - - -).

Discussion

The spectral features observed around 400 nm in the electron absorption and circular dichroism spectra as well as in the photo-action spectrum for the copper release (Fig. 3) can reasonably be attributed to one transition. This transition (like all the others of CuPen) has not been assigned unequivocally as yet.

Due to the different coordination geometries of the copper atoms in the central Cu_8 cube (approximately tetrahedral S_3Cl environment) and those in the peripheral Cu_6 octahedron (square-planar S_2N_2 environment), distinct Cu(I) and Cu(II) centres are assumed, and mixed-valence transitions are not expected at low energy [4].

Every attempt to assign the spectroscopic bands to electronic transitions must account for the Cotton maxima observed in the CD spectrum between 400 and 700 nm. The electronic absorption spectrum features at least four Gaussian components, *i.e.* bands with extinction coefficients per Cu(II) between 700 and 2900 M^{-1} cm^{-1} [11]. The most straightforward way of assignment is shown in Table I. The ligand field band at 637 nm corresponds in position and intensity to a band at 620 nm in a related pentanuclear complex with two $Cu(I)S_3$ and three $Cu(II)N_2S_2$ centres joined by common thiolate groups [12]. The three short wavelength bands of CuPen at

TABLE I. Assignment of Spectral Features of CuPen to Electronic Transitions

λ (nm)	ϵ_d (M^{-1} cm^{-1})	Transition	
427	2200	$S(3p) \rightarrow Cu^{I}$ (4s, 4p)	charge transfer
490	2600	$\sigma S(3p) \rightarrow Cu^{II}$ (3d)	charge transfer
548	2900	$\pi S(3p) \rightarrow Cu^{II}$ (3d)	charge transfer
637	700	$Cu^{II} S_2N_2$	ligand field

427, 490 and 548 nm can be compared with bands at 327, 388 and 460 nm, respectively, which have been assigned in a similar way [12]. The red shift of the transitions in CuPen may be explained by the sharing of the thiolate sulfurs between three Cu atoms instead of two, which reduces their basicity. Moreover, the assignment of the 427 nm band of CuPen to a thiolate → Cu(I) LMCT transition is in good accord with a corresponding transition at 403 nm (in ethanol) in an analogous $Cu(I)_8S_{12}$ complex with $(CN)_2C{=}C(S^-)_2$ ligands [13]. In both cases the thiolate groups are influenced by electron-withdrawing (acid) functions, *i.e.* cyano groups in the latter complex and Cu(II) in CuPen.

This interpretation corresponds well with the photolytic activity observed in the 400 nm range. However, the S → Cu(I) transition might not be the basis for the observed photolytic reaction itself. It cannot be excluded that excited electrons interchange to a neighbouring charge-transfer system with its maximum in the ultraviolet region. The position of the photolytic activity around 400 nm as a marginal shoulder at the long wavelength end of a much more efficient activity in the UV region favours this point. In this way, the low quantum efficiency in the visible region might be explained.

While CuPen is reasonably stable against oxygen and EDTA, during anaerobic irradiation of CuPen solution an EDTA- and/or oxygen-sensitive copper complex having similar magnetic properties as CuPen is generated. The nature of this photochemical product has not been identified and needs further investigation. However, the constant isolated spin concentration in conjunction with an almost total loss of absorbance in the visible region argues for a major reorganisation of the ligand environment of the Cu(II) atoms in the absence of a net oxidative process at the copper atoms. This contrasts with the total reduction of Cu(II) to Cu(I) during thermal degradation of CuPen, as revealed by the absence of any EPR signal.

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