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A specific effect of copper on methylene blue sensitized photodegradation of nucleic acid derivatives

P. MARUTHI MOHAN, J. NIRMALA and K. SIVARAMA SASTRY
Department of Biochemistry, Osmaniu University, Hyderabad 500 007

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Abstract. Among several metal ions tested, Cu^{2+} was unique in slowing down methylene blue sensitized photodynamic breakdown of some nucleic acid bases and nucleosides. The $t_{½}$ values were increased in the case of xanthine and uric acid by Cu^{2+}, but without any alteration in the nature or amounts of photoproducts formed. Xanthine was degraded quantitatively to allantoin and urea.

The breakdown of the sugar moiety of nucleosides was more drastically retarded than that of the purine ring. The decomposition rate and its magnitude was dependent on the concentration of Cu^{2+} as well as the nucleoside. The most profound increase in $t_{½}$ values was found with xanthosine–7min for the purine ring and 65 min for the ribose moiety, at 0.6 mM Cu^{2+}

Hg^{2+} in the case of xanthine, and some paramagnetic metal ions in the case of the nucleosides, slowed down the photobreakdown to a small extent; however, differential effects were not observed unlike with Cu^{2+}. None of the other metal ions tested significantly influenced the process.

Keywords. Photodynamic inactivalion; metal effects; methylene blue; purine photobreakdown.

Introduction

Photodynamic inactivation of nucleic acids is brought about in the presence of oxygen by photosensitizing dyes. In polynucleotides, the base affected is guanine (Sivarama Sastry, 1968). A variety of purine derivatives structurally related to guanine, and several dyes have been studied in model systems with a view to understand the details of this process (Simon and Van Bunakis, 1962; Sussenbach and Berends. 1965; Wacker *et al.,* 1963). It is now evident that this is a very complex phenomenon involving the breakdown of the purine ring, the nature of the photo-products formed depending upon the purine derivatives as well as the dye involved. The nature of the lesions also depends upon the conditions employed. When these are comparatively mild, with moderate dye concentrations and light intensity, no strand cleavage occurs, but coding properties of poly (UG) are destroyed (Simon *et al.,* 1965) and likewise infectivity of TMV-RNA is lost (Sivarama Sastry and Gordon, 1966). Under drastic conditions, extensive depolyrnerization of polynucleotides is brought about photodynamically (Simon and Van Vunakis, 1962: Sivarama Sastry and Gordon. 1964; Freifelder *et al.,* 1961). The mechanisms involved were elucidated by the work of Waskell *et al.,* (1966) who showed that the likely lesion under mild photodynamic conditions is primarily depurination at guanine sites

Abbreviations used; UV. ultraviolet.

following the cleavage of the N-C glycosidic bond and that drastic conditions bring about destruction of ribose attached to guanine which is the probable lesion that results in strand scission.

The influence of metal ions on photodynamic inactivation has not been well studied. Sussenbach and Berends (1963) have shown that paramagnetic metal ions inhibit photodegradation of deoxyguanylic acid sensitized by lumichrome. Sastry and Gordon (1966) have shown that the inactivation of TMV-RNA sensitized by acridine orange is retarded by paramagnetic metal ions. Whether it is a generalized effect on photobreakdown is not known.

In the present study the effect of some metal ions on methylene blue sensitized photodegradation of several purine derivatives has been examined and it will be shown that Cu^{2+} exerts a selectively greater influence in inhibiting ribose and deoxyribose breakdown in nucleosides, in comparison with purine ring degradation.

Materials and Methods

Materials

Xanthine (E. Merck, Bombay), uric acid (Reanal, Budapest, Hungary), guanosine and xanthosine (US Biochemical Corporation, Cleveland, Ohio, USA) and 2′-deoxyguanosine (Fluka AG, Buchs, Switzerland) were used. Zinc-free methylene blue was product of Matheson, Coleman and Bell, New Jersey, USA. Metal salts used were $CuSO_4.5H_2O$, $CoSO_4.7H_2O$, $NiSO_4.7H_2O$, $HgCl_2$ and $MgSO_4.7H_2O$ were of analytical grade (British Drug House, India).

Methods

Irradiation procedures and analytical technique: Irradiation of purine compounds was performed with 2 mg of each compound in 4 ml volume in 50 mM Na_2CO_3-$NaHCO_3$ buffer (pH 9.45) in the presence of 0.03 mM methylene blue and 40,000 lux light intensity in a water jacketted test tube (15 mm internal diameter) with constant circulation of water at 30 ± 1° C and aeration by bubbling air pre-saturated with buffer used, at a predetermined minimal optimal rate. Metal salts were added as required. Under the conditions employed, precipitation did not occur in any of the irradiated mixtures, upto the maximal concentrations of metal ions employed.

The destruction of the purine derivatives was routinely followed by measuring the ultraviolet (UV) absorbance at the following wavelengths; xanthine and xanthosine, 245 nm; 2′-deoxyguanosine, 250 nm; guanosine, 260 nm; and uric acid, 290 nm. Aliquots (0.1 ml) from irradiated mixtures were removed at intervals of time, diluted to 4 ml and UV absorbance measured.

Ribose remaining at various time periods was determined by the orcinol method in 0.1 ml aliquots as in earlier studies (Waskell *et al.,* 1966). Deoxyribose was determined in 0.2 ml aliquots with diphenylamine (Schneider, 1957).

Allantoin in irradiated solutions was estimated by the differential method of Nirmala and Sastry (1972). Uric acid interference was corrected by separately determining the uric acid content of the irradiated mixtures by measuring the absorbance at 290 nm.

Urea was estimated with ∝ -isonitrosopropiophenone (Archibald, 1945).

Residual ultraviolet absorbance was plotted as a function of time and the time for

50% decrease in absorbance ($t_{½}$) was determined graphically. When $t_{½}$ could not be directly measured, it was extrapolated from the time course curves. In the case of nucleosides, ribose/deoxyribose destruction was also plotted graphically and the $t_{½}$ for the sugar moiety determined separately.

Results

Initial experiments showed that Cu^{2+} had a selective effect on retarding ribose breakdown in guanosine in comparison with the degradation of the ring. In view of this finding, the influence of varying concentrations of Cu^{2+}, as well as of other metal ions on photodynamic degradation of guanosine, 2′-deoxyguanosine and xanthosine was studied. The most dramatic effects were obtained with Cu^{2+} (figure 1).

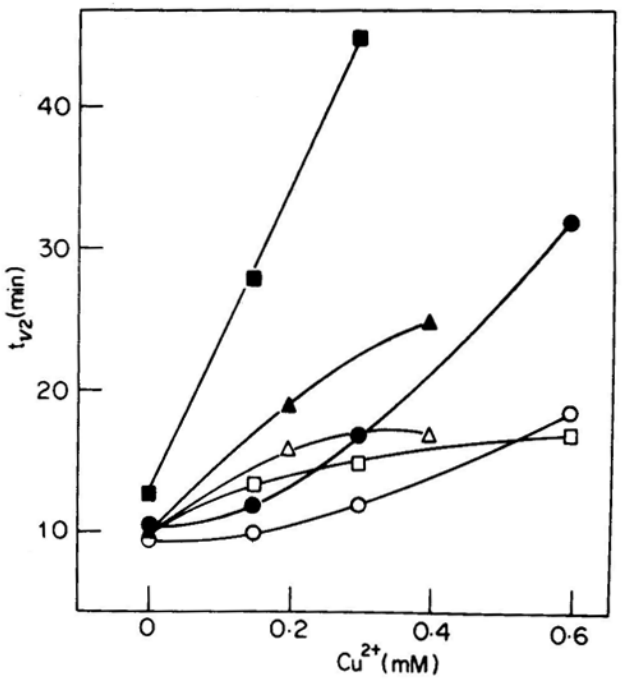

Figure 1. Effect of Cu^{2+} on photodynamic breakdown of guanosine, 2′-deoxyguanosine and xanthosine

Breakdown of the purine ring of guanosine (Δ), 2′-deoxyguanosine (O) and xanthosine (□).

Breakdown of the sugar moiety of guanosine (▲), 2′-deoxyguanosine (●)and xanthosine (■).

Irradiation with 0.3 niM methylene blue in Na_2CO_3-$NaHCO_3$ buffer (50 mM, pH 9.45) at 40,000 lux; Experimental details, see text.

The $t_{½}$ values for guanosine ring breakdown were enhanced from 10 min to 17 min in the range 0-0.4 mM $Cu^{2±}$; in contrast, the increase in $t_{½}$ for the ribose moiety of guanosine was from 10 min to 25 min. Higher concentrations of $Cu^{2±}$ could not be used, due to partial precipitation in the reaction mixtures. In the case of 2′-deoxyguanosine also, a similar concentration dependent effect of $Cu^{2±}$ was seen. With xanthosine the ribose destruction was very markedly inhibited, and $t_{½}$ values for 0.6 mM $Cu^{2±}$ extrapolated from figure 2 gave a $t_{½}$ for ribose of 78 min in contrast to a $t_{½}$ for the purine ring of 17 min. Whereas $t_{½}$ values are nearly identical for the ring and ribose moieties with guanosine and deoxyguanosine, with xanthosine normally ribose breakdown lags a little behind that of the purine ring ($t_{½}$ values are 10 min for the ring and 13 min for ribose).

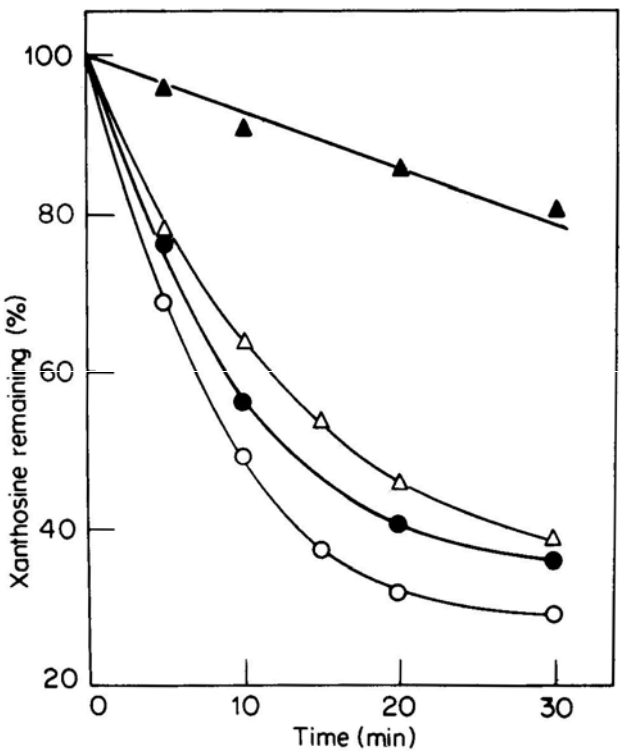

Figure 2. Effect of Cu^{2+} on the time course of photodynamic breakdown of xanthosine.

Xanthosine irradiated in preseigfe of 0.03 mM methylene blue with and without 0.6 mM Cu^{2+} Data indicate the disappearance of: (a) purine ring, control (O), with Cu^{2+} (Δ); (b) ribose moiety, control (●), with Cu^{2+} (▲), other details as in Fig. 1. Experimental details, see text.

Table 1 represents data obtained with various metal ions. Only Cu^{2+} is the most effective, and the other metal ions inhibit only very slightly and ulike-Cu^{2+} do not exhibit differential effects on the disappearence of the ring and sugar moieties.

Table 1. Effect of metal ions on the photodynamic breakdown of guanosine 2′-deoxyguanosine and xanthosine

Compound (mM)	Metal ions	$t_{1/2}$ (min) Purine ring	Ribose or deoxyribose
Guanosine (1.75)	None	10	10
	Cu^{2+}	17	25
	Co^{2+}	15	15
	Ni^{2+}	14	14
	Hg^{2+}	13	13
	Mg^{2+}	10	10
2′-Deoxy-guanosine (1.87)	None	9.5	11
	Cu^{2+}	18	32
	Co^{2+}	16	17
	Ni^{2+}	12	15
	Hg^{2+}	12	12
	Mg^{2+}	11	11
Xanthosine (1.75)	None	10	13
	Cu^{2+}	17	78
	Co^{2+}	14	22
	Ni^{2+}	13	17
	Hg^{2+}	15	17
	Mg^{2+}	11	14

The concentration of metal ions used were 0.4 and 0.6 mM for experiments with guanosine and 2′-deoxyguanosine/xanthosine respectively. Irradiation in the presence of 0.03 mM Methylene Blue in Na_2CO_3-$NaHCO_3$ buffer (50 mM, pH 9.5) was carried out at 40,000 lux. Experimental details, see text.

To determine the effect of metal ions on photodegradable base derivatives, xanthine and uric acid were chosen. Similar experiments with several metal ions were carried out with xanthine and uric acid and the results obtained are shown in table 2. The concentrations of the metal ions used were the maximum possible levels that could be tested, since higher concentrations resulted in precipitation. Only Cu^{2+} was the most potent inhibitor but the nature of photoproducts was not altered by it. Of the other metal ions examined, only Hg^{2+} inhibited the photodegradation of xanthine.

Table 2. Effect of metal ions on photodynamic degradation of xanthine and uric acid

Compound (mM)	Metal ions	$t_{1/2}$ (min)
Xanthine (3.29)	None	17.5
	Cu^{2+}	112
	Co^{2+}	18
	Ni^{2+}	19
	Hg^{2+}	26
	Mg^{2+}	18
Uric acid (3.0)	None	15
	Cu^{2+}	46
	Co^{2+}	16
	Ni^{2+}	13.5
	Hg^{2+}	15
	Mg^{2+}	15

Metal ions were included at 0.06 mM in the case of xanthine and at 0.03 mM for uric acid.
The samples in $Na_2CO_3NaHCO_3$ buffer (50 mM pH 9.45) containing 0.03 mM methylene blue were irradiated at 40,000 lux.

The concentration dependence of the inhibitory effect of Cu^{2+} on the photodegradation of xanthine and uric acid is shown in figure 3. It is interesting that whereas the $t_{1/2}$ of uric acid increased linearly with the concentration of Cu^{2+} through the entire concentration range, with xanthine the effect was not very marked upto about 0.03 mM Cu^{2+}, but thereafter it increased sharply.

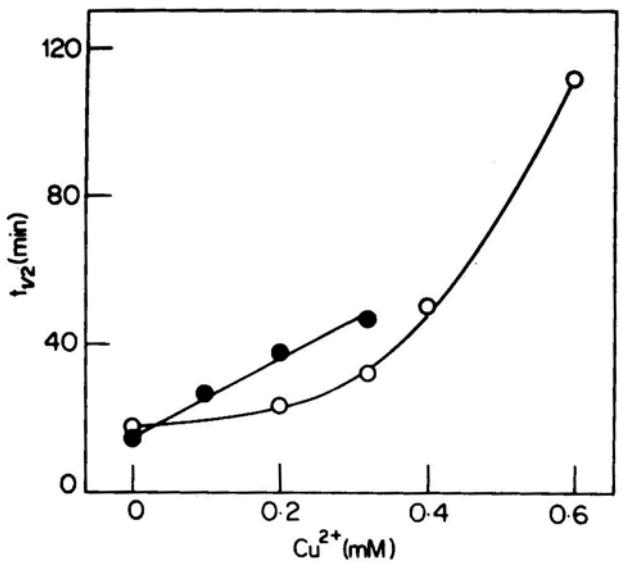

Figure 3. Effect of Cu^{2+} on photodynamic breakdown of xanthine and uric acid.

Uric acid. (●) and xanthine (O) irradiated with 0.03 mM methylene blue in presence of Cu^{2+} Other details as in Fig. 1.

Since the photodynamic breakdown of uric acid sensitized by methylene blue has been shown to result in the formation of allantoin and urea as the two major photo-products (Nirmala and Sivarama Sastry, 1975), it was decided to examine the possible influence of Cu^{2+} on the formation of these two products. Irradiation for 30 min resulted in 95% breakdown of uric acid and 74% of xanthine (table 3).

Table 3. Effect of Cu^{2+} on the photodynamic breakdown of xanthine and uric acid.

Compound (μmol/ml)	Cu^{2+} (mM)	Breakdown	Allantoin formed	Urea formed
			(μmol/ml)	
Xanthine (3.29)	0	2.43	1.81	0.74
	0.03	1.17	0.88	0.26
Uric acid (3.00)	0	2.85	0.70	1.43
	0.03	0.77	0.21	0.48

Irradiation in the presence of methylene blue was done for 30 min with 0.03 mM in Na_2CO_3-$NaHCO_3$ buffer (50 mM, pH 9.45) at 40,000 lux.

From these data, it is evident that the methylene blue sensitized photo-dynamic breakdown of xanthine results in the formation of allantoin and urea as the sole products, since they together fully account for the xanthine degraded.

Discussion

The photodynamic breakdown of nucleic acids is retarded by paramagnetic metal ions (Sivarama Sastry and Gordon, 1964; Sussenbach and Berends, 1963). It had been suggested that this may be due to the metal ions shortening the life-time of the triplet state of the excited species (Sussenbach and Berends, 1963), but this does not completely explain the effects of metal ions observed in this study. Cu^{2+} , in particular, exerts a very unusual effect. Generally Cu^{2+} slows down purine ring breakdown, but with nucleosides, Cu^{2+} differentially and much more powerfully inhibits the photodegradation of the sugar moiety.

The results do not permit the postulation of a detailed mechanism by which Cu^{2+} exerts the very specific effect observed. As suggested earlier, a general effect on the triplet state is not likely to be involved since other parmagnetic metal ions do not have a similar effect. Neither is it likely that interactionof Cu^{2+} with hydroxyls at C_2' and C_3' is the determining factor since guanosine and 2′-deoxyguanosine are degraded similarly.

The Cu^{2+} effect on xanthine and uric acid photodegradation indicates that metal ion interaction with the purine ring is involved. In the case of guanosine, inosine and several related compounds, Cu^{2+} binding to N has been suggested, in addition to chelation with N and O_6 (Tu and Friedrich, 1968) though this is not fully accepted

(Eicchorn, 1973). Hg^{2+} has been thought to bind to N_1 of guanine at high pH and to the amino group at low pH (Simpson, 1964); N_1-O_6 binding for Hg^{2+} has also been suggested (Yamane and Davidson, 1961; Eicchorn and Clark, 1963). Since Cu^{2+} and Hg^{2+} behave quite differently, from a quantitative point of view, the known complexing features imply that the site(s) of binding of metal ions to purine ring (and there could be more than one) under the present conditions also determine to a marked extent the influence of metal ions on photodynamic degradation of purine derivatives.

The degradation of ribose attached to a guanine site is important for an understanding of the mechanism of photodynamic depolymerization of polynucleotides. The present work suggests that metal ions such as Cu^{2+} which protect against photodynamic inactivation, for example with TMV-RNA (Sivarama Sastry and Gordon, 1966), may do so by suppressing ribose breakdown and thereby chain cleavage; protection of guanine residues may be'an additional factor.

Several metal ions (Ni^{2+} , Co^{2+} and Cu^{2+}) are nearly equally effective in protecting TMV-RNA against photodynamic inactivation and these form ternary complexes containing RNA, dye and metal (Sivarama Sastry and Gordon, 1964, 1966). The present work shows that, of these, only Cu^{2+} is a potent inhibitor of photodynamic breakdown of model compounds indicating that the specificity of interaction depends on the metal ion and the purine ring.

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