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Effect of dyes on the photodecomposition of pyridoxine and pyridoxamine

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Erythrosine, eosine Y, rose bengal, mercurochrome, methylene blue, azure A and azure B accelerated the photodecomposition of pyridoxine and pyridoxamine at pH 5–9, but fluorescein and acid red did not. The photodecomposition of pyridoxine in the presence of erythrosine was greatest at pH 9·0. The singlet oxygen generated by light-excited dyes played a role as a mediator in the decomposition of pyridoxine but the degradation products of erythrosine had no effect. The photodecomposition of pyridoxine and pyridoxamine in the presence of dyes was depressed by aminopyrine, sulpyrine and tryptophan. Pyridoxine 3,4′-dioctanoate was stable in the presence of erythrosine.

Mixtures for internal use, liquids for external use, injections and cosmetics containing vitamin B_6 are widely used, and these pharmaceutical products are frequently coloured with dyes. Dyes accelerate the photodecomposition of drugs and chemicals by acting as photosensitizers (Kaminski et al 1979). For example, little photodecomposition of phenylbutazone occurs under irradiation in pH 7·4 buffer solution with a 300-watt incandescent bulb, whereas decomposition is accelerated in the presence of erythrosine (Baugh et al 1977). Photolysis of pyridoxine has been reported to be accelerated in the presence of flavine mononucleotide (FMN) (Mizuno et al 1979), but the effects of dyes other than FMN on pyridoxine photodecomposition have not been reported. This, we now do.

MATERIALS AND METHODS

Materials

Pyridoxine hydrochloride (m.p. 203–204 °C) and pyridoxamine hydrochloride (m.p. 226–227 °C) (Wako Pure Chemical Industries, Ltd.) were recrystallized from ethanol. Pyridoxine 3,4′-dioctanoate (Nikko Chemicals Co.) was recrystallized from hexane (m.p. 69–71 °C). Erythrosine was purchased from San-Ei Chemical Industries, Ltd. Superoxide dismutase from bovine blood and catalase were purchased from Sigma Chemical Co. All other chemicals were of analytical grade.

Irradiation

Photolysis with flood lamp. Buffer of pH 5–9, 30 ml ($\mu = 0{\cdot}1$), containing pyridoxine hydrochloride (0·1 mM) and a dye (0·026 mM) was added to a 50 ml glass-stoppered flask which was immersed in a controlled-temperature water bath of 30 ± 1 °C. Next, the flask was irradiated with a 150 W flood lamp (Tokyo Shibaura Denki Co.) at a distance of 25 cm. The illumination on the surface of the solution was 7300 lx according to a Type 5 Toshiba illuminometer (Tokyo Shibaura Denki Co.).

Photolysis with sunlight. The experiment was done in a laboratory where sunlight illumination was kept at 13000 lx by moving the sample.

Assays

Pyridoxine and pyridoxamine were determined colorimetrically using 2,6-dibromoquinone-chlorimide (Mizuno et al 1968). Pyridoxine 3,4′-dioctanoate was also assayed colorimetrically after being hydrolysed to pyridoxine with 1 M NaOH solution at 100 °C for 20 min (Mizuno et al 1977). Aminopyrine, sulpyrine and tryptophan were measured by high-pressure liquid chromatgraphy (Hitachi Model 635-T). All mobile phases contained 2 mM cetyltrimethylammonium bromide in methanol–phosphate buffer solution (1 : 1, v/v) at pH 3·86. The analyses were carried out at room temperature (20 °C) on a stainless steel column (4 mm i.d. × 15 cm) packed with Lichrosorb RP-18 (5 μm particles) (E. Merck, Darmstat, G. F. R.). The flow rate was 0·3 ml min^{-1}. Aminopyrine, sulpyrin and tryptophan were measured at 260 nm, 270 nm and 277 nm, respectively. Under these conditions the compounds and their photodegradation products could be separated. Fluorescence spectra were measured with a Hitachi MPF-2A type fluorescence spectrophotometer. For degassing, suction under reduced pressure was used and the solution was pipetted into a colorless ampoule under nitrogen.

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RESULTS AND DISCUSSION

Fig. 1 shows the effect of erythrosine on the photodecomposition of pyridoxine. When the solution was irradiated with a flood lamp for 1 h, the photodecomposition rate was 1%, while in the presence of erythrosine, the half life was 18 min. Decomposition did not proceed in the dark and was depressed by degassing. The degradation products of erythrosine did not accelerate the photodecomposition of pyridoxine, nor did oxalic acid, phthalic acid, resorcin, halogens (Kurayuki et al 1963) or hydrogen peroxide (Imamura & Koizumi 1955), which are known to be degradation products of xanthene dyes.

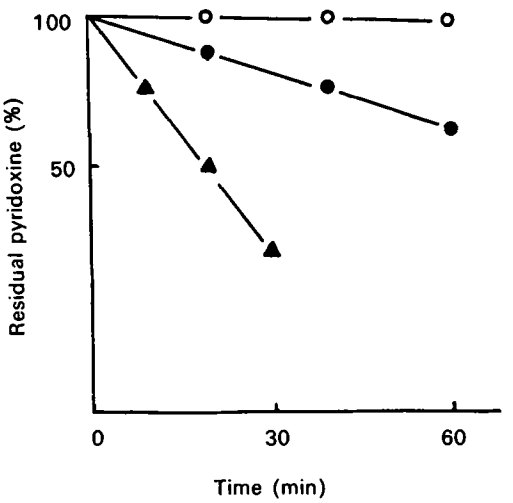

FIG. 1. Effect of erythrosine on the photodecomposition of pyridoxine at pH 7·0 and 30 C. Pyridoxine solution alone was saturated with air (○——○) and pyridoxine solution in the presence of erythrosine was saturated with nitrogen (●——●) or air (▲——▲).

We next studied whether the mechanism of the reaction proceeded via a type I (free radical) or type II (singlet oxygen) mechanism (Moore & Tamat 1980). The addition of potassium iodide and potassium thiocyanate, which are quenchers of the excited triplet (Blok & Verhey 1968), reduced pyridoxine photodecomposition in the presence of erythrosine (Table 1). L-Histidine (Nilsson et al 1972) and sodium azide (Gollnick et al 1972), which are quenchers of singlet oxygen, almost inhibited it. These results indicate that the reaction proceeds via the type II (singlet oxygen) mechanism. Catalase did not inhibit the decomposition, while hydrogen peroxide did not enhance it, therefore, ·OH may be excluded as a mediator in the photolysis (Halliwell 1978), as

Table 1. Effect of quenchers on the photodecomposition of pyridoxine in the presence of erythrosine.

Quencher	Quantity	Photodegradation of pyridoxine as % of control
None (control)		100·0
L-Histidine	$1·0 \times 10^{-2}$ M	10·5
Sodium azide	$2·0 \times 10^{-2}$ M	6·7
Potassium iodide	$1·0 \times 10^{-2}$ M	69·6
Potassium thiocyanate	$1·0 \times 10^{-2}$ M	81·8
Superoxide dismutase	100 units ml^{-1}	105·5
Catalase	400 units ml^{-1}	95·5
Hydrogen peroxide	$3·7 \times 10^{-2}$ M	95·5

The reaction mixtures contained 0·1 mM pyridoxine HCl and 0·026 mM erythrosine in pH 7·0 phosphate buffer.

also can the superoxide ion (O_2^-) since the superoxide dismutase had no effect (McCord & Fridovice 1969).

The mechanism of the action of dyes in pyridoxine photodecomposition was hypothesized to occur as a result of the dyes in the ground state first being transferred to the excited state by light absorption, in which state they react with oxygen to form singlet oxygen (Baugh & Calvert 1977) which reacts with pyridoxine.

In the presence of erythrosine, the ultraviolet maximum peak of pyridoxine at about 326 nm was decreased gradually with irradiation (Fig. 2) and after 1 h, could not be recognized. When either the erythrosine or pyridoxine solution alone was irradiated, the spectra were virtually invariant. The pH-rate profile at 30 °C for the photolysis of pyridoxine in the presence of erythrosine shows a steady rise of k from $1·9 \times$ to $3·8 \times 10^{-2}$ (min^{-1}) between pH 5–8 and then a rapid rise to 10^{-1} (min^{-1}) at pH 9.

The effects of various kinds of dyes on the photodecomposition of pyridoxine and pyridoxamine were also examined (Table 2). The rate constants for the photodecomposition of pyridoxine were larger than those for pyridoxamine. However, fluorescein, acid red, rhodamine B, brilliant blue and indigo carmine did not cause decomposition of either pyridoxine or pyridoxamine. Halogenated xanthene dyes (erythrosine, eosin Y, phloxine and rose bengal) and thiadine dyes (methylene blue, azure A and azure B) have previously been shown to cause photodecomposition (Raju et al 1974); acid red does not (Kido & Watanabe 1977) and there is no report of brilliant blue and indigo carmine having such activity. The

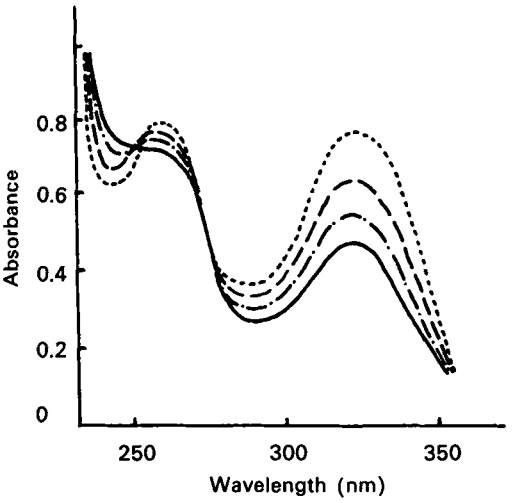

FIG. 2. Changes in the absorption spectra during photodecomposition in the presence of erythrosine at pH 7·0.
------ before irradiation, — — — after 5 min of irradiation, — - — - — after 10 min of irradiation, —— after 15 min of irradiation.

rank of photosensitive activity of the active dyes agreed with the inactivation of chymotrypsinogen (Kido & Watanabe 1977) and their lethal action on bacteria (Inada et al 1970). The interaction among pyridoxine, fluorescein and its halogen substitutions was then studied by measurement of the fluorescence spectra of pyridoxine in it. The size of the maximum peak at pH 7 decreased in the order: rose bengal, phloxine > erythrosine, eosine Y > fluorescein. Halogenated compounds have a quenching action and there appears to be a relationship between this and pyridoxine photodecomposition.

A group of compounds that could prevent photodecomposition of pyridoxine in the presence of dyes was also examined. Aminopyrine, sulpyrine and tryptophan in the presence of erythrosine and methylene blue inhibited the photolysis (Table 3) but other compounds had no effect. Table 4 shows the effects of this group of compounds on pyridoxamine, which were similar to those of pyridoxine. Table 5 shows the rate constants of the photodecomposition of aminopyrine, sulpyrine and tryptophan. The photodecomposition of pyridoxine in the presence of erythrosine was depressed by these compounds (Table 3), while their own photodecomposition was

Table 2. Rate constants for the photodecomposition of pyridoxine and pyridoxamine in the presence of dyes.

Dye (0·026 mM)	Rate constant of pyridoxine $\times 10^2$ (min^{-1})	Rate constant of pyridoxamine $\times 10^2$ (min^{-1})
Control	0·07	0·03
FMN	1·87	0·92
Erythrosine	3·79	0·73
Eosine Y	4·16	0·79
Phloxine	4·82	1·30
Rose bengal	6·11	1·90
Mercurochrome	2·72	0·62
Methylene blue	4·07	0·73
Azure A	2·16	1·02
Azure B	2·28	1·13

Table 3. Effect of drugs on the rate of photodecomposition of pyridoxine in the presence of dyes.

Drug (0·33 mM)	Rate constant $\times 10^2$ (min^{-1}) Erythrosine*	Methylene blue*
Control	3·79	4·07
Tyrosine	3·56	3·66
p-Cresol	2·99	3·09
Sulpyrine	1·17	1·34
Tryptophan	2·27	2·85
Thiourea	3·98	4·27
Sulfisomidine	3·49	3·42
Nicotinamide	4·28	3·95
Caffeine	4·28	4·19
Aminopyrine	1·25	1·63
Sulfadimethoxine	4·05	4·48
Antipyrine	4·70	4·40
4-Isopropylantipyrine	4·51	4·88

* 0·026 mM.

Table 4. Effect of drugs on the rate of photodecomposition of pyridoxamine in the presence of dyes.

Dye (0·026 mM)	Rate constant $\times 10^2$ (min^{-1}) Control	Amino-pyrine*	Sul-pyrine*	Anti-pyrine*	Nicotin-amide*
FMN	0·92	0·17	0·26	1·20	1·11
Erythrosine	0·73	0·12	0·20	0·87	0·90
Eosine Y	0·79	0·25	0·30	0·93	0·91
Phloxine	1·30	0·26	0·33	1·39	1·20
Rose bengal	1·90	0·27	0·23	1·54	1·58
Mercurochrome	0·62	0·17	0·16	0·55	0·51
Methylene blue	0·73	0·13	0·13	0·85	0·85
Azure A	1·02	0·20	0·34	0·87	0·87
Azure B	1·13	0·24	0·26	0·95	0·96

* 0·33 mM.

Table 5. Rate constants of the photodecomposition of aminopyrine, sulpyrine and tryptophan.

Compound (0·33 mM)	Rate constant (min^{-1}) With PN	With PN_+ erythrosine	With PN_+ erythrosine$_+$ sodium azide
Aminopyrine	$1·68 \times 10^{-4}$	$2·62 \times 10^{-3}$	$1·86 \times 10^{-3}$
Sulpyrine	$3·40 \times 10^{-4}$	$5·53 \times 10^{-3}$	$2·29 \times 10^{-3}$
Tryptophan	$1·68 \times 10^{-4}$	$3·46 \times 10^{-3}$	$5·16 \times 10^{-4}$

greater than the control. Pyridoxine photodecomposition was inhibited by sodium azide quenching of the singlet oxygen (Table 1), and photodecomposition of aminopyrine, sulpyrine and tryptophan was also depressed by sodium azide which suggests that the photooxidation of these compounds proceeded via the type II (singlet oxygen) mechanism, thereby protecting pyridoxine. Replacement of pyridoxine by pyridoxine 3,4′-dioctanoate reduces the photodecomposition by erythrosine from 77% (4 h at 7300 lx)–92% (15 min at 13 000 lx) to 0%.

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