Photodynamic Activities of Food Additive Dyes on the Yeast *Saccharomyces cerevisiae*

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Photodynamic cell-inactivating activities of food additive dyes on the yeast *Saccharomyces cerevisiae* were investigated. Activities of dyes not permitted as food additives were also examined. Red No. 105 (rose bengal), Red No. 3 (erythrosine) and Red No. 104 (phloxine), which are permitted as food additives, markedly inactivated yeast cells by photodynamic action. Eosine, matius yellow and guinia green B, which are not permitted, also exhibited moderate cell-inactivating activity by photodynamic action. None of the dyes used in this experiment exhibited petite induction by photodynamic action.

Keywords photodynamic action; food additive dye; cell inactivation; petite induction; yeast; *Saccharomyces cerevisiae*; rose bengal; erythrosine; phloxine; eosine Y

Various kinds of photodamage are induced by a variety of medicines, cosmetics and chemical agents in our environment. In order to protect man and livestock from photodynamic cell damage, a simple and reliable screening system to detect active photosensitizers should be established. In the previous reports[1] we showed that cell inactivation and petite (cytoplasmic respiration-deficient mutant) induction of the yeast *Saccharomyces cerevisiae* DP1 1B/517 are adequate markers of the photodynamic activities of chemicals. Comparison of the photobiological activities of acridine compounds using this experimental system[2] revealed that euflavine and proflavine are the most potent photosensitizers in terms of cell inactivation and petite induction. In this communication, the photobiological activities of food additive dyes on yeast are described. Red No. 105, Red No. 3 and Red No. 104, which are permitted as food additives by the Food Sanitation Law of Japan, exhibited marked cell-inactivating activity by photodynamic action. Eosine, matius yellow and guinia green B, which are not permitted, also have moderate cell-inactivating activity by photodynamic action.

Materials and Methods

Strain and Cultures A haploid yeast strain of *Saccharomyces cerevisiae* DP1 1B/517 (α, his_1, trp_1, ρ^+, ω^+, C^R) was used. Cells were grown for 18 h (late logarithmic phase) in YPD medium [1% yeast extract (Difco), 2% peptone (Difco) and 1% dextrose] at 30 °C with shaking. Cells were washed three times with 1/15 M phosphate buffer (pH 7.0), sonicated to separate clumping cells and adjusted to a cell density of 10^6/ml.

Photodynamic Treatment of Yeast Cells Resuspended cells (10^6/ml) were incubated with 1—100 μM food additive dyes at 30 °C for 60 min in the dark. Aliquots of dye-sensitized cells were taken into Thunberg tubes and irradiated with fluorescent lamps (National, 15 W × 4, 8000 lx), while the remaining cell suspensions were kept in the dark as a control. Cell suspensions, irradiated or unirradiated, were spread on YPD plates after suitable dilution. After 3 d of incubation at 30 °C in the dark, the frequencies (%) of petite colonies were determined by the tetrazolium-overlay method[3] as described in previous reports.[4] Survivals (%) were calculated from the number of colonies relative to the dark control.

Chemicals Food additive dyes were purchased from Tokyo Kasei Organic Chemicals as Food color testing solutions A and B set. All other chemicals were of reagent grade.

Results

Cell Inactivation and Petite Induction by Photodynamic Action of Dyes Permitted as Food Additives Yeast cells treated with 1—100 μM dye for 60 min at 30 °C in the dark were irradiated for 30 min with fluorescent lamps (Table I). Red No. 3 (erythrosine), Red No. 104 (phloxine) and Red No. 105 (rose bengal)-inactivated yeast cells notably by photodynamic action, though survivals were unchanged in the dark control. The other dyes did not inactivate yeast cells. None of the dyes including Red No. 3, Red No. 104 and Red No. 105 used in this experiment induced petites by photodynamic action.

The fact that some red dyes, permitted for use as food additives, may induce various kinds of biological damage

TABLE I. Cell Inactivation and Induction of Mitochondrial Mutation by Photodynamic Action of Food Additive Dyes

Dye	Irradiated			Un-irradiated		
	Survival (%)[a]	Colony number[b]	Petite (%)[c]	Survival (%)[a]	Colony number[b]	Petite (%)[c]
Control	88.1	74±11	0.4±0.8	100	84±19	1.1±0.8
Red No. 2 (amaranth)	78.7	131±19	1.3±0.4	100	166±34	0.9±0.4
Red No. 3 (erythrosine)	0.3	1±1	0±0	100	89±5	0.6±0.8
Red No. 104 (phloxine)	14.4	6±3	8.3±14.4	100	42±10	0±0
Red No. 105 (rose bengal)	0	0±0	0±0	100	191±6	1.0±4.9
Red No. 106 (acid red)	102.4	74±12	0.8±0.7	100	72±13	1.5±1.7
Yellow No. 4 (tartrazine)	89.1	153±18	0.8±0.9	100	172±29	2.2±0.6
Yellow No. 5 (sunset yellow)	106.7	165±6	1.8±0.6	100	154±3	2.8±1.3
Green No. 3 (fast green)	103.2	146±21	2.0±1.3	100	142±26	1.9±1.7
Blue No. 1 (brilliant blue)	114.0	142±25	1.1±0.9	100	124±14	0.5±0.4
Blue No. 2 (indigocarmine)	98.2	150±5	2.2±0.3	100	153±13	1.5±1.4

Yeast cells (10^6/ml) were incubated with 100 μM dye at 30 °C for 60 min in the dark. An aliquot of cell suspension was removed and irradiated for 30 min with fluorescent lamps. *a*) Survivals (%) were calculated from the mean±S.D. of colony numbers of four plates relative to that of the un-irradiated control. *b*) Mean±S.D. of colony numbers of four plates. *c*) Mean±S.D. of petite (%) of four plates.

Table II. Cell Inactivation and Petite Induction by Photodynamic Action of Various Dyes

Dye	Irradiated			Un-irradiated		
	Survival (%)	Colony number	Petite (%)	Survival (%)	Colony number	Petite (%)
Azocompounds						
Ponceau 3R	96.0	106±6	1.2±0.5	100	111±20	1.8±0.9
Ponceau SR	89.9	136±2	0.7±0	100	151±37	0.2±0.7
Ponceau R	123.3	119±19	0.5±0.9	100	96±45	1.9±1.7
Orange I	82.8	104±17	1.3±1.1	100	126±26	0.7±0.7
Orange II	61.9	90±47	2.0±0.7	100	145±9	1.4±0.8
Triphenylmethanes						
Acid magenta	105.2	108±27	1.2±1.1	100	103±41	1.3±0.4
Guinia green B	52.0	86±34	2.9±0.8	100	165±72	2.1±1.4
Brilliant milling green	62.9	104±7	2.3±0.6	100	166±16	1.6±1.5
Patent blue	62.9	76±6	2.5±2.1	100	120±10	0.3±0.5
Azur blue	58.0	97±6	0.7±1.3	100	168±18	2.0±0.9
Acid violet 6B	72.2	110±8	1.6±2.0	100	153±46	0.7±0.9
Light green SF, yellowish	90.6	148±9	2.0±1.1	100	163±27	1.0±0.3
Xanthenes						
Eosine	23.4	29±4	0±0	100	124±9	0.9±0.9
Uranine	62.9	99±6	1.3±1.2	100	157±33	1.9±1.2
Quinolines						
Naphthol yellow S	72.1	100±14	1.7±1.7	100	139±23	0.5±0.4
Matius yellow	48.0	73±8	0.5±0.8	100	153±19	1.3±0.5
Miscellaneous						
Lionol blue	60.1	95±25	1.5±0.9	100	158±16	0.9±0.8

Table III. Cell Inactivation and Petite Induction by Photodynamic Action of Red dyes

Dye	Concn. (μM)	Irradiated		Un-irradiated	
		Survival (%)	Petite (%)	Survival (%)	Petite (%)
Control	—	119.9	1.9±1.9	100	2.1±3.2
Red No. 105	1	121.6	0.5±0.9	100	1.7±1.2
(rose bengal)	10	0.5	6.3±12.5	100	0.5±0.6
	100	0.2	0±0	100	1.0±1.4
Red No. 3	1	77.4	1.5±2.1	100	0.2±0.5
(erythrosine)	10	5.1	0±0	100	1.2±0.9
	100	0.9	1.4±1.7	100	1.8±1.6
Red No. 104	1	75.9	1.1±0.5	100	1.1±1.0
(phloxine)	10	114.2	0.6±1.0	100	1.7±0.9
	100	12.9	1.2±1.5	100	1.4±0.9
Eosine	1	89.3	1.1±0.7	100	0.8±0.9
	10	147.7	0.4±0.5	100	1.2±1.3
	100	39.2	0.1±0.2	100	1.5±1.4

by photodynamic action is noteworthy.

Cell Inactivation and Petite Induction by Photodynamic Action of Various Dyes Not Permitted as Food Additives Photodynamic biological activities of dyes such as azo compounds, triphenylmethanes, xanthenes and quinolines, which are not permitted as food additives, were examined (Table II). There were no dyes which induced petites by photodynamic action. The most potent dye in this group in terms of photoinactivating activity of yeast cells was eosine, and survival decreased to 23.4% after 30 min of irradiation. Survivals of yeast cells photosensitized with guinia green B, azur blue and matius yellow were 52.0, 58.0 and 48.0% respectively. However, cell-inactivating activities of these dyes were generally low compared to those of Food Red dyes.

Photodynamic Cell-Inactivating Activity of Red Dyes Photodynamic cell-inactivating activities of red dyes, permitted or not permitted as food additives, were investigated in detail. As shown in Table III, the most potent photosensitizer was Red No. 105 (rose bengal). It inactivated 99.8% of yeast cells at 100 μM and 99.5% at 10 μM. Red No. 3 (erythrosine) inactivated 99.1% at 100 μM and 94.9% at 10 μM. Red No. 104 was active at 100 μM but not active at less than 10 μM. Eosine, which is not permitted as a food additive, exhibited moderated cell-inactivating activity at 100 μM. Petite induction was not observed. These results suggested that the dyes used here could not be incorporated into the cells.

Discussion

Out of 10 dyes currently used as food additives, 3 dyes were shown to be active photosensitizers for inactivation of yeast cells. Red No. 105 (rose bengal) and Red No. 3 (erythrosine) were effective at 10 μM, though Red No. 104 (phloxine) was effective at 100 μM but not at 10 μM. These dyes have been widely used as coloring agents not only for confectionery and fish paste products but also for medicines and cosmetics. A 68%: 32% mixture of erythrosine and rose bengal is used to obtain a cherry color. Dye contents of products are 0.0002—0.001% in foodstuffs, 0.0005—0.5% in medicines and 0.001—8% in cosmetics.[5] The concentration of 0.001% is equivalent to 100 μM in the case of rose bengal. Therefore dye contents in eye shadow (0.5%), lipstick (1—8%) and rouge (1—3%) may be high enough to induce photodermatological damage.

Rose bengal has already been reported to induce cell inactivation by photodynamic action[6a,7] and photohemolysis.[8] In this work, erythrosine and phloxine were also shown to inactivate yeast cells by photodynamic action. Eosine Y, which is not permitted for use as a food additive dye and is known to be an active photosensitizer,[6a,9] inactivated yeast cells by photodynamic action.

Psoralen,[10] thiopyronine[11] and acridines[1,2,4b] can induce petites (cytoplasmic respiration-deficient mutant) in yeast by photodynamic action, but none of the dyes used here could do so. These dyes may not be incorporated into yeast cells.

The cell inactivation by acridine dyes appears to be mediated largely by singlet oxygen.[1,2,6a] Relative efficiency of $^1O_2$ production and phage $\phi \times 174$ inactivation by different dyes including rose bengal and eosine Y was examined by Houba-Herin *et al.*[12] Gandin *et al.*[13] also compared the quantum yield for $^1O_2$ formation by 11 xanthenes. These results suggest that singlet oxygen scavengers such as α-tocopherol or β-carotene may reduce the photodynamic activities of these dyes. It is difficult to assess the health risk to man and livestock quantitatively. We have to take account of the dye contents in products, intake per day, distribution and exposure to sun or fluorescent light. However, considering the potent photodynamic activities of these red dyes and general use of the dyes at relative high concentrations, it is clear that there is a potential risk, and further research would be warranted.

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