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Petite Induction in Yeast, *Saccharomyces cerevisiae*, by Phenanthridinium Compounds: Promotive Effects of Propidium Iodide on Mutagenesis by Ethidium Bromide or 8-Deaminoethidium Chloride

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Promotive effects of propidium iodide (PI) on petite induction by 8-deaminoethidium chloride (8-DAEC) were examined. 8-DAEC was a potent petite inducer in growing yeast cells but not in resting yeast cells. Addition of PI promoted the petite induction by 8-DAEC throughout the cultivation time at a concentration that was ineffective by itself. In resting cells, petites were scarcely induced by either 8-DAEC or PI or both, even after prolonged incubation. Notable sectored colony induction was observed after prolonged incubation of the yeast cells with either 8-DAEC or PI in phosphate·buffer. The petite induction by ethidium bromide (EB) was not accelerated but was delayed in PI-pretreated cells, whereas the simultaneous presence of PI promoted the EB mutagenesis.

These results suggested that the simultaneous presence of PI and petite inducer in growth medium was required in order to exhibit the promotive effects. Possible mechanisms of the differences in the process of petite induction between resting and growing cells are discussed.

Keywords——petite induction; yeast; *Saccharomyces cerevisiae*; phenanthridinium; promotive effect; propidium iodide; 8-deaminoethidium bromide; ethidium bromide; sectored colony

Ethidium bromide (EB), a phenanthridinium dye, is a potent mutagen for yeast mitochondrial deoxyribonucleic acid (mt DNA) in both growing and resting cells.[1] On the other hand, acriflavine (AF), an acridine dye, is active only in growing cells.[2] However, some phenanthridinium compounds are not mutagenic in either growing or resting cells.[3] Although propidium iodide (PI) is also a phenanthridinium dye (Fig. 1) and non-covalently binds to closed circular DNA as effectively as[4] or more effectively[5] than EB, it is virtually inactive in petite induction.[3a]

Previous studies in this laboratory revealed that PI not only induced petites at high concentrations during prolonged incubation with growing cells[6] or in the presence of sodium dodecyl sulfate,[7] but also induced a large number of sectored colonies.[6b] "Sector" cells, which form sectored colonies on YPD medium, are thought to have unstable "premutational" lesions in mt DNA.[6b] In addition, PI was co-mutagenic for petite induction by EB in growing

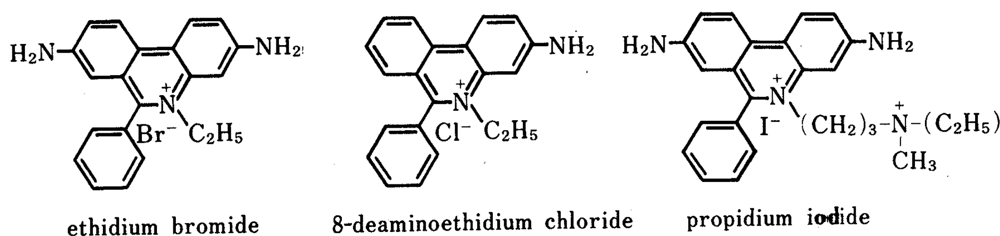

Fig. 1. Chemical Structures of Phenanthridinium Compounds

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cells[8)] and was antagonistic in resting cells.[9)]

In the present work, petite induction by either 8-deaminoethidium chloride (8-DAEC) or PI or both, was examined in growing and resting cells in order to explore the promotive effect of PI on an ethidium analog which only acts on growing cells. 8-DAEC is a potent petite inducer only in growing cells,[3b)] in contrast to the parent compound. PI exhibited a promotive effect on petite induction not only by EB but also by 8-DAEC under conditions where PI itself was not effective for petite induction. Thus, 8-DAEC was considered to be an appropriate model compound for studying ethidium bromide-type petite induction in growing cells.

Materials and Methods

Saccharomyces cerevisiae DP1 1B/517 (α, his_1, trp_1, ρ^+, ω^+, C^R) was used in this study. Yeast cells were grown for 18 h (late exponential phase) at 30°C in 50 ml flasks containing 1% yeast extract (DIFCO), 2% peptone (DIFCO) and either 1% dextrose (YPD medium) or 2% glycerol (YPG medium) with continuous shaking. Cells were harvested, washed 3 times in 1/15 M phosphate buffer (pH 7.0), resuspended in the same buffer, and sonicated for 60 s with a Blackstone sonifier to separate budding daughter cells from mother cells without cell killing.

Cells were suspended in 10 ml of medium (referred to as "growing conditions") or 1/15 M phosphate buffer (pH 7.0) (referred to as "resting conditions") at a cell density of 10^6/ml in 50 ml flasks and incubated with drugs at 30 °C with shaking. Samples were removed at the times indicated and plated onto YPD agar solid medium after suitable dilution. After incubation at 30 °C for 3 d, the proportions of petites in the population were determined by the tetrazolium-overlay method.[10)]

Entirely white colonies were scored as petites, and colonies that had both white and red parts were scored as sectored colonies. More than 600 colonies were examined for each sample, and cell viabilities were estimated from the numbers of colonies on these plates.

PI was purchased from Calbiochem and EB from Sigma Chemical Company. 8-DAEC was synthesized by Yielding *et al.*[3b)] All other chemicals were of reagent grade.

Results

Induction of petite and sectored colonies under growing conditions by either 8-DAEC or

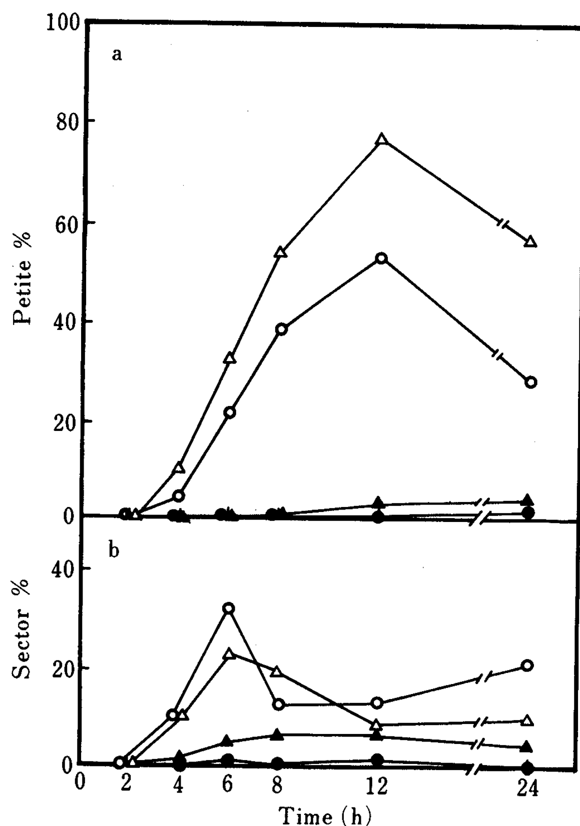

Fig. 2. Petite and Sectored Colony Induction by 8-Deaminoethidium Chloride and Propidium Iodide in Growing Cells

Washed late exponential-phase cells, *Saccharomyces cerevisiae* DP1 1B/517, were suspended in 10 ml of fresh YPD medium at a cell density of 10^6/ml and cultured in a shaking water bath at 30 °C with: 5 μM 8-deaminoethidium chloride (○—○); 100 μM propidium iodide (▲—▲); 5 μM 8-deaminoethidium chloride and 100 μM propidium iodide (△—△); no drug (●—●). Aliquots were removed at the intervals indicated, diluted and plated on YPD agar plates. After incubation for 3 d, petite (a) and sectored (b) colonies were scored by using the tetrazolium salt overlay method.

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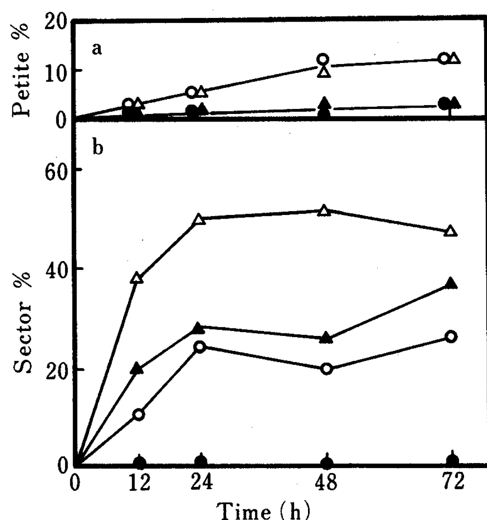

Fig. 3. Petite and Sectored Colony Induction by 8-Deaminoethidium Chloride and Propidium Iodide under Resting Conditions

Washed late exponential-phase cells were suspended in 10 ml of 1/15 M phosphate buffer (pH 7.0) at a cell density of 10^6/ml and incubated in a shaking water bath. Symbols are the same as in Fig. 2.

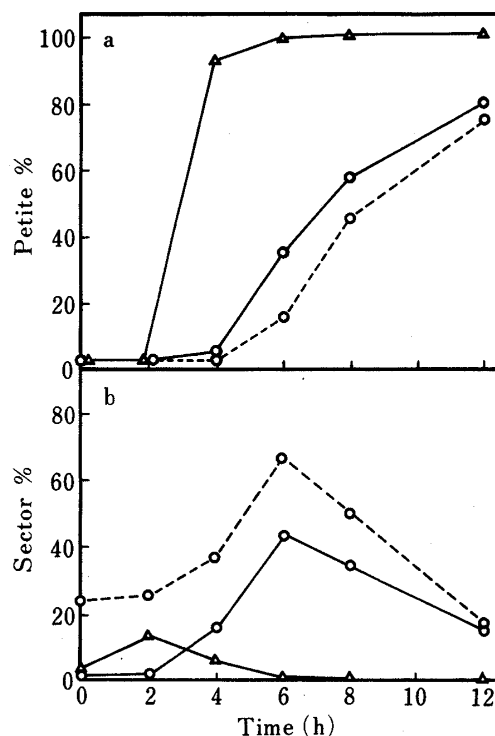

Fig. 4. Effects of Propidium Iodide on Petite and Sectored Colony Induction by Ethidium Bromide

Washed late exponential-phase cells were suspended in 10 ml of 1/15 M phosphate buffer (pH 7.0) at a cell density of 10^6/ml and shaken at 30 °C with (----) or without (——) 100 μM propidium iodide for 12 h. Cells were then centrifuged, resuspended in 10 ml of fresh YPD medium and cultured with: 5 μM ethidium bromide (○); 5 μM ethidium bromide and 100 μM propidium iodide (△).

PI, or both, is shown in Fig. 2. Notable induction of sectored colonies was observed after 6 h of growth with 8-DAEC and the population of "sector" cells, which form sectored colonies on YPD medium plates, was replaced by petite cells at 12 h. PI at 100 μM accelerated and enhanced the petite cells at 12 h. PI at 100 μM accelerated and enhanced the petite induction by 8-DAEC throughout the cultivation time, although PI alone was not effective under the same conditions. Under resting conditions, neither 8-DAEC nor PI was active in petite induction (Fig. 3). However, there was substantial induction of sectored colonies by 8-DAEC alone or in combination with PI after 12 h of incubation. In order to determine whether "sector" cells induced here are converted to petites, the incubation was continued up to 72 h. The petite induction by 8-DAEC, with or without PI, was at most 10% even after 72 h incubation. PI was quite inactive in inducing petites under these conditions. Thus, even with prolonged incubation, "sector" cells induced by PI were not converted to petites. We have described elsewhere[6b,7] sectored and petite colony induction by PI.

Cells incubated with either 8-DAEC or PI or both for 48 h in buffer were plated onto YPG medium to determine whether "sector" cells can grow on a medium which requires mitochondrial respiration. As shown in Table I, the differences between total colony numbers and the sum of petite and sectored colonies on glucose medium correspond to colony numbers on glycerol medium, except for cells which had been treated with PI alone. This result confirmed that "sector" cells exhibit respiration-deficient character at first after plating. In the case of PI-treated cells, many cells which were scored as normal cells on glucose media could

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TABLE I. Comparison of Petite and Sectored Colony Induction with Colony-Forming Ability on Glycerol and Glucose Media Following Treatment with Either 8-Deaminoethidium Chloride or Propidium Iodide or Both under Resting Conditions

Phenanthridinium		Colony number ± S.D.[a]			
8-DAEC (μM)	PI (μM)	Glycerol medium	Glucose medium		
		Total	Total	Petite	Sector
5	0	149 ± 13 (71.6%)	208 ± 30 (100%)	23 ± 2 (11.1%)	41 ± 8 (19.7%)
0	100	6 ± 4 (6.3%)	96 ± 17 (100%)	3 ± 0 (3.1%)	46 ± 6 (47.9%)
5	100	69 ± 11 (47.6%)	145 ± 34 (100%)	13 ± 4 (9.0%)	75 ± 16 (51.7%)

Cells were incubated with either 8-deaminoethidium bromide (8-DAEC) or propidium iodide (PI) or both in phosphate buffer for 48 h. Cell suspensions were diluted adequately and plated on glucose or glycerol medium. Petite and sectored colonies were scored as described in Materials and Methods. *a*) Figures in parenthesis are calculated based on total colony number on glucose medium as 100%. S.D.; standard deviation.

not form colonies on glycerol media. This may indicate rapid recovery or rapid overgrowth of respiratory-competent cells on glucose.

The nature of the PI enhancement was next examined by pretreating yeast cells with PI in the buffer prior to exposure to EB under growing conditions. This experiment addressed the question of whether the "sector" cells induced by PI represent a state of increased sensitivity to EB mutagenesis. Figure 4 shows that 12 h of PI pretreatment decreased the rate and extent of petite induction, in contrast to the enhancement of the rate and extent of induction when both drugs were present simultaneously in the growth medium.

Discussion

Extensive work on the petite induction by ethidium bromide has suggested that two distinct mechanisms are involved. Under growing conditions, EB causes selective inhibition of mt DNA synthesis, which leads to partial or total loss of mt DNA[11] in daughter cells. Under resting conditions, EB binds to mt DNA covalently[3a,12] and stimulates mt DNase, leading to degradation and loss of pre-existing mt DNA.[13] These different events under growing and resting conditions must be considered separately, since chemical modifications of EB lead to partial or complete loss of its mutagenic effects in resting cells.[3] Furthermore, the distinction between these two mechanisms is supported by the finding that the PI is co-mutagenic with EB under growing conditions but anti-mutagenic under resting conditions.[9] It could not be concluded whether PI simply enhanced petite mutation processes during growth or whether it resulted in reactivation of separate processes which operate in resting cells. In the present work, it was found that PI enhances the petite induction not only by EB but also by the 8-deaminated derivative of EB, 8-DAEC, which works only in growing cells. PI itself is known to be a weak petite inducer under growing conditions[6,8] and to be inactive under resting conditions.[3b,6b]

Other experiments have established that PI alone can induce sectored colonies under resting conditions.[6b] This finding is of importance because "sector" cells have been regarded as transient precursors of petites,[14] and it was suggested that PI can provoke transient initial steps on the path to petite mutation. However, initial speculation that PI could exhibit a

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promotive effect owing to an ability to induce such a pre-mutational state seems unlikely in view of the present results that petite induction by EB in PI-pretreated cells was not enhanced but delayed (Fig. 4).

The effects of PI may be explained as follows: under resting conditions, PI binds to mt DNA, initiates mt DNA degradation or disorganization and induces "sector" cells which have unstable premutational lesions in mt DNA. Such damage does not lead to irreversible degradation of all mt DNA, but recovery occurs either through repair or recombination. Delay of petite induction by EB in the PI pretreated cells may be simply the result of occupation by PI of EB binding sites. Under growing conditions, PI binds to mt DNA and inhibits mt DNA synthesis less effectively because of its long charged side chain. Thus, high concentrations of PI are needed for inducing petites. The promotive effects of PI on petite induction by EB may result through increased (co-operative) binding of EB or enhancement of the events which transpire subsequent to EB binding, *i.e.*, inhibition of mt DNA synthesis and stimulation of mt DNase.[8)]

In view of the result that PI reduced the lag time of petite induction (Fig. 4), we may have to consider another possibility, *i.e.*, that PI exerts its promotive effect by changing the membrane structure to permit increased transfer of EB into the cell and mitochondria. In fact, there are convincing data that phenanthridinium dyes do bind to membranes.[15)] These findings of enhancement of the efficacy of a specific drug by the presence of other members from the same family of compounds may represent a mechanism of considerable importance in determining susceptibility.

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