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Massimo D'Aquino, Francesca La Monica, Giovanna Vitali

Mutagenic activity of 1-methyl-2-nitro-5-vinylimidazole on Saccharomyces cerevisiae

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Biologia molecolare. — *Mutagenic activity of 1-methyl-2-nitro-5-vinylimidazole on* Saccharomyces cerevisiae. Nota di MASSIMO D'AQUINO (*), FRANCESCA LA MONICA e GIOVANNA VITALI, presentata (**) dal Socio A. STEFANELLI.

RIASSUNTO. — L'azione mutagena di un composto nitroeterociclico; 1–methil–2–nitro–5 vinylimidazolo, (MEV) è stata saggiata su cellule del lievito *Saccharomices cerevisiae*.

Questo composto viene trasformato in composto attivo in seguito a riduzione del nitrogruppo da parte dei microorganismi, nei quali produce sia una inibizione della crescita che variazioni del genoma. La velocità con la quale la crescita viene inibita dipende dalla aereazione della coltura, dalla concentrazione del MEV e dal tempo di incubazione.

La particolare morfologia delle cellule trattate con il MEV indica difficoltà nel meccanismo di divisione cellulare.

L'aumento di mutanti « petite », anche se è inferiore a quello che si ottiene con altri mutageni quali l'acriflavina, dimostra che il MEV agisce sul DNA mitocondriale.

Non si conosce chiaramente l'effetto del MEV sul DNA nucleare. Si pensa tuttavia che la sua azione sia quella di produrre dei cross–links tra le eliche del DNA.

We have studied the mutagenic action of the nitro-heterocyclic compound 1-methyl-2-nitro-5-vinylimidazole on cells of *Saccharomyces cerevisiae* yeast.

Our interest in this compound stems from the increasing use of its structural analogues in the therapy of bacteria, fungi and protozoa [1, 2]. Compounds such as nitroimidazole, nitrofurazone, furazolidone, nitrofurantoin, etc., are clinically used as antibacterial agents in urinary tract infections and against *Trichomonas vaginalis* (L. Silvestri, personal communication) and their mode of action has been under study for many years.

2-nitrofurazone, a furan derivative, inhibits DNA synthesis in *Escherichia coli*, inducing damage in the double strand which turns into lesions after alkaline treatment [3, 4].

2-nitroimidazole (azomycin) blocks DNA synthesis at the ribonucleoside reductase level [5].

1-methyl-2-nitro-5-vinylimidazole (MEV) assayed in *E. coli* showed an inhibiting activity of the synthesis of new DNA [6]. According to Goldstein *et al.*, [7], the reactive sites in the molecule must be the nitrous part which should be oxidized and the vinylic part which should turn into epoxide by a reduction of the vinyl group. In the absence of microorganisms, these substances are ineffective in the DNA in that needing to be activated.

(*) Present address: Istituto Nazionale della Nutrizione Via dei Lancisi, 29, Roma, Italy.

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MATERIALS AND METHODS

Saccharomyces cerevisiae strain DM42 from the collection of the Institute of General Physiology of the University of Rome.

Culture medium. – The yeast strain was cultured in the following medium: 1 % yeast extract, 2 % peptone, 2 % glucose.

Growth conditions. – Yeast was grown at 28 °C in screw-capped bottles containing 8 ml of culture, in anaerobic conditions.

Mutagenic action. – Samples of a suitably diluted culture of strains DM42 were grown on Petri dishes containing the same culture supplemented with 2 % Agar-agar. For identification of " petite " mutants, the colonies were stained after 48 h with a solution containing 1% Agar-agar, 0.5% glucose and 0.05 % triphenyl-tetrazole.

Chemical compounds. – 1-methyl-2-nitro-5-vinylimidazole (MEV) synthesized by Cavalleri [8] and kindly supplied by Dr. V. Arioli of Lepetit Laboratories, Milano, were sterilized by filtration through GS type Millipore filters (0,22 mμ pore size.).

DNA extraction. – From a 5 lt culture grown at 28 °C to the stationary phase, the DNA was extracted using Marmur's method [9]. Separation of mitochondrial DNA from nuclear DNA was performed by hydroxyapatite chromatographic columns equilibrated with 0.2 M phosphate buffer and washed with 200 ml of phosphate buffer for RNA elimination [10]. The DNA was eluted with a 0.2–0.37 M phosphate buffer gradient ad pH 6.8. 4 μg/ml of DNA were run for 24 h in a Beckman analytical ultracentrifuge, mod E., rotor type TN-D, refraction index 1.3995, at 44,470 rpm (120,000 g). 2 μg/ml of *Micrococcus lisodeikticus* were used as reference. Photograms were obtained at equilibrium with U.V., and diagrams with the Beckman analytrol.

RESULTS

Fig. 1 shows the growth curve of strain DM42 correlated with mevinylimidazole concentration. At a concentration of 2.5 μg/ml there is no growth inhibition and the growth rate does not differ too much from the initial rate. At a concentration of 5 μg/ml the cells reach a density of 10^5 cell/ml and no longer duplicate.

At MEV concentrations of 7 μg/ml and 10 μg/ml the initial cell number was increasing in order to achieve the anaerobic condition necessary for MEV activation more rapidly. In the latter experiment the yeast cells yielded only two generation cycles. At a concentration of 50 μg/ml there was a complete growth inhibition.

In order to evidence the " petite " mutant induction the cells were collected at fixed intervals, layered onto Petri dishes (about 200 cells/dish) and incubated at 28 °C for 48 h.

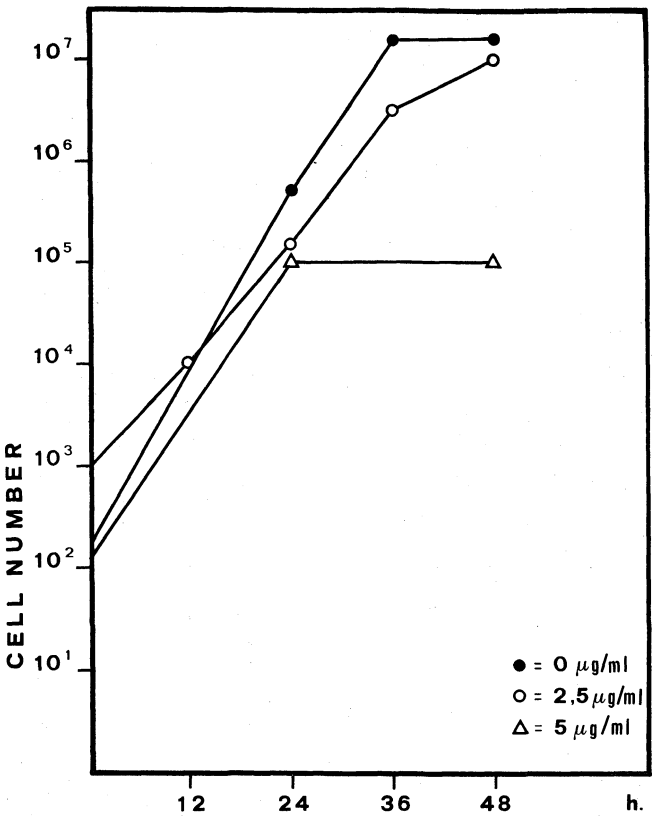

Fig. 1.

Table I indicates the " petite ' mutants at MEV concentrations of 2.5–7–10 μg/ml.

At a concentration of 10 μg/ml there are 2 % MEV-induced " petite " mutants, and 0.5 % spontaneous mutation.

At a MEV concetration of 12.5 % μg/ml the mutation frequency reaches 2 % after 6 h and 4 % after 15 h (Table II). Fig. 2 shows the nuclear DNA and the mitochondrial DNA density in MEV-induced " petite " MF 4 in the analytical ultracentrifuge. The nuclear DNA density is 1.700 g/cm^3 whereas the mitochondrial DNA density is 1.679 g/cm^3. The nuclear DNA weight

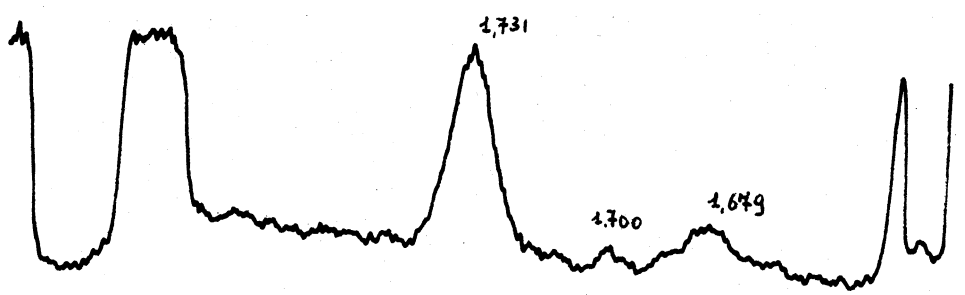

Fig. 2.

TABLE I

MEV	STRAIN	COLONIES	% PETITE
2,5 μg/ml	Saccharomyces cerevisiae	400	1 %
7 μg/ml	" " "	1119	1,7 %
10 μg/ml	" " "	382	2 %
0 μg/ml	" " "	135	0,8 %

TABLE II

MEV	STRAIN	INDUCTION TIME	% PETITE
12,5 μg/ml	Saccharomyces cerevisiae	2 h	2 %
12,5 μg/ml	" " "	4 h	1,5 %
12,5 μg/ml	" " "	6 h	2,5 %
12,5 μg/ml	" " "	15 h	4 %

density of *S. cerevisiae* "wild tipe" DM 42 was 1.700 g/cm³ whereas the mitocnondrial DNA density was 1.685 g/cm³.

CONCLUSIONS

According to our results, 1-methyl-2-nitro-vinylimidazole shows a clear inhibiting action of the growth of *S. cerevisiae* yeast and a fairly good mutagenic effect. The rapidity with which growth is blocked depends on culture aeration, MEV concentration and time of incubation. The anaerobic condition seems to be the one that causes the highest inhibiting effect, owing to the nitro group reduction and subsequent substance activation. The peculiarly long morphology of the yeast cells in the presence of MEV indicates a difficulty in the mechanism of cell division. The increase of the "petite" mutation percentage noticed, even if much lower than the values obtainable with mutagenic substances such as acriflavine [11], shows that MEV acts also on mitochondrial DNA. The mitochondrial DNA density, which is 1.679 g/cm³, indicates a medium G + C content much lower than that of W.T., which is 1.685 g/cm³.

MEV effects on nuclear DNA are so far not known. It has been shown in *E. coli* that 1-methyl-2-nitro-5-vinylimidazole does not inhibit the replication fork progression but acts randomly on the DNA [6]. Since the DNA extracted from MEV-treated cells renatures more rapidly than the DNA extracted from untreated cells, the presence of cross-links was assumed [6].

It may be that the MEV reactive sites able to induce cross-links are the nitro group that in the sensitive microorganisms reduced to the hydroxylamine reactive form [12] and the vinyl group that oxidizing to the epoxide could bind to the DNA [6].

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