Inhibitory effect of 5-bromo-6-azauracil on growth and cell division of *Saccharomyces cerevisiae*

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Summary. The most marked effect of 5-bromo-6-azauracil (BrAzU) on yeast cells is to cause cell lysis. The inhibition of the lytic process is delayed and this delay coincides in time with the capacity of preformed pyrimidines to reverse the effect of BrAzU.

5-Bromo-6-azauracil, first prepared by Handschumacher (see the note in[1]), was shown to inhibit the growth of *Escherichia coli B* cultivated in a defined minimal medium with glucose[2]. Experiments designed to investigate comparatively the concentration dependence of this effect revealed that partial inhibition of growth of procaryotic cells is caused at relatively low concentrations of BrAzU (10 µg/ml). However, even high concentrations of this antimetabolite (1000 µg/ml) did not cause a total inhibition of bacterial growth[2]. In view of these results we undertook an investigation of the effect of BrAzU on single-cell eucaryotes as well as of the antagonistic relationship between this antimetabolite and preformed pyrimidines or their analogs.

Material and methods. BrAzU was prepared according to Chang and Ulbricht[1]. Cytosine, thymine, uracil and 5-bromouracil were furnished by Calbiochem, Ficoll by Pharmacia. *Saccharomyces cerevisiae* U 92 was obtained from the culture collection of the Prague Institute of Chemical Technology. Difco yeast nitrogen base (B 391) with the addition of 1% glucose was used exclusively as the cultivation medium. The above medium was solidified where necessary with 2% Oxoid agar No. 3. Cultivation in liquid medium was carried out under intensive aeration in 15 ml volumes at 28 °C. The medium was inoculated to a concentration corresponding to an OD of 0.05 and the resulting culture was taken to be exponentially growing when attaining OD 0.2. Changes in biomass concentrations were followed turbidimetrically at 450 nm on a Specol spectrophotometer. The total cell number was determined using a Bürker chamber. Formalin (0.4%) was included in the dilutent. Viable counts were made by the plate technique. Dilutions were made in the starvation medium.

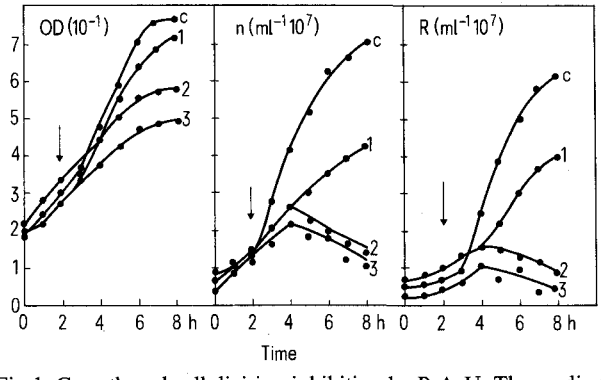

Fig. 1. Growth and cell division inhibition by BrAzU. The medium was supplemented (↓) with BrAzU to final concentrations of 4 mM (1), 8 mM (2) and 12 mM (3). c, control growth without BrAzU; OD, absorbance at 420 nm; n, total cell count; R, colony-forming ability.

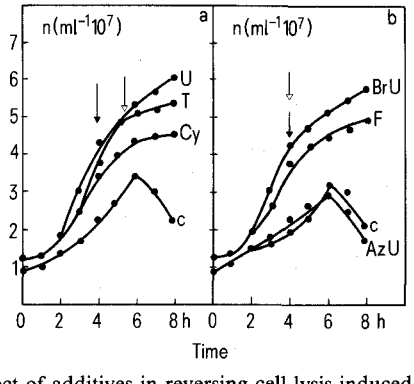

Fig. 2. Effect of additives in reversing cell lysis induced by BrAzU. The medium was supplemented (↓) with BrAzU to a final concentration of 8 mM. Thymine (T), cytosine (Cy), uracil (U), 5-bromouracil (BrU) and 6-azauracil (AzU) were added, respectively, to the same final concentrations (↓). Ficoll (F) was added (↓) to the concentration of 10% (w/v). n, total cell count; c, control growth.

Results and discussion. The concentration dependence of the effect of BrAzU (figure 1) indicates that the approximate minimum concentration causing significant inhibition of growth and cell division is 8 mM, if BrAzU is added at the early exponential growth. Higher concentrations had no greater inhibitory effect. Another fact to be noted is that between the application of BrAzU and the onset of its inhibitory manifestation there is always a delay of 120 min. The effect of BrAzU is completely removed by preformed pyrimidines (thymine, cytosine and uracil) if they are added 90 min after BrAzU (figure 2, a). On the other hand, there is no difference in the inhibitory effect of BrAzU if the pyrimidine bases are added simultaneously with the analog. However, simultaneously added 5-bromouracil completely antagonized BrAzU. 6-Azauracil applied in the same way neither reversed nor enhanced the effect of BrAzU which was, however, eliminated if the analog was applied to a yeast population in a medium which provided osmotic protection (figure 2, b). Microscopic observation of the cells throughout the interval of significant decrease in total cell count disclosed no morphological changes that would indicate the loss of cell wall rigidity and gradual protoplast formation. The picture that emerges from these results suggests that BrAzU stimulates a rapid lytic process. Nevertheless, the delay in the initiation of this process does not eliminate the possibility that cell lysis is directly or indirectly induced by a derivative or a breakdown product of BrAzU. In this connection, the capacity of pyrimidine bases to reverse the effect of BrAzU only 90 min after its addition suggests that a derivative of BrAzU is available at that time and is competitively suppressed by the preformed pyrimidines. On the other hand, the supposed transformation of BrAzU might not take place in the presence of pyrimidine bases. In connection with the unusual character of the inhibitory effect of BrAzU on yeast cells our further study will be focussed on a comparative investigation of the suppressive effects of other 5-halogenated 6-azauracil derivatives as potential fungicides.

1 P.K. Chang and T.L.V. Ulbricht, J. Am. chem. Soc. *80*, 976 (1958).

2 J. Gut, J. Morávek, C. Párkányi, M. Prystaš, J. Škoda and F. Šorm, Colln Czech. chem. Commun. Engl. Edn *24*, 3154 (1959).