Interaction of Ethidium Bromide with the Transport System for Monovalent Cations in Yeast

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Summary. Ethidium was found to be taken up by yeast cells in a process that, at certain concentrations has the main following characteristics: a) a substrate is required; b) it presents cooperative kinetics, with $n$, according to the Hill equation $\approx 3$; c) ethidium can be concentrated more than 100-fold; d) the uptake is inhibited by Ca$^{2+}$; e) the uptake of the dye is inhibited by monovalent cations with a selectivity pattern similar to that observed in their transport by yeast; f) ethidium inhibits the uptake of K$^+$, and, at concentrations up to about 250 $\mu$M produces a competitive inhibition on the uptake of Rb$^+$; and g) ethidium produces the same effects as K$^+$ on respiration and the extrusion of H$^+$. It is concluded that ethidium is taken up by yeast cells in a selective way by the same transport system normally employed for monovalent cation uptake.

In the course of some experiments designed to study the effects of some cationic dyes on K$^+$ transport in yeast, it was found that ethidium bromide, as well as other cationic dyes were able to inhibit the uptake of K$^+$. In view of this, and the fact that this dye has been so extensively studied in relation to its fluorescent characteristics, it was considered important to determine the kind of interaction of the molecule with the monovalent cation transport mechanism in yeast cells. This paper presents the results of such studies.

Materials and Methods

Saccharomyces cerevisiae cells from a pure strain kindly donated by La Azteca, S.A., were prepared as described previously (Peña, 1975).

The methods for the measurement of K$^+$ uptake, pH recording, oxygen consumption and $^{86}$Rb uptake were also described previously (Peña, 1975).

Fluorescence changes of ethidium were followed in a Farrand, Mark I spectrofluorometer at 530–590 nm. Narrow band filters of 530 and 590 nm were placed additionally between the excitation monochromator and the sample, and between this and the analyzer monochromator, respectively.

The uptake of ethidium bromide was followed in two ways; in the first, the cells were added to the incubation mixture in centrifuge tubes previously equilibrated to the temperature
of a water bath (30 °C); after the incubation, the tubes were cooled in an ice water bath for 2 min, and then centrifuged at 3,000 rpm for 3 min. After centrifuging, the supernatants were decanted to measure the ethidium concentration. In the second method, after mixing the cells with the medium at 30 °C, aliquots were withdrawn at fixed intervals, rapidly placed in tubes of a Beckman Microfuge and centrifuged for 10 sec. The supernatant was then separated with a Pasteur pipette to measure its ethidium concentration. The ethidium concentration of the supernatants obtained was determined by measuring the fluorescence of adequate dilutions in a 20 mM maleate-triethanolamine buffer, pH 6.0. The wavelengths employed were 330 nm, excitation, and 600 nm, emission. The results were compared each time with a standard curve of ethidium bromide from 4 to 40 µM at the same pH.

Results

Ethidium bromide inhibits $K^+$ uptake in yeast at rather low concentrations; concentrations of 100 µM are enough to produce a clear inhibition (Fig. 1). As reported for alkyl guanidines (Peña, 1973), the dye does not produce at this concentration practically any effect on the expulsion of $H^+$. At higher concentrations, the addition of the dye produces an actual exit of $K^+$, and a strong inhibition of the $H^+$ expulsion from the cells.

The fact that ethidium produces an inhibition of the transport of $K^+$ can be due in a first analysis to the possible binding of the molecule to the surface of the cell, in a similar way to that described for ANS by Fortes and Hoffman (1974). The first efforts to measure binding to the cells by fluorescent techniques gave negative results at the concentrations of the dye usually employed in these studies (10 to 40 µM). An experiment was carried out using higher concentrations of ethidium, and measuring its disappearance from the medium by centrifugation in the microfuge, to follow the time course of the phenomenon; the results are shown in Fig. 2. In the presence of glucose as substrate, the initial entrance of ethidium is very fast, and the rate seems to be related to the concentration of the dye employed. The results showed also that although in the absence of a substrate, some uptake exists, much higher concentrations have to be used in order to observe a significant uptake of the dye by the cells. By assuming an internal water content of approximately 50 percent of the wet weight for the cells, as measured before (Peña et al., 1967), internal concentrations of ethidium between 20 and 30 mM can be calculated. Calculating the final ratio between this and the external concentration, values higher than 100 can be observed in the presence of substrate. Without substrate, at 1.333 mM ethidium, internal concentrations around 15 mM can be calculated, and concentration ratios close to 20 have been estimated. This is of course by assuming