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Effect of ruthenium red upon Ca^{2+} and Mn^{2+} uptake in *Saccharomyces cerevisiae*. Comparison with the effect of La^{3+}

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The initial rate of both Ca^{2+} and Mn^{2+} uptake is inhibited by ruthenium red to about the same extent as by equivalent concentrations of La^{3+}. The inhibition of Ca^{2+} uptake, however, is relieved during further incubation with ruthenium red. On preincubating the cells with ruthenium red even a stimulation of divalent cation uptake can be found. Relieve of the inhibition of divalent cation uptake is accompanied by K^+ efflux. Both ruthenium red and La^{3+} displace Ca^{2+} very effectively from binding sites at the cell surface. The inhibition of initial Ca^{2+} uptake is accompanied by a reduction in the binding of Ca^{2+}.

Introduction

Borbolla and Peña [1] have reported that ruthenium red is a poor inhibitor of Ca^{2+} uptake in *Saccharomyces cerevisiae*. At low ruthenium red concentrations the uptake of Ca^{2+} was even slightly enhanced, although raising the ruthenium red concentration up to 10 μM did inhibit Ca^{2+} uptake. Higher concentraitons of ruthenium red up to 30 μM, however, did not affect Ca^{2+} uptake anymore. Lanthanum appeared to be a more efficient inhibitor showing a progressive decrease in the rate of Ca^{2+} uptake on increasing the La^{3+} concentration. Also in the yeast *Schizosaccharomyces pombe* [2] ruthenium red and La^{3+} influenced Ca^{2+} uptake quite differently. The effect of the ruthenium red concentration on the Ca^{2+} uptake in this yeast was very complicated. At low ruthenium red concentrations the uptake of Ca^{2+} was slightly inhibited. At medium ruthenium red concentrations uptake of ^{45}Ca was greatly stimulated, whereas at high ruthenium red concentrations a reduction in the rate of uptake was again found. We will now show that the low sensitivity of *S. cerevisiae* to ruthenium red is only apparent and should be ascribed to the fact that ruthenium red has a dual effect upon Ca^{2+} uptake.

Methods

The yeast, *S. cerevisiae* (2% w/v, Konings gist from Gist-Brocades) was starved by aeration for 24 h in distilled water. In this way non-metabolizing cells were obtained. Uptake of ^{45}Ca in these cells was virtually zero. For that reason these cells were used for the study of binding of ^{45}Ca to the yeast cells. This binding was studied by determining both the radioactivity of 0.5 ml of the supernatant (AS) and the radioactivity of 0.5 ml of the

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total suspension (AT). Binding (B) is given by

$$B = 1.02AT - AS \quad (1)$$

The factor 1.02 corrects for the volume occupied by the yeast in the suspension. The relative binding F is given by

$$F = B/AS \quad (2)$$

Uptake of ^{45}Ca in yeast was studied with metabolizing cells. After harvesting the non-metabolizing cells, these cells were preincubated with 3% w/v glucose in 45 mM Mes/Tris buffer (25°C, pH 6.5) for 15 min before adding ^{45}Ca. Nitrogen was bubbled through the suspension. Uptake of ^{45}Ca or ^{54}Mn in *S. cerevisiae* was studied according to Ref. 3. Unless otherwise stated, either ruthenium red or lanthanum chloride were added together with the radioactive isotopes.

Efflux of K^+ from a 10 ml suspension of 2% w/v metabolizing cells was determined by means of a K^+-selective glass electrode (Philips, G15-K) at 25°C and pH 6.5. The reference electrode was a calomel electrode filled with 100 mM $NaNO_3$.

The radioactivity of ^{45}Ca was determined by means of liquid scintillation, that of ^{54}Mn was determined by means of a gamma counter.

Chemicals. ^{45}Ca and ^{54}Mn were obtained from Amersham International (Amersham, U.K.), ruthenium red and lanthanum chloride were from Sigma (Taufkirchen, F.R.G.).

Results

Fig. 1 shows that 50 μM ruthenium red inhibited the uptake of Ca^{2+} greatly. This inhibition, however, was transient. After 2.5 min the uptake of ^{45}Ca was approximately the same as that of the control and after 4 min the uptake was even 15% higher. Relief of the inhibition was accompanied by efflux of K^+ from the cells.

From the time-course of uptake of ^{45}Ca in the presence of ruthenium red, such as illustrated in Fig. 1, it was difficult to obtain initial rates of uptake. We therefore developed another more suitable method based upon the following considerations. Theoretically the dependence of the uptake of ^{45}Ca (A) upon the time (t) can be described by

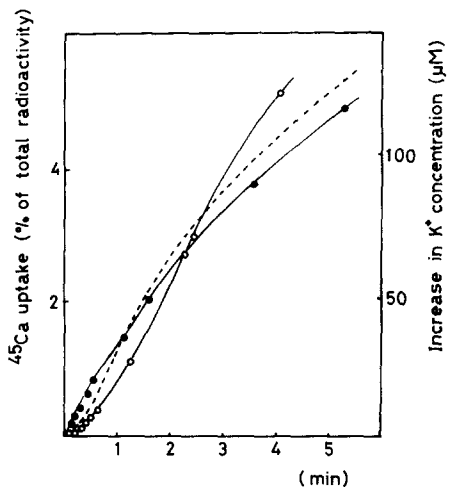

Fig. 1. Time-course of ^{45}Ca uptake at pH 6.5 in metabolizing cells. Efect of 50 μM ruthenium red upon both ^{45}Ca uptake and K^+ efflux. ^{45}Ca uptake in the absence of ruthenium red (●) or in the presence of ruthenium red (○). K^+ released to the medium in the presence of ruthenium red (dotted line). This K^+ release was continuously measured by means of a K^+-sensitive glass electrode in the medium. In the absence of ruthenium red no K^+ was released. The uptake of ^{45}Ca in the cells is expressed as a percentage of total radioactivity present in the suspension.

an nth order function, see Eqn. 3.

$$A = A_0 + A_1t + A_2t^2 + A_3t^3 + \ldots A_nt^n \quad (3)$$

At values of $t < 40$ s a plot of $(A - A_0)/t$ against t gave a straight line, see Fig. 2. This means that third-order terms were negligibly small within that time period. Initial rates of uptake could be calculated from the intercepts (A_0) with the Y-axis of plots like those presented in Fig. 2. We also determined the time-course of the inhibition of ^{45}Ca uptake by La^{3+}. It appeared that in the presence of this trivalent cation the initial inhibition was not relieved. The uptake isotherms were linear for at least 60 s (data not shown).

Ca^{2+} is strongly bound to the yeast cells by which the available Ca^{2+} in the medium is decreased [1]. Both ruthenium red and La^{3+} replaced ^{45}Ca effectively from the binding places on the cell as shown in Fig. 3. The effect of the two cations upon binding of ^{45}Ca was approximately the same.

In Fig. 4 the effects of ruthenium red and La^{3+} upon the initial rates of ^{45}Ca uptake were com-

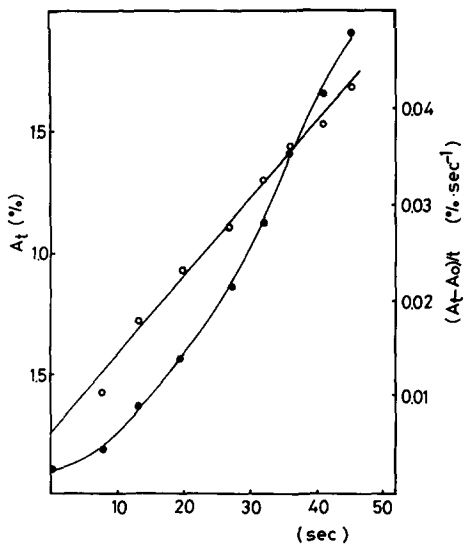

Fig. 2. Determination of the initial rate of uptake of ^{45}Ca uptake in metabolizing cells in the presence of 10 μM ruthenium red. Plot of radioactivity accumulated into the cells (A_t) against the time of incubation (t) (●) and plot of $(A_t - A_0)/t$ against t (○). A_0 is the radioactivity at zero time. A and A_0 are expressed as a percentage of the total radioactivity present in the yeast suspension.

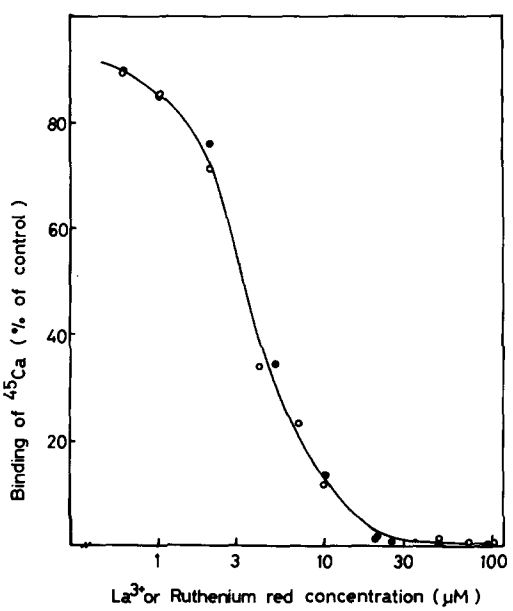

Fig. 3. Dependence of the binding of ^{45}Ca to non-metabolizing cells upon added ruthenium red or La^{3+}. Ruthenium red (○); La^{3+} (●).

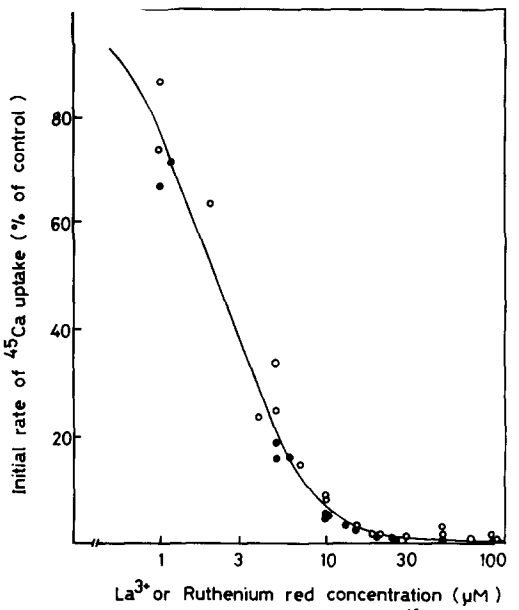

Fig. 4. Dependence of the initial rate of ^{45}Ca uptake in metabolizing cells upon the concentration of added ruthenium red or La^{3+}. The initial rates were corrected for the decrease in free ^{45}Ca in the medium due to binding of the cation to the cells. Ruthenium red (○); La^{3+} (●).

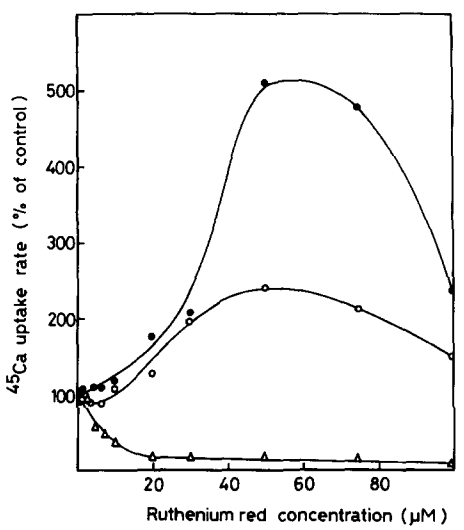

Fig. 5. Comparison of the effect of ruthenium red upon the rate of ^{45}Ca uptake determined in different ways. Initial rates of uptake of ^{45}Ca (△, ●); uptake of ^{45}Ca after 3 min incubation of the cells with both ruthenium red and ^{45}Ca (○); ruthenium red added together with ^{45}Ca (△, ○) or 10 min prior to the ^{45}Ca (●). Uptake of ^{45}Ca in the absence of ruthenium red was taken as 100%. The rates of uptake were not corrected for changes in binding of ^{45}Ca.

pared. The initial rates of uptake derived from the uptake isotherms were multiplied by $(1 + F)$, see Eqn. 2, in order to account for the decrease in available ^{45}Ca in the suspension owing to binding of ^{45}Ca to the yeast cells. Just as was found for the reduction in the binding of ^{45}Ca, La^{3+} and ruthenium red had almost the same effect upon the uptake of ^{45}Ca.

The dependence of the uptake of Ca^{2+} upon the ruthenium red concentration found after incubation of the cells for 3 min with both ruthenium red and ^{45}Ca was quite different from the concentration dependence observed for the initial rates of uptake, see Fig. 5. A small decrease in the uptake was found at concentrations of ruthenium red below 5 μM. Above that concentration the uptake was increased. Maximal uptake was found at 50 μM. When the cells were preincubated for 10 min with different amounts of ruthenium red before the addition of ^{45}Ca, the initial rates of ^{45}Ca uptake appeared to be far greater than those found on adding ^{45}Ca simultaneously with ruthenium red. These rates were also higher than the mean uptake rates calculated from the uptake after 3 min incubation of the cells with both ruthenium red and ^{45}Ca. On the other hand the concentration dependence of these initial rates was similar to the concentration dependence found for uptake of ^{45}Ca after 3 min incubation.

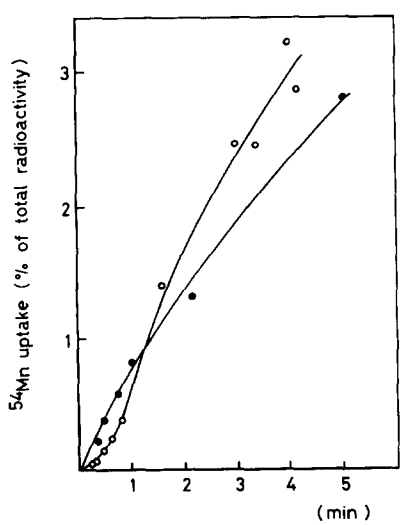

Fig. 6. Effect of ruthenium red upon the uptake of 10 μM Mn^{2+} at pH 6.5. Mn^{2+} uptake in the absence of ruthenium red (●); in the presence of ruthenium red (○).

Finally, we examined whether the effect of ruthenium red upon Ca^{2+} uptake was specific to Ca^{2+}. Fig. 6 shows that uptake of Mn^{2+} was also initially inhibited by ruthenium red and that this inhibition was relieved during incubation of the cells with ruthenium red. The extent of the initial inhibition was of the same order of magnitude as that found for ^{45}Ca uptake.

Discussion

Obviously ruthenium red is an effective inhibitor of Ca^{2+} uptake into yeast cells. There is no significant difference between the effectiveness with which La^{3+} inhibits the uptake of Ca^{2+} and the effectiveness with which ruthenium red affects the initial rate of Ca^{2+} uptake. That Borbolla and Peña [1] did not find an appreciable inhibition of Ca^{2+} uptake by ruthenium red is apparently due to the fact that they took their yeast samples 3 min after the addition of the Ca^{2+} at which time the inhibition of Ca^{2+} uptake is already almost completely relieved. Similar reasons apply to the reported lack of an appreciable inhibition of ^{45}Ca uptake by ruthenium red in the yeast *S. pombe* [2]. In that case the cells have been preincubated for 15 min with ruthenium red before the addition of ^{45}Ca and furthermore the yeast samples have been taken 15 min after the addition of ^{45}Ca. As shown by us, on preincubating the cells with ruthenium red prior to the addition of Ca^{2+} no initial inhibition of the uptake of ^{45}Ca is found anymore.

Apparently ruthenium red has a dual effect upon the yeast cells: an initial inhibition of Ca^{2+} uptake which it shares with La^{3+} and a subsequent stimulatory effect upon Ca^{2+} uptake which is specific to ruthenium red. This secondary effect proceeds with a small lag time and is accompanied by a concomitant release of K^+ from the cells. In that respect the effect of ruthenium red resembles that of Cd^{2+} upon Ca^{2+} uptake. Cd^{2+} also enhances Ca^{2+} uptake and gives rise to concomitant efflux of K^+ from the cells [4,5]. Moreover both the enhancement of Ca^{2+} uptake and the release of K^+ is not immediately maximal but increase initially during incubation of the cells with Cd^{2+}. A large number of organic poisons which are all inhibitors of the plasma membrane ATPase of yeast also enhance both divalent cation uptake

and K^+ efflux in yeast [2,5–8]. In almost all the cases examined the efflux of K^+ shows a small lag period. Furthermore there are indications that this is also true for the enhancement of divalent cation uptake by these compounds (Borst-Pauwels, unpublished experiments). Recently we have argued that the effects of the plasma membrane ATPase inhibitors including Cd^{2+} are probably due to an increase in cation permeability of the yeast plasma membrane [5,9]. This may also apply to the effect of ruthenium red upon the yeast cells.

The reduction in the binding of ^{45}Ca to non-metabolizing yeast cells and the inhibition of the initial ^{45}Ca uptake into metabolizing cells by ruthenium red or La^{3+} show approximately the same concentration dependence. Apparently the rate of ^{45}Ca influx is closely related to the extent of binding of this cation to the yeast exterior. As shown previously by us the rate of Ca^{2+} uptake depends upon the surface potential [10]. It is to be expected that binding of ruthenium red or La^{3+} to the yeast cell exterior will reduce this surface potential, which offers a reasonable explanation for the correlation between binding and the influx rate of ^{45}Ca.

The lack of any specificity between the effect of ruthenium red upon the initial rate of Mn^{2+} uptake and upon the initial rate of Ca^{2+} uptake is in accordance with the view that the decrease in the initial rate of uptake is due to a decrease in the surface potential. Apparently ruthenium red also leads to an increase in Mn^{2+} uptake after the initial reduction in the rate of uptake. Also in this respect there is no difference between Mn^{2+} and Ca^{2+} at all.

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