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9-AMINOACRIDINE, A FLUORESCENT PROBE OF THE THIAMINE CARRIER IN YEAST CELLS

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The uptake of 9-aminoacridine is studied in the yeast *Saccharomyces cerevisiae* by fluorescence and absorbance measurements of the dye. Uptake of the dye proceeds via two pathways. One pathway consists of a diffusion of the non-protonated form. At high pH (7.5) this pathway is the predominant one, and the dye distributes between the cell inner and the medium according to the ratio of the proton concentrations in the two compartments. In other words, at high pH 9-aminoacridine behaves as a probe of the H^+ gradient across the yeast cell membrane. At low external pH (4.5) a second pathway is involved. Much greater accumulation ratios for the dye are observed than can be accounted for by the H^+ gradient across the membrane. The transport system predominantly responsible for the great accumulation of the dye appears to be inducible, to require metabolic energy and to be saturable. This transport system is competitively inhibited by thiamine, and also by dibenzyldimethylammonium and thiaminedisulfide, two specific inhibitors of the thiamine carrier in the yeast. On the other hand, the thiamine uptake by the yeast cells is competitively inhibited by 9-aminoacridine. In addition, uptake of 9-aminoacridine is greatly reduced in the thiamine transport-negative mutant of *S. cerevisiae*, PT-R2. It is concluded that at low pH 9-aminoacridine is taken up by yeast via the thiamine carrier of the cell and that, consequently, the dye may be applied as a probe of this transport system.

Introduction

Since its first use by Schuldiner et al. [1] 9-aminoacridine has been widely used to probe the ΔpH of living cells in suspension. The distribution ratio of the dye is simply related to the ΔpH, provided intracellular adsorption of the dye is neglectable. A prerequisite for the applicability of the 9-aminoacridine method is that the dye is able to pass the cellular membrane exclusively in its unprotonated form, and is trapped inside the cells by protonation. In view of this notion, no appreciable trapping of the dye inside the cells is expected, if the intracellular pH (pH_i) is more alkaline than the pH of the medium (pH_e). Because of the high pK_a of the dye (9.99) [1], an additional unfavourable condition for dye uptake is a low pH_e.

In accordance with this notion, at pH_e 4.5 only a slow and small uptake of 9-aminoacridine by starved yeast cells (pH_i 6.2 [2]) could be detected [3]. On adding the cells to the dye solution only binding of the dye to the outerside of the cells occurred. This binding depended on the yeast surface potential [3]. However, in preliminary experiments we found a great accumulation of the dye in cells that were preincubated anaerobically for 1 h in the presence of glucose. An accumulation due to an acidification of the cell interior should be considered unlikely as during the 1 h fermentation period pH_i even was raised to 6.8 [2]. Thus, under conditions unfavourable for uptake of 9-aminoacridine driven by the difference between pH_e and pH_i (ΔpH), still an accumulation of the

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dye inside the cells could be observed.

The present investigation is aimed to characterize this transport of 9-aminoacridine into yeast cells. In this paper, we described evidence that the uptake of the dye at pH_e 4.5 is mediated by an inducible transport system of physiological significance, notably the thiamine carrier of the yeast cell. The usefulness of our finding for the study of the kinetic features of the thiamine carrier is discussed.

Material and Methods

Pretreatment of cells. Phosphate-starved pressed yeast cells, *Saccharomyces cerevisiae* Delft II, were aerated for 20 h at room temperature in distilled water in order to exhaust endogeneous substrates. Subsequently, the yeast was three times washed with distilled water by centrifugation. The starved cells (10%, w/v) were resuspended in 45 mM Tris/succinate of the desired pH, provided with or without 5% (w/v) glucose. The cells were incubated for varying periods at 25°C. Nitrogen was bubbled through the suspension. Binding and uptake of 9-aminoacridine were measured either by absorbance or by fluorescence assays.

Absorbance assays. After the various preincubation periods the suspension was diluted with an equal volume buffer, also containing 9-aminoacridine (110–120 μM) and glucose (10%, w/v). At appropriate times, the dye concentration in the supernatant was measured spectrophotometrically at 298 nm, after spinning down the cells. A correction was made for the presence of 4% inaccessible volume in a 5% (w/v) cell suspension [8], in the calculations of the initial concentration of the dye.

Fluorescence assays. The phenomenon of fluorescence quenching observed when 9-aminoacridine is bound or taken up by living cells (see, for example, Refs. 1,4–7) was explored for the study of the concentration dependence of the acridine uptake in yeast. Assays were performed at room temperature. Portions of the preincubated cells were diluted with an equal volume of buffer of the same pH, also containing glucose (10%, w/v) to a final volume of 2 ml. At zero time 10 μl 9-aminoacridine of appropriate concentration (where indicated, provided with an extra addition) was quickly mixed with the cell suspension in the cuvet. Relative fluorescence was measured with an Aminco SPF 500 spectrofluorimeter, using the wavelength pairs for excitation and emission, 400 nm and 454 nm, respectively. Changes in the fluorescence were recorded with an Aminco SPF 500 *XY*-recorder.

Initial rates of the acridine uptake were determined from the slopes of the tangents to the fluorescence quenching curves at zero time. The relative fluorescence of 100% was determined by adding 10 μl acridine solution to 1.92 ml buffer.

Preparation and characterization of permeabilized cells. After a fermentation period of 1 h at pH 4.5 (see above) the cells were collected by centrifugation and resuspended in ice-cold acetone. This acetone treatment was repeated for a second time and the permeabilized cells were collected by centrifugation and air dried. Per gram of pressed intact cells 0.26 g dry weight was obtained in this way. The same value for the dry weight was obtained if one gram of intact pressed yeast cells was dried.

In accordance with earlier findings [8] the acetone treatment made the cells stainable by the dye Bromophenol blue. The permeabilized cells also had a 42% smaller cellular volume than the intact and starved cells. This was measured with a Coulter Counter, Model ZF (Coulter Electronics Ltd., Harpender, Herts, U.K.).

The permeabilized cells were resuspended to a density of 66 g dry wt./l suspension in 45 mM Tris/succinate pH 8, provided with 250 mM KCl and stored at 4°C until further use.

Intracellular binding of 9-aminoacridine. In order to mimic intracellular conditions of intact cells (with respect to pH and ionic strength), the concentration dependence of the dye binding in permeabilized cells was performed in the pH range 6.3–7.1 and in the presence of 250 mM KCl (the intracellular K^+ concentration of intact cells [9]).

One volume of permeabilized cells was added to nine volumes of ice-cold 45 mM Tris/succinate of pH 6.3, 6.65 and 7.15, respectively, also containing 9-aminoacridine at the desired concentration (20–250 μM). Immediately after mixing, the cells were separated from the medium by centrifugation and the concentration of 9-aminoacridine in the supernatants was determined by the absorbance

assay. The initial dye concentration was determined after dilution of 9 ml of the dye-containing buffers with 0.95 ml of buffer. In this way a correction was made for the volume occupied by the dry mass of the cells, assuming a specific density of 1.3 $g \cdot l^{-1}$.

For the determination of the binding of the dye to the outer surface of the cells, the same procedure was followed. However, instead of permeabilized cells a 25% (w/v) suspension of intact starved yeast cells was used. The initial dye concentration was determined after dilution of 9 ml of the dye-containing buffer with 0.80 ml of buffer.

Calculations. The amount of acridine bound to intracellular constituents was calculated according to Eqn. 1,

$$B_i = \frac{F_{o,0} - F_o}{\delta} - \frac{\alpha F_o}{\delta} - (\beta - 1)\,\gamma F_o \qquad (1)$$

where B_i is the amount of dye absorbed in the cell (μmol/g dry weight of yeast). The dye concentration is denoted by the symbol F and is expressed in μM. $F_{o,0}$ is the initial dye concentration before the addition of yeast and F_o its concentration in the supernatant of the yeast suspension. The difference $F_{o,0} - F_o$ represents the amount of dye both trapped inside the cells and adsorbed at the outerside of the cells. δ is the relative amount of dry weight of cells, expressed in $g \cdot l^{-1}$ suspension. α is a factor representing the ratio $(F_{o,0} - F_o)/F_o$, as measured after binding of the dye to starved intact yeast cells. The term $\alpha F_o/\delta$ represents a correction for binding of the dye to the outer membrane surface and the cell wall [3]. β is the Donnan ratio for a monovalent cation amounting to 1.06 (Boxman, personal communication) and γ is the amount of cell water per unit dry weight. γ was $10^{-3}\ l \cdot g^{-1}$ and $1.6 \cdot 10^{-3}\ l \cdot g^{-1}$ for acetone-treated and intact starved yeast cells, respectively (as measured according to Ref. 8).

Uptake of [^{14}C]thiamine, ^{86}Rb and 9-aminoacridine in cultivated yeast cells. Part of the experiments was performed with cultivated yeast cells. The thiamine-transport-lacking mutant of *S. cerevisiae*, PT-R_2 [10] and the Delft II yeast were grown on Wickerham's medium without thiamine on a reciprocal shaker during 20 h at 30°C. The cells were collected by centrifugation, and three times washed with distilled water. Subsequently, the cells (10%, w/v) were resuspended in 45 mM Tris/succinate (pH 4.5) provided with 5% (w/v) glucose. The cells were incubated for 10 min at 25°C anaerobically. After this preincubation period uptake of [^{14}C]thiamine and $^{86}Rb^+$ was determined according to Barts et al. [11]. In addition, the uptake of 9-aminoacridine was determined. The fluorescence of the dye was measured immediately after the addition of 2.6 μM 9-aminoacridine and 5% glucose (w/v) to the yeast suspension (5%, w/v) at zero time.

Chemicals. All chemicals were reagent or analytical grade and obtained from commercial sources. [^{14}C]thiamine and ^{86}Rb were purchased from the Radiochemical Centre, U.K. The mutant PT-R_2 was kindly provided by Dr. Iwashima from the Prefactural University of Medicine at Kyoto, Japan.

Results

Induction of 9-aminoacridine uptake in yeast during fermentation of the cells

The ability of yeast cells to accumulate the cationic dye 9-aminoacridine at pH 4.5, markedly depended on the length of anaerobic preincubation of the cells in the presence of glucose, as is shown in Fig. 1. Addition of cells to the dye solution resulted in an instantaneous decrease in the free dye concentration in the medium, reflecting an electrostatic binding of the acridine to the outer surface of the cells [3]. This binding was independent of the anaerobic pretreatment period with glucose. In the absence of glucose a slight further decrease in the free acridine concentration occurred during a 2 h incubation. However, if the starved cells were suspended in the acridine solution, also containing glucose, after about 40 min a great accumulation of the dye by the cells was observed. The lag-phase in the time course of this uptake process could be shortened by preincubating the cells with glucose prior to adding the cells to the dye solution. If the cells were preincubated longer than 40 min, no lag-phase in the dye uptake was observed anymore.

Saturability of the transport system. The involvement of the thiamine carrier

The uptake of 9-aminoacridine showed satura-

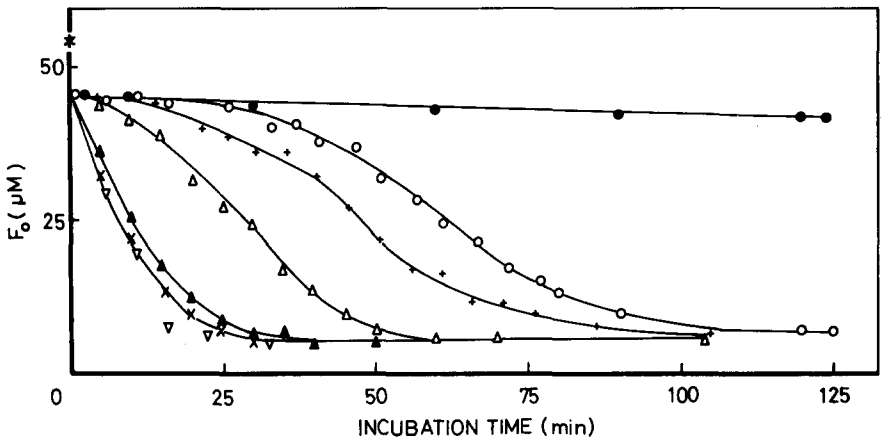

Fig. 1. The time course of the uptake of 9-aminoacridine after varying periods of anaerobic preincubation of yeast cells in the presence of glucose. Preincubation period in the presence of 5% glucose at pH 4.5 and 25°C: ●, no preincubation, no glucose added; ○, no preincubation, glucose added at zero time; +, 10 min; △, 30 min; ▲, 1 h, ×, 2 h; ▽, 3 h. (*) Initial dye concentration in the medium 52.6 μM. Data were obtained by absorbance assays of the supernatants of the yeast suspension at appropriate times. F_o, dye concentration in the supernatant.

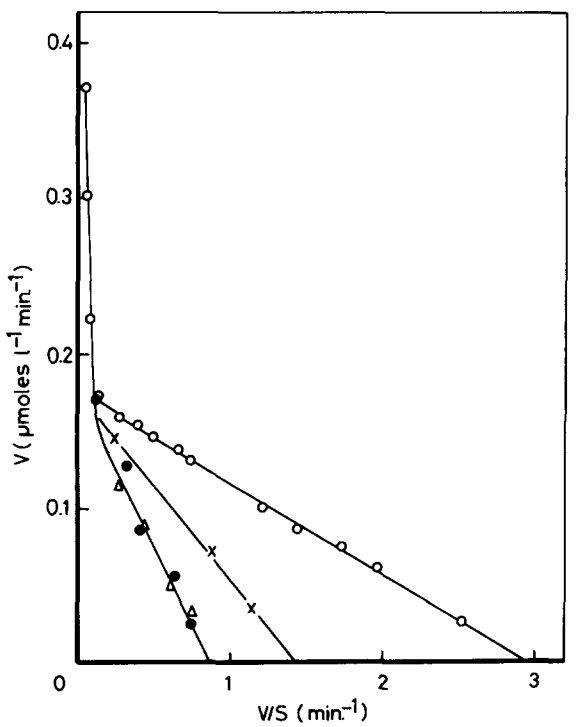

Fig. 2. Hofstee plots of the 9-aminoacridine uptake at pH 4.5. ○, control; ●, in the presence of 0.55 μM thiamine; △, in the presence of 4.3 μM dibenzyldimethylammonium; ×, in the presence of 0.08 μM thiaminedisulfide. The cells were preincubated for 1 h in the presence of 5% glucose. v, initial uptake rate; s, 9-aminoacridine concentration in the medium measured by the fluorescence assays. Each point represents the mean of triplicates.

tion characteristics. A Hofstee plot [12] revealed a biphasic curve, suggesting a low and a high affinity uptake system, working simultaneously (Fig. 2). Similar kinetic features were also found for the uptake of dibenzyldimethylammonium in yeast by Barts et al. [11]. This unnatural compound appeared to be taken up by yeast cells via the thiamine carrier. We have now examined the possible involvement of this physiological transport system also in the uptake of the acridine. Indeed, uptake of the acridine via the high affinity system was competitively inhibited by thiamine, dibenzyldimethylammonium and also by thiaminedisulfide, a specific inhibitor of thiamine transport in yeast [13]. Conversely, uptake of thiamine was inhibited competitively by 9-aminoacridine (data not shown).

In Table I, a comparison is made of dissociation and inhibition constants, referring to the high affinity uptake system of the acridine and the thiamine uptake at pH 4.5. In addition, in the literature reported K_i values of thiaminedisulfide and dibenzyldimethylammonium in thiamine transport are included. There is a good correspondence between the K_i values of inhibitors of the acridine uptake on the one hand, and the thiamine uptake on the other hand. In addition, the K_i value of the dye in thiamine uptake was the same as its K_m value. After correction for extracellular

TABLE I

COMPARISON OF K_i VALUES OF COMPETITIVE INHIBITORS OF THE 9-AMINOACRIDINE UPTAKE AND THIAMINE UPTAKE AT pH 4.5

Numbers between square brackets denote references to reported K_i values.

Inhibitor	9-Aminoacridine uptake $K_m = 0.06$ μM K_i (μM)	Thiamine uptake $K_m = 0.16$ μM K_i (μM)	Ref.
Thiamine	0.18 [a]	–	
9-Aminoacridine	–	0.06	
Thiamine-disulfide	0.08	0.06	[13]
Dibenzyl-dimethyl-ammonium	1.8	3.5	[11]

[a] From the data in Fig. 2 a K_i value of thiamine in 9-aminoacridine uptake of 0.23 μM was calculated. Correcting for binding of thiamine to the cells resulted in the tabulated value of 0.18 μM. Binding was calculated from the instantaneous decrease in the thiamine concentration in the medium after the addition of cells. The absorbance of thiamine was measured in supernatants of the yeast suspension at 240 nm.

binding, also the K_i value of thiamine in acridine uptake was the same as its K_m value.

Dependence of 9-aminoacridine uptake on metabolic energy and the cell pH

In Fig. 3 the effect of iodoacetic acid, an inhibitor of glycolysis [14] is shown. 3 mM iodoacetic acid, sufficient to block glycolysis completely, added 4 min prior to the initiation of the dye uptake, almost completely prevented the accumulation of the dye into the cells. Addition of iodoacetic acid during the course of the dye uptake gave rise to a slow efflux of the accumulated dye. The effect of butyric acid is also shown in Fig. 3. In metabolizing cells an acidification of the cell inner is achieved by the addition of this acid to the suspension [2]. If added 4 min prior to the initiation of the dye uptake, a time period sufficiently large for accumulation of butyric acid into the cells to an appreciable extent, it did not affect the initial rate of the dye uptake. The steady-state accumulation, however, was greatly reduced. The addition of butyric acid during the course of the

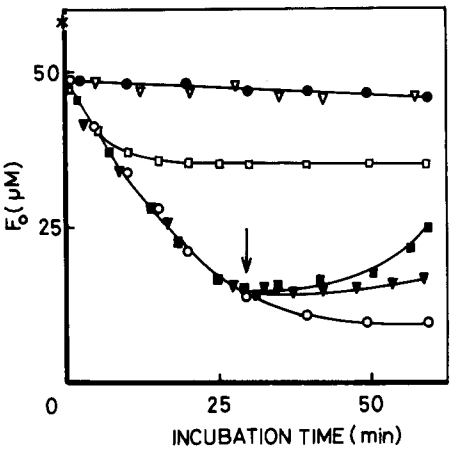

Fig. 3. Effect of iodoacetic acid and butyric acid on 9-aminoacridine uptake at pH 4.5. The cells were preincubated for 1 h in the presence of 5% glucose, except (●), representing the uptake of the dye in starved cells in the absence of added glucose. ○, control; ▽, 3 mM iodoacetic acid added after 56 min preincubation; ▼, the same as the control but 3 mM iodoacetic acid added at 30 min (indicated by the arrow); □, 3.4 mM butyric acid added after 56 min preincubation; ■, the same as the control but 3.4 mM butyric acid added at 30 min (indicated by the arrow). See also legend to Fig. 1.

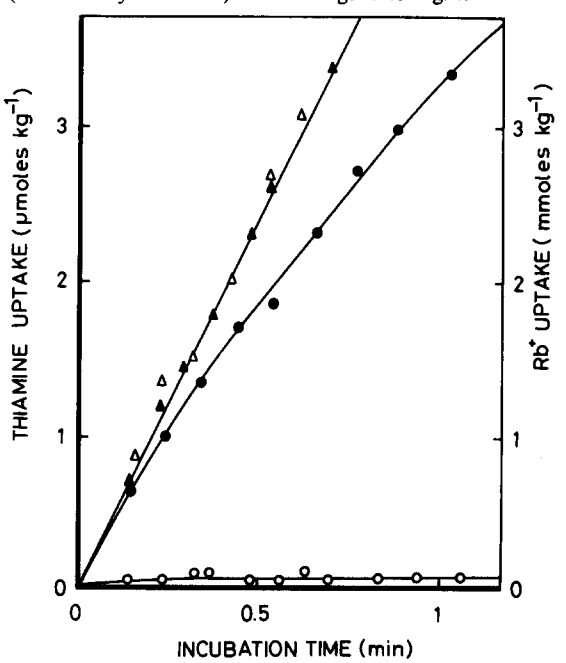

Fig. 4. The time course of the Rb^+ and thiamine uptake by cultivated PT-R_2 cells and Delft II cells at pH 4.5. △, Rb^+ uptake at 1 mM unlabelled RbCl in the medium; ○, thiamine uptake at 0.33 nM thiamine in the medium. The cells were preincubated for 10 min in the presence of 5% (w/v) glucose. Each point represents the mean of triplicates. Open symbols refer to uptake in the mutant PT-R_2, closed symbols to the Delft II yeast.

dye uptake resulted in an efflux of dye already being accumulated.

If the acridine and thiamine share the same transport system, it is expected that in the mutant PT-R_2, in which the thiamine transport capacity is almost completely lost (Fig. 4), also the dye uptake will be greatly reduced. In agreement with this notion the large fluorescence quenching of 9-aminoacridine which developed in time with the Delft yeast was not observed in the mutant (data not shown). Uptake of Rb^+, however, was not different in the two strains studied, as is shown in Fig. 4.

Absence of an energy dependent and inducible uptake of 9-aminoacridine at pH 7.5

According to Iwashima et al. [15] thiamine uptake is maximal around pH 4.5 and almost completely absent at pH 7.5. It was therefore expected that at the high pH also no uptake of the acridine via the thiamine carrier would occur. At this high pH, however, uptake of the dye via a diffusion of the unprotonated acridine will be greatly favoured. Because of its high pK_a (9.99) the concentration of the unprotonated form will be 1000-times higher than at pH 4.5. In addition, at pH 7.5 the intracellular pH_i is lower than the external one [2], which also favours an accumulation driven by the difference in cell pH and medium pH [1]. Confirming our expectations, the uptake of the dye at pH 7.5 did not depend on the presence of a metabolic energy source, or upon the period of anaerobic preincubation and was insensitive for thiaminedisulfide, the selective inhibitor of the thiamine carrier (data not shown). Typically, the immediate decrease in the concentration of the dye observed on adding the cells, was appreciably greater than at pH 4.5, in agreement with the notion that this binding depends on the yeast surface potential, which at pH 7.5 is significantly more negative than at pH 4.5 [16].

Intracellular binding of 9-aminoacridine

The possibility that the greater part of the dye trapped inside the cells was due to binding to intracellular constituents was examined in this experiment. Binding data obtained with acetone-permeabilized cells were corrected for binding of the dye to the outer surface of the cells and also for

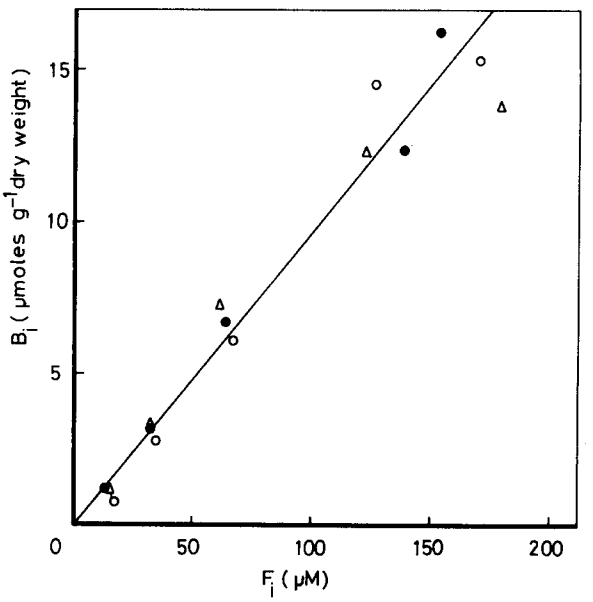

Fig. 5. Intracellular adsorption of 9-aminoacridine (B_i) as a function of the free intracellular concentration of the dye (F_i) and of the pH. ●, pH 6.3; ○, pH 6.65; △, pH 7.15. The values of B_i and F_i were calculated according to Eqn. 1 from data obtained by absorbance assays. See also text.

accumulation of the dye inside the permeabilized cells due to the Donnan potential of the cell interior. The intracellular binding of the dye was calculated by making use of Eqn. 1. It appeared that in the pH range applied and probably because of the high ionic strength, both the electrostatic binding of the dye to the outerside of the cells and the Donnan ratio were rather low ($\alpha = 0.01$ and $\beta = 1.06$, corresponding to a Donnan potential of -1.5 mV). The decrease in the dye concentration in the medium after the addition of the permeabilized cells, was therefore mainly due to intracellular binding of the acridine.

Fig. 5 shows that, by approximation, the intracellular binding of the dye B_i was linearly related to F_i ($= \beta F_o$), according to Eqn. 2, and was independent of the pH.

$$B_i = 0.096 F_i = \gamma \cdot f_{ads} \cdot F_i \qquad (2)$$

where f_{ads} is the intracellular adsorption coefficient of the dye. As for the acetone-treated yeast $\gamma = 10^{-3}\ l \cdot g^{-1}$, f_{ads} amounted to 96. The free

intracellular dye concentrations under steady-state conditions of the dye uptake by intact yeast cell could be calculated according to Eqn. 3.

$$F_i = \left(\frac{F_{o,0} - F_o(1+\alpha)}{\delta} \right) / (\gamma(1+f_{ads})) \quad (3)$$

For these calculations the data of the accumulation of the dye at pH_e 4.5 (Fig. 1) and pH_e 7.5 (data not shown) were taken and we took into account that for intact yeast cells $\gamma = 1.6 \cdot 10^{-3}$ $l \cdot g^{-1}$ and that α was 0.20 and 0.64 at pH_e 4.5 and pH_e 7.5, respectively. The values of α were now much higher because of the low ionic strength of the media. In addition α raised with pH, because of the increase in the yeast surface potential [3].

From the corresponding distribution ratio (F_i/F_o) a ΔpH of 0.54 ($pH_i = 3.96$) and of 0.32 ($pH_i = 7.18$) at pH_e 4.5 and 7.5, respectively, could be calculated, according to the method described by Schuldiner et al. [1].

Discussion

Just as in many other cell types [1,4,17,18], the unprotonated acridine probably is able to pass the plasma membrane of yeast cells freely, whereas this membrane represents an effective barrier for the protonated, positively charged form of the dye. If this is the only way by which the acridine can pass the cell membrane, the accumulation of the dye inside the cells would depend on the ΔpH, as has been pointed out by Schuldiner et al. [1]. At pH_e 4.5, however, yeast cells have a relatively high cellular pH_i (6.2–6.8) depending on the pretreatment of the cells [2]. An accumulation of the acridine according to the ΔpH ($pH_i > pH_e$!) is therefore not favoured under these conditions. Still, a great uptake of the dye at pH_e 4.5 occurs (Fig. 1). However, a minimal fermentation period of approx. 40 min is required to enable the cells to accumulate the dye. Once the cells are able to take up the dye, the dye is accumulated by the cells to an extent that is independent of the anaerobic preincubation period applied. The rate of uptake, however, increases with the length of this preincubation period and reaches a maximal value with cells that are preincubated 2 h.

The concentration dependence of the dye uptake at pH_e 4.5 (Fig. 2) shows an involvement of a saturable transport system with a high affinity ($K_m = 0.06$ μM) for 9-aminoacridine. This transport system is competitively inhibited by thiaminedisulfide and dibenzyldimethylammonium (Fig. 2). According to Iwashima et al. [13] thiaminedisulfide, and according to Barts et al. [11] dibenzyldimethylammonium are competitive inhibitors of the thiamine carrier of the yeast cell. As a matter of fact thiamine and acridine also inhibit each others uptake at pH_e 4.5 competitively. The K_i values correspond to their K_m values. Also the K_i values of the other inhibitors applied are almost the same for the two uptake processes examined (Table I). Because of these kinetic similarities, and the fact that thiamine, and dibenzyldimethylammonium and thiaminedisulfide, as well, are positively charged species, it is considered that the protonated form of the acridine is accumulated by the yeast cells via the physiological thiamine carrier. Apart from kinetic properties, the dye uptake and the thiamine uptake appear to share also other properties. A characteristic feature of this transport system, namely its inducibility [19], is also observed in the uptake of 9-aminoacridine (Fig. 1). In addition, just as the thiamine uptake [15], the accumulation of the dye at pH_e 4.5 depends on metabolic energy. The uptake of the dye is namely completely blocked by iodoacetic acid (Fig. 3), an effective inhibitor of glycolysis.

Iwashima et al. [15] showed that short-chain fatty acids, butyrate included, strongly inhibited the thiamine accumulation in yeast. With caproate, for instance, an efflux of thiamine from preloaded cells was observed. The reduction of the dye accumulation, and its efflux from preloaded cells in the presence of butyrate (Fig. 3) is therefore also compatible with an involvement of the thiamine carrier in the acridine uptake at pH 4.5. The data in Fig. 3 also show that 9-aminoacridine cannot be applied to probe the ΔpH of the yeast cell at pH_e 4.5. This conclusion is based on the finding that the acidification of the cell inner leads to a decreased accumulation of the dye, instead of to an increased one. The latter is namely expected if the dye accumulation is driven by the ΔpH. As a matter of fact, a pH_i of 3.96, calculated from the dye distribution greatly differs from the pH_i de-

termined according to Ref. 8, being 6.8 [2].

The inducibility of the dye uptake, its dependence on metabolic energy and the effects of competitive inhibitors, support the notion that the uptake of 9-aminoacridine at pH_e 4.5 proceeds via the thiamine carrier. Accordingly, the uptake of the dye in the thiamine transport mutant PT-R_2 is reduced considerably. This reduction in the dye uptake is not due to an affected metabolic energy supply in the PT-R_2 strain. The Rb^+ uptake, also strongly depending on metabolic energy (see for example Ref. 2), is namely not different from that in the Delft yeast (Fig. 4).

Besides uptake of the protonated form of 9-aminoacridine via the thiamine carrier also uptake of the dye occurs under conditions that the thiamine carrier is not induced, namely at high pH without preincubating the cells. In agreement with this notion, dye uptake at pH 7.5 is not inhibited by thiaminedisulfide. The uptake at pH 7.5 may be ascribed to diffusion of the uncharged acridine, into the cells. Namely, at this pH a pH_i of 7.18 is calculated from the distribution ratio, a value that corresponds well with the pH_i of 7.05 [2] determined according to Ref. 8.

We presented evidence that 9-aminoacridine may be taken up by yeast cells via the thiamine carirer and also via a diffusion of its uncharged form. The concave Hofstee plot (Fig. 2) might be the reflection of the simultaneous occurrence of both these transport processes. It should be remarked, however, that such a concave Hofstee plot can also come to the fore in the case of a two-site transport mechanism [20].

Conclusion

The presented results show that 9-aminoacridine cannot be applied as a simple probe of the pH in yeast cells, unless at high medium pH. The dye, however, may find an other useful application. Applied at low pH, it may be used to probe kinetic features of the thiamine carrier of the yeast cell. By measuring its fluorescence changes, the transport of 9-aminoacridine via the thiamine carrier can be monitored directly during transport. This may be of advantage under certain conditions, instead of studying the characteristics of this transport system of physiological significance, by applying radioactively labelled thiamine. In addition, use may be made of the fluorescent properties of the dye during the isolation of proteins involved in thiamine transport.

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