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APPLICATION OF 9-AMINOACRIDINE AS A PROBE OF THE SURFACE POTENTIAL EXPERIENCED BY CATION TRANSPORTERS IN THE PLASMA MEMBRANE OF YEAST CELLS

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The applicability of 9-aminoacridine as a probe of the surface potential of yeast cells is examined. Yeast cells are found to quench the fluorescence of the dye and it is shown that this quenching is caused by a decrease in the dye concentration in the bulk aqueous phase. Consistent with predictions of the Gouy-Chapman theory the dye is displaced from the surface of the yeast cells by addition of salts, the effectiveness of the salts being related to the valency of the cation: $C^{3+} > C^{2+} > C^{1+}$. It is shown that 9-aminoacridine is predominantly bound by the plasma membrane of the cells. Only a minor part of the binding occurs in the cell wall, in line with the finding that enzymic removal does not significantly affect the binding of the dye to the cells. A single relationship for the distribution ratio of the dye between cells and medium with the ζ potential of the cells is found, irrespective of the way the ζ potential is changed, either by varying the pH or the Ca^{2+} concentration. It is argued that the electrostatic potentials probed by the dye are much higher than the corresponding ζ potentials and are of the same order of magnitude of the presumed discrete charge potentials experienced by cation transporters in the plasma membrane. It is concluded that 9-aminoacridine may be applied as a convenient and almost quantitative probe of the surface potential that effects the kinetics of ion uptake by the yeast cells.

Introduction

Previous work in this laboratory has revealed the importance of the surface potential of yeast cells in controlling the transport of ions across their plasma membrane (see Ref. 1 and references therein and Refs. 2 and 3)). We have shown that due to the influence of the surface potential on ion uptake the interpretation of transport kinetic data may be completely different from that applicable to enzyme kinetics. For example, polyvalent cations caused an increase in K_m of the Rb^+ uptake. Within the concept of enzyme kinetics this effect would be interpreted as a competitive inhibition. However, the inhibition appeared to be caused by reduction in the surface potential by the polyvalent cations [4,5]. An other important effect of the surface potential on ion uptake was involved in the concentration dependence of the Ca^{2+} uptake. The process showed saturation kinetics, which, however, could be ascribed to the progressive reduction of the surface potential on raising the Ca^{2+} concentration [3]. In other words, due to the reduction in surface potential caused by Ca^{2+} itself, the diffusion of Ca^{2+} into the cells behaved kinetically as being mediated by a two-carrier system.

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The above conclusions were reached by making use of the ζ potential as a measure for the surface potential. The comparison of transport kinetic data and the ζ potential measured by means of cell electrophoresis, however, revealed that the electrostatic potential that would effect the kinetics of ion uptake is greatly underestimated by the ζ potential [2,3]. This is possible if the negative surface charges are not uniformly distributed over the surface of the membrane and the cation transporters are located in close proximity of these negatively charged groups. A relatively high discrete charge potential will then be experienced by the transporters. The ζ potential, which depends on the average charge density, may consequently be appreciably lower (see for example Ref. 6). In this connection it is of great importance to have a technique available that would enable us to probe the negative surface charges intimately. Such a technique has been described by Chow and Barber [7]. They showed that a variety of negatively charged surfaces quench 9-aminoacridine fluorescence and presented evidence for the idea that the quenching of 9-aminoacridine fluorescence is brough about by the attraction of this monovalent cationic dye molecule into the diffuse double layer adjacent to the negatively charged surface. They also presented evidence for the applicability of 9-aminoacridine as a probe of the surface potential in localized regions of the membrane surface.

We have now examined the applicability of the 9-aminoacridine method to probe the surface potential experienced by cation transporters in the yeast cell membrane quantitatively. In addition, we have compared the effect of pH and of calcium ions on the dye/cell interaction with their effect on the ζ potential of the cells.

Materials and Methods

Preparation of cells, protoplasts and cell walls. A 4% (w/v) suspension of yeast cells, *Saccharomyces cerevisiae* strain Delft II in distilled water was starved overnight by aeration. After aeration, the cells were washed three times with distilled water and resuspended in distilled water to a density of 10% (w/v). Cells of the nonflocculent brewing yeast *S. cerevisiae* A294 (obtained from Whitbread and Co., Ltd., Luton, U.K.) were grown as described in Ref. 8. After harvesting in the exponential phase of growth by centrifugation, the cells were washed thrice with distilled water and resuspended in distilled water to a density of 10% (w/v).

Protoplasts of the strain A294 were prepared as described by Theuvenet and Bindels [8]. The protoplasts were suspended in 0.82 M sorbitol provided with 1 mM $CaCl_2$ at a cell density of $0.5 \cdot 10^9$ protoplasts per ml, which was equivalent to 39 mg dry weight of intact cells per ml.

Cell walls were isolated from the Delft II strain according to Ref. 9. In order to remove remnants of membranes the cells were delipidized in an ethanol/diethyl ether (3 : 1, v/v) mixture, according to Ref. 10, Subsequently the cell walls were washed ten times by centrifugation in ice-cold distilled water and resuspended in distilled water at a density of 20 mg dry weight/ml. Per g dry weight of cells 173 mg dry weight of cell walls was obtained.

Fluorescence assays. The suspensions of yeast cells, cell walls and protoplasts were diluted with an equal volume of 90 mM Tris/succinate buffer of the desired pH, also containing 9-aminoacridine. In some cases a 20 mM Tris/succinate buffer was used as indicated in the legend to the figures. The buffer used to dilute the protoplast suspension also contained sorbitol as an osmotic stabilizer. The concentration of sorbitol was properly adjusted to assure a constant osmolarity in the protoplast suspension of 865 mosM. Routinely, the buffers contained 2 μM 9-aminoacridine, unless stated otherwise. Rapidly after dilution, the suspensions were centrifuged and the supernatants collected. The fluorescence of the supernatants, if needed after appropriate dilution, were measured with an Aminco SPF 500 spectrofluorimeter, using the wavelength pairs for excitation and emission, 400 nm and 454 nm, respectively, and the dye concentration, F, was calculated by making use of a calibration curve. In one experiment the fluorescence of the suspension was measured, both before and after the addition of 100 mM $MgCl_2$, in addition to the measurement of the fluorescence of the supernatants. The fluorescence of a 1 : 1 dilution of the 9-aminoacridine-containing buffers with distilled water was also measured. From these data, after applying a correction for the presence

of a volume in the suspension that was occupied by either the yeast cells, the protoplasts or the cell walls, and thus was inaccessible for the dye (see Ref. 11), the initial concentration of the dye, F_i was calculated. The inaccessible volume occupied by cell walls was calculated from their dry weight, assuming a specific density of $1.3 \cdot 10^3\ g \cdot l^{-1}$. The amount of acridine bound to either the cells, the protoplasts or the cell walls, B, was calculated according to Eqn. 1,

$$B = \frac{F_i - F}{\delta} = \frac{\Delta C}{\delta} \quad (1)$$

where δ represents the dry weight of an equivalent amount of intact cells per unit volume of suspension and is expressed in $g \cdot l^{-1}$; F_i, F and ΔC are in μM and B in $\mu mol \cdot g^{-1}$. Unless stated otherwise, the binding of the dye was measured at 25 °C.

Cell electrophoresis. Cell electrophoretic mobilities were measured at 25 °C using a rectangular cuvette and apparatus as described by Fuhrmann et al. [12]. From the electrophoretic mobility, the zeta potential was calculated by using the Helmholtz-Smoluchowski equation [13].

Chemicals. 9-Aminoacridine was purchased from Sigma. All other chemicals were reagent or analytical grade and obtained from commercial sources.

Results

Recently we reported [11] that the addition of yeast cells to a 9-aminoacridine solution causes an instantaneous and significant quenching of the fluorescence of the dye. Depending on the pretreatment of the cells and on the pH of the medium the instantaneous quenching may be followed by a further decrease in dye fluorescence. The primary quenching was interpreted to reflect the interaction of the dye with the extracellular surface of the cells, while the secondary decrease in the fluorescence was attributed to the transport of the dye into the cells. In this article attention has been focussed on the primary quenching phenomenon. It will be shown that this primary quenching is related to a surface potential-dependent binding of the dye by the cells.

It appeared that addition of 100 mM $MgCl_2$ to the yeast suspension, which is expected to reduce the negative surface potential considerably, instantaneously released the quenching of the dye and concomitantly increased the fluorescence of the supernatant of the suspension (data not shown). Fig. 1 shows that the salt provoked release of the quenching of the dye could be accounted for quantitatively by the increase in fluorescence of the supernatant. Apparently, only the species of the dye which remained present in the bulk solution contributed to the fluorescence, whereas the molecules bound by the yeast cells had become nonfluorescent. The different levels of fluorescence quenching release were obtained by variation of the cell density. Although the decrease in dye concentration in the bulk solution (ΔC) increased

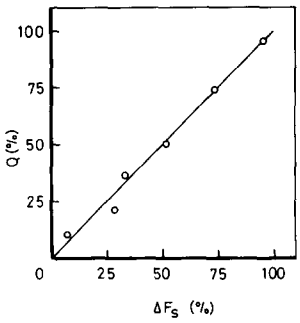

Fig. 1. Relationship between the fluorescence quenching release in the yeast suspension Q and the increase in fluorescence of the supernatant ΔF_s provoked by 100 mM $MgCl_2$ at pH 6.0. The data points were obtained by varying the cell density from 1 to 10 mg dry weight per ml. The 9-aminoacridine concentration was 4 μM. Q and ΔF_s are expressed in the same relative fluorescence units. The 100% value corresponds to the fluorescence of a 1 μM dye solution.

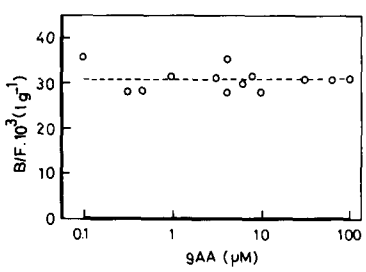

Fig. 2. Dependence of B/F on the dye concentration in the bulk aqueous phase F at pH 6.0.

Fig. 3. Arrhenius plot of B/F. Data were obtained at pH 6.0.

with the density of the cells (δ), the relative amount of dye bound to the cells ($B = \Delta C/\delta$) decreased (data not shown). The ratio of B and the dye concentration in the supernatant, F, however, appeared to be independent of the cell density between 1 and 50 mg dry weight of yeast/ml (data not shown). For convenience all further experiments have been performed at a cell density of 12 mg/ml and B/F values have been estimated from measurements of the acridine concentration in supernatants of the cell suspension.

The value of B/F also appeared to be independent of the initial dye concentration. Fig. 2 shows that this applies to a wide range of acridine concentrations (0.1–100 μM).

The experiments described in this article were all carried out at 25 °C. The necessity to measure the binding of 9-aminoacridine by yeast cells at a properly specified and constant temperature is illustrated in Fig. 3. The binding of the dye showed a peculiar temperature dependence. The binding was increased approximately 2-fold on lowering the temperature from 40 °C to 5 °C.

Not only $MgCl_2$ diminished the binding of 9-aminoacridine by the yeast cells. Also other salts caused a decrease in the B/F value. Fig. 4 shows the dependence of B/F on the concentration of a salt added. All the salts applied gave similar effects though over different concentration ranges

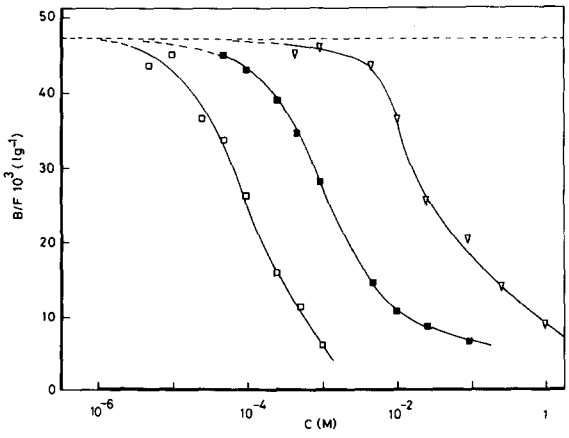

Fig. 4. Dependence of B/F on the presence of mono-, di- and trivalent cations in the yeast suspension at pH 6.0. The concentrations, denoted C, refer to the nominal concentrations of salt added. The final buffer concentration was 10 mM Tris/succinate. (□) $LaCl_3$, (■) $MgCl_2$ and (▽) LiCl.

TABLE I

RELATIVE EFFECTIVENESS OF SALTS IN REDUCING THE BINDING OF 9-AMINOACRIDINE BY YEAST CELLS

The salt additions were made to a yeast suspension in 10 mM Tris/succinate buffer pH 6.0 containing 1 μM 9-aminoacridine. The $C_{1/2}$ values given are the concentration of salts producing a 50% reduction in B/F.

Addition	$C_{1/2}$ (mM)
KCl	21
CsCl	21
NaCl	32
LiCl	40
$MnCl_2$	0.80
$BaCl_2$	0.80
$CaCl_2$	1.05
$SrCl_2$	1.20
$MgCl_2$	1.57
$MgSO_4$	1.55
$LaCl_3$	0.13
$TEC \cdot Cl_3$ [a]	0.18

[a] TEC^{3+}, tris(ethylenediamine)-cobaltic cation.

depending mainly on the valency of the cation. In comparison to salts of monovalent cations those of trivalent cations were on the average 200-times more effective, whereas salts of divalent cations were approx. 25-times more effective. The nature of the anion appeared to be unimportant. Salts containing the same cation but different anions, like $MgCl_2$ and $MgSO_4$, were equally efficient (see Table I). Table I shows the concentration of salt needed to reduce the B/F value to half that in the absence of the salt ($C_{1/2}$).

Fig. 5 shows the pH dependence of the 9-aminoacridine binding by both intact yeast cells and isolated walls of the cells. For comparison the pH dependence of the ζ potential of the cells, determined by means of cell electrophoresis, is also given in this figure. Both the binding of the dye to the intact cells and their ζ potential were appreciably reduced on lowering the pH. The data show that only a minor part of the dye bound by intact cells (approx 6%) can be attributed to 'binding' of the dye to the cell wall. Apparently, the dye was predominantly bound to the outer surface of the plasmamembrane. Indeed, enzymic removal of the wall did not much affect the binding of the dye

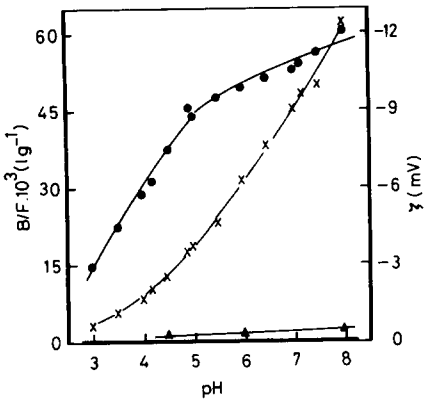

Fig. 5. pH dependence of 9-aminoacridine binding to intact cells and isolated cell walls and the ζ potential of the cells. The binding of the dye to intact cells (●) and to cell walls (▲) is expressed in values of B/F. ×, the ζ potential of the cells.

by the cells, neither did it affect the effectivity of Ca^{2+} in reducing this binding (see Fig. 6). Fig. 6 also shows that the ζ potential of the cells is appreciably reduced by Ca^{2+}.

It should be remarked that the results in Fig. 6 were obtained with the *S. cerevisiae* strain A294 and not with the Delft II strain used throughout this article. The A294 strain was chosen because from this strain, in contrast to the Delft strain, easily viable protoplasts could be prepared. Compared to the Delft strain, cells of S. cerevisiae A294 appeared to have a somewhat higher B/F value for the dye and the cells also exhibited a slightly more negative ζ potential. (Compare the data with those in Fig. 5 at pH 4.5).

By making use of the data from Figs. 5 and 6 we have constructed Fig. 7. In this figure also other data obtained with the Delft strain have been included. Data on the pH dependence of B/F and the ζ potential in 10 mM instead of in 45 mM Tris/succinate buffer and on the effect of Ca^{2+} in 45 mM Tris/succinate (pH 6.0) have been used, as well. In addition data are included obtained with cells which were washed with 300 mM KCl adjusted to pH 3.0 with HCl. Both the binding of the dye to the cells and their ζ potential were increased by this washing. To a good ap-

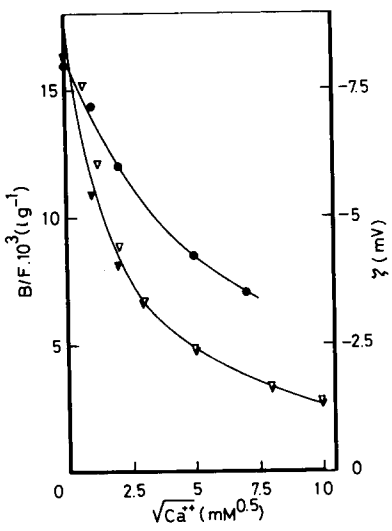

Fig. 6. Dependence of 9-aminoacridine binding to intact cells and protoplasts of the strain A294 and the ζ potential of the cells upon the Ca^{2+} concentration in the suspension at pH 4.5. The binding of the dye to intact cells (▼) and to protoplasts (▽) is expressed in values of B/F. (●) the ζ potential of the cells. Aimed at obtaining a better distribution of data points in the figure B/F and ζ are plotted against $\sqrt{Ca^{2+}}$ instead of against Ca^{2+} directly.

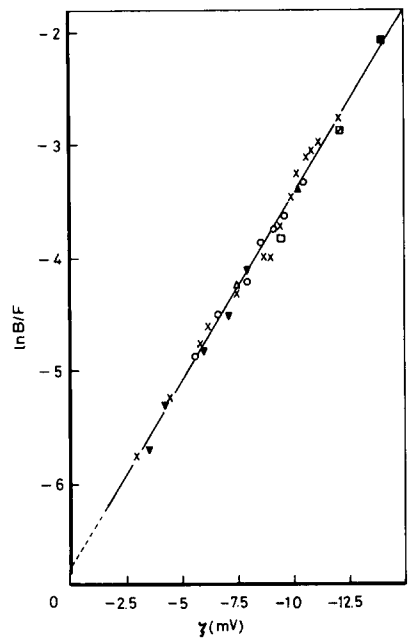

Fig. 7. Relationship between B/F and the ζ potential of the cells. Data on the effect of pH (×) and Ca^{2+} at pH 4.5 (▼) given in Figs. 5 and 6, respectively, are used. Also not shown data are included: (○) effect of Ca^{2+} at pH 6.0; effect of washing the cells with an acid KCl solution. B/F and the ζ potential of the cells were determined at pH 4.5. (△) before and (▲) after the washing; effect of pH in 10 mM Tris/succinate buffers (□) pH 4.0, (▨) pH 6.0 and (■) pH 8.0.

proximation, a single relationship between B/F and the ζ potential was found, irrespective of the way the changes in these parameters were brought about. The relationship between B/F and the ζ potential is described by Eqn. 2:

$$\ln B/F = I + \gamma\zeta \quad (2)$$

where I is the intercept of the extrapolated line with the ordinate and γ is the slope of the line. It appeared that $I = -6.74$ and $\gamma = 0.329\ \mathrm{mV}^{-1}$.

Discussion

The results presented in this article are compatible with the idea that the quenching of 9-aminoacridine fluorescence is brought about by the attraction of this positively charged dye molecule ($pK_a = 9.99$ [14]) into the diffuse double layer adjacent to the negatively charged surface. This implicates that 9-aminoacridine may be used as a probe of the surface potential. Fig. 1 makes clear that the dye molecules associated with the cells become nonfluorescent, in line with observations made by other investigators (see for example Refs. 15–17).

The acridine dye fulfils the prerequisites needed for a suitable probe of the surface potential. The distribution ratio of the dye between cells and medium, expressed by its B/F value is independent of the dye concentration and of the density of the cells. This means that the dye itself does not affect the potential to be probed. Consistent with predictions of the Gouy-Chapman theory [18,19], displacement of the dye from the surface of the yeast cells can be achieved by the addition of salts (Fig. 4). The addition of salts increases the dye concentration in the supernatants. The effectiveness depends on the valency of the cation used: $C^{3+} > C^{2+} > C^{1+}$, in line with the expected decrease in the surface potential of the cells.

Within one class of cations of the same valency small differences in their potency in reducing the binding of the dye by the cells are observed (Table I). This probably reflects the ability of the cations to reduce the surface potential not only by means of a charge screening effect, but in addition, by binding to the fixed charges. Then namely, the surface potential will be reduced more effectively by the cation with a higher affinity for the fixed charges. As a matter of fact, similar differential effects of the divalent cations have been observed on the ζ potential of the cells [20]. Also effects of divalent cations on Rb^+ uptake, which are attributed to a reduction in the surface potential showed the same pattern [4].

It is well known that the cell wall of yeast also contains negatively charged groups [21,22]. However, only 6% of the binding of 9-aminoacridine by intact yeast cells can be accounted for by binding of the dye in the cell wall (Fig. 5). Part of this binding may even be apparent, being due to the negative Donnan potential between cell walls and medium by which the dye will be concentrated inside the cell walls. It thus appears that the negative charges of the cell wall are of minor importance for the binding of 9-aminoacridine by intact cells. Further support for this notion comes from the experiment with protoplasts (Fig. 6). Enzymic removal of the cell wall does not significantly affect the binding of the dye. It may be concluded therefore that the binding of the dye is mainly determined by the negative charges of the plasma membrane, i.e. by the negative surface potential of the cells.

Fig. 6 also shows that the effectivity of Ca^{2+} in reducing the binding of 9-aminoacridine by the yeast cells remains the same after enzymic removal of the cell wall. This means that also the effects of divalent cations mainly reflect a decrease in the negative surface potential of the yeast cell, and that possible interactions of the divalent cations with the cell wall are only of minor importance.

Unexpected is the large temperature dependence of the 9-aminoacridine binding (Fig. 3). According to the theory of the electrical double layer [18,19] only a slight increase in the binding is expected on lowering the temperature. The great increase in the binding is probably a general feature of acridines in their interaction with cellular surfaces. The binding of substituted aminoacridines, ACMA (9-amino-6-chloro-2-methoxyacridine) and atebrin by thylakoids, for example, is also enlarged appreciably at lower temperatures [23,24]. The strong dye interaction at low temperature could be due to an increase in charge density of the membrane. From the Gouy-Chapman equation [18,19] it can be deduced that then the con-

centration of a divalent cation that produces a 50% reduction in B/F will be decreased. It appeared, however, that the binding of 9-aminoacridine at 5 °C is reduced by Ca^{2+} as effectively as at 25 °C (data not shown). The increased binding of the dye at lower temperatures is therefore unlikely due to a higher surface charge density. Possibly, the increased binding is due to an increased number of binding sites as is recently found for atebrin by Schuurmans et al. [24] or to an enhanced dye aggregation at the membrane surface as has been suggested by Torres-Pereira [23]. Another possibility would be that the affinity of binding sites on the membrane for the dye is increased on lowering the temperature.

In previous publications we have used the ζ potential of the cells as a diagnostic tool in evaluating effects of the surface potential in ion uptake. Indeed the ζ potential appears to be a reasonable probe of the surface potential. Fig. 7 shows that the ζ potential is directly related to B/F, a parameter determined by the surface potential. Irrespective of the way the surface potential is changed, a single relationship between B/F and the ζ potential is found. The finding of this relationship not only gives credibility to our arguments with regards to the use of the ζ potential as a diagnostic tool in the study of electrostatic effects in ion uptake, it also enables us to determine the value of B/F at zero surface potential and to correlate the measured B/F values with the surface potential ψ_o experienced by the dye molecules. This is argued below.

Recently, Huang et al. [16] have shown that the quenching of acridine dyes in the presence of biological membranes is due to complex formation between the dye and the plasma membrane. Under conditions that the adsorption of the dye has a negligible effect on the net charge density of the membrane the relationship between the surface density of dye molecules adsorbed to the membrane per unit dry weight of cells, B, and the dye concentration in the medium, F, respectively, may be described by a combination of the Langmuir equation and the Boltzmann equation [25];

$$B = \frac{B_{max} \cdot F_o}{K_D + F_o} = \frac{B_{max} \cdot F \cdot y}{K_D + F \cdot y} = \frac{B_{max} \cdot F}{(K_D/y) + F} \qquad (3)$$

where K_D is the dissociation constant of the complex, B_{max} is the maximum surface density of adsorbed dye molecules, F_o is the concentration of the dye in the aqueous phase adjacent to the membrane, $y = (\exp - F^* \psi'_o / RT)$ and F^*, R and T have their usual meaning.

Under the experimental conditions applied in the present study the distribution of 9-aminoacridine between cells and medium (B/F) is independent of the dye concentration (Fig. 2). Apparently $K_D/y \gg F$ and, consequently Eqn. 3 reduces into

$$B/F = \frac{B_{max}}{K_D} \cdot y = I \cdot y \qquad (4)$$

and after rearrangement, into

$$\psi_o = 25.6 \, (\ln I - \ln B/F) \qquad (5)$$

where $\ln I = -6.74$, and represents the value of the intercept of the extrapolated line in Fig. 7 with the ordinate, assuming that at zero ζ potential also ψ'_o is zero. Eqn. 5 predicts that a reduction of 59 mV in ψ'_o causes B/F to decrease 10-fold. Fig. 7 shows that a 10-fold decrease in B/F is achieved on reducing the ζ potential 7 mV. Thus apparently, $\psi'_o = 8.4 \, \zeta$. Also the electrostatic potential experienced by cation transporters is an order of magnitude higher than the corresponding ζ potential [2,3]. It thus appears that 9-aminoacridine indeed is able to probe the surface potential adjacent to the cation transporters of the yeast cell almost quantitatively. The latter potential has been considered to represent a discrete charge potential [2,3]. Its almost quantitative detection with 9-aminoacridine therefore supports the notion that this dye is able to probe localized charged areas on a biological membrane surface [7].

Though several other fluorescent probes for the surface potential are described in literature, we feel that 9-aminoacridine is one of the most suitable probes. Because of its high pK_a it is almost quantitatively ionized up to pH = 8. In this respect it is more favourable than other acridines like 9-amino-6-chloro-2-methoxyacridine (ACMA) which has a pK_a of 8.5 [26]. 1-Anilino-8-naphthalene sulfonate (ANS) may also be used as surface potential probe of biological membranes [27]. Orienting experiments, however, have shown that this compound is not suitable for our purpose (Barts, P.W.J., unpublished experiments). The ap-

plication of 9-aminoacridine is restricted to yeast cells which do not have developed the thiamine carrier system. As we have recently shown [11], the dye is accumulated into the yeast cells by means of this derepressible system. In exhausted non-metabolizing cells, as applied by us, this system is not active anymore. After addition of glucose, however, the thiamine transport system is developed within 30 min. But even with metabolizing yeast, information about the surface potential can be obtained by either suppressing metabolic uptake of the dye by addition of thiamine disulfide, a potent specific inhibitor of the thiamine carrier [11], or by extrapolating uptake data to 'zero' time by which only the rapid almost immediate binding of the probe to the external surface of the cells will be obtained.

In view of the presented results we conclude that 9-aminoacridine may be applied as a convenient probe of the surface potential of yeast cells. Changes in the surface potential that effect the kinetics of ion uptake by the cells can be probed almost quantitatively by this acridine dye, provided the temperature is kept constant.

Acknowledgements

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References

1 Borst-Pauwels, G.W.F.H. (1981) Biochim. Biophys. Acta 650, 88–127
2 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1983) Biochim. Biophys. Acta 734, 62–69
3 Borst-Pauwels, G.W.F.H. and Theuvenet, A.P.R. (1984) Biochim Biophys. Acta 771, 171–176
4 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Biochim. Biophys. Acta 426, 745–756
5 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Bioelectrochem. Bioenerg. 3, 230–240
6 Sauvé, R. and Ohki, S. (1979) J. Theor. Biol. 81, 157–179
7 Chow, W.S. and Barber, J. (1980) Biochim. Biophys. Acta 589, 346–352
8 Theuvenet, A.P.R. and Bindels, R.J.M. (1980) Biochim. Biophys. Acta 599, 587–595
9 Lyons, T.P. and Hough, JS. (1970) J. Inst. Brew. 76, 564–571
10 Wanka, F. (1962) Planta 58, 594–609
11 Theuvenet, A.P.R., Van de Wijngaard, W.M.H. and Borst-Pauwels, G.W.F.H. (1983) Biochim. Biophys. Acta 730, 255–262
12 Fuhrmann, G.F., Granzer, E., Bey, E. and Ruhenstroth-Bauer, G. (1964) Z. Naturforsch. 196, 613–621
13 Smoluchowski, M. (1903) Bull. Acad. Sci. Cracovic, p. 182
14 Schuldiner, S., Rottenberg, H. and Avron, M. (1972) Eur. J. Biochem. 25, 64–70
15 Searle, G.F.W. and Barber, J. (1978) Biochim. Biophys. Acta 502, 309–320
16 Huang, C.-S., Kopacz, S.J. and Lee, C.-P. (1983) Biochim. Biophys. Acta 722, 107–115
17 Kraayenhof, R. (1970) FEBS Lett. 13, 161–165
18 Gouy, M. (1910) J. Phys. Radium 9, 457–468
19 Chapman, D.L. (1913) Philos. Mag. 25, 475–481
20 Theuvenet, A.P.R. (1978) Thesis, Nijmegen
21 Jayatissa, P.M. and Rose, A.H. (1976) J. Gen. Microbiol. 96, 165–174
22 Eddy, A.A. and Rudin, A.D.l (1958) Proc. R. Soc. B. 148, 419–432
23 Torres-Pereira, J.M.G. (1983) Thesis, Amsterdam
24 Schuurmans, J.J., Veerman, E.C.I., Francke, J.A., Torres-Pereira, J.M.G. and Kraayenhof, R. (1984) Plant Physiol. 74, 170–175
25 McLaughlin, S. (1977) in Current Topics in Membranes and Transport (Bonner, F. and Kleinzeller, A., eds.), Vol. 9, pp. 71–144, Academic Press, New York
26 Kraayenhof, R. and Arents, J.C. (1977) in Electrical Phenomena at the Biological Membrane Level (Roux, E., ed.), pp. 493–504, Elsevier, Amsterdam
27 Haynes, D.H. (1974) J. Membrane Biol. 17, 341–366