Biochimica et Biophysica Acta, 1028 (1990) 261–267
Elsevier

BBAMEM 75005

Proton-linked transport systems as sensors of changes in the membrane surface potential

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(Received 3 April 1990)

Key words: Membrane potential; Surface potential; Proton linked transport

The kinetic properties of proton linked transport systems and their relation to the membrane surface potential were studied in yeast cells. (1) The negative surface potential of cells rich in anionic phospholipids was found to be 2-times higher than that of control cells; in agreement with their 2-fold increase in the anionic/zwitterionic phospholipid ratio (A/Z). (2) At low external concentration of substrates (high-affinity systems), higher uptake activities were observed for the anions, glutamate, aspartate and phosphate; the zwitterion glycine and the cations lysine and arginine, in both phosphatidylserine and phosphatidylinositol rich cells when compared to control cells. (3) On the other hand, at high external concentration of substrates (low-affinity systems), lower uptake activities were observed for glutamate, aspartate, phosphate and glycine in the cells rich in anionic phospholipids. (4) A decrease in K_m without significant alteration in V_{max} was found in the high-affinity transport systems that can be explained by the increase in proton concentration at the interface caused by the enhancement in negative surface charge of the cells rich in anionic phospholipids. (5) The mechanisms of the high-affinity proton linked transport systems are compatible with a model which is necessarily ordered, protons before anions. The low-affinity transport systems, on the other hand, follow a random order of binding. The transport systems studied behave as sensors of the changes in surface potential. The reduction of the surface potential reversed the transport alterations with the following sequence: monovalent cations < divalent cations < cationic local anesthetics.

Introduction

The cell plasma membrane contains negatively charged phospholipids which produce a negative electrostatic surface potential in the aqueous phase adjacent to the membrane. This negative surface potential causes the local accumulation of cations and depletion of anions in solution and results in a diffuse double layer of charge at equilibrium [1].

An important question concerns to the effect of surface charge on the proteins responsible for membrane functions. We have recently shown that phosphoinositide-rich cells (PI-rich) have an increased membrane surface potential and exhibit an enhanced transport of phosphate and glycine entering the cells accompanied by protons [2]. The present paper intends to provide a more detailed discussion on the effect of the membrane surface potential on proton linked-transport systems. We decided to study the kinetic properties of the transport systems for the anions phosphate, glutamate and aspartate, for the cations arginine and lysine and the zwitterion glycine, and the relation they might have with the membrane surface potential. We compared normal cells with those rich in phosphatidylserine (PS-rich) in addition to PI-rich cells, to know whether PS can substitute PI in their contribution to the membrane surface potential and in their effect on the transport systems. We also studied the effect of monovalent cations, divalent cations and cationic local anesthetics on the membrane surface potential and on the kinetic properties of proton-linked transport systems. We found that the cells rich in anionic phospholipids show a negative surface potential higher than normal cells, which activates the high-affinity proton-linked transport systems and inhibits the transport of anions from high external concentrations. The reduction of the surface potential by counterions reverses completely the above-mentioned transport alterations. These effects can be conveniently interpreted as an increased concentration of protons in the vicinity of the carrier proteins as predicted by the Gouy-Chapman double layer theory,

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and indicate that the anionic phospholipids travel through the membrane and contribute to the external surface charge.

Materials and Methods

Organisms

All the strains used were derived from *Saccharomyces carlsbergensis* ATCC-9080. PI-rich cells were obtained by growing the cells in a medium containing 10 mM arsenate, as previously described [2], and PS-rich cells by adding hydroxylamine (10 mM final concentration) to growing cells (absorbance, $A = 0{,}25$). The cultures were further continued 10–18 h. As previously reported, hydroxylamine at 10 mM caused the maximum increment in PS content of cells without affecting viability [3]. All cells were grown at 37 °C in nutrient broth containing 1% (w/v) yeast extract.

Analysis of phospholipids

Phospholipids were extracted from yeast cultures uniformely labelled with [^{32}P]P_i, by chloroform/methanol (2 : 1) and separated by thin-layer chromatography, as described previously [4].

Binding of 9-aminoacridine

The dye binds to negatively charged surfaces and quenches its fluorescence [5]. The cells were washed and resuspended in 20 mM Tris-citrate buffer (pH 6.0) containing 9-AA. Immediately after exposure, the suspensions were centrifuged and the fluorescence of the supernatants measured with an Aminco-Bowman spectrofluorometer, using the wavelength pairs 400 nm and 454 nm, for exitation and emission, respectively. The dye concentration was calculated from a calibration curve. The amount of dye bound to the cells, due to surface charge effects, was calculated from the decrease of fluorescence in the supernatant and the recovery of fluorescence observed after the addition of 100 mM $MgCl_2$ to the cell suspensions in duplicate experiments. The addition of 100 mM $MgCl_2$ released the cells-bound 9-AA due to a reduction of the negative surface potential [6]. The binding of the dye was measured at 23°C. Routinely the buffer contained 2 μM 9-AA.

Surface charge density

9-Aminoacridine at 2 μM was added to 3 mM Tris-citrate buffer (pH 6.0) and the fluorescence was adjusted to 80 units on the recorder. The cells (1.0 mg dry wt.) were added; this brought the fluorescence down to a minimum. The fluorescence was now brought back up by titrating with either KCl or methyl viologen $(MV)Cl_2$ used as divalent cation. At the end of each experiment 20 mM $MgCl_2$ was added to give the maximum fluorescence release (F_{max}). The relative fluorescence values were plotted and the figure used to determine concentrations of KCl and $(MV)Cl_2$, which gave the same relative fluorescence.

The surface charge density (σ) was calculated from these values as in Ref. 7 for different levels of fluorescence quenching.

Transport assays

As previously described [2], the yeasts were harvested, washed twice with water and suspended for 20 min in 1% (w/v) glucose at 23°C. The yeasts were collected, water-washed and used at once as follows. The uptake of substrates was assayed at 23 °C. The cells were resuspended (1.0 mg dry wt./ml) in prewarmed 20 mM Tris-citrate solution (23°C) containing 200 μg/ml cycloheximide; adjusted to the desired pH with HCl or citric acid. Values of pH 6.8 for phosphate and pH 5.0 for the amino acids were the optimum pH values found experimentally (data not shown). Cells were incubated for 5 min before the substrate was added. The uptake of the labelled compounds by the cells was determined as a function of time. At the desired times the cells were separated from the suspension medium by centrifugation through dibutylphthalate in a Beckman E microfuge ($1200 \times g$, acceleration 6 s), instead of filtering through Millipore filters. Radioactivity on the cell pellet was determined by scintillation counting in a Packard TriCarb scintillation counter. Aquasol was utilized as scintillation liquid. All cpm values were corrected with the use of an external standard and the channel ratio method. The intracellular concentration was calculated by assuming a value of 2.3 μl intracellular water/mg dry wt. [8]. The K_m and V_{max} values were obtained from double reciprocal plots optimized by linear regresion analysis. The rates of uptake appear linear for at least 1 min. For the determination of K_m the reaction was terminated after 30 s.

Chemicals

[^{32}P]P_i as orthophosphoric acid, L-[2,3-^{3}H]glutamic acid, L-[2,3-^{3}H]aspartic acid and L-[2,3-^{3}H]arginine; L-[4,5-^{3}H]lysine and [2-^{3}H]glycine were purchased from New England Research Products. The other reagents used: 9-aminoacridine, methyl viologen, 1,1′-dimethyl 4,4′-bipyridinium dichloride $(MV)Cl_2$, solvents, salts, etc. were purchased from Sigma, J.T. Baker, Mallinckrodt and E. Merck; A.C., dibutylphthalate was purchased from Eastman Kodak Co.

Results and Discussion

Phospholipid composition and surface potential

Table I shows the lipid composition in normal PS-rich and PI-rich yeast-cells. The ratio of anionic phospholipids to zwitterionic phospholipids (A/Z) increased in the PS-rich and in the PI-rich cells. Table I also illustrates the changes in 9-aminoacridine binding that

TABLE I

Phospholipid composition and surface potential in yeast cells

The phospholipid composition was expressed by percentage distribution of [^{32}P]P_i. The ratio A/Z is the relation anionic/zwitterionic phospholipids. 9-Aminoacridine (9-AA) binding is expressed in nmol/g dry wt. of cells. (a) The cells were cultivated in the presence of [^{32}P]P_i. Hydroxylamine was added when the absorbance reached 0.3 at 600 nm and the incubation continued 18 h more. Final concentration of hydroxylamine was 10 mM. (b) The cells were cultivated in presence of [^{3P}]P_i in a medium containing 10 mM sodium-arsenate for 48 h. The data represent the average of five separate experiments by duplicate. S.D. was never higher than 10%.

	Phospholipid composition				Ratio A/Z	9-AA Binding
	PI	PS	PC	PE		
Normal cells	12.0	8.6	51.4	27.9	0.26	27.5
(a) PS-rich cells	13.0	20.9	49.8	16.3	0.51	60.3
(b) PI-rich cells	23.7	9.1	47.2	20.0	0.49	63.7

occurred when the proportion of anionic phospholipids (PS and PI) increased. The amount of 9-AA bound was 2.2-times higher and similar to the increment in the ratio A/Z, which was also around 2-times higher for the cells rich in anionic phospholipids.

Surface charge density measurements

9-AA fluorescence changes, as a measure of membrane surface charge density, have been reported [9]. When suspended in a low cation-containing medium, yeast cells quench the fluorescence from 9-AA [6]. Relief of this quenching is achieved by adding cations to the suspension medium, with the order of effectiveness being $C^{3+} > C^{2+} > C^{+}$, indicating that the fluorescence acts as an indicator of the membrane surface electrical potential [5,6]. Fig 1 and Table II show the results of typical experiments where the surface charge density was determined on normal cells. The addition of the cells rich in anionic phospholipids to the weak buffer (3 mM), caused a larger quenching than the same amount of normal cells. The titration with either KCl (monovalent cation) or methyl viologen $(MV)Cl_2$ (divalent cation) gave S-shaped curves (on a semilogarithmic plot) similar to those in Ref. 7. When the surface charge densities were calculated using corresponding concentration of KCl and $(MV)Cl_2$ from the figures, increasing values were obtained with increasing salt concentrations, as also found in Ref. 7. Due to the form of Eqn. 3 in Ref. 7, σ can not be calculated as long as the KCl concentration added is less than or equal to the concentration of monovalent cation in the medium (below 0.41 F/F_{max}). High F/F_{max} values are also not suitable, since on decreasing the surface potential (high ionic strength) the binding of protons to the cell surface decreases and the charge density increases. F/F_{max} values from 0.49 to 0.70 were used for all further comparisons. The σ of cells rich in anionic phospholipids was

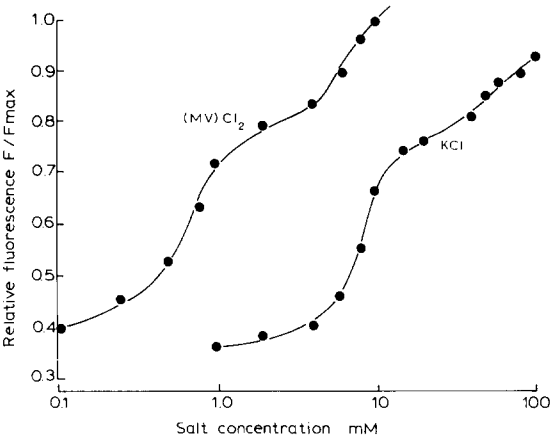

Fig. 1. Dependence of 9-aminoacridine fluorescence on the presence of mono and divalent cations in yeast suspensions. The concentrations refer to the nominal concentrations of salt added. The buffer concentration was 3.0 mM Tris-succinate at pH 6.0. The concentration of 9-aminoacridine was 2 μM and cells concentration was 0.4 mg dry wt. of cells/ml. The osmolarity was adjusted to 300 mosM with mannitol. F is the fluorescence yield under a particular experimental condition and F_{max} is the maximum fluorescence intensity observed on adding 20 mM $MgCl_2$.

higher (1.7–1.9-times) than that of normal cells, as shown in Table III.

The concentration of the cations was not corrected for binding. The association constants of the alkaline earth cations with PS are all extremely low (< 0.1 l/mol) and the decrease they produce in the magnitude of the surface potential may be mainly attributed to a screening process (see McLaughlin et al. [9]).

Effect of surface potential on transport systems

In comparison to normal cells, the anionic phospholipid-rich cells have an increased negative surface charge.

TABLE II

The calculation of the net negative surface charge density on normal yeast cells using 9-aminoacridine fluorescence

C_1^+ and C_2^+ were calculated by linear interpolation from Fig. 1. σ was calculated as in Ref. 7. The yeast concentration was 0.4 mg dry wt./ml and 9-aminoacridine concentration 2.0 μM. F is the fluorescence yield of a particular experimental condition and F_{max} is the level obtained on adding 20 mM divalent cation. MV, methyl viologen.

F/F_{max}	$(MV)Cl_2$ (mM)	KCl (mM)	σ (C/m^2)
0.40	0.10	3.5	0.0193
0.45	0.24	5.5	0.0189
0.50	0.42	6.8	0.0169
0.55	0.52	7.8	0.0167
0.60	0.66	8.8	0.0168
0.65	0.79	9.8	0.0168
0.70	0.95	11.0	0.0165
0.75	1.30	17.0	0.0230
0.80	2.30	33.0	0.0343
0.90	6.00	80.0	0.0507

TABLE III

Estimated charge density for yeast cells

F/F_{max}	Surface charge density (C/m^2)		
	normal cells	PS-rich cells	PI-rich cells
0.48	0.0180	0.0260	0.0340
0.51	0.0166	0.0287	0.0343
0.54	0.0170	0.0286	0.0328
0.61	0.0169	0.0290	0.0328
0.67	0.0168	0.0314	0.0342
0.70	0.0165	0.0295	0.0329

The concentration of cations at their membrane surface is higher, whereas for anions the reverse is true. Therefore, apparent K_m values should be elevated for anions and decreased for cations, and should remain without change for the zwitterionic substrates. However, transport of anions across biological membranes often occurs as a cotransport with one or more cations, mainly protons. This is the case for phosphate and glutamate in yeast [10]. The effect of the surface potential on the transport kinetics of such a cotransport system will be complex, since the interfacial concentration of protons and anions are affected in an opposite way. The uptake kinetics for all substrates tested show a deviation from simple Michaelis Menten kinetics. Biphasic curves of the Lineweaver-Burk plots (Fig. 2) and concave upward curves of the Hofstee plots (not shown) revealed the existence of two kinetic systems. In the low-affinity transport systems (Table IV), as anticipated theoretically, the apparent K_m values were increased for the anionic substrates in the cells with higher negative surface potential. K_m increased 3–4-times for phosphate and up to 2.6–2.9-times for glutamate and aspartate, respectively. A small tendency of the K_m values to decrease was found for arginine and lysine (cations), but the zwitterionic glycine behaved as an anion, since its K_m value increased up to 2.6-times. With regard to V_{max} values, a small increase of 1.25-times was observed only for phosphate and aspartate.

On the other hand, at low substrate concentrations (high-affinity systems) lower K_m values (2–3-times) were found for all substrates tested without significant alterations in V_{max} in the cells with higher surface potential when compared to normal cells (Table V). This event was independent of the charge of the substrate tested. Nevertheless, the magnitude of the increase in affinity was more marked when the substrate was a cation (arginine and lysine) where K_m decreased 3-times. The decreased K_m values observed for phosphate, glutamate and aspartate (anions) and glycine (zwitterion) can be explained only if protons are the first substrates to bind to the translocators (for a detailed theoretical discussion see Roomans and Borst-Pauwels [11]). The results indicate that the low-affinity transport systems are inhibited by increasing the negative surface potential, while the high-affinity transport systems are activated; that is, the mechanisms of transport are different for the same substrate (anionic or zwitterionic). It has been found that fewer protons are absorbed when the concentration of anions (phosphate

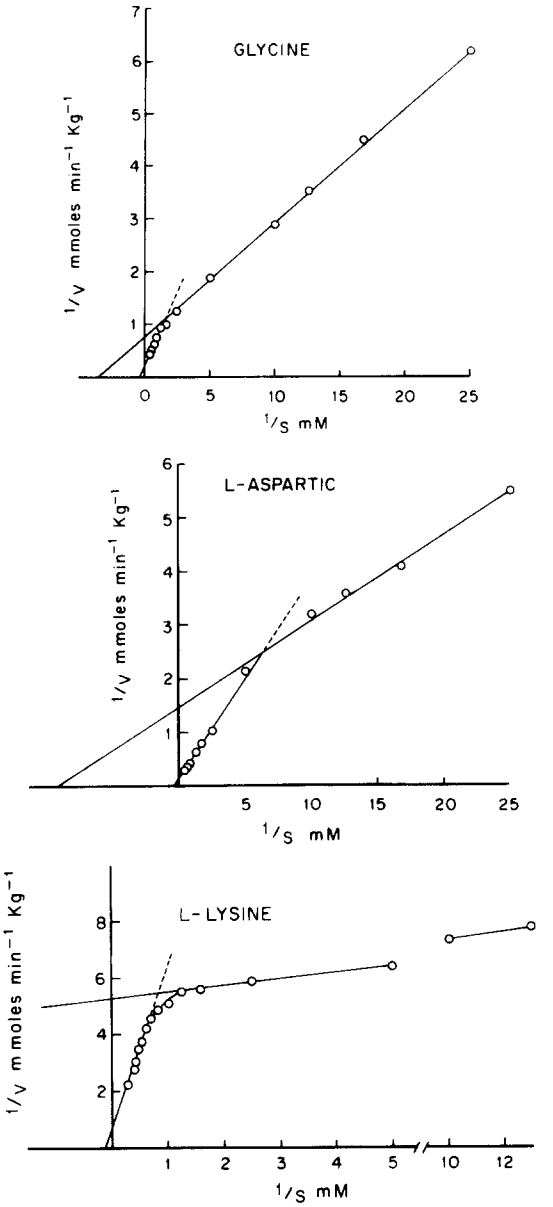

Fig. 2. Lineweaver-Burk plots of amino acid uptake (optimized by linear regression). Cells were kept for 20 min at 23°C in 1% glucose, water washed twice and resuspended in 20 mM Tris-citrate (pH 5.0). After 5.0 min the substrate was added (1.0 μM to 3.0 mM). The uptake was stopped by centrifugation through dibutylphthalate in a microfuge. Data were calculated using initial rate of uptake (30 s). The correlation coefficients for regression were: 0.987 and 0.989 (glycine, A); 0.988 and 0.993 (aspartate, B); 0.991 and 0.994 (lysine, C) for low- and high-affinity transport systems, respectively.

TABLE IV

Effect of PS or PI enrichment on K_m and V values of the low-affinity transport systems

The K_m (M) and V (mmol/kg dry wt. cells per min) values of the various transport systems were calculated from Lineweaver-Burk plots optimized by linear regression analysis. Various external concentrations of the different substrates ranging from 1 μM to 3 mM were used. All values are the average of three separate experiments by triplicate. S.D. was never higher than 15%. The correlation coefficients for regression were around 0.98 (phosphate), 0.97 (glutamate), 0.96 (aspartate), 0.93 (arginine), 0.97 (lysine) and 0.96 (glycine).

Transport systems	Normal cells		PS-rich cells		PI-rich cells	
	K_m ($\times 10^3$)	V	K_m ($\times 10^3$)	V	K_m ($\times 10^3$)	V
Phosphate	1.53	16.99	4.44	21.61	6.11	23.01
Glutamate	1.47	5.55	2.32	6.48	3.80	5.80
Aspartate	1.14	6.66	3.01	8.12	3.33	9.10
Arginine	5.91	1.39	5.00	1.05	4.93	1.42
Lysine	6.12	1.00	6.04	1.12	5.20	1.18
Glycine	1.57	4.84	4.11	4.44	2.94	4.94

TABLE V

Effect of PS or PI enrichment on K_m and V values of the high-affinity transport systems

The K_m (M) and V (mmol/kg dry wt. cells per min) values of the various transport systems were calculated from Lineweaver-Burk plots optimized by linear regression analysis. Various external concentrations of the different substrates ranging from 1 μM to 3 mM were used. All values are the average of three separate experiments by triplicate. S.D. was never higher than 15%. n.d. not detected. The correlation coefficients for regression were around 0.99 (phosphate), 0.97 (glutamate), 0.96 (aspartate), 0.98 (arginine), 0.98 (lysine) and 0.99 (glycine).

Transport systems	Normal cells		PS-rich cells		PI-rich cells	
	K_m	V	K_m	V	K_m	V
Phosphate	$1.22 \cdot 10^{-4}$	2.07	$5.55 \cdot 10^{-5}$	1.89	$5.01 \cdot 10^{-5}$	1.93
Glutamate	$1.82 \cdot 10^{-4}$	0.66	$8.00 \cdot 10^{-5}$	0.58	$9.85 \cdot 10^{-5}$	0.73
Aspartate	$2.21 \cdot 10^{-4}$	0.73	$1.01 \cdot 10^{-4}$	0.70	$9.98 \cdot 10^{-5}$	0.77
Arginine	$1.01 \cdot 10^{-4}$	0.25	$3.21 \cdot 10^{-5}$	0.29	$3.49 \cdot 10^{-5}$	0.27
Lysine	$1.49 \cdot 10^{-4}$	0.19	$4.00 \cdot 10^{-5}$	0.17	$3.33 \cdot 10^{-5}$	0.19
Glycine	$9.02 \cdot 10^{-4}$	1.75	n.d.	n.d.	$3.85 \cdot 10^{-4}$	1.67

TABLE VI

Effect of reducing the negative surface potential on K_m and V of the high-affinity transport systems of the cells rich in anionic phospholipids

The K_m (M) and V (mmol/kg dry wt. cells per min) values of the various transport systems were calculated from Lineweaver-Burk plots optimized by linear regression analysis. All values are the average of three separate experiments by triplicate. $MgCl_2$ and tetracaine were added 5 s before the addition of substrate. The correlation coefficients for regression in the presence of tetracaine or $MgCl_2$ were similar to those in the footnote of Table V.

	Normal medium		+ Tetracaine 0.1 mM		+ $MgCl_2$ 1 mM	
	K_m	V	K_m	V	K_m	V
Phosphate						
PS-rich	$5.55 \cdot 10^{-5}$	1.89	$1.78 \cdot 10^{-4}$	4.51	$1.33 \cdot 10^{-4}$	2.68
PI-rich	$5.01 \cdot 10^{-5}$	1.93	$1.23 \cdot 10^{-4}$	2.60	$1.06 \cdot 10^{-4}$	2.04
Glutamate						
PS-rich	$8.00 \cdot 10^{-5}$	0.58	$2.01 \cdot 10^{-4}$	0.59	$1.57 \cdot 10^{-4}$	0.67
PI-rich	$9.85 \cdot 10^{-5}$	0.73	$1.76 \cdot 10^{-4}$	0.85	$1.55 \cdot 10^{-4}$	0.81
Arginine						
PS-rich	$3.21 \cdot 10^{-5}$	0.29	$2.32 \cdot 10^{-4}$	0.24	$1.31 \cdot 10^{-4}$	0.31
PI-rich	$3.49 \cdot 10^{-5}$	0.27	$2.51 \cdot 10^{-4}$	0.23	$1.61 \cdot 10^{-4}$	0.29
Glycine						
PI-rich	$3.85 \cdot 10^{-4}$	1.67	$8.42 \cdot 10^{-4}$	1.73	$8.00 \cdot 10^{-4}$	1.79

or glutamate) is increased [10] and that there is inhibition of transport of several amino acids in PS-rich cells when they are used in the millimolar range (low-affinity systems [12].

Effect of reducing the surface potential on transport

An increase in cations should lower the magnitude of the surface potential, reduce the concentration of the permeant protons at the outer membrane solution interface and decrease the rate of transport. In order to determine whether this simple interpretation was adequate, the effect of $MgCl_2$ (1.0 mM) and the cationic local anesthetic tetracaine (0.1 mM), which are known to reduce significantly the negative surface potential of artificial lipid membranes, was studied. The later $MgCl_2$ concentration was chosen since it released 80% of the 9-AA bound to yeast cells (data not shown). On the other hand, local anesthetics are 1 to 2 orders of magnitude more efficient than Mg^{2+} in reducing the surface potential of artificial lipid bilayers [13]. Cationic local anesthetics change the surface potential not only by screening the negative surface charge, but by becoming anchored to the bilayer [14] and allowing the displacement of polyvalent cations from the interface [15]. It is apparent from the results shown in Table VI, that tetracaine 0.1 mM is more efficient than $MgCl_2$ 1.0 mM in reducing the apparent affinity of the transport systems (increasing K_m), in agreement with their higher capacity to reduce the surface potential [15]. As anticipated, the increase in K_m was more clearly seen for the cationinc arginine where K_m increases 4-times with 1.0 mM $MgCl_2$ and 7-times with 1.0 mM tetracaine. For the anionic phosphate and glutamate and the zwitterionic glycine K_m increased 2- to 3-times. The increases in K_m observed upon reduction of the surface potential were similar in both PS-rich and PI-rich cells; the only exception was the high-affinity glycine transport system, which was not detected in PS-rich cells. A direct effect of hydroxylamine upon the glycine carrier can be suspected.

Proton-linked transport systems as sensors of changes in surface potential

The above-mentioned results lead to the conclusion that the state of the diffuse layer surrounding the cell membrane surface is well represented by the Gouy-Chapman theory and show that the proton-linked transport systems behave as sensors of changes in membrane surface charge. Having found that K_m decreases when the negative surface charge increases from 0.0170 (C/m^2) in the normal cells to 0.029–0.032 (C/m^2) in the anionic phospholipid-rich cells, we decided to study whether proton-linked transport systems can detect smaller changes in the membrane surface potential. With this purpose, the kinetic parameters of the high-affinity transport system of phosphate in PS-rich cells,

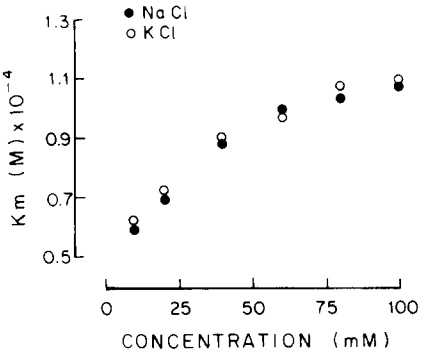

Fig. 3. Relation between surface potential and K_m of the phosphate high affinity transport system. The K_m values of the phosphate high-affinity transport system of PS-rich cells were calculated from Lineweaver-Burk plots. The surface potential was reduced by adding either NaCl (●) or KCl (○) to the suspension medium (10 mM Tris-succinate buffer pH 6.8). K_m values were obtained from two experiments in triplicate for each cation.

were determined while decreasing the negative surface potential by varying the concentration of monovalent cations (C^+) in the suspension media (from 10 to 100 mM KCl or NaCl). The effects of surface potential on K_m can be seen in Fig. 3. The apparent K_m of the PS-rich cells increased with increasing the monovalent cation concentration (C^+) approaching a value similar to that of the normal cells. There were no significant differences between NaCl and KCl. Note that K_m decreases while the negative surface potential increases (the C^+ decreases). These results give experimental support to the suggested cotransport mechanism in which protons bind before the anion. A 1 anion-2 protons cotransport mechanism can be proposed, since when the substrate concentration is in the micromolar range 2 protons are absorbed per phosphate [10] or amino acid equivalent [16]. V_{max} was independent of the surface potential (1.731 ± 0.15).

Opposite to the above-mentioned results, the reduction of the surface potential by 100 mM Tris decreases K_m 5-times in the low-affinity transport system of phosphate from a K_m of $6.1 \cdot 10^{-3}$ M in 20 mM Tris (Table IV, PS-rich cells) $1.22 \cdot 10^{-3}$ M in 100 mM Tris (data not shown). This behavior is compatible with a model of random binding proposed theoretically [11] where K_m and V_{max} increase while increasing the surface potential and F_1 (V_{max}/K_m) decreases. The results also show that proton-linked transport systems behave surprisingly well as sensors of changes in membrane surface potential. In other words, the surface potential regulates the function of the transport systems with high precision.

Our interpretation of the lipid effects is based on the difference of surface potential of the normal, PS-rich or PI-rich cells, which coincide with the increment in the A/Z ratio.

An important contribution of the proteins to the increment in surface charge is unlikely, since the cells

maintain their protein/phospholipid ratio. The total phospholipid phosphorous does not change significantly in the PI-rich cells when compared to normal cells [4].

Furthermore, the increment of the surface potential in the anionic phospholipid rich cells disappears when the proportion of anionic phospholipid returns back to that of the normal cells. It was observed that the lipid effects are mediated strictly by charge effects and are not due to differences in the head groups of the lipids. For the present results, we can conclude that the carriers studied experience a similar surface potential in a PI-rich than in a PS-rich membrane. This may mean that the lipids are located in the same domain of the carrier protein. In the absence of structural information regarding the location of proton binding sites in the carrier proteins with respect to the membrane surface, it is surprising that all the carriers studied sense almost the same increase in the surface potential. The binding sites should be at a similar distance lateral or normal to the membrane.

Equivalent effects have been observed on the conductance of a calcium-activated potassium channel in planar lipid bilayers [17]. At low K^+ concentrations the channel conductance was higher in PS membranes, but approached the same conductance observed in phosphatidylethanolamine (PE) above 0.4 M KCl. The difference in conductance observed in the two lipids was explained by the negative surface charge of PS compared to the neutral PE membrane. Another interesting observation was that increases in the concentration of anionic phospholipids in vivo, resulted in their translocation to the external surface of the plasma membrane, since a more negative surface potential was produced.

Acknowledgements

The authors are grateful to Dr. A. Darszon for reading the manuscript. This research was supported in part by a grant from CONACYT (PCEXCNA-050160).

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