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Effect of pH and Different Substrates on the Electrokinetic Properties of (Na^+, K^+)-ATPase Vesicles

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Abstract. Some biophysical properties of a (Na^+, K^+)-ATPase preparation from guinea-pig kidney have been analysed. The recently developed technique of laser Doppler spectroscopy was applied to measure particle mobility under electrophoretic conditions. The following results were obtained:

1. magnesium ions at pH 7.3 decrease the mobility of the ATPase containing vesicles by binding to negatively charged surface groups. At pH 3.3 the competitive binding of protons causes a shift of the mobility vs. $[Mg^{2+}]$ curve to higher values of $[Mg^{2+}]$,
2. binding of ATP at pH 7.3 ($K_d = 0.9 \times 10^{-4}$ M for (mM 1 NaCl, 0.2 KCl, 0.1 $MgCl_2$, 0.1 *Tris*) was measured as an increase in particle mobility depending also on $[Mg^{2+}]$. At pH 3.3 also unspecific ATP-binding occurred,
3. ITP and GTP had the same K_d value as ATP; ADP a slightly lower one ($K_d = 1.2 \times 10^{-4}$ M). *Tris*-H_3PO_4 ($K_d = 2.6 \times 10^{-4}$ M) was also able to increase particle mobility, but only at higher concentrations and not to the same extent as ATP; AMP induced only very small changes,
4. from the mobility-pH curve an isoelectric point of 4.1 is derived (buffer: 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris*). In the presence of 0.9 mM ATP the isoelectric point is shifted to 3.2.

As the electrophoretic mobility is directly proportional to the net charge of the vesicles, the results may be interpreted as changes in surface charge density, originating from both a conformational change of the ATPase polypeptide and a decrease in vesicle size.

Key words: (Na^+, K^+)-ATPase – Laser Doppler electrophoresis – Surface charge – Conformational changes

Introduction

It is a well established fact that cytoplasmic membranes of intact cells have charged surfaces. The charge density may dominate physico-chemical properties

of the cell, like ion transport, ligand binding to receptors or vesicle adhesiveness and deformability.

Binding of a ligand to its membrane located receptor usually takes place by ionic, Van der Waals or dipole interactions. Binding by ionic forces would result in changes of the surface charge and is influenced by various ions and their concentrations.

It has been confirmed that the (Na^+, K^+)-ATPase is the enzyme of the active sodium and potassium transport across cell membranes (Dahl and Hokin 1974; Skou 1975; Schwartz et al. 1975). The enzyme transports cations against a concentration gradient by hydrolysing ATP to ADP and P_i. For this process sodium, potassium and magnesium ions are necessary. The ATP, Mg^{2+}, and Na^+ binding sites are located on the inside surface of the cell, while potassium and cardiac glycosides are known to interact with the enzyme from the outside.

The preparation of (Na^+, K^+)-ATPase leads to spherical, closed vesicles which contain the ATPase polypeptide as protruding structures from the vesicle surface (Maunsbach et al. 1980; Schlieper et al. 1981). It is generally assumed that the vesicles are mainly inside out with the catalytic site of the enzyme accessible to substrates. Therefore ATPase containing vesicles seem to be suited to study ligand binding to surface active groups.

Surface charges may be analysed by various electrophoretic methods which have been applied to investigate liposomes, lysosomes, liver mitochondria and microsomes, synaptic vesicles and erythrocytes (Hauser et al. 1976; McDonald and Bangham 1972; Davenport 1964; Plummer 1965; Hannig 1969; Heidrich et al. 1970; Ryan et al. 1971; Blad-Holmberg 1979; Haydon and Seaman 1967). One of the most common technique which has been used is the slow and tedious procedure of microscopic observation of the bioparticles, moving in an uniform electric field.

Ware and Flygare (1971) and Uzgiris (1974) developed a much more rapid and accurate method for the measurement of particle mobilities, based on light scattering and the Doppler principle. This method has successfully been applied to characterize the surface groups of red blood cells (Uzgiris and Kaplan 1976), chromaffin granules (Siegel et al. 1978), synaptic vesicles (Siegel and Ware 1980), mast cells (Petty et al. 1980) and recently by us to study (Na^+, K^+)-ATPase vesicles (Schlieper et al. 1981).

In this report the influence of pH on substrate binding to the enzyme and the effect of magnesium are investigated measuring the electrophoretic mobility of ATPase vesicles by laser Doppler spectroscopy.

Materials and Methods

Enzyme Preparations

The extraction procedure for (Na^+, K^+)-ATPase from guinea-pig kidney was the same as described before (Schlieper et al. 1981).

Radioactive Binding Studies

To the buffer media (composition is listed in Table 2), 1.5 ml, 50 µl phosphate buffer, 100 µl Na_2 ATP (10 µM final concentration), 200 µl of enzyme suspension (containing 500–600 µg protein) and 10^5 cpm γ-^{32}P-ATP have been added. The suspension was stirred for 10 s at room-temperature. The reaction was stopped by adding 4 ml of ice-cold 5% trichloroacetic acid. The sample was transferred to millipore filters with a pore size of 0.4 µm and washed subsequently three times with ice-cold trichloroacetic acid. Before counting (Beckman LS 9000 counter) the filters were dissolved in 1 ml of 99% ethyleneglycolmonoethylether and then the scintillation liquid was added. Control values were obtained by following the whole experimental procedure but in the absence of the enzyme. The results are given as per cent increase of radioactivity above controls. Each value is the mean of two experiments with a deviation of maximally 15%.

Materials

All substances were obtained from Boehringer, Mannheim, F.R.G. Guanosine-(GTP) and inosinetriphosphate (ITP) were used as trisodium-salts, adenosine-tri- and adenosinediphosphate (ATP and ADP) as disodiumsalts and adenosinemonophosphate (AMP) was used as free acid. The substances were dissolved in deionized water and then titrated to the corresponding pH, at which the experiment was performed. The γ-^{32}P-ATP was from New England Nuclear and had a specific activity of 24.0 Ci/mmol. All other chemicals were analytical reagent grade.

Methods

The instrumental setup for the measurement of electrophoretic mobilities has been described elsewhere (Schlieper et al. 1981; Mohan et al. 1976). The mathematical equations for evaluation of electrophoretic mobility, zeta potential, charge density and particle size are found in Schlieper et al. (1981).

Results

Biochemistry

Specific activities of (Na^+, K^+)-ATPase preparations from guinea-pig kidneys have been measured under various ionic conditions and also at different pH with the coupled spectrophotometric assay. In order to compare the results to those of the electrophoretic mobility measurements, (Na^+, K^+)-ATPase activity was

Table 1. Activity of the (Na^+, K^+)-ATPase, suspended in different electrolyte media at different pH and temperature. The activity of Mg^{2+}-ATPase in this preparation was determined to be about 2% of total activity

Buffer composition [mM]	Activity [μM PO_4/mg Prot/h]		Incubation temperature
	pH 7.4	pH 3.3	
100 NaCl, 20 KCl, 5 $MgCl_2$, 50 *Tris*	309.4		37° C
10 NaCl, 2 KCl, 1 $MgCl_2$, 1 *Tris*	86.8		
100 NaCl, 20 KCl, 5 $MgCl_2$, 50 *Tris*	13.2	4.5	20–23° C
10 NaCl, 2 KCl, 1 $MgCl_2$, 1 *Tris*	3.1	1.8	

Table 2. Percentage increase of radioactivity bound to (Na^+, K^+)-ATPase, which is suspended in different buffers of different pH

Buffer solutions [mM]	% increase in radioactivity	
	pH = 7.4	pH = 3.3
1 NaCl, 1 *Tris*	34	117
1 NaCl, 1 $MgCl_2$, 1 *Tris*	72	128
1 KCl, 1 *Tris*	–	108
1 KCl, 1 $MgCl_2$, 1 *Tris*	22	112
1 NaCl, 0.2 KCl, 0.1 $MgCl_2$, 0.1 *Tris*	–	101
5 NaCl, 1 KCl, 0.5 $MgCl_2$, 0.5 *Tris*	11	92
10 NaCl, 2 KCl, 1 $MgCl_2$, 1 *Tris*	24	89

measured under similar ionic conditions. The results are listed in Table 1. It can be seen that the activity strongly depends on the temperature, the ionic concentrations and the pH value of the incubation medium. The activity is high, when the ATPase is incubated in electrolytes of high concentration at 37° C. The activity is reduced, when the electrolyte concentration is reduced to one tenth. Low activities are measured when the experiments are performed at room-temperature. At pH 3.3 almost no activity can be detected.

The radioactive binding studies (Table 2, binding of γ-^{32}P-ATP to the ATPase under various conditions) show that in sodium buffer there is a phosphorylation at pH 7.4, compared to potassium, where no binding could be detected. Addition of $MgCl_2$ to the buffers increases binding. At pH 3.3 binding is higher, however the dependence of the binding on the ion composition of the medium could no longer be observed.

Electrophoresis in Different Media

The electrophoretic mobility of the (Na^+, K^+)-ATPase containing vesicles was measured by the laser Doppler technique to study the influence of different electrolyte media on the surface properties of these particles. No difference in

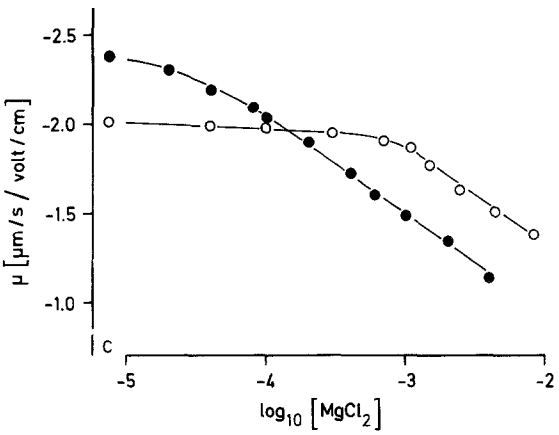

Fig. 1. Mobility of (Na^+, K^+)-ATPase containing vesicles as a function of magnesium concentration. The particles were suspended in 1 mM KCl, 1 mM *Tris*-HCl and buffered to pH 7.3 (filled circles) and pH 3.3 (open circles)

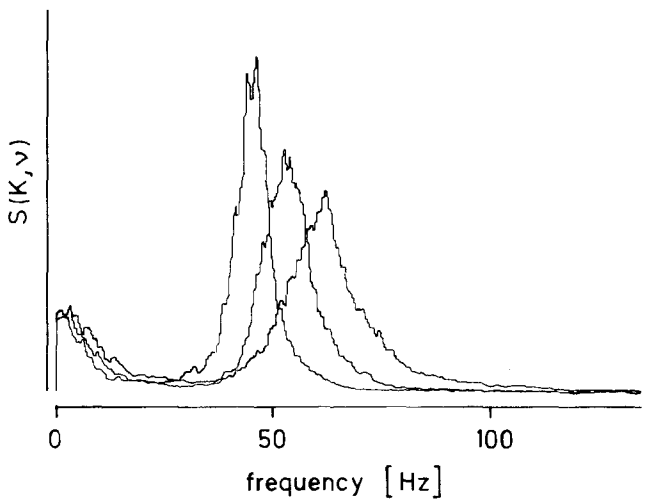

Fig. 2. Experimental light scattering spectra of (Na^+, K^+)-ATPase vesicles in electrophoresis under control conditions (left curve), under the influence of 100 μM ATP (intermediate curve) and under 900 μM ATP (right curve). The vesicles were suspended in: 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris* pH 7.3

electrophoretic mobility could be seen in pure sodium and pure potassium buffers from 1 to 10 mM each at pH 7.3 as has been reported before (Schlieper et al. 1981). A typical vesicle mobility was $\mu = 2.35$ μm/s/volt/cm for 2 mM total buffer concentration (1 mM NaCl, 1 mM *Tris*-HCl). When magnesium was added to the sample, the mobility decreased significantly. Figure 1 shows the results of two experiments, where small amounts of $MgCl_2$ were successively added to the (Na^+, K^+)-ATPase, suspended in 1 mM KCl, 1 mM *Tris*-HCl, buffered to pH 7.3 (filled circles) and 3.3 (open circles). There is a decrease in electrophoretic mobility under increasing concentrations of $MgFl_2$ at the two pH values. The Mg-effect is smaller when the experiment is performed at pH 3.3. The effect-concentration curve is shifted to higher concentration values.

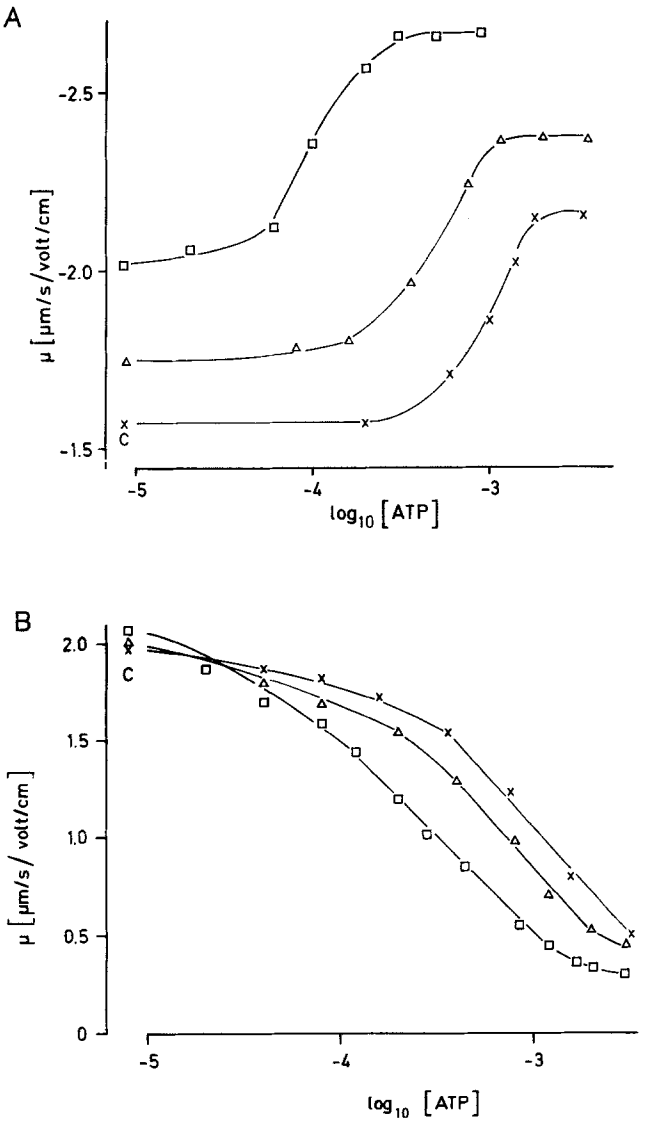

Fig. 3A and B. Mobility of (Na^+, K^+)-ATPase vesicles as a function of ATP concentration at pH 7.3 (**A**) and at pH 3.3 (**B**). The particles were suspended in 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris*-HCl (□); 5 mM NaCl, 1 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM *Tris*-HCl (△); 10 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM *Tris*-HCl (×)

Effect of ATP and Other Substrates on Particle Mobility

Typical Doppler spectra of (Na^+, K^+)-ATPase vesicles under control conditions and in the presence of two concentrations of ATP are shown in Fig. 2. ATP causes an increase in electrophoretic mobility of these particles. At the same time the linewidth of the Doppler peak is broadened. The effect is only observed,

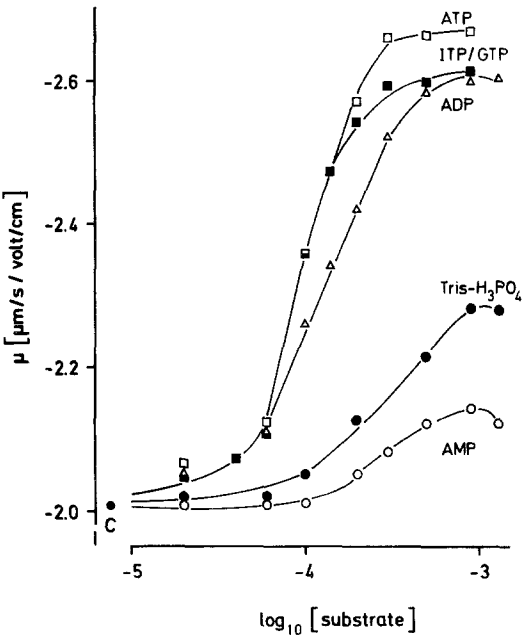

Fig. 4. Mobility of (Na^+, K^+)-ATPase vesicles as a function of substrate concentration. The particles were suspended in 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris*-HCl, pH 7.3. The substrates used were: adenosinetri- (ATP), -di- (ADP) and -monophosphate (AMP), inosine- (ITP) and guanosine-triphosphate (GTP) and *Tris*-phosphate

when the enzyme is suspended in a magnesium containing medium. Figures 3A and B show the change in particle mobility when the ATP concentration is gradually raised. The ATPase vesicles were suspended in three different buffer solutions of pH 7.3 (Fig. 3A) and 3.3 (Fig. 3B). At pH 7.3 sigmoidal effect-concentration curves are obtained as would be expected for binding of ATP to the vesicles. The mean effective concentrations (EC_{50}) were determined by fitting the logit function to the experimental data using the least squares method (Hafner et al. 1977): $EC_{50} = 0.9 \times 10^{-4}$ M (for 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris*); 4.2×10^{-4} M (for 5 mM NaCl, 1 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM *Tris*); 10.3×10^{-4} M (for 10 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM *Tris*). The effect-concentration curves look different, when the experiments are performed at pH 3.3 (Fig. 3B). Here, the particle mobility is decreased under increasing concentrations of ATP. At pH 7.3 saturation levels are reached within a short range of ATP concentration, whereas at pH 3.3 mobility decreases linearly over a wide range of substrate concentration, until the particles almost reach zero mobility. EC_{50} values were determined for the same set of electrolytes: 1.6×10^{-4} M, 3.5×10^{-4} M, 6.0×10^{-4} M.

Other substrates were tested on the particle mobility at pH 7.3. As shown in Fig. 4 ATP is the most effective agent in increasing vesicle mobility, followed by ITP and GTP. These three substrates have the same EC_{50} value of 0.9×10^{-4} M. ADP also increased the particle mobility with a slightly higher $EC_{50} = 1.2 \times 10^{-4}$ M. *Tris*-phosphate only showed half the effect of ATP ($EC_{50} = 2.6 \times 10^{-4}$ M) and AMP only a slight increase in mobility at higher concentrations.

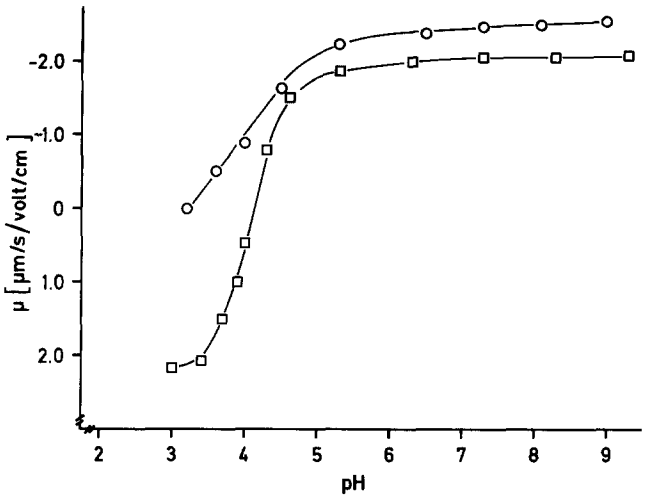

Fig. 5. Mobility of (Na^+, K^+)-ATPase vesicles as a function of pH in the presence (○) and absence (□) of 9×10^{-4} M ATP. The particles were suspended in 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris*-HCl

Electrophoresis at Different pH

Mobility measurements at different pH should give information about surface active ionic groups. Figure 5 shows the mobility as a function of the pH of suspended enzyme particles in 1 mM NaCl, 0.2 mM KCl, 0.1 mM $MgCl_2$, 0.1 mM *Tris*. On the alkaline side of the pH scale, mobilities are almost constant at different pH values. Towards acidic pH, mobilities strongly decrease to zero at pH of about 4 and then increase again for reversed direction of motion. The isoionic point is 4.1. When ATP in a concentration of 9×10^{-4} M is added to the suspension, the mobility-pH curve is shifted to higher mobility values. At pH 3.2 the particles have zero mobility.

Discussion

Electron micrographs of ATPase vesicles (Schlieper et al. 1981) clearly demonstrated that a clean preparation was used consisting of spherical, closed and homogeneously distributed vesicles. This finding is consistent with the spectra in Fig. 2, which prove that the ATPase preparation contains vesicles with the same type of surface charge. The protein structures protruding from the vesicle surface, also found by other investigators (Maunsbach et al. 1980; Deguchi et al. 1977), indicate that broken membrane parts form closed vesicles during the extraction procedure in a inside-out manner. These protein structures contain the ATP binding site (Deguchi et al. 1977). Any change in the charge of the vesicle results in a change of its electrophoretic mobility. Primarily charges at the vesicle surface are monitored by electrophoresis, whereas charges deeper in

the membrane core possibly resulting from charged lipids like phosphatidylserine are probably balanced by groups with opposite charges and do not contribute to electrophoretic mobility. The biochemical studies (Table 1) demonstrate that the activity of the ATPase measured by the free P_i generation strongly depends on the temperature, electrolyte concentration and pH of the incubating medium. As found by other workers (Dahl and Hokin 1974; Skou 1975; Schwartz et al. 1975) highest activity is observed in highly concentrated buffer solutions at pH 7.4 and 37° C. The activity decreases when the ATPase is suspended in buffers of low ionic concentration at low temperatures and at an acidic pH. This means for the electrophoretic measurements which were performed in electrolytes of low concentration and at room-temperature that the enzyme was in a state of low activity. Buffer solutions of low concentrations had to be chosen for the electrophoretic measurements to avoid Joule heating of the sample by the resulting ionic currents at the 100 V used. The binding studies (Table 2) show that γ-^{32}P-ATP is significantly bound to the ATPase in NaCl buffer and not in KCl. However addition of $MgCl_2$ strongly increased binding in NaCl and also caused binding in KCl at pH 7.4. At pH 3.3 binding was higher, but no influence of magnesium ions on the binding properties could be observed.

In electrophoretic experiments ATP increased the mobility of the ATPase vesicles. This increase in mobility can be attributed to a higher charge density on the particle surface which could result: 1. from the bound ATP molecule itself, 2. from the phosphorylated protein and 3. from conformational changes in the ATPase polypeptide. As can be deduced from the titration experiments with magnesium (Fig. 1) and from variation of pH (Fig. 5) the ATPase vesicles are negatively charged.

The ATP effect could only be observed in the presence of magnesium ions. In sodium buffer as well as in potassium buffers only slight increases in particle mobility could be measured. Being within the experimental error they could not be taken as significant. Addition of ATP to either NaCl and $MgCl_2$ or to KCl and $MgCl_2$ solutions at equal concentrations lead to the same significant increase in particle mobility. These results are contradictory to the ones obtained from radioactive binding. Therefore in answer to Point 1 mentioned above, the increase in charge density does not result from mere binding of the substrate. Concerning Point 2, it is generally accepted that in potassium buffers no phosphorylation can take place (Dahl and Hokin 1974; Skou 1975; Schwarz et al. 1975); potassium causes only dephosphorylation of the phosphorylated enzyme. Conformational changes of the ATPase polypeptide are probably responsible for the increase in surface charge density. Such conformational changes have been proposed by many authors and have been investigated in detail with the aid of fluorescent probes (Harris and Stahl 1977; Karlish and Yates 1978; Jorgensen and Karlish 1980), antibody reactions (Koepsell 1979A) and trypsin digestion (Koepsell 1979B). Sodium, magnesium and ATP or magnesium and P_i convert the ATPase protein from a E_1Na state to a E_2P state (for a review of the different enzyme states: Dahl and Hokin 1974; Skou 1975; Schwartz et al. 1975; Sen et al. 1969). This conformational change is related to a large increase in fluorescence (Harris and Stahl 1977; Karlish and Yates 1978). As the yield of fluorescent

probes is also influenced by the charge density (Eisenberg et al. 1979; McLaughlin 1977) an increased observed fluorescence should correspond to a higher surface potential, i.e., a higher charge density. Our results are in agreement with the fluorescence data. Karlish and Yates (1978) could not only detect an increase in fluorescence by adding ATP (transitions from E_1Na to E_2K or from E_1Na to E_2P) but also by adding KCl (transition from E_1Na to E_2K). In our experiments only a slight decrease in particle mobility (less than 1%) could be observed when KCl (1 mM) was added to the ATPase suspended in *Tris*-HCl. The same effect could be observed by adding 1 mM NaCl instead of KCl. These are screening effects of electrolytes, according to the Gouy-Chapman theory (for reviews see McLaughlin 1977; Aveyard and Haydon 1973) and are not due to conformational changes.

In Fig. 3A the effect concentration curves are shifted with increasing Mg^{2+} concentrations in the suspending medium. This effect might reveal an antagonistic behavior of magnesium against ATP, although some Mg^{2+} is required for the ATP effect. It may be derived from these curves that the best substrate for ATPase activity is ATP/Mg^{2+} in equimolar concentrations. Skou points out that magnesium in a non optimal concentration inhibits the enzyme (Skou 1974). The saturation mobility determined for high ATP concentration even at lowest ionic strength exceeds that of a control in the absence of magnesium.

This means that ATP is not chelating Mg^{2+}, reversing the mobility decreasing effect of magnesium ions (Fig. 1) but is increasing surface charges by inducing conformational changes of the enzyme. Further support for this hypothesis comes from experiments, where the effect of EDTA alone on the vesicle mobility was investigated. Up to a concentration of 1 mM, particle mobility was not changed by EDTA, indicating that the ATPase preparation was not contaminated by divalent cations.

The low dissociation constants, derived from these curves indicate that the ATP effect results from the interaction with low-affinity sites on the ATPase polypeptide. Binding studies revealed an ATP dissociation constant of 0.1–0.3 μM (Hegyvary and Post 1971; Norby and Jensen 1971), while from enzyme kinetics a dissociation constant of 100–400 μM was determined (Robinson 1976; Glynn and Karlish 1976; Matsui and Schwartz 1966). Therefore a high-affinity binding site being responsible for outward Na^+ transport (Glynn and Karlish 1976) and a low-affinity site for inward K^+ transport (Simons 1974) have been postulated. For activating the low-affinity site magnesium ions are always required. Possible conformational changes of the high-affinity site could not be detected by our method. As discussed in detail by Robinson (Robinson 1976) the two ATP binding sites are supposed to be situated on two different monomers, forming the ATPase polypeptide. Our results seem to suggest that only the charges of the low affinity binding site contribute to electrophoretic mobility.

The concentration effect curves at pH 3.3 are shown in Fig. 3B. A stronger binding of ATP can be suggested from these curves. This effect has already been observed in the radioactive binding experiment. As pH 3.3 is beyond the isoelectric point (see Fig. 5) most of the negatively charged groups on the surface of the ATPase vesicles are protonated. An unspecific binding of ATP to

protonated polar groups may also occur at this pH. A possible ATP induced conformational change at this pH is probably screened off by the neutralizing effect of ATP on protonated groups. In experiments not shown here, ATP at pH 3.3 also reduced particle mobility in the absence of Mg^{2+}, indicating that possibly protons substitute Mg^{2+}.

Other nucleotides like ITP and GTP show similar effects to ATP with the same dissociation constants (Fig. 4). ADP shows a slightly lower dissociation constant. These findings seem to be in contradiction to the results of Hegyvary and Post (1971) obtained for a similar ATPase preparation. These authors find clear differences in the dissociation constants of various substrates: ATP shows the lowest (K_d = 0.2 μM) followed by ADP and the one for ITP and GTP is three orders of magnitude higher. But these results were obtained for the high-affinity binding site and as discussed above we are dealing with the low-affinity site. Obviously other substrates than ATP are able to induce similar conformational changes. The low-affinity site may also serve for the binding of P_i. As shown in Fig. 4, *Tris*-phosphate is able to change the mobility of the ATPase vesicles by inducing more charges, but it does not seem to be as effective as ATP. Again the question arises whether the substrate induced charges are due to the binding of the charged molecules or to conformational changes. There is some evidence that also during P_i-phosphorylation a conformational change of the enzyme is taking place (Schuurmans Stekhoven et al. 1976, 1980. For that case we could postulate that the P_i-induced conformational change is different from the one observed under ATP. Schuurmans Stekhoven et al. (1980) investigated the P_i- and the ATP-binding site by successive phosphorylation and found that a maximum of 1 mol phosphate/mol enzyme can be accepted by the ATPase, either from P_i or ATP or from both. The authors interpret their results by postulating "either a single common phosphorylation site for P_i and ATP or a conformational change of the enzyme following phosphorylation by P_i, which excludes phosphorylation by ATP". Similar studies by us principally confirmed the results of Schuurmans Stekhoven et al.: additional application of small amounts of ATP on the P_i treated enzyme led to the fully phosphorylated protein; the same particle mobility could be measured as obtained by saturational concentrations of ATP alone.

Magnesium ions are required for the phosphorylation of the ATP low-affinity site (Skou 1974). The effect of $MgCl_2$ on ATPase particle mobility has been studied. The results in Fig. 2 demonstrate that at pH 7.3 Mg^{2+} bind to the negatively charged vesicle surface and reduce the charge density. At an acidic pH the mobility-Mg^{2+}-concentration curve is shifted to higher concentrations. Because of protonization of the negative surface groups magnesium exerts its effect via simple screening mechanisms (McLaughlin 1977; Aveyard and Haydon 1973; D'Arrigo 1978).

From Fig. 5 an isoelectric point of 4.1 can be derived. This value almost corresponds to the pK of the β-carboxyl group of aspartic acid which is supposed to be the site for phosphorylation (Post and Orcutt 1973; Degani and Boyer 1973). ATP shifts the whole curve to higher mobility values from which follows a shift of the isoelectric point to a more acidic pH.

In our recent paper (Schlieper et al. 1981) we have discussed the ATP induced reduction in vesicle size as still another explanation for the increase in

surface charge, i.e., vesicle mobility. In light scattering theory, peak linewidth is a direct measure of particle size. Under the realistic assumption the preparation stays homogeneous, the spectra in Fig. 2 clearly show that peak linewidth is increased under ATP action, indicating a decrease in the size of the ATPase vesicles. This decrease was also observed under GTP, ITP, and ADP and could perhaps be attributed to an active extrusion of ions. However no change in linewidth could be detected at pH 3.3 or after treatment with ouabain at pH 7.4. With ouabain and obviously at pH 3.3 ATPase activity is completely blocked. Linewidth measurements under electrophoretic conditions only allow a qualitative estimate of the particle size. The size change observed under ATP action should be investigated in more detail.

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