Biochimica et Biophysica Acta, 644 (1981) 147–152
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71519

A (Ca^{2+}, Mg^{2+})-ATPase ACTIVITY IN PLASMA MEMBRANE FRAGMENTS ISOLATED FROM SQUID NERVES

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(Received March 11th, 1981)

Key words: (Ca^{2+}, Mg^{2+})-ATPase; Ca^{2+} transport; Ca^{2+} regulation; Squid optical axons

A (Ca^{2+}, Mg^{2+})-ATPase activity and a (Ca^{2+}, Mg^{2+})-dependent phosphorylation from ATP have been found in plasma membrane fragments from squid optical nerves under conditions where contamination by intracellular organelles is unlikely. The properties of this (Ca^{2+}, Mg^{2+})-ATPase activity are almost identical to those of the ATP-dependent uncoupled Ca^{2+} efflux observed in dialyzed squid giant axons. This gives further support to the notion that the mechanism responsible for maintaining the low levels of ionized Ca concentration in nerves at rest is not a Na^+-Ca^{2+} exchange system but an ATP-driven uncoupled Ca^{2+} pump.

There is presently a controversy as to how nerve cells manage to maintain an exceedlingly low [Ca_i^{2+}] against very large electrochemical Ca^{2+} gradients. It has been proposed that the responsible mechanism is a Na_o^+-Ca_i^{2+} countertransport, where the energy derived from the Na^+ electrochemical gradient (which favours net Na^+ gain) is used to expel Ca^{2+} out of the cells (for review see Ref. 1). Although a passive Na^+-Ca^{2+} exchange is supported by experimental evidences obtained in cells with high [Ca_i^{2+}], it cannot account for a series of data recently obtained in axons with normal levels of [Ca_i^{2+}]. Thus, in squid axons with physiological [Ca_i^{2+}], Ca efflux is almost completely insensitive to external Na^+, Ca^{2+} and Mg^{2+} [2–5]; in addition, ATP appears as an absolute requirement to promote an efflux of Ca^{2+} above the leak levels and to keep the balance of Ca^{2+} fluxes [5]. This ATP-dependent uncoupled Ca^{2+} efflux is also present at high Ca_i^{2+} concentrations [5], and can be demonstrated in the absence of any ionic gradients [6]. This has lead to the suggestion [5,6] that nerve cells possess an ATP-driven Ca^{2+}-pump mechanism which is responsible for maintaining a large electrochemical gradient for [Ca^{2+}] under resting conditions. If this latter hypothesis is true, it should be possible to demonstrate a Ca^{2+}-dependent ATPase activity in plasma membrane isolated from squid nerves. In addition, the properties of that ATPase should resemble those of the ATP-dependent Ca^{2+} transport in intact cells. The experiments we report here indicate that plasma membrane fragments isolated from fibers of the squid optical nerve do have a (Ca^{2+}, Mg^{2+})-ATPase activity. The apparent affinities of this enzyme for ATP and Ca^{2+} are the same as those observed for Ca^{2+} transport in dialyzed squid giant axons.

The experiments were performed on membrane fragments isolated from the optical nerve of the squid *Sepiotheutis sepiodea*. The optical nerve was chosen because the giant axons do not provide with enough material for the assays. For similar reasons the *Sepiotheutis sepiodea*, instead of the *Dorytheutis plei*, was selected. The optical nerve of this species consists of several bundles of about 1 cm length and less than 1

mm in diameter. Each bundle is composed of thousands of unmyelinated nerve fibers surrounded by one single layer of Schwann cells. The proportion of fibrous tissues is negligible. Accordingly, the dissected material has a much higher membrane area due to nerve fibers than to Schwann cells. Plasma membranes were isolated following the method of Fischer et al. [7] with modifications [8]. The usual yield was about 0.4 g of nerve per squid and about 1 mg of membrane protein per gram of nerve tissue. The membrane fraction I (see Ref. 8) was used throughout this work. The experimental evidence is consistent with this fraction being composed mostly of nerve plasma membrane fragments, whereas fragments derived from the Schwann cells are present in the so called Fraction II [8]. The membrane fragments were stored at −70°C (5–8 mg protein/ml) in a solution containing 0.78 M sucrose, 30 mM Tris-HCl, pH 7.4 and 1 mM EDTA. Before used the membranes were washed twice with 30 mM Tris-HCl, pH (20°C) 7.4. Succinate dehydrogenase activity was assayed by the method of Slater and Bonner [9] and glucose-6-phosphatase activity by the method of Swanson [10]. ATP hydrolysis was determined either from the release of inorganic phosphate, by a modification of the method of Fiske and SubbaRow [11], or using (γ-^{32}P)-labelled ATP with isobutanol extraction of the ^{32}P radioactivity. Protein was determined by the method of Lowry et al. [12]. The experiments were performed at 25°C and pH 7.4. All chemicals were reagent grade.

The purity of the membrane fragments was evaluated by (1) enzymatic and (2) electronmicroscopic studies. (1) Three enzyme marker activities were assayed: glucose-6-phosphatase (endoplasmic reticulum), succinate dehydrogenase (mitochondria) and ($Na^+ + K^+$)-ATPase (plasma membrane). Glucose-6-phosphatase activity was below the detection limits of the method and succinate dehydrogenase activity was less than 1% of that found in pools of purified mitochondria. On the other hand there was a strong ($Na^+ + K^+$)-ATPase activity (see Table I); this activity was fully inhibited by 10^{-4} M ouabain. When checked, the specific activity of the ouabain sensitive ($Na^+ + K^+$)-ATPase activity was 10.2 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ in the initial homogenate and 30.5 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ in the final membrane fraction. The percentage yield was rather small amounting to 7% of the activity initially present in the homogenate. The assays of intermediary pellets and supernatants indicated that the low yield was not due to denaturation and/or inactivation but to an actual loss of cell membranes during the isolation procedure. (2) Electron microscopy: at the electron microscope (results not shown) we found vesicles of the type and size described by Fischer et al. [7] and Barnola et al. [8]. Also, in coincidence with their results, we found no indication of contamination by mitochondria, ribosomes (either free or bound) or any structure of fibrillar nature. On the basis of both criteria for purity used here (see also other enzymatic tests applied by Fischer et al. [7]) it seems unlikely that the ATPase activities reported in this paper come from structures other than plasma membrane fragments.

Table I summarizes the different ATPase activities found in membrane fragments isolated from squid optical nerves. In all the cases the values correspond to maximal, or near maximal, activities. The ($Na^+ + K^+$)-ATPase represents 75% of the total, whereas the

TABLE I

ATPase ACTIVITIES IN PLASMA MEMBRANE FRACTION ISOLATED FROM SQUID OPTICAL NERVES

The ($Na^+ + K^+$)-ATPase activity was calculated as the difference between the activity found in the presence of 50 mM KCl/460 mM NaCl/30 mM Tris-HCl and that found in the absence of Na^+ and K^+ with 510 mM Tris-HCl. The (Ca^{2+}, Mg^{2+})- and the Mg^{2+}-ATPase activities were determined in media containing 50 mM KCl/460 mM Tris-HCl. The (Ca^{2+}, Mg^{2+})-ATPase was taken as the difference between the activity observed in the presence of 0.6 mM $CaCl_2$ and that found with no added $CaCl_2$. In all cases the incubation media contained 3 mM ATP (Tris salt), 3 mM $MgCl_2$ and 0.5 mM EGTA. Temperature was 25°C and pH 7.4. The incubation time lasted 0.5 h to 1 h. All activities were linear with time and the total ATP hydrolyzed was always less than 15%. The release of phosphate was determined by a modification of the method of Fiske and SubbaRow [11]. The number of experiments (n), carried out in triplicate, is given.

	Activity (μmol $P_i \cdot mg^{-1} \cdot h^{-1}$)		
	($Na^+ + K^+$)-ATPase	(Ca^{2+}, Mg^{2+})-ATPase	Mg^{2+}-ATPase
Mean ± S.E.	27.3 ± 0.6	0.56 ± 0.07	8.34 ± 0.48
n	3	7	7

Mg^{2+}-ATPase is about 23%. The (Ca^{2+}, Mg^{2+})-dependent activity is rather small (less than 2% of the total and about 7% of the (Na^+, K^+)-independent activity) but close to that reported by Robinson [13] in fresh rat brain microsomes, where the Ca^{2+}-dependent activity was about 20% of the basal Mg^{2+}-ATPase. The optimum pH of the (Ca^{2+}, Mg^{2+})-ATPase was found between 7.3 and 8.0, with the activity declining more markedly towards the acid than the basic side of the pH curve. The presence of sodium or potassium ions, tested up to 200 mM concentration, had no effect on the Ca^{2+}-dependent activity. In all cases the ionic strength was in the range of that used in dialyzed squid axons. It is obvious that a difficulty of this work is the very small activity of the (Ca^{2+}, Mg^{2+})-ATPase relative to the Mg^{2+}-ATPase. However, in every single experiment performed the difference

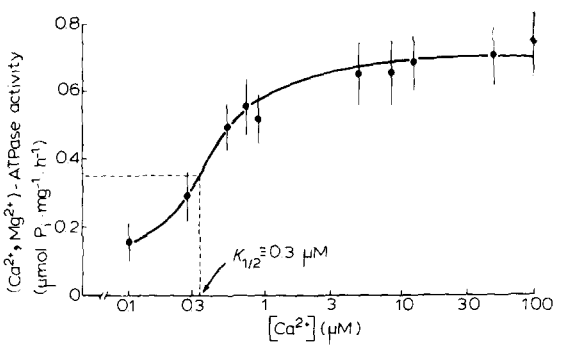

Fig. 1. Activation by Ca^{2+} of the (Ca^{2+}, Mg^{2+})-ATPase in membrane fragments of squid optical nerve. The incubation solutions had the following composition (mM): KCl 50; Tris-HCl, 450; $MgCl_2$, 0.5; ATP-Tris, 3; and EGTA 0.5. Enough $CaCl_2$ was added to give the desired ionized Ca concns., which were calculated from the multiple equilibria involving Mg, Ca, ATP and EGTA. The dissociation constants of the different complexes used for the computations were taken from estimations for our experimental conditions of pH, temperature and ionic strength (similar to those of squid axoplasm). The values used were: Mg · ATP, 0.7 mM (DeWeer, unpublished results); Mg · EGTA, 30 mM [32]; Ca · ATP, 1.4 mM (assuming it changes with pH, temperature and ionic strength in the same way as the K_d for Mg · ATP); Ca · EGTA, 0.15 μM [33]. Temperature was 25°C and pH 7.4. The incubation time lasted 0.5 h and the total ATP hydrolyzed never exceeded 15%. Phosphate was determined by a modification of the method of Fiske and SubbaRow [11]. The (Ca^{2+}, Mg^{2+})-ATPase activity was taken as the difference between the activity in the presence and absence of Ca^{2+}. Each point is the mean ± S.E. of three to four determinations. The total incubation volume was 1 ml.

between both activities was highly significant. The errors listed in Table I are not those of a single experiment but correspond to the dispersion of the means of all the experiments performed. Genuine differences between preparations seem the logical explanation for the fact that the absolute error of the Mg^{2+}-ATPase is nearly as large as the absolute value of the (Ca^{2+}, Mg^{2+})-ATPase.

Fig. 1 shows the activation by Ca^{2+} and the (Ca^{2+}, Mg^{2+})-ATPase in the range from 0.1 μM to 100 μM. The $K_{1/2}$ for Ca^{2+} from Fig. 1 is about 0.3 μM. The concentrations of Mg^{2+} and ATP were non limiting.

The effects of Mg^{2+} on the Mg^{2+}- and the (Ca^{2+}, Mg^{2+})-ATPases are shown in Fig. 2. Between parenthesis in the insert of the Figure are the values for free Mg^{2+} and the Mg · ATP concentrations. The different Mg^{2+} requirements for both ATPases is striking. For the Mg^{2+}-ATPase saturation is not reached even at 500 μM total $MgCl_2$ (experiments not shown indicate that the $K_{1/2}$ is about 1–2 mM) whereas for the Ca^{2+}-dependent component (insert in Fig. 2) the $K_{1/2}$ for $MgCl_2$ is close to 50 μM. Magnesium appears as an absolute requirement for the Ca^{2+}-dependent ATP hydrolysis. At 500 μM Mg^{2+} becomes slightly inhibitory; however, this small inhibition remained unchanged when $MgCl_2$ concentrations were increased up to 3 mM (not shown). It has been suggested [14] that if sarcoplasmic reticulum Mg^{2+} has two modes of action on the (Ca^{2+}, Mg^{2+})-ATPase, (i) it activates the enzyme directly, and (ii) it forms part of the Mg · ATP substrate. If Mg^{2+} are activators, the $K_{1/2}$ from Fig. 2 is about 20 μM. However, as an absolute value this $K_{1/2}$ for Mg^{2+} may not mean much because, although the total ATP concentration was kept constant at 2 mM, the concentration of Mg · ATP increased together with those of $MgCl_2$ and Mg^{2+}. If the substrate for the reaction is Mg · ATP [14], its concentration was also rate limiting (see Fig. 3) and this would make impossible to dissociate the effects of Mg^{2+} from those of Mg · ATP.

Fig. 3 represents an experiment on the ATP dependence of (Ca^{2+}, Mg^{2+})-ATPase where the total ATP concentration was varied from 10 μM to 160 μM and the total $MgCl_2$ concentration was kept constant at 0.5 mM. The experimental points were fitted with an expression following Michaelian kinetics. From a total of three experiments the value of K_m averages 43 ± 10 μM for total ATP and 18 ± 4 μM when Mg ·

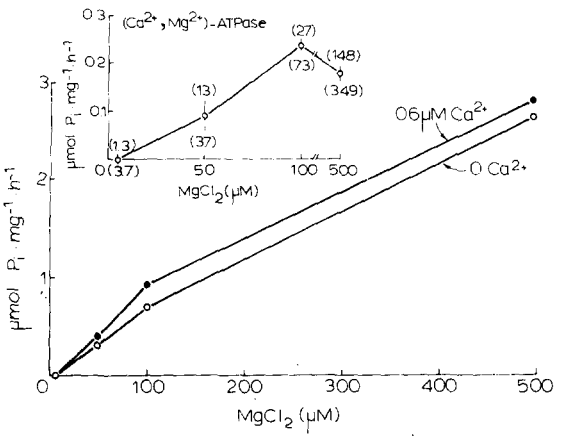

Fig. 2. Effects of Mg^{2+} on the Mg^{2+}- and (Ca^{2+}, Mg^{2+})-ATPase activities in membrane fragments of squid optical nerve. The incubation solutions had the following composition (mM): KCl, 50, Tris-HCl, pH (25°C) 7.4, 450; ATP-Tris, 2; EGTA, 0.5, $CaCl_2$, none or enough to give a Ca^{2+} concentration of 0.6 μM. The $MgCl_2$ concentrations are indicated in the figure. At zero $MgCl_2$ concentration tubes with and without EDTA were used, and both gave the same results. The incubation lasted 0.5 h at 25°C. Phosphate was determined by a modification of the method of Fiske and SubbaRow [11]. The calculation of free and bound species was done using the dissociation constants given in the legend to Fig. 1. The (Ca^{2+}, Mg^{2+})-ATPase was taken as the difference between the activity in the presence and absence of Ca^{2+}. Each point is the mean ± the S.E. of three determinations. The values between parenthesis in the insert represent the ionized magnesium (above) and the MgATP (below) concentrations. The total incubation volume was 1 ml.

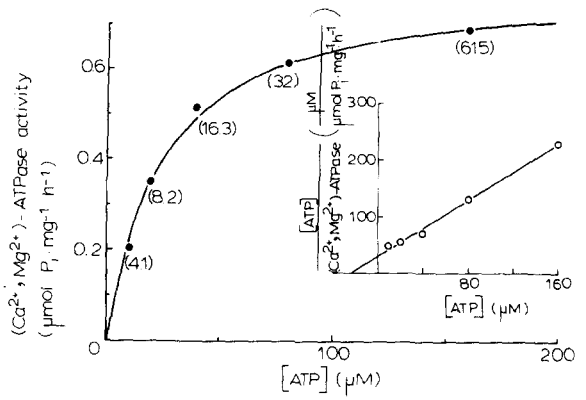

Fig. 3. The effects of ATP on the (Ca^{2+}, Mg^{2+})-ATPase activity in membrane fragments of the squid optical nerve. The solutions had the following composition (mM): KCl, 50; Tris-HCl, pH (25°C) 7.4, 450; $MgCl_2$, 0.5; EGTA, 0.5; $CaCl_2$ none or 0.55. The total ATP concentration is indicated in the ordinate axis of the figure and the insert. The Mg · ATP concentration (in μM) is between parenthesis in the figure. The incubation time varied from 10 to 30 min and the temperature was 25°C. A mixture of non radioactive and (γ-^{32}P)-labelled ATP was used as substrate. The reaction was started by transferring the tubes from an ice cold bath into a bath at 25°C, and was ended by returning the tubes to the ice bath. After 15 min in the cold 5 ml of ammonium molybdate 0.5% in 7% (w/v) perchloric acid were added, followed by 5 ml of isobutanol. The tubes were vigorously mixed by hand for 20 s and centrifuged at 1500 × g for 30 s. About 4 ml of the supernatant (isobutanol) were mixed with 5 ml of scintillator and counted. For the estimation of 100% hydrolysis, 0.01 ml of incubation solution were mixed with 1 ml of HCl 1 N and heated at 100°C for 30 min. The phosphate extraction was identical to that of the incubation tubes. The (Ca^{2+}, Mg^{2+})-ATPase was estimated as the difference between the activities found in the presence and absence of Ca^{2+}. The points are the means of duplicate determinations. The line through the points is the theoretical line corresponding to an hyperbola with K_m of 25 μM and V of 0.8 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$. A plot of substrate concentration/activity vs. substrate concentration is shown in the insert. The estimation of the Mg · ATP concentration was done as explained in the legend to Fig. 1. The total sample volume was 1 ml.

ATP is considered to be the substrate. The theoretical V is 0.68 ± 0.06 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$, which is within the range seen under conditions of ligands saturation in Table I and Fig. 1. It is also clear from Fig. 3 that, as the ATP concentration was varied, the free Mg^{2+} concentration did not change by more than 13% and remained always saturating. It is extremely unlikely that the results of these experiments are influenced by so small changes in free Mg^{2+}.

Other characteristics of the (Ca^{2+}, Mg^{2+})-ATPase activity described in this paper (not shown) are its insensitivity to ouabain (up to 10^{-3} M) and to oligomycin (up to 10 μg/ml) and the fact that it is completely inhibited by vanadate concentrations in the micromolar range. In addition, we were able to demonstrate in these membrane fragments a Ca^{2+}-dependent phosphorylation from ATP in the presence of Mg^{2+} and a fast spontaneous dephosphorylation rate. The phosphorylated protein was acid stable, indicating that the fast turnover rate is enzymatically produced.

In order to assess the physiological relevance of this (Ca^{2+}, Mg^{2+})-ATPase activity one must establish (i) if the fragments used indeed derive from plasma membrane of nerve fibers, (ii) what degree of contamination can be expected from intracellular orga-

nelles and Schwann cell membranes, and (iii) how do the properties of this Ca^{2+}-dependent ATPase correlate with those of the ATP dependent uncoupled Ca efflux in squid axons.

The fact that we were dealing with plasma membrane fragments is evidenced by the strong ouabain-sensitive ($Na^+ + K^+$)-ATPase activity present in the preparation. The histological characteristics of the optical nerve (see above) as well as previous works performed with this and similar methodologies [7,8] suggest that the contamination by Schwann cell plasma membranes is very small. In addition, the kinetic properties of the ($Na^+ + K^+$)-ATPase activity (activation by K^+, dependence on ATP concentration and inhibition by ouabain) were the same as those found for the active Na^+-K^+ transport in dialyzed squid axons [15]. In turn, this indicates that it is fair to compare the Ca^{2+}-dependent ATPase activity in these membrane fragments with the Ca^{2+} transport in dialyzed squid axons. The experiments with enzymatic markers together with the electromicroscopic studies suggest that contamination by Ca^{2+}-sequestering intracellular organelles (endoplasmic reticulum [16] and mitochondria [17]) is minimal or absent. The absence of any mitochondrial Ca^{2+}-ATPase is also indicated by two properties of the (Ca^{2+}, Mg^{2+})-ATPase described here: its high apparent affinity for Ca^{2+} and its insensitivity to oligomycin [18].

The main properties of the ATP-dependent uncoupled Ca^{2+} efflux in dialyzed squid axons [5,26] and the Ca^{2+}-dependent ATPase activity in membrane fragments from squid optical nerve superimpose to each other. Almost identical high apparent affinity for Ca^{2+} ($K_{1/2}$ of 0.2–0.3 μM) and intermediate high apparent affinity for MgATP ($K_{1/2}$ about 20 μM), the presence of Mg^{2+} is essential for both systems to operate. In addition, both mechanisms are insensitive to ouabain and oligomycin and are fully inhibited by vanadate acting with high apparent affinity. Furthermore, the ATP-dependence curve can be fitted in both systems with rectangular hyperbolae (Ref. 5, and DiPolo, R. and Beaugé, L., unpublished results). If we accept that the preparation used in this work represents mainly the behaviour of enzymes present in the plasma membrane of nerve fibers, the data strongly suggest that the ATP-dependent uncoupled Ca^{2+} efflux and the (Ca^{2+}, Mg^{2+})-dependent ATPase are closely related mechanisms. This gives further support to the notion that an uncoupled Ca^{2+} pump, similar to that of red cells [19] is responsible for maintaining the low levels of ionized Ca concentrations in squid axons under resting conditions.

It could be argued that if EGTA and/or Ca · EGTA excert some complex effects on the membrane [20] it is possible that the behaviour of the Ca^{2+}-pump is different in the presence and absence of EGTA. However, even if this were the case, a comparison between the results presented in this paper with those of Ca^{2+} efflux in dialyzed squid axons should still be valid because in both instances the regulation of Ca^{2+} concentration was obtained with EGTA. In addition, no appreciable differences in the behaviour of the (Ca^{2+}, Mg^{2+})-ATPase were observed when the EGTA concentration used was 0.5 mM or 1 mM. Obviously, the estimations for free Ca^{2+} are influenced by the Ca · EGTA stability constants employed.

A (Ca^{2+}, Mg^{2+})-ATPase activity with high affinity for Ca^{2+} has also been described in plasma membrane fragments from several tissues. It has been found in fat cells [21] together with an active Ca^{2+}-transport mechanism [22], rat brain [13], bovine brain [23], myometrium [24] and skeletal muscle [25,26]. The existence of (Ca^{2+}, Mg^{2+})-ATPase activity and ATP-dependent Ca^{2+}-pump has also been found in heart sarcolema [26–28], a tissue which is exitable and has a well established Na^+-Ca^{2+} exchange mechanism [30]. All of this indicates that the ATP-driven Ca^{2+}-pump is not confined to intracellular organelles and red cells, but must be considered a generalized mechanism present perhaps in the plasma membrane of all animal cells, being directly involved in the regulation of the intracellular Ca^{2+} concentration.

This work was aided by Grants from CONICET (Argentina) and S-1144 from CONICIT (Venezuela). We wish to thank Dr. Agustin Aoki, from the Centro de Microscopia Electronica, Universidad Nacional de Cordoba for the electron microscopic studies.

References

1 Requena, J. and Mullins, L.J. (1979) Q. Rev. Biophys. 12, 371–460
2 Brinley, F.J., Spangler, S.G. and Mullins, L.J. (1975) J. Gen. Physiol. 66, 223–250
3 Baker, P.F. (1972) Progr. Biophys. Mol. Biol. 24, 177–223
4 Baker, P.F. and McNaughton, P. (1978) J. Physiol. 276, 127–150

5 DiPolo, R. and Beaugé, L. (1979) Nature 278, 271–273
6 DiPolo, R. (1978) Nature 274, 390–392
7 Fischer, S., Cellino, M., Zambrano, F., Zampighi, G., Tellez-Nagel, M., Marcus, D. and Canessa-Fischer, M. (1970) Arch. Biochem. Biophys. 138, 1–15
8 Barnola, F.V., Villegas, R. and Camejo, G. (1973) Biochim. Biophys. Acta, 298, 84–94
9 Slater, E.C. and Bonner, W.D. (1952) Biochem. J. 52, 185–196
10 Swanson, M.A. (1955) in Methods in Enzymology (Colowick, S.P. and Kaplan, N.O., eds.), Vol. 2, pp. 541–543, Academic Press, New York
11 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1971) J. Biol. Chem. 193, 265–275
13 Robinson, J.D. (1976) Arch. Biochem. Biophys. 176, 366–374
14 Makinose, M. and Boll, W. (1979) in Cation Flux Across Biomembranes, (Mukohata, Y. and Packer, L., eds.), pp. 85–102, Academic Press, San Francisco
15 Beaugé, L. and DiPolo, R. (1981) J. Physiol., in the press
16 Baker, P.F. and Schlapfer, W.W. (1978) J. Physiol. 276, 103–125
17 DeBuve, C., Wattiaux, R. and Blaudhuin, P. (1962) Adv. Enzymol. 24, 291–299
18 Scarpa, A. (1979) in Membrane Transport in Biology (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), Vol. 2, pp. 263–355, Springer Verlag, New York
19 Schatzmann, H.S. (1975) in Currents Topics in Membrane Transport, (Bronner, G. and Kleinzeller, A., eds.), pp. 125–168, Academic Press, New York
20 Gill, D.L., Grollman, E.F. and Kohn, L.D. (1981) J. Biol. Chem. 256, 184–192
21 Pershadsingh, H.A. and McDonald, J.M. (1980) J. Biol. Chem. 255, 4087–4093
22 Clausen, T. and Martin, B.R. (1977) Biochem. J. 164, 251–255
23 Saemark, T. and Vilhard, H. (1979) Biochem. J. 181, 321–330
24 Akerman, K.E. and Wikström, M.K. (1979) FEBS Lett. 97, 283–287
25 Sulakhe, P.V., Drummond, G.I. and Ng, D.C. (1973) J. Biol. Chem. 248, 4150–4157
26 Sulakhe, P.V., Drummond, G.I. and Ng, D.C. (1973) J. Biol. Chem. 248, 4158–4162
27 Caroni, P. and Carafoli, E. (1980) Nature 283, 765–767
28 Hui, C., Drummond, M. and Drummond, G. (1976) Arch. Biochem. Biophys. 173, 415–427
29 St. Louis, P. and Sulakhe, P.V. (1976) Can. J. Biochem. 54, 946–956
30 Reuter, H. and Seitz, M. (1968) J. Physiol. 195, 451–470
31 DiPolo, R., Rojas, H. and Beaugé, L. (1979) Nature 281, 228–229
32 Brinley, F.J., Scarpa, A. and Tiffert, T. (1977) J. Physiol. 266, 545–564
33 DiPolo, R., Requena, J., Brinley, F.J., Mullins, L.J., Scarpa, A. and Tiffert, T. (1976) J. Gen. Physiol. 67, 433–467