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Binding of manganese ions to the Na^+/K^+-ATPase during phosphorylation by ATP

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The aim of the present work was to study the Mg^{2+}–Na^+/K^+-ATPase interaction that was proposed to lead to the formation of a stable Mg-enzyme complex during phosphorylation from ATP. Instead of Mg we used Mn, which can replace Mg as essential activator of Na^+/K^+-ATPase activity. The amounts of steady-state Mn bound to the enzyme were estimated at 0 °C on the basis of the ^{54}Mn remaining in the effluent after passing the reaction mixture through a cation exchange resin column. As a function of the $MnCl_2$ concentration, the amount of Mn retained by the enzyme in the absence and presence of ATP showed a saturable and a linear component; the slope of the linear component was the same in both instances (0.016 nmol/mg per μM). The ATP-dependent Mn binding could be adjusted to a hyperbolic function with a K_m of 0.76 μM. The ratio [ATP-dependent E-Mn]/[E-P] measured at 5 μM $MnCl_2$ and 5 μM ATP was not different from 1.0, both in native ($Mn\text{-}E_2\text{-}P$) as well as in a chymotrypsin treated enzyme ($Mn\text{-}E_1\text{-}P$). When the Mn · E-P complex was allowed to react with KCl (E_2-P form) or ADP (E_1-P form), the enzyme was dephosphorylated and simultaneously lost the strongly bound Mn in such a way that the ratio [ATP-dependent E-Mn]/[E-P] remained 1:1. These results show the existence of strongly bound Mn ions to Na^+/K^+-ATPase during phosphorylation by ATP. That binding is (i) of high affinity for Mn, (ii) probably on a single site, and (iii) with a stoichiometry Mn-P_i of 1:1.

Introduction

Since the discovery of the Na^+/K^+-ATPase, it was known that that enzymatic activity required Mg^{2+} ions as an essential activator [1]. In addition, it was found that the optimal $MgCl_2$ concentrations were equal to those of ATP for the Na^+-ATPase activity whereas they doubled ATP when hydrolysis took place in the presence of Na + K [1]; this could indicate that, besides forming the Mg · ATP complex, Mg might have an activating role of its own. Based on the fact that the Mg concentrations required for ATP–ADP exchange were lower than those required for Na^+/K^+-ATPase activity, it was suggested that there was indeed a stimulating effect of Mg^{2+} that took place beyond the phosphorylation step accelerating the $E_1(P)$–$E_2(P)$ phosphoenzyme conversion [2,3]. This hypothesis was later considered unlikely [4], but the relationships between the Mg · ATP complex, free Mg^{2+} and free ATP with the Na^+,K^+-dependent ATP hydrolysis remained undefined and up to the present times there is no

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agreement about what ATP form is the real substrate for the reaction [5–10]. On the other hand, there are overwhelming evidences that Mg ions per se indeed have effects on the enzyme; these effects are activating as well as inhibitory, and the Mg^{2+} concentrations required depend on the reactions or ligand interactions being looked at. Thus, Mg^{2+} stimulates with high affinity (micromolar range) ATPase activity [7], enzyme phosphorylation from ATP [8] and ATP–ADP exchange [2,11,12] and with low affinity (millimolar range) phosphatase activity [13], phosphorylation by inorganic phosphate [14], vanadate binding [15] and the induction of conformational changes [16]. On the other hand, Mg^{2+} inhibition of ATP–ADP exchange takes place in the micromolar range [12,17] whereas other inhibitory actions (ATPase activity [18–21], active Na^+/K^+ transport [22] and nucleotide binding [23]) show low affinity for Mg^{2+}.

All the experimental evidence that has been described so far addresses to the questions about the number and identity of the Mg^{2+} binding sites present in the ATPase molecule. Different schemes for Mg^{2+} inhibition of ATPase activity have been proposed [17,18–21], but even in those cases where the inhibitor could act at more than one step in the reaction cycle [18–21] the possibility remains that the 'geographical' site is the same with some of its properties changed. On the basis of physical evidences a single Mg^{2+} binding site has been recognized in the enzyme [24]. Very recently, it has been proposed that during phosphorylation by inorganic phosphate Mg^{2+} is bound to a site which is different from that involved in E-P formation from ATP [10]. On the other hand, there is indirect evidence that in the ATPase cycle E-P has Mg tightly bound to it [25], although there is no indication about the number of Mg ions per phosphorylating units.

The results of these experiments, part of which have already been communicated elsewhere [26], confirm the existence of a strong binding of Mn^{2+} to Na^+/K^+-ATPase during ATP promoted phosphorylation. That binding is (i) of high affinity for Mn^{2+}, (ii) very likely on a single site, and (iii) with a Mn^{2+}-inorganic phosphate stoichiometry of one to one. While this work was in process, D.E. Richards (Ref. 27, and personal communication) using cobalt as Mg^{2+} substitute also found a phosphorylation dependent Co^{2+} binding to this enzyme.

Methods

Pig kidney Na^+/K^+-ATPase partially purified according to Jørgensen [28] was used throughout; the specific activity was 15–20 units/mg and it remained stable when the preparation was stored (3–5 mg protein/ml) at −85°C in 25 mM imidazole (pH (20°C) 7.5)/2 mM EDTA/10% sucrose. Immediately before used, and in order to remove any tightly bound Mg, the enzyme was washed five times (1 : 10, v/v) and resuspended in a solution containing 100 mM NaCl/10 mM imidazole without sucrose and EDTA.

ATPase activity was assayed as in Ref. 29 following the release of $[^{32}P]P_i$ from $[\gamma\text{-}^{32}P]ATP$ labelled according to the method of Glynn and Chappell [30], slightly modified [31]. Protein was determined by the method of Lowry et al. [32].

The amount of manganese bound to the enzyme was determined at 0°C on the basis of the $^{54}Mn^{2+}$ remaining in the effluent after the reaction mixtures were passed through a 0.5 ml Dowex 50–8–400 cation-exchange columns using the same system described for Rb^+ occlusion experiments [29]. Aliquots of 0.05 mg enzyme were preincubated for 30 s with variable concentrations of $^{54}MnCl_2$, 100 mM NaCl and 70 mM imidazole (pH (20°C) 7.5); when KCl was also present (20 mM) the amount of imidazole was reduced accordingly. After 20 s of adding 0.005 mM ATP, or equal volume of buffer, the reaction mixture was passed through the cation exchange resin column; the time that the enzyme spent in contact with the resin was about 0.7 s. In parallel experiments where what was measured was the amount of phosphoenzyme formation, the protocol was identical but unlabelled $MnCl_2$ and $[\gamma\text{-}^{32}P]ATP$ were used instead. In these cases, the effluent was collected in 2 ml of ice cold solution containing 20% (w/v) trichloroacetic acid/1 mM ATP/5 mM inorganic phosphate; after 15 min in the cold the denatured protein was retained in Whatman GF/F glass fiber filters, washed with 5% trichloroacetic acid/5 mM inorganic phosphate and counted in a liquid scintillation counter.

The effects of KCl and ADP on the release of labelled inorganic phosphate or Mn from Mn · E-P were investigated using the two syringes, mixing chamber and resin column already described in detail for Rb^+ occlusion experiments [33] with slight modifications. In this case, in order to remove external Mn before the KCl or ADP challenge, there was a 0.3 ml resin column in the syringe containing the phosphorylated Na^+/K^+-ATPase. On the other hand, to reduce the time of exposure to the last column to a minimum, that column was only 0.2 ml in volume and was inserted directly below the mixing chamber (without connecting tube); with this arrangement, an excellent performance could be attained with a total contact time of 0.15 s. In this set up, one of the syringes contained the phosphorylated enzyme (in the same solution as before) with either $^{54}Mn^{2+}$ and unlabelled ATP or unlabelled Mn^{2+} and [γ-^{32}P]ATP; the other syringe contained the same solution without Mn^{2+} and ATP with the addition of KCl or ADP at twice the desired final concentration. The effects of ADP were studied on Na^+/K^+-ATPase subjected to partial chymotrypsin inactivation prior to phosphorylation from ATP; this procedure is described in Ref. 34. The temperature during dephosphorylation was between 0 and 4°C.

All solutions were made with de-ionized bidistilled water. NaCl and KCl were of spectrometric grade; the other chemicals were of reagent grade. Ouabain, imidazole, ATP (vanadium-free), ADP and chymotrypsin were from Sigma Chemical Co, U.S.A. $^{54}Mn^{2+}$ was purchased from New England Nuclear and [^{32}P]P_i from the Comisión Nacional de Energía Atómica of Argentina.

Radioactivity assays were performed in a Beckman liquid scintillation counter using a toluene based scintillator; counting times were long enough to obtain standard errors of approx. 1%. When required, curve fitting was performed with a non-linear regression computer program.

Results

In a preliminary group of experiments (not shown) we confirmed results reported by Robinson [6] in the sense that manganese was able to substitute for magnesium as essential activator of both Na^+/K^+- and Na^+-ATPase activities (3 mM ATP and 130 mM NaCl with and without 20 mM KCl), and that that effect was concentration dependent. At 0.1 mM or less, the levels of activities were practically the same with both divalent cations; above that concentration Mn^{2+} was less effective than Mg^{2+} and above 2 mM it became inhibitory. These results enabled us to use Mn^{2+} in the micromolar concentration range as Mg^{2+} analogue.

Fig. 1 summarizes the results of a series of experiments where the amount of $^{54}Mn^{2+}$ retained by Na^+/K^+-ATPase after passing through a cation-exchange column was measured as a function of $MnCl_2$ concentration in the absence and presence of ATP. The concentrations of $MnCl_2$ ranged from 0.1 μM to 50 μM whereas that of ATP was kept constant at 5 μM; temperature was 0°C. Prior to the addition of the nucleotide (or equal volume of buffer) the enzyme was equilibrated with all ligands (100 mM NaCl, 70 mM imidazole-HCl and $^{54}MnCl_2$). With and without ATP a saturable and a linear component were observed; the linear component had the same slope in both cases (0.016 nmol/mg per μM). On the other hand, the addition of ATP markedly

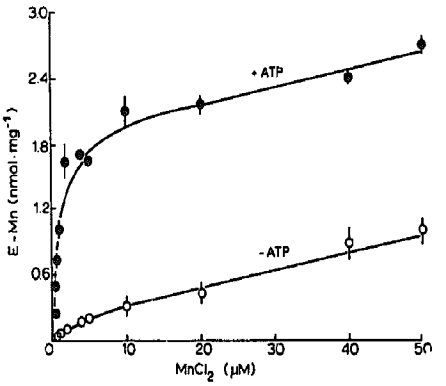

Fig. 1. Binding of ^{54}Mn to partially purified Na^+/K^+-ATPase as a function of $^{54}MnCl_2$ in the absence (open circles) and presence (filled circles) of phosphorylation from ATP. The composition of the incubation solutions was (mM): NaCl, 100; imidazole (pH 7.4 at 20°C), 30; ATP 0 or 0.005 and $^{54}MnCl_2$ 0.0001 to 0.05. Temperature throughout the experiment was 0°C. Binding was estimated from the ^{54}Mn remaining in the effluent after passing the reaction mixture through a Dowex cation exchange resin column. Each point is the mean ± S.E. of three to five different experiments. For details see Methods.

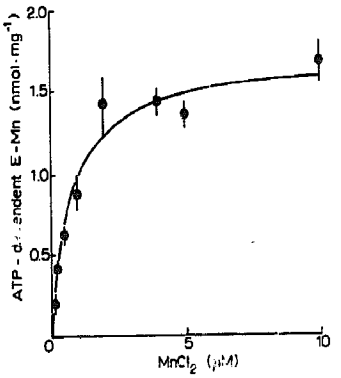

Fig. 2. ATP-dependent Mn binding to partially purified Na^+/K^+-ATPase. The data corresponds to the experiments illustrated in Fig. 1. The line through the points is the fit to an hyperbolic function with a K_m of 0.75 ± 0.13 μM and a maximal binding of 1.71 ± 0.09 nmol Mn/mg protein.

increased the saturable component of bound Mn. When that ATP-stimulated binding was plotted as a function of $MnCl_2$ concentration (Fig. 2), the points could be fitted reasonably well to a hyperbolic function with a K_m of 0.76 ± 0.13 μM and a maximal binding of 1.71 ± 0.09 nmol Mn^{2+}/mg protein.

The following group of experiments was designed to test the stoichiometry between the number of Mn^{2+} ions bound and acid-stable phosphorylated sites. Preliminary results (not shown) indicated that whereas acid denaturation of the enzyme preserved the phosphorylated intermediate it resulted in a complete loss of manganese. For this reason we tackled the problem running parallel experiments on ^{54}Mn trapping (with and without unlabelled ATP) and phosphorylation from [γ-^{32}P]ATP (with unlabelled Mn) keeping all other conditions constant; in the latter case the effluent from the columns was collected in ice-cold acid media. The concentrations of $^{54}MnCl_2$ and ATP were both 5 μM. The results of five different experiments, carried out over a period of several months and with different batches of enzyme, are summarized in Table I. They clearly indicate that the ratio between the ATP-dependent E-Mn and the amount of phosphoenzyme formation is 1 : 1. The upper part of Table II shows complementary experiments were Mn^{2+} binding and phosphoenzyme formation were compared in the absence and presence of 30 mM KCl in the incubation solution. Without KCl the results repeated those of Table I; with KCl there was no detectable phosphorylation and the ATP-dependent Mn^{2+} binding was non existing.

All phosphorylating conditions explored so far were likely to result in the formation of phosphoenzymes in the E_2 state. In order to see if the same behavior was observed in phosphoenzyme formed from ATP but remaining in the E_1 state, we explored ATP-dependent Mn^{2+} binding and phosphorylation in Na^+/K^+-ATPase partially inactivated with chymotrypsin. The lower part of Table II indicates that also under these conditions there is an ATP-dependent Mn^{2+} binding with a 1 : 1 stoichiometry in relation to E-P. In addition, the presence of 0.02 mM ADP (just four times the ATP concentration) led to a complete absence of phosphorylation and ATP-dependent Mn^{2+} binding.

The next steps explored the release of inorganic phosphate and Mn^{2+} from the Mn · E-P complex when the enzyme was in the E_1 and E_2 conformations. The rationale was the following; if the release of Mn^{2+} following dephosphorylation is slow enough, it might be possible to separate it from E-P breakdown and to study the effects of related ligands (K^+ and its congeners, different nucleotide, etc.) on the stability of E-Mn after dephos-

TABLE I

RELATIONSHIP BETWEEN THE AMOUNTS OF ATP-DEPENDENT E-Mn COMPLEX AND PHOSPHOENZYME FORMATION FROM ATP

The experiments were performed as described in the legend to Fig. 1 and Methods. Each entry is the mean ± S.E. of triplicate determinations using different batches of enzyme. The concentrations of ATP and $MnCl_2$ were 0.005 mM. Temperature was 0 °C.

Expt. No.	ATP-dependent E-Mn (nmol/mg)	E-P (nmol/mg)	ATP-dependent E-Mn/E-P
1	0.97 ± 0.03	0.90 ± 0.02	1.08
2	0.95 ± 0.08	0.91 ± 0.04	1.03
3	1.50 ± 0.15	1.49 ± 0.03	1.00
4	1.40 ± 0.10	1.74 ± 0.02	0.80
5	1.80 ± 0.10	1.71 ± 0.02	1.06
Mean ± S.E.	1.32 ± 0.16	1.35 ± 0.19	1.00 ± 0.05

TABLE II

EFFECTS OF KCl AND ADP IN THE INCUBATION MIXTURE ON THE AMOUNTS OF ATP-DEPENDENT E-Mn AND PHOSPHOENZYME FORMATION FROM ATP IN NATIVE AND CHYMOTRYPSINE TREATED Na^+/K^+-ATPase

The experiments were performed as described in the legend to Fig. 1 and Methods. All ligands were present at the beginning of phosphorylation. The concentrations of ATP and $MnCl_2$ were 0.005 mM and the temperature was 0°C. Each entry is the mean ± S.E. of triplicate determinations.

Enzyme pretreatment	Incubation solution					ATP-dependent E-Mn (nmol/mg)	E-P (nmol/mg)
	NaCl (mM)	KCl (mM)	$MnCl_2$ (mM)	ATP (mM)	ADP (mM)		
None	100	–	0.005	0.005	–	1.62 ± 0.10	1.55 ± 0.06
None	100	30	0.005	0.005	–	0.08 ± 0.07	0.05 ± 0.03
Chymotrypsin	100	–	0.005	0.005	–	1.32 ± 0.07	1.37 ± 0.04
Chymotrypsin	100	–	0.005	0.005	0.02	0.10 ± 0.07	0.07 ± 0.04

phorylation. To attain this aim, we modified the two syringes and chamber system in such a way that the contact time of the reaction mixture with the cation exchange columns was approx. 150 ms (see Methods). Just like before, $Mn \cdot E_2$-P was obtained with native enzyme whereas $Mn \cdot E_1$-P with enzyme partially inactivated with chymotrypsin. The results of these experiments, illustrated in Figs. 3 and 4, indicate that within the resolution of the method it is not possible to separate the release of inorganic phosphate from that of Mn. Therefore, the 1 : 1 stoichiometry between ATP-dependent E-Mn and E-P was maintained regardless of the phosphorylation levels. It is important to point out that the total dephosphorylation time consisted of approx. 0.05 s in the mixing chamber plus 0.15 s in the resin column. In addition

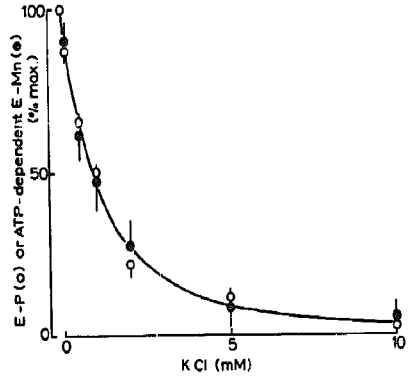

Fig. 3. Effects of different concentrations of KCl on the levels of E-P (open circles) and ATP-dependent E-Mn (filled circles). The enzyme was in contact with KCl after 20 s phosphorylation in 100 mM NaCl with 0.005 mM [γ-^{32}P]ATP and 0.005 mM unlabelled $MnCl_2$ or the same concentrations of unlabelled ATP and $^{54}MnCl_2$. In these experiments two syringes, a mixing chamber and a 0.2 ml Dowex resin column were used. The exposure to potassium lasted about 0.2 s at a temperature between 0 and 4°C. Each point is the mean ± S.E. of triplicate determinations and expresses E-P and E-Mn as percentage of those observed when KCl was omitted; the 100 percent values were 1.65 ± 0.01 nmol/mg and 1.62 ± 0.05 nmol/mg for the ATP-dependent E-Mn and E-P, respectively. For details see Methods.

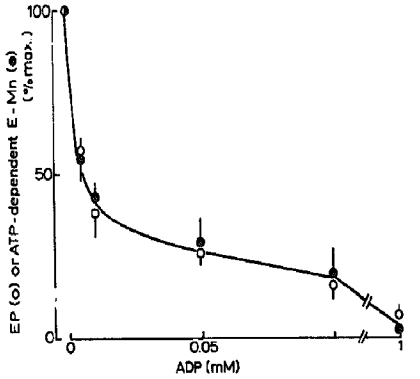

Fig. 4. Effects of different concentrations of ADP on the levels of E-P (open circles) and ATP-dependent E-Mn (filled circles). The general technique was similar to that described in the legend to Fig. 3 except that the enzyme had been previously partially inactivated with chymotrypsin and ADP replace KCl. Each point is the mean ± S.E. of triplicate determinations and represents E-P and E-Mn as percentage of those observed when ADP was omitted; the 100 percent values were 1.28 ± 0.08 nmol/mg and 1.22 ± 0.03 nmol/mg for the ATP-dependent E-Mn and E-P, respectively. See Methods for details.

dition, the temperature of dephosphorylation, although constant through the experiment, could have been as high as 4°C.

Discussion

The experiments on manganese binding have confirmed the hypothesis [14,25] that in the early stages of the cycle of ATP hydrolysis, more precisely at the moment of enzyme phosphorylation from ATP, the essential divalent cation activator becomes tightly bound to the Na^+/K^+-ATPase, and it remains so as long as the enzyme stays phosphorylated. In addition, these results show that the stoichiometry between the number of ATP-dependent Mn^{2+} bound and the acid-stable phosphorylated sites is 1:1 (see also Ref. 27); this suggests that during the ATPase cycle the MgE-P complex has the same number of Mg^{2+} ions and inorganic phosphate attached to it. Furthermore, the same ratio is obtained whether the E-P intermediate is in the E_1 or E_2 conformation, an indication that the E_1–E_2 transition of the phosphoenzyme does not affect its affinity for Mn^{2+} (Mg^{2+}). Looking at Fig. 1 one sees that in the absence of ATP the Mn collected in the effluent as a function of [$MnCl_2$] still has a small saturable component (approx. 10% of the total at 5 μM $MnCl_2$). The nature of that component is not clear, but it seems reasonable to consider it an expression of the Mn^{2+} originally bound to the enzyme which will become part of the ATP-dependent MnE-P complex upon phosphorylation; actually, if instead of taking the difference between the Mn^{2+} bound in the presence and absence of ATP, one subtracts the linear component from the total, both in the presence of ATP, the [E-Mn]/[E-P] ratio increases by less than 10% at the lowest saturable [$MnCl_2$]; within the errors of measurement this is not different from 1:1. Obviously, this explanation specifically excludes any Mg^{2+} site other than that where the ion acts as essential activator for phosphorylation, at least during the early stages of the reaction cycle. The K_m value for $MnCl_2$ is less than 1 μM at 0°C. However, in curves like that of fig. 2, the Mn · ATP complex and free-Mn^{2+} coexist as a sum in the abscissa; therefore there is no way to assess the participation of each species in the overall affinity constant. Moreover, the enzyme form involved (E_1, E_1Na, E_1ATP) cannot be identified.

When E_2-P was dephosphorylated by K or E_1-P by ADP we were unable to separate the release of inorganic phosphate from that of Mn^{2+}, i.e. the [E-Mn]/[E-P] ratio was kept constant for all [E-P] (Figs. 3 and 4) even when the contact time with the resine was reduced to the minimum compatible with an acceptable Mn^{2+} removal. Two interpretations are possible: (i) Mn^{2+} and inorganic phosphate are simultaneously released, or (ii) Mn^{2+} is debound after dephosphorylation has taken place, but that process is an extremely rapid one. In line with the second interpretation are studies on inhibition kinetics of Na^+- and Na^+/K^+-ATPase activities which are consistent with an orderly release where inorganic phosphate comes out first [20] and indications that the release of Mg^{2+} from $MgE_2(K)$, the intermediate that follows K^+-stimulated dephosphorylation, is indeed a very fast reaction [10].

The fact that the relationship between [Mn^{2+}] and [E-P] is hyperbolic does not necessarily imply a single site, for that relationship can also be obtained with multiple sites as long as they are identical. However, taken together with the [E-Mn]/[E-P] ratio of one, the existence of more than one Mg^{2+} site in $E_1(Na)$ seems rather unlikely.

Acknowledgments

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References

1 Skou, J.C. (1957) Biochim. Biophys. Acta 23, 394–401.
2 Fahn, S., Koval, G.J. and Albers, R.W. (1966) J. Biol. Chem. 241, 1882–1889.
3 Post, R.L., Kume, S., Tobin, T., Orcutt, B. and Sen, A. (1969) J. Gen. Physiol. 54, 306s–326s.
4 Skou, J.C. (1974) Biochim. Biophys. Acta 339, 245–257.
5 Hexum, T., Samson, F.E. and Himes, R.H. (1970) Biochim. Biophys. Acta 212, 322–331.

6 Robinson, J.D. (1974) Biochim. Biophys. Acta 341, 232–247.
7 Garrahan, P.J., Rossi, R. and Rega, A. (1982) Ann. N.Y. Acad. Sci. 402, 239–252.
8 Mardh, S. and Post, R.L. (1977) J. Biol. Chem. 252, 633–638.
9 De Weer, P. (1983) Curr. Topics Membr. Transp. 19, 599–623.
10 Forbush, B. (1987) J. Biol. Chem. 262, 11116–11127.
11 Beaugé, L. and Glynn, I.M. (1979) J. Physiol. 289, 17–31.
12 Beaugé, L. and Campos, M. (1986) J. Physiol. 375, 1–25.
13 Robinson, J.D. (1969) Biochemistry 6, 3250–3258.
14 Post, R.L., Toda, G. and Rogers, F.N. (1975) J. Biol. Chem. 250, 691–701.
15 Robinson, J.D. and Mercer, R.W. (1981) J. Bioenerg. Biomembr. 13, 205–218.
16 Hegyvary, C. and Jørgensen, P.L. (1981) J. Biol. Chem. 256, 6296–6303.
17 Covarrubias, Y.G.M., Breitwieser, G. and De Weer, P. (1987) J. Gen. Physiol. 90, 14a.
18 Plesner, L. and Plesner, I.W. (1981) Biochim. Biophys. Acta 643, 449–462.
19 Plesner, I.W., Plesner, L., Norby, J.C. and Klodos, I. (1981) Biochim. Biophys. Acta 643, 483–494.
20 Pedemonte, C.H. and Beaugé, L. (1983) Biochim. Biophys. Acta 784, 245–253.
21 Pedemonte, C.H. and Beaugé, L. (1986) Arch. Biochem. Biophys. 244, 596–606.
22 Flatman, P.W. and Lew, V.L. (1981) J. Physiol. 315, 421–446.
23 Robinson, J.D. (1980) J. Bioenerg. Biomembr. 12, 165–174.
24 Grisham, C.M. (1981) J. Inorg. Biochem. 14, 45–57.
25 Fukushima, Y. and Post, R.L. (1978) J. Biol. Chem. 253, 6853–6862.
26 Campos, M. and Beaugé, L. (1987) XVI Reunion Cientifica de la Sociedad Argentina de Biofisica, 57Abs.
27 Richards, D.E. (1987) J. Physiol. 386, 112P.
28 Jørgensen, P.L. (1974) Biochim. Biophys. Acta 356, 36–52.
29 Beaugé, L., Berberian, G. and Campos, M. (1984) Biochim. Biophys. Acta 773, 157–165.
30 Glynn, I.M. and Chappell, J.B. (1964) Biochem. J. 90, 147–149.
31 De Meis, L. (1974) Biochemistry 11, 2460–2465.
32 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
33 Glynn, I.M., Howland, J.L. and Richards, D.E. (1985) J. Physiol. 368, 453–469.
34 Beaugé, L., Berberian, G., Campos, M. and Pedemonte, C. (1985) in The Sodium Pump (Glynn, I.M. and Ellory, C., eds.), pp. 321–333, The Company of Biologists, Cambridge.