Biochimica et Biophysica Acta 939 (1988) 1–7
Elsevier

BBA 73914

Effect of alkaline earth ions on the movement of mobile charges in *Valonia utricularis*

L. Walter, K.-H. Büchner and U. Zimmermann

Lehrstuhl für Biotechnologie der Julius-Maximilians-Universität Würzburg, Würzburg (F.R.G.)

(Received 14 October 1987)

Key words: Barium ion effect; Mobile charge; Turgor pressure; Potassium ion transport; (*V. utricularis*)

An attempt was made to further characterise the pressure-dependent translocation of mobile charges which are probably linked to the pressure-dependent K^+ transport system in the membranes of the giant marine alga *Valonia utricularis*. It could be shown that concentrations of 10 mM $BaCl_2$ added to the external sea water specifically and reversibly reduce the pressure dependence of the translocation rate of the mobile charges, leaving their total concentration unaffected. No such effect could be observed with other alkaline earth ions. The results are discussed on the basis that either Ba^{2+} decreases membrane compressibility or that the transport system may consist of a pressure-independent and a pressure-dependent part and that Ba^{2+} competes with K^+ for specific sites on the latter.

Introduction

Elucidation of the molecular mechanisms involved in the turgor pressure-dependent transport of K^+ across the membranes of the giant algal cells of *Valonia utricularis* is fundamental to the understanding of turgor pressure- (and cell volume-) control in plants [1–4]. Recently mobile negative charges within the membranes of *V. utricularis* and *Halicystis parvula* have been identified as possible candidates for the transformation of turgor pressure signals into K^+ transport [5–9]. These mobile charges are presumably connected to highly specialised integral proteins such as channels, pumps and/or carriers which may be involved in the K^+ transport [7]. The rise in the translocation rate of the mobile charges with increasing turgor pressure in the turgor pressure interval between 0 MPa and 0.2 MPa, in which the K^+ fluxes are pressure-dependent [10], is assumed to result from membrane thinning [5,9]. Such transient changes in the membrane thickness and in the translocation rate of the mobile charges could modulate K^+ transport and coupled fluxes of other ions which ultimately regulate the internal osmotic pressure.

Although various approaches can be considered to explore the nature and the role of these mobile charges in turgor pressure regulation of *V. utricularis*, studies of the specific interactions of certain ions and uncharged low-molecular weight compounds with the mobile charge system represent a suitable method of obtaining insight into the events and processes on the molecular level.

Recently, we have shown that some anaesthetics at very low sub-clinical concentrations significantly increase the translocation rate of the mobile charges. This observation supports the assumption that the mobile charges are ligands of proteins which exhibit pressure-sensitive sites [8].

In this communication we report on the in-

Correspondence: U. Zimmermann, Lehrstuhl für Biotechnologie der Julius-Maximilians-Universität Würzburg, Röntgenring 11, D-8700 Würzburg, F.R.G.

fluence of various concentrations of alkaline earth ions on the translocation rate of the mobile charges. We could demonstrate that Ba^{2+} ions very specifically suppress the pressure dependence of the translocation rate of the mobile charges within the membranes of *V. utricularis*. The results can be explained either by the assumption that the mobile charge system represents a heterogeneous transport system consisting of pressure-independent and pressure-dependent parts on which Ba^{2+} and K^+ compete as ligands or by assuming that Ba^{2+} specifically decreases membrane compressibility.

Materials and Methods

Giant marine algal cells of *Valonia utricularis*, collected from the Gulf of Naples were cultivated in natural sea water at a salinity of 1200 mosmol/kg [5,9]. For the investigation with the charge pulse technique well-rounded cells with volumes between 10 and 53 μl were employed. Single cells were fixed in a small perspex chamber perfused with artificial sea water (ASW), containing 545 mM NaCl, 12 mM KCl, 11 mM $CaCl_2$ and 10 mM $MgCl_2$. The pH was buffered at 8.0 to 8.2 with 10 mM carbonate or 10 mM Hepps (4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid) and the temperature was kept at $20 \pm 1°$ C. The turgor pressure was varied by addition of NaCl or distilled water.

The charge-pulse technique has been described in several publications in detail, [5,9,11], therefore, only a short description of the experimental set up will be given. Two microcapillaries were inserted into the cell under investigation. The first (current electrode) was for the application of the charge pulse, the second, an Ag/AgCl-electrode filled with 3 M KCl, was for the measurement of the voltage relaxation. The current electrode was combined with a pressure transducer (CQS 140, Kulite Semiconductors, Ridgefield, NJ) for recording the cell turgor pressure. The membranes of the cells were charged to 20–40 mV with a charge pulse of 1 μs duration generated by a fast pulse generator (Hewlett Packard 214 B) and recorded across a series resistor (10 Ω) using an HP 7633 storage oscilloscope. Membrane discharge was monitored with a Nicolet Explorer III digital storage oscilloscope and evaluated by a DEC Minc 1.1 Lab Computer or an Olivetti M24.

The decay of the initial voltage with time across the membrane of *Valonia utricularis* can be described by the mobile charge concept [5,9]. The two exponential relaxations might be attributed to the transport of mobile negative charges in the membrane, possibly as part of a potassium transport system. From an analysis of the experimental results in terms of the proposed model, the translocation rate, k, the concentration of the mobile charges, N_t, as well as the specific membrane resistance, R_m, could be evaluated. From the relaxations the following relations can be calculated as functions of the time constants of the biphasic relaxation τ_1 and τ_2 and their relative amplitudes a_1 and a_2 ($a_1 + a_2 = 1$):

$$k = (1/2)(a_1/\tau_2 + a_2/\tau_1) \tag{1}$$

$$R_m = (1/C_m)(a_1\tau_1 + a_2\tau_2) \tag{2}$$

$$N_1 = (4RTC_m/F^2)\left(a_1a_2(\tau_1 - \tau_2)^2/(R_mC_m)^2\right) \tag{3}$$

where F = Faraday constant, R = gas constant, T = absolute temperature and $C_m = Q/U_o$ (Q is the amount of charge needed to generate the initial voltage U_o).

For the investigation of the influence of alkaline earth ions on the translocation rate of the mobile charges in the membranes of *V. utricularis*, the cells were exposed to artificial sea water containing various chlorides: $BaCl_2$ (puriss, p.a.) was obtained from Fluka, CH; $SrCl_2$, $CaCl_2$, $MgCl_2$ from Merck, F.R.G. other chemicals NaCl, KCl, Na_2CO_3, and $NaHCO_3$ (p.a.) were obtained from Merck, F.R.G., and Hepps from Sigma, F.R.G.

Results

The translocation rate k of the mobile charges in the membranes of *Valonia utricularis* increases by a factor of about 2 in the pressure range between 0 and about 0.2 MPa, whereas towards higher values it assumes a constant maximum value (see also below and [5,9]).

In order to explore possible effects of alkaline earth ions on the translocation rate and to exclude simultaneously interference of pressure effects, the effect of various concentrations of Mg^{2+}, Ca^{2+},

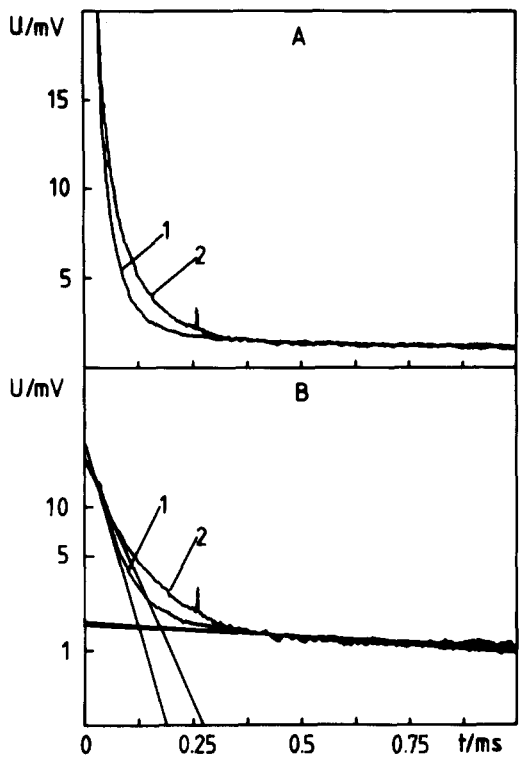

Fig. 1. Voltage relaxations of a cell of *V. utricularis* ($V = 20$ µl, $A = 0.36$ cm^2). Fig. 1A shows the actual voltage decay of a cell in artificial sea water (ASW) (trace 1) and after incubation in ASW containing 10 mM $BaCl_2$ (trace 2), at a turgor pressure of 0.39 MPa. In Fig. 1B the resulting semilogarithmic plots are displayed. The charge pulse of 1 µs duration and 4.5 nA·s magnitude resulted in an initial voltage $U_o = 25$ mV in ASW (22 mV in ASW + 10 mM $BaCl_2$) across the membrane. Using a least-squares fit, the best straight line through the curve for longer times was found. Hence the parameters (a_2, τ_2) of the slower decay were calculated and then subtracted from the experimental curve. Subsequently the corrected data were fitted again and the parameters a_1 and τ_1 were obtained. From the above curves the following values were calculated: $a_1 = 0.93$, $\tau_1 = 50$ µs, $\tau_2 = 3007$ µs for the voltage relaxation in ASW, and $a_1 = 0.92$, $\tau_1 = 76$ µs, $\tau_2 = 2739$ µs for the relaxation in 10 mM $BaCl_2$.

Sr^{2+} and Ba^{2+} ions on the translocation rate of the mobile charges were studied in the first set of experiments at turgor pressure values above 0.2 MPa.

Fig. 1A shows typical voltage–relaxation curves recorded on the same cell in the absence and presence of 10 mM $BaCl_2$ at a turgor pressure of 0.39 MPa. Both relaxations could be fitted by two exponentials (Fig. 1B), indicating that the total relaxation process consisted of fast and slow components (with time constants τ_1 and τ_2) both in the presence of and in the absence of $BaCl_2$. Addition of $BaCl_2$ obviously resulted in a significant increase of the relaxation time τ_2 of the fast relaxation process. The relaxation time τ_1 of the slow process and the relative amplitudes a_1 and a_2 of the two relaxation processes as well as the specific capacity C_m and the specific resistance R_m seemed to be unchanged – at least within the limits of accuracy. Calculations of k, N_t, and R_m from these data according to Eqns. 1–3 revealed that the translocation rate decreased by about 23% compared to the control value, whereas the other parameters remained fairly constant (see Table I, No. 1). Further independent experiments on 10 different algal cells supported these findings (see Table I). Performance of relaxation studies at various times after the addition of $BaCl_2$ to the external sea water showed that the effect of this alkaline earth ion on the fast relaxation and, in turn, on k was very rapid as compared to effects of pH or pressure changes [9]. After about 5 to 10 min the final values could be recorded. The effect of Ba^{2+} ions on the fast relaxation was almost completely reversible when the sea water containing Ba^{2+} was replaced by normal sea water (ASW, see Table I). However, about 15 to 30 min were required before the original voltage–relaxation curves could be recorded. The reversibility of the barium effect on k is particularly obvious from Table I. As can be seen the translocation rate is reduced between 45% (No. 4) and 12% (No. 2) in the presence of 10 mM Ba^{2+} ions.

Table II shows the influence of turgor pressure on k in the absence and presence of 10 mM Ba^{2+}. It is evident that addition of $BaCl_2$ nearly suppressed the increase of k with increasing turgor pressure. Measurements on various algal cells over the whole turgor range between 0 and 0.4 MPa confirmed this result.

Only in the very low concentration range could a dependence of the translocation rate upon the concentration of Ba^{2+} be found (Fig. 2). Measurements were performed on the same cell exhibiting a constant turgor pressure of 0.27 MPa. It is obvious that a concentration of only 0.1 mM was sufficient to induce a significant reduction in k which became more pronounced with increasing concentration up to 1 mM. However, above a

TABLE I

TEN TYPICAL RESULTS FROM MEASUREMENTS OF THE BARIUM EFFECT (10 mM) ON DIFFERENT CELLS OF *V. UTRICULARIS* IN THE HIGHER TURGOR PRESSURE RANGE (> 0.25 MPa)

The results show a reversible increase in the fast relaxation time τ_1. The relative amplitude a_1, the slower relaxation time τ_2 and the membrane capacity C_m remained unaffected. From these parameters R_m, N_t and k could be calculated. It is evident that addition of $BaCl_2$ decreased the translocation rate and the other parameters showed no significant response. Further information is given in the text.

No.	Medium	P (MPa)	a_1	τ_1 (μs)	τ_2 (μs)	R_m ($10^{-4}\ \Omega \cdot m^2$)	C_m ($10^{-4}\ \mu F \cdot m^{-2}$)	N_t (nmol·m^{-2})	k (s^{-1})
1	ASW		0.93	50	3007	523	0.50	44.3	876
	10 mM $BaCl_2$	0.39	0.92	76	2739	499	0.56	37.5	678
	ASW		0.94	53	2789	433	0.53	47.4	785
2	ASW		0.94	47	1956	456	0.36	29.3	900
	10 mM $BaCl_2$	0.39	0.93	62	1857	469	0.38	24.5	792
	ASW		0.93	50	1858	428	0.40	29.1	932
3	ASW		0.94	58	2268	526	0.38	29.1	763
	10 mM $BaCl_2$	0.25	0.93	81	2523	624	0.41	26.0	639
	ASW		0.93	63	2646	577	0.41	32.0	717
4	ASW		0.90	74	4543	1160	0.44	31.0	776
	10 mM $BaCl_2$	0.34	0.91	152	3838	1152	0.43	21.0	426
	ASW		0.90	86	4785	1189	0.47	32.3	665
5	ASW		0.88	49	6009	1525	0.51	33.4	1344
	10 mM $BaCl_2$	0.48	0.88	58	5500	1401	0.50	32.8	1101
	ASW		0.88	49	5762	1475	0.50	33.2	1316
6	ASW		0.81	75	11880	3268	0.71	29.5	1311
	10 mM $BaCl_2$	0.49	0.85	101	9106	2633	0.56	28.1	807
	ASW		0.84	77	9388	2645	0.60	29.2	1115
7	ASW		0.92	73	2277	404	0.63	37.2	769
	10 mM $BaCl_2$	0.52	0.91	109	2577	453	0.70	34.4	578
	ASW		0.92	74	2625	437	0.62	40.9	707
8	ASW		0.88	142	6081	1592	0.55	28.5	506
	10 mM $BaCl_2$	0.42	0.90	196	5075	1458	0.47	22.4	345
	ASW		0.88	143	5928	1650	0.50	26.7	492
9	ASW		0.85	105	10722	2280	0.74	38.7	761
	10 mM $BaCl_2$	0.41	0.85	142	13951	2956	0.75	38.7	572
	ASW		0.87	105	11762	2537	0.64	38.9	667
10	ASW		0.86	201	37774	8638	0.62	37.6	355
	10 mM $BaCl_2$	0.41	0.87	275	41326	9775	0.58	36.1	253
	ASW		0.85	220	34507	8189	0.66	35.5	360

concentration of about 1 mM a further decrease of k could not be achieved even up to a concentration of 100 mM (not shown). The dependence of k on the Ba^{2+} concentration curve could be roughly fitted to a binding curve first order with respect to Ba^{2+} concentration (not shown), from which a K_i value of 0.3 mM was estimated.

In order to investigate possible interference of Ca^{2+} ions with the effect of the Ba^{2+} ions the calcium concentration in sea water was lowered from 11 mM (the normal concentration in the sea water used) to 1 mM and the effect of two concentrations of barium ions (0.5 mM and 10 mM) on the voltage relaxations was studied. The experiments were performed at high turgor pressures of 0.27 and 0.4 MPa, respectively. As shown in several

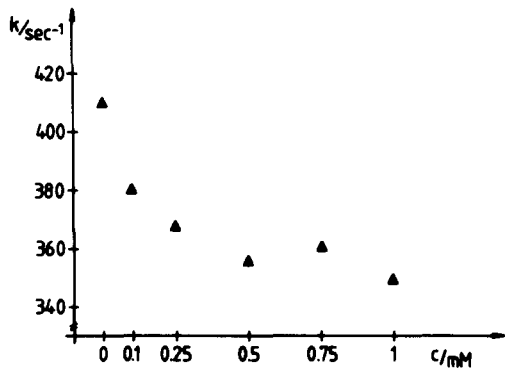

Fig. 2. The dependence of the translocation rate of the mobile charges on the concentration of Ba^{2+} ions in the external seawater. The translocation rate decreased as the concentration of $BaCl_2$ was increased from 0.1 to 1 mM. The measurements were performed on a cell ($V = 53$ µl, $A = 0.92$ cm^2) exhibiting a constant turgor pressure of 0.27 MPa. Further information is given in the text.

experimental runs the low Ca^{2+} concentration did not lead to qualitative or quantitative changes of the barium effect on the translocation rate. Significantly higher Ca^{2+} concentrations than 11 mM could not be tested because of the low solubility of the calcium salt at pH 8.2. The effects of Ca^{2+} concentrations lower than 1 mM were difficult to investigate because of membrane damage occurring after some hours of exposure. However, some short-time experiments in the absence of Ca^{2+} ions showed that within the first 10 min no changes in the voltage relaxations could be recorded, supporting strongly the assumption that Ca^{2+} did not interfere with the Ba^{2+}.

It is also interesting to note that Sr^{2+} and Mg^{2+} had no effect on the translocation rate during a time period of up to 30 min within the limits of accuracy (not shown). In these experiments concentrations of Sr^{2+} up to 10 mM (above this concentration $SrCl_2$ was insoluble at pH 8.2) and of $MgCl_2$ up to 100 mM were used. In all these measurements the turgor pressure was above 0.25 MPa.

TABLE II

INFLUENCE OF BARIUM IONS ON THE TRANSLOCATION RATE IN A SINGLE CELL OF *V. UTRICULARIS* ($V = 24$ µl, $A = 0.51$ cm^2)

As can be seen, incubation in 10 mM $BaCl_2$ causes a significant decrease in the translocation rate at higher turgor pressures, whereas in the low pressure range, where the translocation rate is decreased anyway, no additional effect of barium could be detected within the limits of accuracy.

Medium	P (MPa)	a_1	τ_1 (µs)	τ_2 (µs)	k (s^{-1})
ASW	0.36	0.90	151	4662	421
10 mM $BaCl_2$	0.34	0.90	316	4532	258
ASW	0.36	0.90	156	5144	406
ASW	0.11	0.91	154	4619	403
10 mM $BaCl_2$	0.12	0.90	477	5071	194
ASW	0.12	0.90	165	3235	433
ASW	0.02	0.88	322	18732	214
10 mM $BaCl_2$	0.02	0.88	324	14793	222
ASW	0.02	0.88	321	15281	223

Discussion

Millimolar concentrations of barium have been found to specifically block inward and outward rectifying K^+ channels in muscle cells, starfish eggs, heart cells and guard cell protoplasts [12–22]. In contrast, Mg^{2+}, Ca^{2+} and Sr^{2+} ions showed – if any – only slight blocking effects [15]. On the other hand, Ba^{2+} ions have been found to open potassium channels in algal membranes at very low concentrations (0.1–100 µM), but showing the normal blocking effect at higher concentrations (0.1–5 mM) [23].

The charge-pulse experiments presented here also demonstrate a highly specific effect of Ba^{2+} on the voltage relaxation pattern of the membranes of *Valonia utricularis*. Ba^{2+} suppressed nearly completely the pressure-dependence of the translocation rate, which was observed at external K^+ concentrations of 12 mM. The other alkaline earth ions showed no effect. This finding coincides with the sequence of size of the ions. The ionic radius of Ba^{2+} is at 0.134 nm only slightly greater than that of K^+ (0.133 nm). In contrast Sr^{2+}, Ca^{2+} and Mg^{2+} have with 0.112, 0.099, and 0.066 nm, respectively, much smaller radii [24]. Thus, the ionic radius of Ba^{2+} relative to that of K^+ is probably one of the important parameters which influence the translocation rate of the mobile charges. Preliminary experiments at high external K^+ concentrations of 100 mM confirm this assumption. In this case the translocation rate was decreased in the higher pressure range. No additional effect of Ba^{2+} could be detected (unpublished).

The results can be interpreted in different ways. It can be assumed that the mobile charge system is a heterogeneous transport system which is composed of a pressure-independent and of a pressure-dependent part. In this case Ba^{2+} competes with K^+ ions at specific sites of the pressure-dependent part [16]. As the affinity of the mobile charges for Ba^{2+} seems to be higher than for K^+ this leads to an almost complete decrease in the pressure dependence of the translocation rate. Thus we could assume K^+ channels to which the mobile charges are linked if we accept the interpretation of Ba^{2+} effects on the cell systems mentioned above [14–21].

However, it is not necessary to assume a heterogeneous transport system. We can still proceed with the homogeneous model previously introduced for the interpretation of the pressure dependence of the translocation rate of the mobile charges (and of the K^+ transport system) [5,9]. The increase in the translocation rate was explained as mentioned above in terms of an electromechanical compression of local areas of the membranes. Thinning of the membrane by compression leads to a lowering of the energy barrier in the center of the membrane according to the change in the Born energy [5], resulting ultimately in an increase in the translocaton rate. Because of its ionic radius and of its divalency, Ba^{2+} may specifically cross-link membrane components at sites of the membranes where the mobile charge system is located or which are connected to the mobile charge system – with the consequence that the compressibility of this local membrane area is decreased. Therefore, increase of turgor pressure leads in the presence of Ba^{2+} only to a minor change in membrane thickness and Born energy and thus in the translocation rate. On the basis of the theoretical considerations and data given elsewhere [9,25] and from the pressure dependence of the translocation rate observed here in the presence of Ba^{2+} we can calculate that the elastic modulus of the membrane has to be increased by about 10% as compared to the untreated membrane in order to explain the effect of Ba^{2+} ions.

At the present stage of information it is difficult to decide which of these two interpretations is correct. Certainly, patch clamp experiments on membrane droplets of *V. utricularis* cells and studies of the effect of Ba^{2+} on radioactively labelled K^+ fluxes are required to get more insight into the complex nature of this and other pressure-dependent transport systems.

Acknowledgements

The authors would like to thank Professor R. Benz and Dr. W.M. Arnold for critically reading the manuscript and many helpful discussions. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 176, B4) to K.-H. B. and U. Z.

References

1 Zimmermann, U. (1977) in Integration of activity in the higher plants (Jennings, D., ed.), pp. 117–154, Cambridge University Press, Cambridge.
2 Zimmermann, U. (1978) Annu. Rev. Plant Physiol. 29, 121–148.
3 Zimmermann, U. and Steudle, E. (1974) in Membrane Transport in Plants (Zimmermann, U. and Dainty, J., eds.), pp. 64–71, Springer Verlag, Berlin, Heidelberg, New York.
4 Zimmermann, U., Steudle, E. and Lelkes, P.I. (1976) Plant Physiol. 58, 608–613.
5 Benz, R. and Zimmermann, U. (1983) Biophys. J. 43, 13–26.
6 Benz, R., Büchner, K.-H. and Zimmermann, U. (1987) Planta, in press.
7 Büchner, K.-H., Rosenheck, K. and Zimmermann, U. (1985) J. Membr. Biol. 88, 131–137.
8 Büchner, K.-H., Walter, L. and Zimmermann, U. (1987) Biochim. Biophys. Acta, 903, 241–247.
9 Zimmermann, U., Büchner, K.-H. and Benz, R. (1982) J. Membr. Biol. 67, 183–197.
10 Steudle, E., Zimmermann, U. and Lelkes, P.I. (1977) in Transmembrane Ionic Exchange in Plants (Thellier, M. and Dainty, J., eds.), pp. 123–132, CNRS, Paris.
11 Benz, R. and Läuger, P. (1977) Biochim. Biophys. Acta 468, 245–258.
12 Armstrong, C.M. and Taylor, S.R. (1980) Biophys. J. 30, 473–488.
13 Assmann, S.M., Simoncini, L. and Schroeder, J.I. (1985) Nature 318, 285–287.
14 Eaton, D.C. and Brodwick, M.S. (1980) J. Gen. Physiol. 75, 727–750.
15 Hagiwara, S. et al. (1978) J. Physiol. 279, 167–185.
16 Miller, C., Latorre, R. and Reisin, I. (1987) J. Gen. Physiol. 90, 427–449.
17 Moffett, D.F. and Koch, A.R. (1985) J. Membr. Biol. 86, 89–97.
18 Sakmann, B. and Trube, G. (1984) J. Physiol. 347, 659–683.
19 Schroeder, J.I., Hedrich, R. and Fernandez, J.M. (1984) Nature 312, 361–362.

20 Schroeder, J.I., Rascke, K. and Neher, E. (1987) Proc. Natl. Acad. Sci. USA 84, 4108–4112.
21 Spalding, B.C., Swift, J.G. and Horowicz, P. (1986) J. Membr. Biol. 93, 141–156.
22 Vergara, C. and Latorre, R. (1983) J. Gen. Physiol. 82, 543–568.
23 Thaler, M., Steigner, W., Köhler, K., Simonis, W. and Urbach, W. (1987) FEBS Lett. 219, 355–359.
24 Hollemann-Wiberg (1985) Lehrbuch der anorganischen Chemie, pp. 926–954, de Gruyter, Berlin, New York.
25 Zimmermann, U., Beckers, F. and Coster, H.G.L. (1977) Biochim. Biophys. Acta 464, 399–416.