Volume 303, number 2,3, 229–232 FEBS 11121 June 1992

Regulation of a major cloned voltage-gated K^+ channel from human T lymphocytes

Bernard Attali, Eric Honoré, Florian Lesage, Michael Lazdunski and Jacques Barhanin

Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, Sophia Antipolis 06560 Valbonne, France

Received 30 March 1992; revised version received 21 April 1992

When expressed into *Xenopus* oocytes, HLK_3 K^+ channel (Kv1-3) induced a slowly inactivating voltage-dependent K^+ current. We have studied the modulation of this K^+ current by co-expressing a cloned 5-HT_2 receptor together with HLK_3 K^+ channel protein. Application of 5-HT caused a long-lasting inhibition of the voltage-gated K^+ current. This inhibitory modulation was mimicked by intracellular injection of inositol triphosphate or Ca^{2+}, as well as by incubation with phorbol esters or diacylglycerol analogs. Oocytes pretreatment with staurosporine and EGTA fully prevented 5-HT inhibitory action. Elevation of cAMP and cGMP levels into oocytes did not produce any detectable effect on the current recorded in the absence or the presence of 5-HT. These data suggest that the second messengers generated by phospholipase C activation may be important modulators of HLK_3 K^+ channels in the immune and the central nervous systems.

T cell; Jurkat; Serotonin; Kinase C; Calcium

1. INTRODUCTION

K^+ channels are ubiquitous membrane proteins which are centrally involved in multiple functions in both excitable and non-excitable cells [1]. In neurons, K^+ channel diversity influences action potential shaping and firing frequency [1,2]. In non-excitable cells, such as lymphocytes or glial cells, the expression of a given K^+ channel repertoire seems to be correlated with a specific functional stage of the cell development [3,4]. The diversity of K^+ channel electrophysiological properties reflects a large set of diverse molecular structures. The cloning of several families of voltage-dependent K^+ channel genes in *Drosophila*, *Aplysia*, frog and mammals have been reported (for review see [5,6]).

By use of probes from *Shaker*-related K^+ channels we have isolated the gene called HLK_3 (corresponding to Kv1-3 according to the nomenclature proposed by Chandy et al. [7]) from a T lymphocyte cell line cDNA library [8]. Functional expression of in vitro synthesized RNA of this clone demonstrates that it encodes the so called n-type K^+ channel commonly found in human T lymphocytes [8]. This K^+ channel type is also expressed in the central nervous system [9]. Two genomic clones, MK3 and RGK5, corresponding to the mouse and rat counterpart of HLK_3, have also been found to direct the expression of n-type K^+ current in *Xenopus* oocytes [10,11].

In many neurons and other excitable cells channel modulation by the action of neurotransmitter- or hormone receptor-activated second messenger systems has been recognized as an important mechanism which adapts cells for particular functions [12–14]. In this report we investigate the modulation of HLK_3 activity expressed in *Xenopus* oocytes, either alone or in association with a 5-HT_2 receptor [15].

Correspondence address: E. Honoré, Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France. Fax: (33) 93 95 77 04.

2. MATERIALS AND METHODS

Cloning of HLK_3, RNA synthesis, oocyte isolation, injection and electrophysiology have been described elsewhere [8,16]. The Chinese hamster 5-HT_2 receptor cDNA clone was isolated by Chambard et al. [15].

3. RESULTS

Under voltage-clamp conditions oocytes injected with HLK_3 cRNA (0.1 ng per oocyte) exhibited outward K^+ currents that were activated at membrane potentials positive to −50 mV, and which were inactivated by more than 80% at the end of a 3 s depolarizing pulse (Fig. 1B). The biophysical properties of HLK_3 channel have been recently described [8]. A cloned Chinese hamster 5-HT_2 receptor [15] was co-expressed with HLK_3 K^+ channel in *Xenopus* oocytes. In this cell system stimulation of 5-HT receptors results in G-protein activation of phospholipase C [17]. Application of 5-HT (1 μM) resulted in a profound inhibition of the voltage-dependent K^+ current (Fig. 1A) within 2 min. The peak amplitude of the K^+ current was decreased maximally by 5-HT after about 20 min. No significant change in the kinetics or the voltage dependence of HLK_3 K^+ current was observed following 5-HT application (Fig.

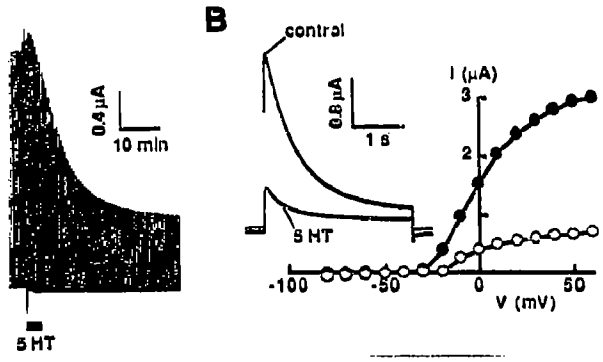

Fig. 1. Coexpression of 5-HT_2 receptor with HLK_3 K^+ channel in *Xenopus* oocytes. (A) Chart recording showing that 5-HT (1 μM) applied for 2 min reduced the amplitude of the HLK_3 K^+ currents recorded during iterative depolarizations from a holding potential of −80 mV to test potentials of 30 mV. (B) I–V relationships in control (●) and 25 min after the 5-HT application (○).

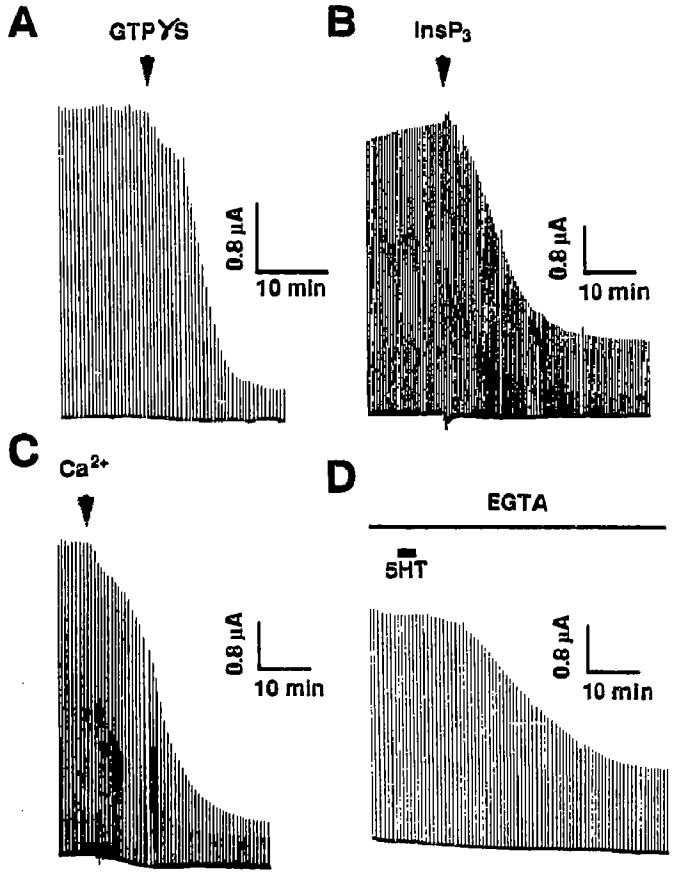

Fig. 2. Intracellular Ca^{2+} regulates HLK_3 channel. (A) Intracellular microinjection of GTPγS (final concentration 1 μM) depressed HLK_3 K^+ current. (B) Intracellular injection of $InsP_3$ (final concentration 1 μM) inhibited HLK_3 current. (C) Intracellular injection of Ca^{2+} (final concentration 1 μM) inhibited HLK_3 current. (D) Intracellular EGTA injection (final concentration 5 mM) delayed the 5-HT (1 μM) inhibition. Holding potential was −80 mV and test potential 30 mV.

1B). The inhibitory action was long-lasting and, at 1 μM 5-HT, the effect was practically irreversible.

In oocytes, as in most cells, activated phospholipase C generates $InsP_3$ and diacylglycerol, resulting in the subsequent release of Ca^{2+} from intracellular stores and activation of protein kinase C. The involvement of these second messengers in mediating the 5-HT inhibition of HLK_3 K^+ current was therefore examined. Fig. 2A shows that intracellular injection of GTPγS, which activates phospholipase C via G-proteins, mimicked the 5-HT-induced depression of K^+ currents. Intracellular application of $InsP_3$ into the oocyte potently inhibited K^+ channel activity, and direct injection of Ca^{2+} produced a similar depression with a time-course close to that observed following 5-HT treatment (Fig. 2B,C). Prior injection with EGTA slowed the 5-HT inhibition onset kinetics by a factor of about two (Fig. 2D).

The phorbol ester, PMA, and the diacylglycerol analog, OAG, also produced a long-lasting blockade of HLK_3 K^+ current (Fig. 3A,B). Interestingly, following PMA inhibition a subsequent application of 5-HT further depressed K^+ channel activity (Fig. 3C). Staurosporine pretreatment alone did not prevent 5-HT-in-

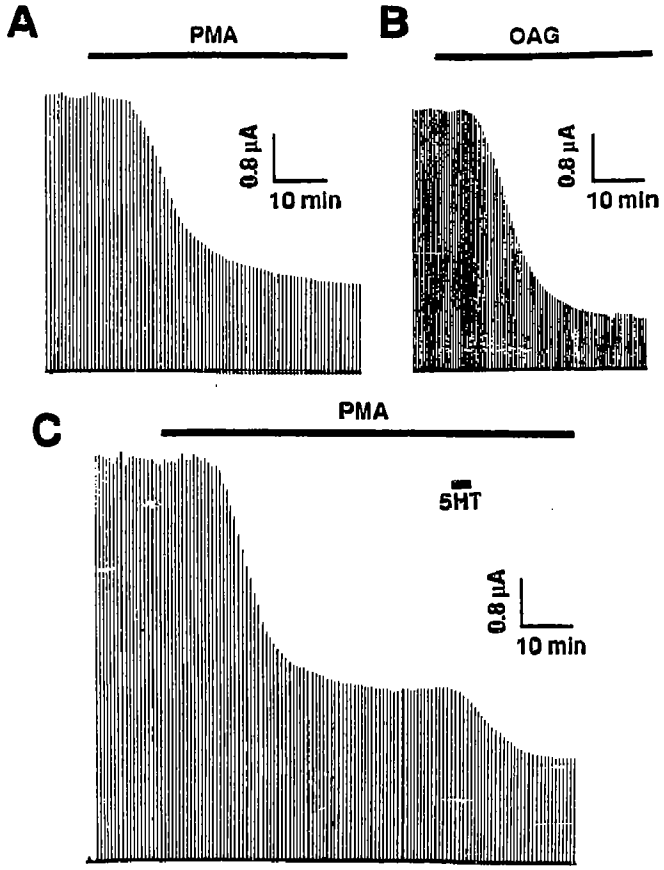

Fig. 3. Protein kinase C activation inhibits HLK_3 current. (A) 30 nM PMA inhibited HLK_3 current. (B) 30 μM OAG inhibited HLK_3 current. (C) 5-HT (1 μM) inhibition was additive with PMA (30 nM). Holding potential was −80 mV and test potential 30 mV.

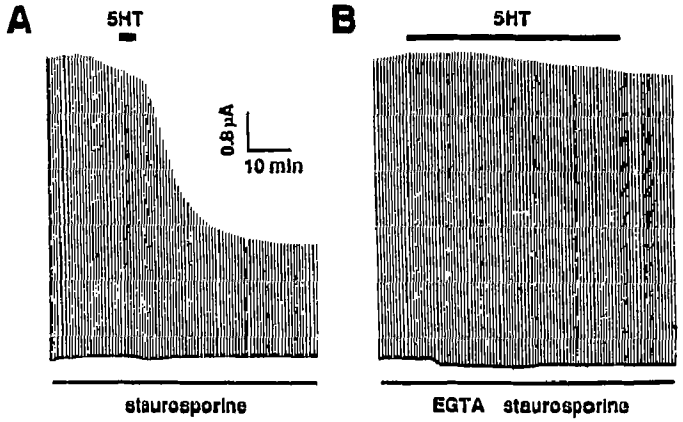

Fig. 4. Phospholipase C products inhibit HLK_3 current. (A) Staurosporine (0.1 µM) pretreatment for 4 h did not reverse the inhibition by 5-HT. (B) Intracellular injection of EGTA (final concentration 5 mM) and pretreatment with 0.1 µM staurosporine for 4 h reversed the 5-HT inhibition. Holding potential was −80 mV and test potential 30 mV.

duced inhibition (Fig. 4A), but together with EGTA microinjection it completely blocked 5-HT action (Fig. 4B). The K^+ current and the action of 5-HT were unaffected by extracellular application of 1 mM 8-(4-chlorophenylthio)3',5'-cAMP, 1 mM 8-Br-cGMP, as well as by treatment with the calmodulin antagonists, W7 or calmidazolium, and by microinjection with calcineurin and alkaline phosphatase.

We have recently observed [18] that injection into oocytes of different levels of mRNA generates voltage-gated K^+ currents with different biophysical and pharmacological properties. On the one hand a low cRNA concentration produces an inactivating K^+ current with inwardly-rectifying properties (Fig. 5A). On the other hand high levels of cRNA express a non-inactivating K^+ current that rectifies outwardly (Fig. 5B). To test whether different levels of HLK_3 expression may influence the modulation of channel properties by intracellular Ca^{2+} and by protein kinase C activation we have examined the effects of Ca^{2+} injection, as well as those of PMA exposure, to oocytes injected with two different HLK_3 cRNA concentrations. Our results show that the modulation produced by both effectors is not affected by the level of mRNA expression (Fig. 5).

4. DISCUSSION

This work demonstrates that the human voltage-gated K^+ channel, HLK_3 (Kv1-3 [8]), can be depressed by activation of a co-expressed 5-HT_2 receptor. The coupling between the receptors and the channel appears to be mediated by the inositol phospholipid second messenger pathway via a G-protein, since activation of this pathway evokes a similar modulation. When G-proteins are activated by GTPγS, thus bypassing the need for receptor stimulation, the K^+ current was also potently

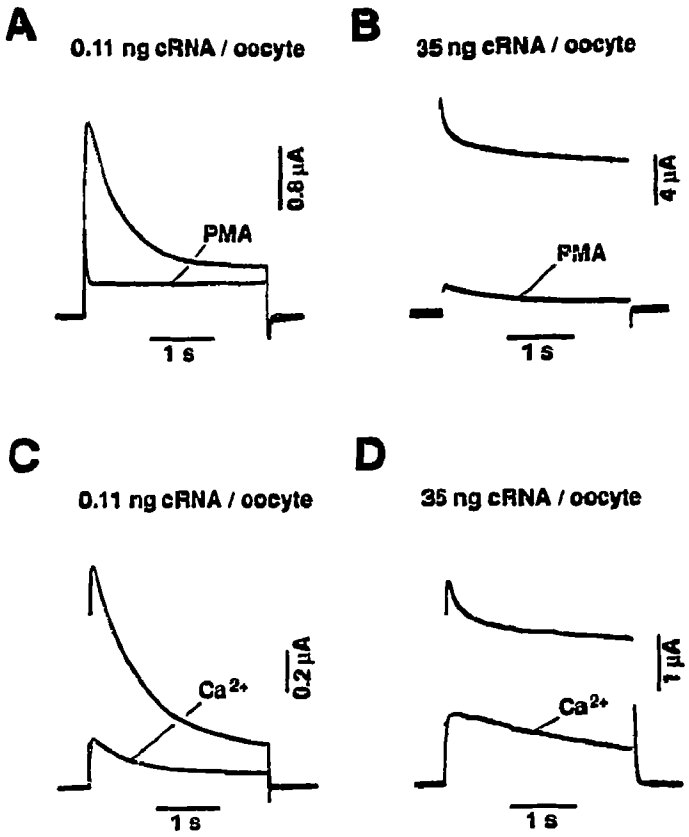

Fig 5. Regulation of HLK_3 channel remained constant whatever the RNA concentration used. (A) The transient K^+ current recorded in an oocyte injected with 0.11 ng cRNA was inhibited with 30 nM PMA. (B) The sustained K^+ current recorded in an oocyte injected with 35 ng cRNA was inhibited with 30 nM PMA. (C) Inhibition by internal Ca^{2+} (final concentration 1 µM) of the transient current. (D) Inhibition by Ca^{2+} of the sustained current.

blocked, indicating that the 5-HT action is mediated by a G-protein. Direct G-protein activation of HLK_3 channels seems unlikely because direct coupling generally occurs within milliseconds (for a review see [19]). We have examined whether phospholipase C products alter HLK_3 K^+ currents. Our results indicate that the two second messenger products, diacylglycerol and $InsP_3$, have the potential to converge signalling by inhibiting the voltage-gated K^+ current. Intracellular injection of $InsP_3$ or Ca^{2+} shows that an increase in intracellular Ca^{2+} concentration leads to a decreased K^+ channel activity. Recent reports have also observed a similar modulation by Ca^{2+} of mouse (MBK1) and rat (RBK1) delayed rectifier K^+ channels cloned from brain [20,21]. Direct gating of the channel by intracellular Ca^{2+} is unlikely since the modulation of K^+ currents affected by free Ca^{2+} levels usually occurs in the millisecond range. The Ca^{2+} regulatory action on HLK_3 K^+ current may involve a change in the phosphorylation state of the channel. However, alkaline phosphatase and calcineurin microinjections did not prevent Ca^{2+} inhibition nor 5-HT action. Alternatively, the action of a Ca^{2+}-

activated protease cannot be excluded. Further experiments are clearly needed to address this problem.

Our data show that the modulation of the HLK_3 K^+ channel by protein kinase C activation is also inhibitory. In this regard a recent report [22] has shown that protein kinase C can phosphorylate a *Drosophila Shaker* K^+ channel at a site located in the S4–S5 intracellular loop and which is conserved in the HLK_3 amino acid sequence [8]. The question now is to understand how results obtained with the cloned channel relate to observations which have been made in studies on the regulation of n-type K^+ channels in lymphocytes. First, the potent and long-lasting inhibition of the cloned voltage-sensitive K^+ channel observed in this work in the presence of phorbol esters and diacylglycerol has not been described for human circulating T-lymphocytes [23], as if subunits other than the HLK_3 subunit prevented phosphorylation and inhibition by kinase C (for example K^+ channels also have β subunits [24]). Second, the lack of a cAMP effect observed in this work is consistent with the absence of cAMP-mediated inhibition of n-type K^+ currents in human T cells [25,26], although a partial inhibition (due to an acceleration of inactivation) of probably similar K^+ channels has been observed in B lymphocytes [27]). Finally, the inhibitory effect of intracellular Ca^{2+} on the cloned HLK_3 channel expression in oocytes is very similar to that found for the n-type K^+ current in T-lymphocytes [25].

Lymphocytes bear receptors for a variety of transmitters that influence cellular functions [28]. Whether the opening of n-type K^+ channels is crucial for T-cell activation still remains unsolved [8,29]. The modulation of K^+ fluxes by several agents, including 5-HT [27], isoproterenol [30,31] and substance P [32], have been regarded as an important pathway for neural regulation of immune function. Knowledge of the action of diverse transmitters on individual cloned channels will certainly help to clarify this issue. Similarly, the regulation of HLK_3 K^+ channels in the central nervous system might be of great significance. Second messengers such as $InsP_3$ and diacylglycerol, by depressing the amplitude of this K^+ current, could serve to increase action potential duration and to decrease neuronal firing frequency.

Acknowledgements: We are grateful to Dr. E. Van Obberghen-Schilling for providing the 5-HT_2 receptor clone. We thank M.-M. Larroque, C. Roulinat and F. Aguila for skillful technical assistance. B.A. is a recipient of a fellowship from the Association pour la Recherche contre le Cancer. This work was supported by the Centre National de la Recherche Scientifique, the Association pour la Recherche contre le Cancer and the Association pour la Recherche sur la Sclérose en Plaques.

REFERENCES

[1] Rudy, B. (1988) Neurosciences 25, 729–749.
[2] Hille, B. (1984) Ionic Channels of Excitable Membranes, Sinauer, Sunderland, MA.
[3] Lewis, R.S. and Cahalan, M.D. (1988) Trends Neurosci. 11, 214–218.
[4] Barres, B.A., Chun, L.L.Y. and Corey, D.P. (1990) Annu. Rev. Neurosci. 13, 441–474.
[5] Rudy, B., Kentros, C. and Vega-Saenz De Miera, E. (1991) Mol. Cell. Neurosci. 2, 89–102.
[6] Philipson, L.H. and Miller, R.J. (1992) Trends Pharmacol. Sci. 13, 8–11.
[7] Chandy, K.G., Douglass, J., Gutman, G.A., Jan, L., Joho, R., Kaczmarek, L., McKinnon, D., North, R.A., Numa, S., Philipson, L., Ribera, A.B., Rudy, B., Salkoff, L., Swanson, R., Steiner, D., Tanouye, M. and Tempel, B.L. (1991) Nature 354, 26.
[8] Attali, B., Romey, G., Honoré, E., Schmid-Alliana, A., Mattéi, M.G., Lesage, F., Ricard, P., Barhanin, J. and Lazdunski, M. (1992) J. Biol. Chem. (in press).
[9] Stühmer, W., Ruppersberg, J.P., Schröter, K.H., Sakmann, B., Stocker, M., Giese, K.P., Perschke, A.A.B. and Pongs, O. (1989) EMBO J. 8, 3235–3244.
[10] Grissmer, S., Dethlefs, B., Wasmuth, J.J., Goldin, A.L., Gutman, G.A., Cahalan, M.D. and Chandy, K.G. (1990) Proc. Natl. Acad. Sci. USA 87, 9411–9415.
[11] Douglass, J., Osborne, P.B., Cai, Y.C., Wilkinson, M., Christie, M.J. and Adelman, J.P. (1990) J. Immunol. 144, 4841–4850.
[12] Kaczmarek, L.K. and Levitan, I.B. (1987) Neuromodulation: The Biochemical Control of Neuronal Excitability, Oxford University Press, New York.
[13] Choquet, D. and Korn, H. (1988) Biochem. Pharmacol. 37, 3797–3802.
[14] Rehm, H., Pelzer, S., Cochet, C., Chambaz, E., Tempel, B.L., Trautwein, W., Pelzer, D. and Lazdunski, M. (1989) Biochemistry 28, 60455–60460.
[15] Chambard, J.C., Van Obberghen-Schilling, E., Haslam, R.J., Vouret, V. and Pouysségur, J. (1990) Nucleic Acids Res. 18, 5282.
[16] Honore, E., Attali, B., Romey, G., Heurteaux, C., Ricard, P., Lesage, F., Lazdunski, M. and Barhanin, J. (1991) EMBO J. 10, 2085–2118.
[17] Dascal, N. (1987) CRC Crit. Rev. Biochem. 22, 317–387.
[18] Honoré, E., Attali, B., Romey, G., Lesage, F., Barhanin, J. and Lazdunski, M. (1992) EMBO. J. (submitted).
[19] Brown, A.M. (1991) FASEB J. 5, 2175–2179.
[20] Hoger, J.H., Walter, A.E., Vance, D., Yu, L., Lester, H.A. and Davidson, N. (1991) Neuron 6, 227–236.
[21] Kavanaugh, M.P., Christie, M.J., Osborne, P.B., Busch, A.E., Shen, K.Z., Wu, Y.N., Seeburg, P.H., Adelman, J.P. and North, R.A. (1991) Biochem. J. 277, 899–902.
[22] Isacoff, E.Y., Kimmerly, W., Jan, Y.N. and Jan, L.Y. (1992) Biophys. J. 61, 73 (Abstr.).
[23] Deutsch, C., Krause, D. and Lee, S.C. (1986) J. Physiol. 372, 405–423.
[24] Rehm, H. and Lazdunski, M. (1988) Proc. Natl. Acad. Sci. USA 85, 4919–4923.
[25] Bregestovski, P., Redkozubov, A. and Alexeev, A. (1986) Nature 319, 776–778.
[26] Lee, S.C. and Deutsch, C. (1990) Biophys. J. 57, 49–62.
[27] Choquet, D. and Korn, H. (1988) Proc. Natl. Acad. Sci. USA 85, 4557–4561.
[28] Plaut, M. (1987) Annu. Rev. Immunol. 5, 621–669.
[29] Price, M., Lee, S.C. and Deutsch, C. (1989) Proc. Natl. Acad. Sci. USA 86, 10171–10175.
[30] Choquet, D., Sarthou, P., Primi, D., Cazenave, P.A. and Korn, H. (1987) Science 235, 1211–1214.
[31] Soliven, B. and Nelson, D.J. (1990) J. Memb. Biol. 117, 263–274.
[32] Schumann, M.A. and Gardner, P. (1989) J. Memb. Biol. 111, 133–139.