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Minireview

The functional role of the β-subunit in the maturation and intracellular transport of Na,K-ATPase

Käthi Geering

Institut de Pharmacologie et Toxicologie, Université de Lausanne, CH-1005 Lausanne, Switzerland

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The minimal functional enzyme unit of Na,K-ATPase consists of an α–β complex. The α-subunit bears all functional domains of the enzyme and so far a regulatory role for the β-subunit in the catalytic cycle has not been established. On the other hand, increasing experimental evidence suggests that the β-subunit is an indispensable element for the structural and functional maturation of the enzyme as well as its intracellular transport to the plasma membrane. This brief review summarizes the experimental data supporting the hypothesis that assembly of the β-subunit is needed for the α-subunit to acquire the correct, stable configuration necessary for the acquisition of functional properties and its exit from the ER.

Na-K-ATPase; Adenosine triphosphatase; β-Subunit; Protein oligomerization; Subunit assembly; Maturation; Intracellular transport

1. INTRODUCTION

The Na,K-pump or its molecular equivalent the Na,K-ATPase is a ubiquitous plasma membrane transporter found in animal cells (for reviews see [1–3]). Its principal function is related to the maintenance of cellular ion homeostasis, e.g. high intracellular K and low Na concentrations. The Na,K-pump moves K and Na against their chemical gradients by using the energy provided by ATP hydrolysis. The Na gradients resulting from the enzyme's activity are crucial for the efficient functioning of other Na-coupled transport systems and in consequence the Na,K-ATPase becomes a key enzyme implicated in many general and specialized cellular functions.

At the molecular level, Na,K-ATPase is composed of two non-covalently linked subunits. Hydropathy analysis of amino acid sequences [4–7], chemical labeling or proteolysis [8–10] and studies on antibody reactivity [11–15] of Na,K-ATPase subunits have provided the necessary information to deduce the putative membrane topology of the 2 enzyme components. The α-subunit is a complex membrane protein of class IV, with several membrane-spanning domains. Na,K-ATPase α-subunit shares this membrane organization with all P-ATPases (for review see [1]) as well as with proteins such as adrenergic receptors, subunits of the acetylcholine receptor or the human glucose transporter (for review see [16]). The β-subunit on the other hand belongs to the class II membrane proteins characterized by one membrane-spanning domain and the N-terminus exposed to the cytoplasmic side (for review see [16]).

The α- or catalytic subunit bears all domains related to the enzyme's activity, namely binding sites for cations, ATP and phosphate as well as for the specific cardiac glycoside inhibitors. At least 3 α-isoforms exist which exhibit a tissue-specific distribution and differences in functional properties (for review see [17,18]).

The putative role of the β-subunit, the second component of the functional enzyme expressed at the plasma membrane, remains obscure. However, recently, β1- [5,7,19–22] β2- [23,24] and β3-isoforms [25] have been identified, a fact which strongly suggests that β-subunits like α-subunits belong to a family of proteins, each having a distinct functional property. Since β-subunits do not participate in an obvious, direct way in the catalytic cycle, nor do they determine ouabain sensitivity of the enzyme [26–29], β-subunits might rather be discrete indirect modulators of the enzyme's activity. This new interesting concept is indeed supported by the recent observation that β2-isoforms exhibit characteristics of an adhesion molecule [23].

The fine tuning of the Na,K-ATPase activity by the β-subunit or its role as a cellular recognition signal awaits further confirmation. On the other hand, there is increasing experimental evidence that the β-subunit or more specifically its assembly to the catalytic α-subunit

Correspondence address: K. Geering, Institut de Pharmacologie et Toxicologie, Université de Lausanne, CH-1005 Lausanne, Switzerland. Fax: (41) (21) 3132775.

might be a major determinant in the structural and functional maturation of the enzyme as well as for its intracellular transport. In the following, I would like to summarize what we know so far on the main steps implicated in the biosynthesis, the posttranslational processing and the cellular sorting of Na,K-ATPase with special emphasis on the putative role of the β-subunit in these events.

2. BIOSYNTHESIS, SUBUNIT ASSEMBLY AND POSTTRANSLATIONAL MATURATION OF Na,K-ATPase

Some but not all details of the biosynthesis and the membrane integration of the two Na,K-ATPase subunits have been established (for recent review see [30]). The available information predicts that these processes are governed by certain general mechanisms which are similar for most membrane proteins (for recent review see [31]). Briefly, the subunits of Na,K-ATPase are synthesized from distinct mRNAs and can insert into membranes of the endoplasmic reticulum (ER) independently of each other [32,33]. Integration of the two subunits occurs cotranslationally [32–35] and eventually depends on the interaction with the signal recognition particle (SRP) [35,36] and probably the SRP receptor [32]. Neither of the two subunits has a cleavable signal sequence. In the α-subunit, the first four transmembrane segments [37] and in the β-subunit, the single membrane-spanning domain [35] apparently include internal signals necessary for membrane insertion. Finally, in the course of translation, the β-subunit acquires core sugars [33] and most likely forms the three disulphide bonds which have been identified in the purified enzyme [38–40].

The actual data on the biosynthesis and membrane integration of Na,K-ATPase do not support a previously proposed idea that β-subunits might act as receptors for membrane integration of the α-subunits [41]. Nevertheless, studies of the biosynthesis performed in cultured cells, in transfected animal or yeast cells or in *Xenopus* oocytes injected with cRNA (mRNA prepared from cloned cDNA by transcription in vitro) reveal that the β-subunit certainly plays an important role in the efficient expression of newly synthesized α-subunits and ultimately in functional α–β complexes at the cell surface. Noguchi et al. [42] were the first to show that an increase in functional pumps exposed at the plasma membrane of *Xenopus* oocytes injected with cRNA critically depends on the concomitant expression of α- and β-subunits. Similarly, both α- and β-subunits were required to increase the Na,K-ATPase activity and ouabain-binding in transfected yeast cells which unlike animal cells lack an endogenous Na,K-ATPase [43]. Finally, in certain experimental systems, an increase in cellular Na,K-pump molecules or activity is achieved by a selective upregulation of β-mRNA alone [44,45].

Several lines of evidence now suggest that the basis for the interdependence of α- and β-subunits is the necessity of subunit oligomerization in the structural and functional maturation of the enzyme. Actually, several arguments are in favour of the hypothesis that assembly of the β-subunit is needed to impose a correct, stable membrane organization on the newly synthesized α-subunit. A first interesting observation related to this issue was made in cultured cells treated with tunicamycin. This inhibitor of protein core glycosylation indeed produced a significant and specific decrease in the amount not only of the newly synthesized glycoproteinic β-subunit but also of the α-subunit which is not a glycoprotein [46,47]. This result pointed to the possibility that a decreased synthesis of the β-subunit or its destabilization due to glycosylation-inhibition might ultimately prevent an efficient cellular accumulation of α-subunits. The importance of subunit assembly for the structural and functional maturation of the enzyme is also supported by the temporal and regional coincidence of the two processes. Indeed, subunit assembly occurs rapidly after completion of synthesis at the level of the ER [48]. Similarly, the α-subunit is processed from a trypsin-sensitive form into a trypsin-resistant form when still residing in the ER [49]. Finally, the α-subunit acquires some of its functional properties in parallel with and most likely as a consequence of its structural maturation. Within 10–20 min after synthesis, the enzyme becomes able to change its configuration in response to Na and K [49] or to bind ouabain in the presence of Na, Mg and ATP [50].

More direct evidence for the role of the β-subunit in the correct membrane assembly of the α-subunit is provided by recent biosynthesis studies in the *Xenopus* oocyte. Interestingly, these cells in contrast to most other animal cells, do not synthesize stoichiometric amounts of α- and β-subunits but accumulate an excess of α-subunits over β-subunits [51]. The overexpressed α-subunit is highly trypsin-sensitive and resembles, in this respect, the immature α-subunit found in cultured *Xenopus* kidney (A_6) cells shortly after synthesis [49]. Significantly, the sole injection of β-cRNA into *Xenopus* oocytes renders the trypsin-sensitive α-subunit population trypsin-resistant [51]. Similarly, an increased protection against trypsinolysis is also observed for exogenous α-subunits concomitantly synthesized with β-subunits in oocytes injected with cRNA [52,53].

Coexpression of the two subunits not only renders the α-subunit trypsin-resistant, but also leads to an increased cellular accumulation of the catalytic subunit [53,54]. This result suggests that assembly of the β-subunit has a stabilizing effect on the α-subunit. This hypothesis was tested by measuring the half-life of newly synthesized α-subunits that were expressed in *Xenopus* oocytes either alone or together with β-subunits. Such experiments reveal that exogenous α-subunits are rapidly degraded ($t_{1/2}$ about 2 h) in

Xenopus oocytes unless they are expressed concomitantly with β-subunits ($t_{1/2}$ about 20 h) [55]. Since the transit time of Na,K-ATPase from the ER to the Golgi is rather slow in *Xenopus* oocytes ($t_{1/2}$ about 10 h) it is likely that degradation of the α-subunit takes place at the level of the ER or in a nearby compartment [55]. Overall, the actual experimental data support the hypothesis that assembly of the β-subunit to the α-subunit, rapidly after synthesis, is a pre-requisite for the catalytic subunit to acquire a correct, stable membrane organization, likely to be needed for its functional maturation. Thus Na,K-ATPase must meet similar requirements for a correct folding as many other oligomeric proteins studied (for review see [56]).

Interestingly, Noguchi et al. [57] recently reported that β-subunits do not need to be concomitantly synthesized with α-subunits to be able to form active α–β complexes. Newly synthesized α-subunits in *Xenopus* oocytes can associate with pre-existing β-subunits which had been synthesized from injected cRNA before expression of α-subunits. This result indicates that assembly is not necessarily a cotranslational event and provides a further argument that the β-subunit is the limiting factor for the formation of functional α–β complexes. This hypothesis is further supported by the observation that β-subunits synthesized alone in *Xenopus* oocytes have a longer half-life ($t_{1/2}$ about 9 h) than unassembled α-subunits ($t_{1/2}$ about 2 h) [55]. Since very little is known about the mechanisms which govern oligomerization of multisubunit proteins, we can only speculate that the longer half-life of the β-subunit might be potentially important for an efficient subunit assembly to occur.

3. SUBUNIT ASSEMBLY AND INTRACELLULAR TRANSPORT OF Na,K-ATPase

Injection of β-cRNA into *Xenopus* oocytes leads not only to an increased trypsin resistance of the overexpressed endogenous α-subunit, but also to the expression of more functional pumps at the plasma membrane as assessed by ouabain binding [51]. This result led to the assumption that in analogy to other oligomeric proteins (for review see [56]), subunit assembly might be required for intracellular transport of the enzyme to the plasma membrane, in particular for its exit out of the ER. The experimental evidence so far available is indeed in favour of this hypothesis since as discussed above, the α-subunit synthesized in the absence of β-subunit in *Xenopus* oocytes has a very short half-life which is incompatible with transit through the Golgi [55]. Furthermore, Takeyasu et al. [58] have observed in immunofluorescence studies that most of an avian α-subunit population overexpressed in transfected mouse cells accumulated intracellularly, most likely in the ER.

Interestingly, in *Xenopus* oocytes, β-subunits despite their longer half-life, appear to also remain confined to the ER in the absence of concomitant α-subunit synthesis. This result was deduced from the observation that β-subunits are kept in a core-glycosylated form if expressed alone. Acquisition of complex type sugars, an indicator of passage through a trans-Golgi compartment, is only observed when the β-subunit is expressed together with α-subunits [55].

At present, there is no definite proof that the observation made in *Xenopus* oocytes on the transport incompetence of unassembled β-subunits is of general validity. Indeed, by combining pulse-chase experiments with cellular fractionation of MDCK cells, it was recently reported that sucrose gradient fractions enriched in basolateral membranes contain an excess of β-subunits over α-subunits [44]. In addition, Marxer et al. [59] were able to reveal a β-subunit-like antigen in the brush border of rat distal colon which cross-reacted with a monoclonal β-antibody but which did not co-distribute with α-subunits. These results could indicate that in certain cell types, β-subunits might escape the transport constraint observed in the *Xenopus* oocyte. However, this hypothesis can only be validated if it could be shown that β-subunits are not associated with another non-α-subunit protein which might permit their intracellular transport.

4. POSTTRANSLATIONAL PROCESSING OF THE β-SUBUNIT AND ITS ROLE IN CELL SURFACE EXPRESSION OF FUNCTIONAL Na,K-ATPase

So far, it has become evident that the assembly process of the β-subunit to the α-subunit at the level of the ER is of crucial importance for the maturation and transport competence of Na,K-ATPase. The question arises whether the β-subunit or in particular the post-translational modifications to which it is subjected as a glycoprotein might additionally be implicated in the correct targeting of the enzyme to the cell surface or in the acquisition of some functional properties.

The β-subunit of Na,K-ATPase is a heavily glycosylated polypeptide. *N*-linked glycosylation processing of the β-subunit is much the same as for other glycoproteins (for review and references see [30]). In brief, the β-subunit acquires core sugars during its synthesis on consensus asparagine residues which are progressively trimmed during the transport of the polypeptide to the Golgi. Finally, complex-type sugars are added in a trans-Golgi compartment before the enzyme reaches the plasma membrane. Interestingly, besides the previously discussed observation that inhibition of core glycosylation leads to a significant decrease in the cellular accumulation of newly synthesized β- and α-subunits, no obvious impediment to the enzyme's sorting or to its activity could be demonstrated when glycosylation of the β-subunit was perturbed. Indeed, neither structural nor functional properties of the

catalytic α-subunit are affected after inhibition of correct terminal glycosylation [46]. In addition, even unglycosylated Na,K-ATPase produced by inhibition of core glycosylation with tunicamycin reaches the plasma membrane and exhibits similar catalytic activities as does the fully glycosylated enzyme [47,48,50]. Thus the role of the sugar moiety of Na,K-ATPase remains an enigma as is the case for many other glycoproteins. In view of the recent observation that certain β-isoforms might have a putative role in cell adhesion [23] it is tempting to speculate that complex type sugars of the β-subunit might be implicated in cellular recognition. Differences in the number [52] and certainly also in the chemical composition of sugar moieties observed in different β-isoforms could provide a high signal specificity.

5. PERSPECTIVES

In this short review, I have summarized the available information which supports the notion that one of the basic functions of the β-subunit of Na,K-ATPase is to confer a stable, correct configuration on the catalytic α-subunit which permits the structural, and in consequence the functional, maturation as well as the exit from the ER of the Na,K-ATPase. Since failure to assemble into α–β complexes leads to the retention and degradation of the catalytic α-subunit at the level of the ER, it appears that Na,K-ATPase is subjected to the same quality control of the ER as other multisubunit proteins (for reviews see [56,61,62]). It will now be important to study what the determinants are in the β- and the α-subunits which control an efficient assembly and thus abrogate ER degradation. To localize specific putative assembly domains in the two subunits it might be helpful to check the ability of various isoform combinations to assemble into stable α–β complexes. It will also be most interesting to test whether the recently identified β-subunit from an other P-ATPase, the H,K-ATPase [63–66] might act as a surrogate for Na,K-ATPase β-subunits.

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REFERENCES

[1] Jørgensen, P.L. and Andersen, J.P. (1988) J. Membrane Biol. 103, 95–120.
[2] Nørby, J.G. (1989) Biochem. Soc. Trans. 17, 806–808.
[3] Skou, J.C. (1990) FEBS Lett. 268, 314–324.
[4] Kawakami, K., Noguchi, S., Noda, M., Takahashi, H., Ohta, T., Kawamura, M., Nojima, H., Nagano, K., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. and Numa, S. (1985) Nature 316, 733–736.
[5] Noguchi, S., Noda, M., Takahashi, H., Kawakami, K., Ohta, T., Nagano, K., Hirose, T., Inayama, S., Kawamura, M. and Numa, S. (1986) FEBS Lett. 196, 315–320.
[6] Shull, G.E., Schwartz, A. and Lingrel, J.B. (1985) Nature 316, 691–695.
[7] Shull, G.E., Lane, L.K. and Lingrel, J.B. (1986) Nature 321, 429–431.
[8] Jørgensen, P.L., Karlish, S.J.D. and Gitler, C. (1982) J. Biol. Chem. 257, 7435–7442.
[9] Dzhandzhugazyan, K.N. and Jørgensen, P.L. (1985) Biochim. Biophys. Acta 817, 165–173.
[10] Ohta, T., Yoshida, M., Nagano, K., Hirano, H. and Kawamura, M. (1986) FEBS Lett. 204, 297–301.
[11] Farley, R.A., Miller, R.P. and Kudrow, A. (1986) Biochim. Biophys. Acta 873, 136–142.
[12] Farley, R.A., Ochoa, G.T. and Kudrow, A. (1986) Am. J. Physiol. 250, C896–C906.
[13] Girardet, M., Geering, K., Frantes, J.M., Geser, D., Rossier, B.C., Kraehenbuhl, J.P. and Bron, C. (1981) Biochemistry 20, 6684–6691.
[14] Kano, I., Satoh, K., Nagai, F., Ushiyama, K., Nakao, T., Hara, Y. and Kano, K. (1990) Biochem. Cell Biol. 68, 1262–1267.
[15] Ovchinnikov, Y.A., Luneva, N.M., Arysstarkhova, E.A., Gevondyan, N.M., Arzamazova, N.M., Kozhich, A.T., Nesmeyanov, V.A. and Modyanov, N.N. (1988) FEBS Lett. 227, 230–234.
[16] Heijne, G.V. and Gavel, Y. (1988) Eur. J. Biochem. 174, 671–678.
[17] Sweadner, K.J. (1989) Biochim. Biophys. Acta 988, 185–220.
[18] Lingrel, J.B., Orlowski, J., Shull, M.M. and Price, E.M. (1990) Progr. Nucleic Acid Res. Molec. Biol. 38, 37–89.
[19] Bhattacharyya, K.K., Bergstrom, E.E. and Hokin, L.E. (1990) FEBS Lett. 269, 233–238.
[20] Brown, T.A., Horowitz, B., Miller, R.P., McDonough, A. and Farley, R.A. (1987) Biochim. Biophys. Acta 912, 244–253.
[21] Kawakami, K., Nojima, H., Ohta, T. and Nagano, K. (1986) Nucleic Acids Res. 14, 2833–2844.
[22] Ovchinnikov, Y.A., Modyanov, N.N., Broude, N.E., Petrukhin, K.E., Grishin, A.V., Arzamazova, N.M., Aldanova, N.A., Monastyrskaya, G.S. and Sverdlov, E.D. (1986) FEBS Lett. 201, 237–245.
[23] Gloor, S., Antonicek, H., Sweadner, K.J., Pagliusi, S., Frank, R., Moos, M. and Schachner, M. (1990) J. Cell Biol. 110, 165–174.
[24] Martin-Vasallo, P., Dackowski, W., Emanuel, J.R. and Levenson, R. (1989) J. Biol. Chem. 264, 4613–4618.
[25] Good, P.J., Richter, K. and Dawid, I.B. (1990) Proc. Natl. Acad. Sci. USA 87, 9088–9092.
[26] Emanuel, J.R., Schulz, J., Zhou, X.M., Kent, R.B., Housman, D., Cantley, L. and Levenson, R. (1988) J. Biol. Chem. 263, 7726–7733.
[27] Hara, Y., Nikamoto, A., Kojima, T., Matsumoto, A. and Nakao, M. (1988) FEBS Lett. 238, 27–30.
[28] Kent, R.B., Emanuel, J.R., Neriah, Y.B., Levenson, R. and Housman, D.E. (1987) Science 237, 901–903.
[29] Takeyasu, K., Tamkun, M.M., Siegel, N.R. and Fambrough, D.M. (1987) J. Biol. Chem. 262, 10733–10740.
[30] Geering, K. (1990) J. Membrane Biol. 115, 109–121.
[31] Lazdunski, C.J. and Benedetti, H. (1990) FEBS Lett. 268, 408–414.
[32] Cayanis, E., Bayley, H. and Edelman, I.S. (1990) J. Biol. Chem. 265, 10829–10835.
[33] Geering, K., Meyer, D.I., Paccolat, M.P., Kraehenbuhl, J.P. and Rossier, B.C. (1985) J. Biol. Chem. 260, 5154–5160.
[34] Caplan, M.J., Palade, G.E. and Jamieson, J.D. (1986) J. Biol. Chem. 261, 2860–2865.
[35] Kawakami, K. and Nagano, K. (1988) J. Biochem. 103, 54–60.
[36] Geering, K. (1988) in: The Na^+,K^+-Pump Part B: Cellular Aspects, (Skou, J.C., Nørby, J.G., Maunsbach, A.B. and

Esmann, M. eds) Progress in Clinical and Biological Research, Vol. 268B, pp. 19-33, A.R. Liss, New York.
[37] Homareda, H., Kawakami, K., Nagano, K. and Matsui, H. (1988) in: The Na^+,K^+-Pump. Part B: Cellular Aspects (Skou, J.C., Nørby, J.G., Maunsbach, A.B. and Esmann, M. eds) Progress in Clinical and Biological Research Vol. 268B, pp. 77-84, A.R. Liss, New York.
[38] Gevondyan, N.M., Gevondyan, V.S., Gavrilyeva, E.E. and Modyanov, N.N. (1989) FEBS Lett. 255, 265-268.
[39] Kirley, T.L. (1989) J. Biol. Chem. 264, 7185-7192.
[40] Miller, R.P. and Farley, R.A. (1990) Biochemistry 29, 1524-1532.
[41] Hiatt, A., McDonough, A.A. and Edelman, I.S. (1984) J. Biol. Chem. 259, 2629-2635.
[42] Noguchi, S., Mishina, M., Kawamura, M. and Numa, S. (1987) FEBS Lett. 225, 27-32.
[43] Horowitz, B., Eakle, K.A., Scheiner-Bobis, G., Randolph, G.R., Chen, C.Y., Hitzeman, R.A. and Farley, R.A. (1990) J. Biol. Chem. 265, 4189-4192.
[44] McDonough, A.A., Geering, K. and Farley, R.A. (1990) FASEB J. 4, 1598-1605.
[45] Taormino, J.P. and Fambrough, D.M. (1990) J. Biol. Chem. 265, 4116-4123.
[46] Zamofing, D., Rossier, B.C. and Geering, K. (1988) J. Membrane Biol. 104, 69-79.
[47] Zamofing, D., Rossier, B.C. and Geering, K. (1989) Am. J. Physiol. 256, C958-C966.
[48] Tamkun, M.M. and Fambrough, D.M. (1986) J. Biol. Chem. 261, 1009-1019.
[49] Geering, K., Kraehenbuhl, J.P. and Rossier, B.C. (1987) J. Cell Biol. 105, 2613-2619.
[50] Caplan, M.J., Forbush III, B., Palade, G.E. and Jamieson, J.D. (1990) J. Biol. Chem. 265, 3528-3534.
[51] Geering, K., Theulaz, I., Verrey, F., Häuptle, M.T. and Rossier, B.C. (1989) Am. J. Physiol. 257, C851-C858.
[52] Jaunin, P., Good, P., Corthésy, I., and Geering, K. (1991) in: The Sodium Pump: Recent Developments, The Rockefeller University Press, New York (in press).
[53] Noguchi, S., Higashi, K. and Kawamura, M. (1990) J. Biol. Chem. 265, 15991-15995.
[54] Geering, K. (1991) in: The Sodium Pump: Structure, Mechanism and Regulation, The Rockefeller University Press, New York (in press).
[55] Ackermann, U. and Geering, K. (1990) FEBS Lett. 269, 105-108.
[56] Hurtley, S.M. and Helenius, A. (1989) Annu. Rev. Cell Biol. 5, 277-307.
[57] Noguchi, S., Higashi, K. and Kawamura, M. (1990) Biochim. Biophys. Acta 1023, 247-253.
[58] Takeyasu, K., Tamkun, M.M., Renaud, K. and Fambrough, D.M. (1988) J. Biol. Chem. 263, 4347-4354.
[59] Marxer, A., Stieger, B., Quaroni, A., Kasgarian, M. and Hauri, H.-P. (1989) J. Cell Biol. 109, 1057-1069.
[60] Takeda, K., Noguchi, S., Sugino, A. and Kawamura, M. (1988) FEBS Lett. 238, 201-204.
[61] Lodish, H.F. (1988) J. Biol. Chem. 263, 2107-2110.
[62] Rose, J.K. and Doms, R.W. (1988) Annu. Rev. Cell Biol. 4, 257-288.
[63] Canfield, V.A., Okamoto, C.T., Chow, D., Dorfman, J., Gros, P., Forte, J.G. and Levenson, R. (1990) J. Biol. Chem. 265, 19878-19884.
[64] Okamoto, C.T., Karpilow, J.M., Smolka, A. and Forte, J.G. (1990) Biochim. Biophys. Acta 1037, 360-372.
[65] Reuben, M.A., Lasater, L.S. and Sachs, G. (1990) Proc. Natl. Acad. Sci. USA 87, 6767-6771.
[66] Shull, G.E. (1990) J. Biol. Chem. 265, 12123-12126.