Biochimica et Biophysica Acta, 1023 (1990) 247–253
Elsevier

BBAMEM 74803

Assembly of the α-subunit of *Torpedo californica* Na^+/K^+-ATPase with its pre-existing β-subunit in *Xenopus* oocytes

Shunsuke Noguchi, Ken Higashi and Masaru Kawamura

Department of Biology and Biochemistry, University of Occupational and Environmental Health, Yahatanishi, Kitakyushu, 807 (Japan)

(Received 23 October 1989)

Key words: ATPase, Na^+/K^+-; cDNA expression; Antisense oligonucleotide; *Xenopus* oocyte; (*T. californica*)

The α- and β-subunits of *Torpedo californica* Na^+/K^+-ATPase were expressed in turn in single oocytes by alternately microinjecting the specific mRNAs for the α- and β-subunits. The mRNA first injected was degraded prior to the injection of the second mRNA by injecting the antisense oligonucleotide specific for the first mRNA. The pre-existing β-subunit, which had been synthesized by injecting mRNA for the β-subunit, could assemble with the α-subunit expressed later in the single oocytes and the resulting αβ complex acquired both ouabain-binding and Na^+/K^+-ATPase activities. On the other hand, formation of the αβ complex was not detected when the α-subunit was expressed first, followed by the β-subunit. These data suggest that the β-subunit acts as a receptor or a stabilizer for the α-subunit upon the biogenesis of Na^+/K^+-ATPase.

Introduction

Sodium- and potassium-dependent ATPase (Na^+/K^+-ATPase) is a membrane-bound enzyme catalyzing ATP-driven transport of Na^+ and K^+. It is composed of two heterologous subunits: a catalytic α-subunit of about 110 kDa, which spans the membrane 6 to 8 times, [1–3] and an N-linked glycoprotein β subunit (molecular mass of its protein moiety is about 35 kDa), which spans the membrane once [4–6]. While the α-subunit carries all of the catalytic properties of the enzyme so far known (for review see Ref. 7), the precise role of the β-subunit remains to be solved. However, Hiatt et al. [8] have suggested that the β-subunit has an anchoring role when the nascent α-subunit is incorporated into the membrane. Tamkun and Fambrough [9] have claimed that the assembly of the α- and the β-subunits occurs during or immediately after the synthesis of the polypeptides. Moreover, Noguchi et al. [10] have recently reported that the expression of functionally active Na^+/K^+-ATPase in *Xenopus* oocytes can be achieved only when mRNAs for both the α- and β-subunits have been injected. These observations suggest some functional roles of the β-subunit in the membrane insertion or the maturation of the enzyme.

In view of these data, we investigated when the assembly of the α- and β-subunits occurs or whether the β-subunit assists the α-subunit to become correctly and stably expressed in the cell. For this purpose, we have induced the expression of the α- and β-subunits of *Torpedo californica* Na^+/K^+-ATPase in *Xenopus* oocytes. The oocyte system allows the specific programmed synthesis of different subunits in a cell by alternately injecting individual mRNAs.

Here we report that the active αβ complex was formed in oocytes when the injection of the mRNA for the β-subunit was followed by the injection of the mRNA for the α-subunit; the active complex was also formed when both mRNAs were injected simultaneously. These data suggest that the β-subunit acts as a receptor for the newly synthesized α-subunit and facilitates the accumulation of the enzyme in the membrane.

Materials and Methods

Xenopus oocytes and microinjection. Gravid females of *Xenopus laevis* (Fukuda, Kumamoto, Japan) were anesthetized by immersion in ice water. Their ovarian lobes were surgically removed and collected into modified Barth's medium [11]. Stage IV or V oocytes, distinguished by size, were then manually dissected away

Correspondence: M. Kawamura, Department of Biology, University of Occupational and Environmental Health, Yahatanishi, Kitakyushu, 807 Japan.

from the lobes and stored at 19° C in modified Barth's medium containing 100 μg/ml each of ampicillin, streptomycin, and cefmenoxime and 15 μg/ml nystatin. The recombinant plasmids used for in vitro synthesis of mRNAs for the subunits of *Torpedo californica* Na^+/K^+-ATPase were constructed as described previously [10]. Oocytes were microinjected with 20 nl of mRNA and incubated at 19° C for 3 days. A synthetic oligodeoxynucleotide that was complementary to the coding sequence for amino acid residues near the N-terminus of the α- or β-subunit (3′-(3)CCCCTTTCCC-CGACGTTCACTCTTC(27)-5′ for the α-subunit and 3′-(78)CCCGTCCTGGCCGTGCTCGACCAAG(102)-5′ for the β-subunit) was injected to inhibit further expression of the subunit in oocytes [12]. Oocyte proteins were metabolically labeled with L-[4,5-^{3}H]leucine by incubating oocytes in modified Barth's medium containing [^{3}H]leucine (142 Ci/mmol, 0.6 mCi/ml; Amersham) at 19° C for the last 3 days of the incubation period, irrespective of the total incubation time.

Immunoprecipitation and fluorography. Immunoprecipitation of the α- and the β-subunits was carried out with antisera prepared against purified subunits from the electric organ of *T. californica* [10]. The homogenates (corresponding to 18 labeled oocytes each) were brought to 1% with respect to Triton X-100 and cleared by centrifugation at 160000 × *g* for 30 min. Antiserum was added to the supernatant and incubated for 8 h at 4° C, after which Protein A-Sepharose CL-4B was added. The resulting immunoprecipitate was analyzed by electrophoresis on 0.1% SDS, 10% polyacrylamide gels [13], followed by fluorography.

Blot hybridization analysis of mRNA. Tolal oocyte RNA was extracted by the guanidium/cesium chloride method [14] from 80 oocyte. For blot analysis [15], samples (10 μg) of total oocyte RNA were denatured with glyoxal, electrophoresed in 1.5% agarose gel, transferred to Biodyne membrane (Pall, East Hills, NY), and hybridized to nick-translated probes [14].

Membrane preparation and assay of ATPase activity. About 400 oocytes for each preparation were homogenized in a 6 ml solution containing 250 mM sucrose, 50 mM imidazole-HCl buffer (pH 7.5) and 1 mM EDTA (solution A). The homogenate was centrifuged at 7000 × *g* for 10 min on a 50% sucrose cushion (1 ml); then the supernatant was removed and further centrifuged at 160000 × *g* for 30 min. The resulting pellet was suspended and incubated at 20° C for 1 h in 4 ml of a solution containing 1 M NaSCN, 125 mM sucrose, 25 mM imidazole-HCl buffer (pH 7.5) and 0.5 mM EDTA to reduce ouabain-insensitive ATPase activity [16]. The suspension was then centrifuged at 160000 × *g* for 30 min. The pellet was washed twice with solution A and suspended in 0.4 ml solution A; and ATPase activity was then assayed at 37° C in a reaction mixture (0.2 ml) containing 50 mM imidazole-HCl buffer (pH 7.5), 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$, 1 mM ATP and microsomes (20–80 μg protein) in the presence or absence of 1 mM ouabain. Na^+/K^+-ATPase activity was obtained by subtracting the ATPase activity measured in the presence of ouabain from that measured in its absence.

Assay of [^{3}H]ouabain-binding activity. For the assay of [^{3}H]ouabain-binding activity on the cell surface, 40 oocytes were treated with 1 mg/ml collagenase and then manually dissected away from the surrounding layers of follicular cells [17]. Defolliculated oocytes were incubated at 19° C for 6 h with 0.1 μM [21,22-^{3}H]ouabain (7.8 mCi/mmol) in modified Barth's medium from which KCl was omitted. The amount of [^{3}H]ouabain-binding measured in the presence of 1 mM unlabeled ouabain was subtracted to estimate the specific binding.

Materials. Enzyme used for construction of recombinant plasmids and in vitro transcription were purchased from Takara Shuzo, Nippon Gene and Toyobo. Other chemicals and biochemicals were obtained from Nacalai Tesque and of reagent or higher grade.

Results

Repression of the expression of Na^+/K^+-ATPase subunits by antisense oligonucleotides

We have shown that injection of mRNAs specific for the α- and β-subunits of *Torpedo* Na^+/K^+-ATPase into *Xenopus* oocytes leads to the synthesis of the subunits and the incorporation of functional *Torpedo* Na^+/K^+-ATPase in the oocyte surface membrane [10,18]. Dash et al. [12] have demonstrated that injection of small oligonucleotides into *Xenopus* oocytes leads to complete degradation of complementary mRNA by means of an RNase-H-like activity. To study whether an antisense oligonucleotide could degrade mRNA for the Na^+/K^+-ATPase subunit, the oligonucleotide complementary to a part of the mRNA was injected 3 days after injection of the mRNA and the time-course and degree of mRNA degradation following antisense oligonucleotide injection were examined by using blot hybridization analysis. The results are shown in fig. 1. The injected mRNA was recovered with total oocyte RNA at 1, 3, 6, 12 and 24 h after injection of oligonucleotide, electrophoresed and transferred to Biodyne membrane for hybridization to either α-subunit (Fig. 1, lanes 1–6) or β-subunit (Fig. 1, lanes 7–12) cDNA probes. In both cases, the mRNAs were almost completely degraded 12–24 h after the injection of the oligonucleotides. Therefore, we routinely injected the second mRNA at the earliest 12 h after the injection of oligonucleotide in order to avoid the expression of the first-injected mRNA while the second mRNA was translated.

To confirm the repression of mRNA translation by specific antisense oligonucleotide, the translation prod-

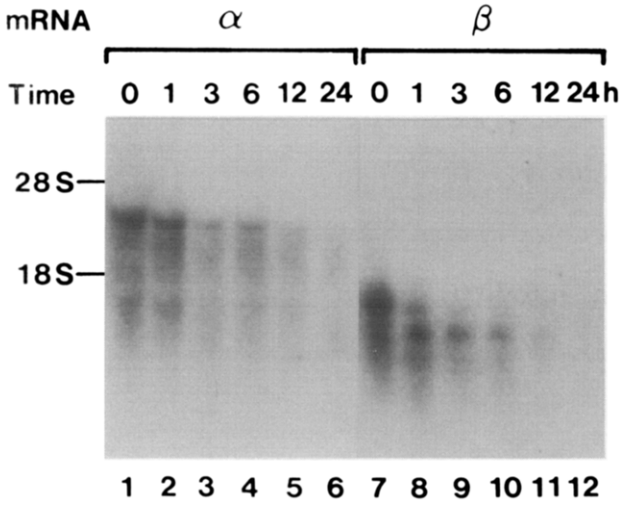

Fig. 1. Blot hybridization analysis of the α- and β-subunit mRNAs of Na^+/K^+-ATPase. The oocytes were injected with either mRNA (0.5 μg/μl, 20 nl/oocyte) for the α- or the β-subunit and incubated at 19°C for 3 days. The oocytes were then injected with the antisense oligonucleotide specific for either the α-subunit (1.9 μg/μl, 20 nl/oocyte) or the β-subunit (3.3 μg/μl, 20 nl/oocyte). The amounts of the antisense oligonucleotides injected corresponded to a 500-times molar excess of the respective mRNA that had been injected into an oocyte. At 0, 1, 3, 6, 12 and 24 h after injection of the antisense oligonucleotide, approx. 10 μg of extracted oocyte RNAs were electrophoresed, blotted and probed with a radioactive α-subunit (lanes 1–6) or β-subunit (lanes 7–12) cDNA probes.

ucts were examined by immunoprecipitation followed by fluorography (Fig. 2). The oocytes injected first with mRNA for the α-subunit, incubated for 3 days and then injected with antisense oligonucleotide complementary to mRNA for the α-subunit synthesized no detectable amount of the α-subunit during a second 3 day incubation with [^{3}H]leucine after the antisense oligonucleotide injection (Fig. 2, lane 2), while the distinct amount of the α-subunit was detected in the oocytes without the antisense oligonucleotide injection (Fig. 2, lane 1). The same was true in the case of mRNA for the β-subunit (Fig. 2, lanes 4 and 6). (The faint band seen at the position of βm in lane 6 was unlikely to be the product of mRNA for the β-subunit, judging from the facts that βc was completely diminished in lane 6 and that occasionally a nonspecific band appeared near the position of βm, which are observed, for examples, in lanes 1, 2 and 3 of Fig. 2.) Moreover, the oligonucleotide specific for the α-subunit mRNA did not affect the expression of the β-subunit and vice versa (Fig. 2, lanes 3 and 5). These results indicate that an antisense oligonucleotide blocks the translation of mRNA in a specific manner.

The facts that the oocytes injected with mRNA but not followed by antisense oligonucleotide injection still synthesized the subunit under the direction of the injected mRNA (Fig. 2, lanes 1 and 4) indicated that the mRNA was still active even after 3 days incubation.

Sequential injection of mRNAs and antisense oligonucleotides

We first expressed the α-subunit followed by expression of the β-subunit in single oocytes or in the reverse order by alternately injecting the respective mRNA. The translation of the mRNA that had been injected first was blocked by the injection of the antisense oligonucleotide specific for that mRNA prior to the second injection of the other mRNA. The schedules for the time of injection and the period of labeling are shown in Fig. 3a, and analysis of the immunoprecipitated oocyte proteins is shown in Fig. 3b. This type of experiment was performed several times and reproducible results were obtained. Oocyte injected first with mRNA for the β-subunit, then with the antisense oligonucleotide specific for the β-subunit mRNA, and finally with mRNA for the α-subunit containing a high level of the labeled α-subunit (Fig. 3b, lanes 16 and 17), whereas any detectable labels were not incorporated into either the coreglycosylated(βc) subunit or the fully glycosylated β-subunit (βm) of this oocyte (Fig. 3b, lanes 16 and 18), suggesting that the α-subunit but not the β subunit was synthesized during the second 3-day in-

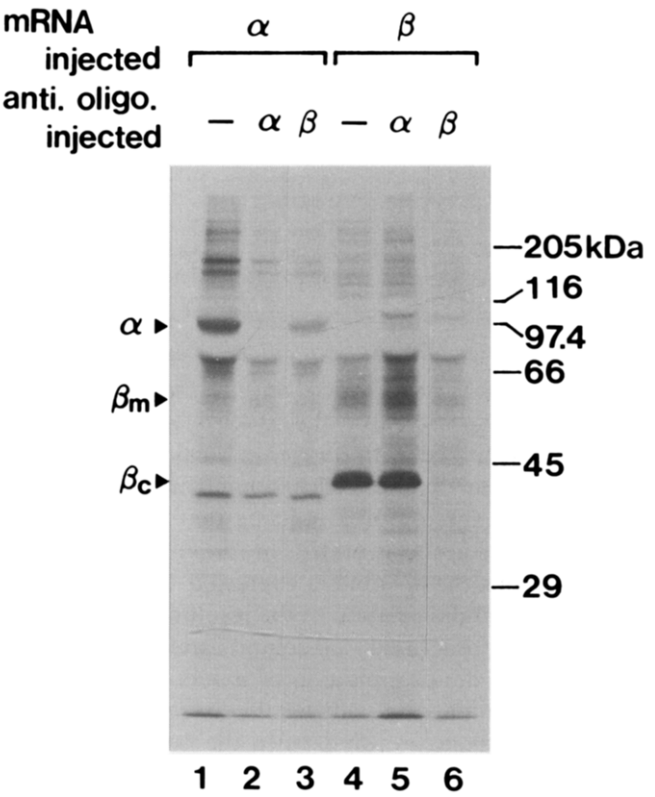

Fig. 2. Repression of Na^+/K^+-ATPase subunit expression by an antisense oligonucleotide. The oligonucleotide complementary to the mRNA for the α- and β-subunit were injected into oocytes 3 days after injection of respective mRNA as described in the legend for Fig. 1 and incubated for further 3 days in the presence of [^{3}H]leucine. The extracts from the oocytes were immunoprecipitated with antiserum against the α-subunit (lanes 1–3) or the β-subunit (lanes 4–6) and analyzed by gel electrophoresis followed by fluorography.

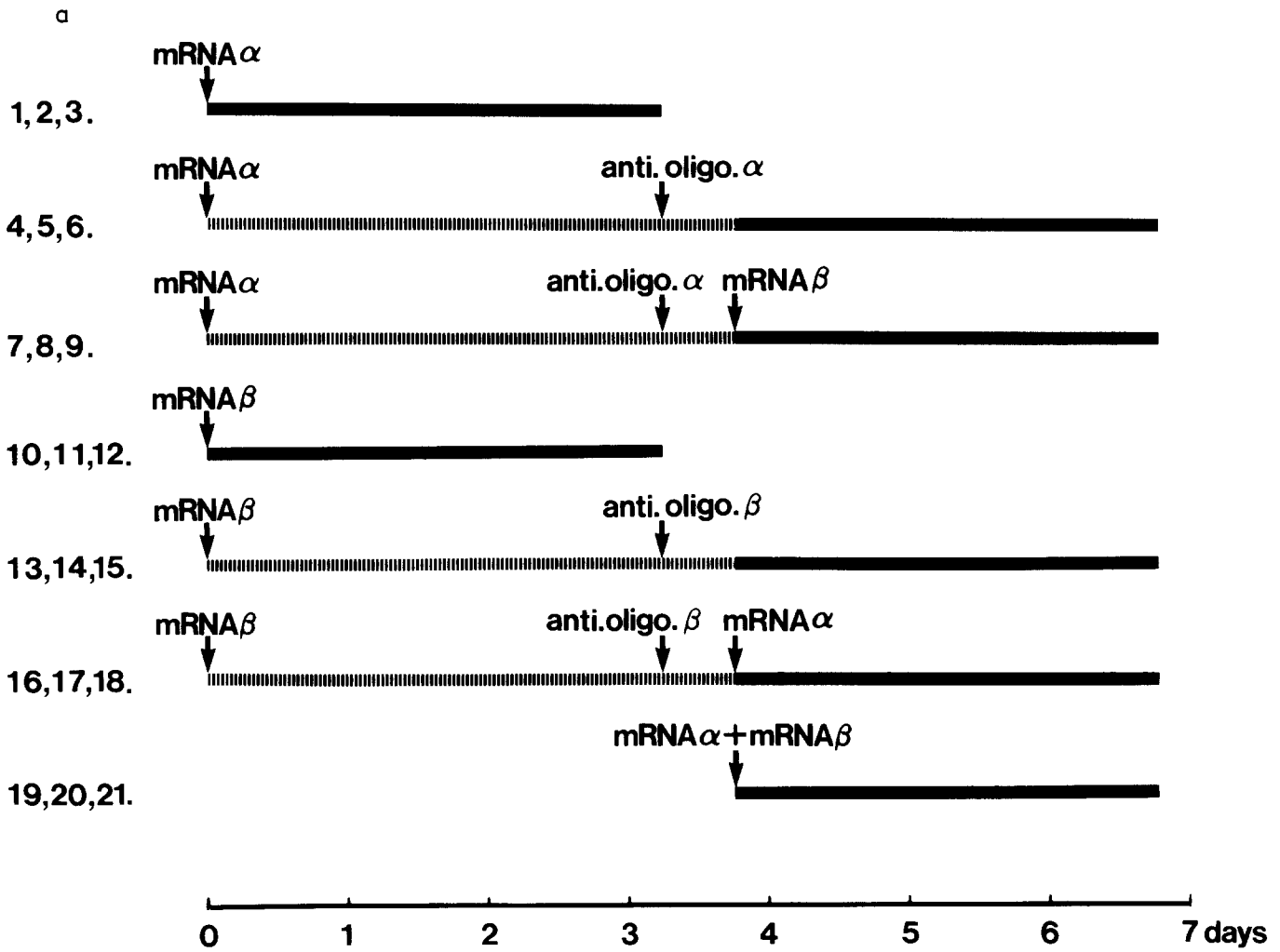

Fig. 3. (a) Time schedule for the injection of mRNAs and antisense oligonucleotides. The figures on the left side correspond to the lane numbers in the fluorograms shown in (b). Broken and solid lines represent the periods when the oocytes were incubated in the absence and the presence of [^{3}H]leucine, respectively.

cubation, which was carried out in the presence of [^{3}H]leucine. Nevertheless, the labeled α-subunit was immunoprecipitated with antiserum against the β-subunit, though the precipitated α-subunit was slight (Fig. 3b, lane 18). These results indicate that the β-subunit which has been incorporated in the membrane can assemble with the α-subunit expressed later in single oocytes. Partial dissociation of the resulting αβ complex caused by Triton (used for solubilization of oocytes) might lead to the precipitation of a very small amount of the α-subunit with anti-β-subunit serum. In fact, even the α-subunit synthesized in the oocytes injected with both mRNAα and mRNAβ simultaneously was precipitated very slightly with anti-β-subunit serum (Fig. 3b, lane 21). This was also the case for the precipitation of the β-subunit with anti-α-subunit serum (Fig. 3b, lane 20).

The oocytes injected first with the mRNA for the α-subunit, then with an antisense oligonucleotide specific for the α-subunit and finally with the mRNA for the β-subunit produced a high level of the β-subunit (Fig. 3b, lanes 7 and 9). Contrary to the former case, any detectable β-subunit was not immunoprecipitated with antiserum against the α-subunit (Fig. 3b, lane 8), suggesting that very little, if any, of the αβ complex is formed in this case. In these oocytes, a faint band of labeled α-subunit was observed (Fig. 3b, lanes 7 and 8). At present, we are unable to explain the basis for this observation.

ATPase and ouabain-binding activities in oocytes injected with mRNAs

The Na^+/K^+-ATPase and ouabain-binding activities of the oocytes were assayed to determine whether the αβ complexes formed through the alternate injections of mRNAs were enzymatically active. Oocytes injected with mRNAs for the α- and β-subunits simultaneously were used as positive controls, and noninjected oocytes were used as controls to assess the endogenous Na^+/K^+-ATPase activity. The results are shown in

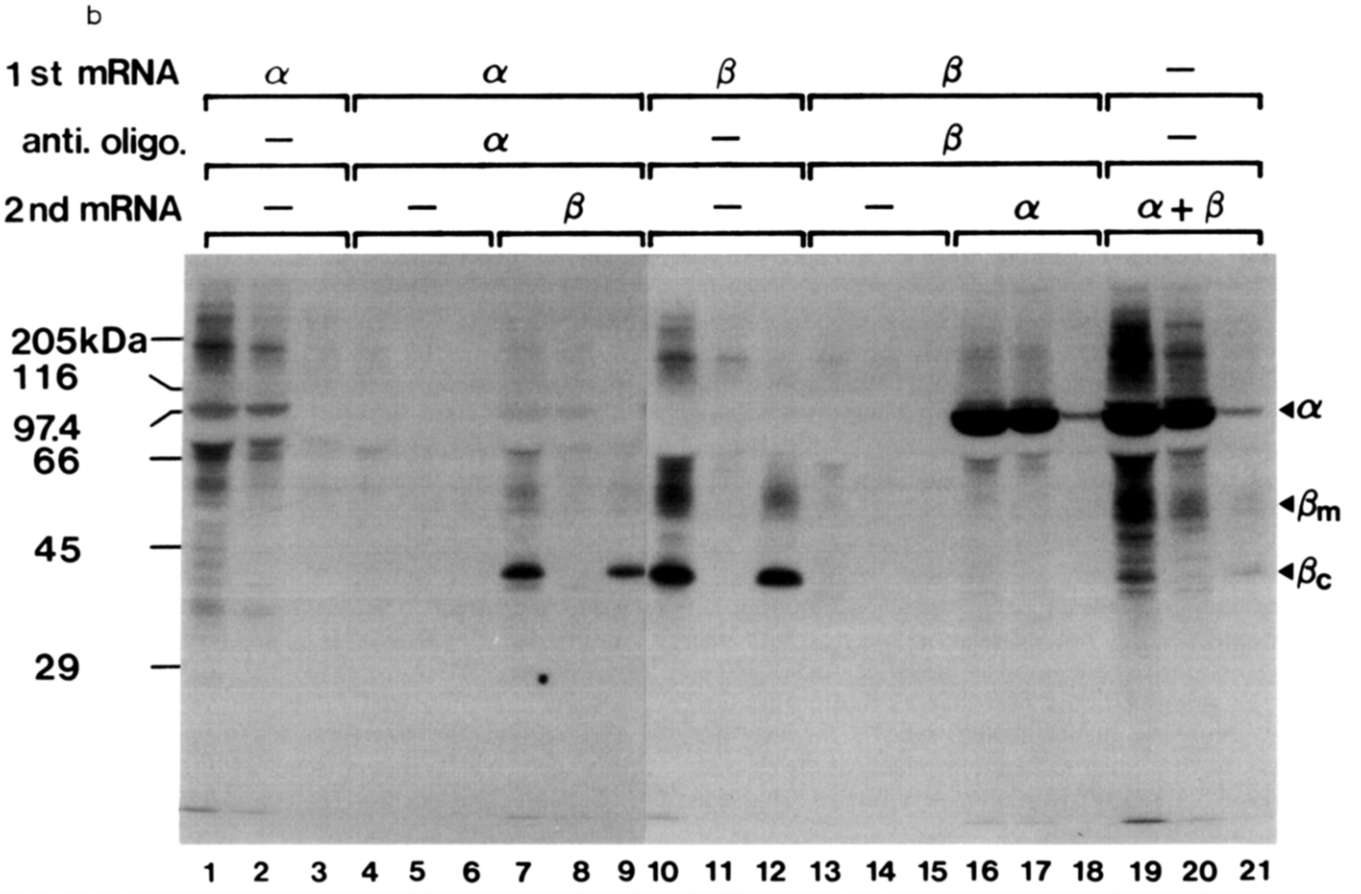

Fig. 3. (b) Expression of Na^+/K^+-ATPase subunits in mRNA-injected oocytes. Oocytes were injected with mRNAs and antisense oligonucleotides in turn as illustrated in fig. 3a. The concentrations used for injections were as follows: both mRNAs, 0.5 μg/μl each; antisense oligonucleotide for the α-subunit, 1.9 μg/μl; antisense oligonucleotide for the β-subunit, 3.3 μg/μl. 20 nl of each was injected into an oocyte. The extracts were immunoprecipitated with antiserum against the α-subunit (lanes 2, 5, 8, 11, 14, 17 and 20), antiserum against the β-subunit (lanes 3, 6, 9, 12, 15, 18 and 21), or with a mixture of both sera (lanes 1, 4, 7, 10, 13, 16 and 19) and analyzed by gel electrophoresis followed by fluorography.

Table I. Both the ATPase and ouabain-binding activities in oocytes injected first with mRNA for the β-subunit then with antisense oligonucleotide for the β-subunit and finally with mRNA for the α-subunit were obviously higher than those in noninjected oocytes, although they were lower than those in oocytes injected with both mRNAs simultaneously. The oocytes first injected with mRNA for the α-subunit then with antisense oligonucleotide for the α-subunit and finally with mRNA for the β-subunit showed little increase in both activities from those of noninjected oocytes.

Discussion

This paper describes the sequential expression of the α- and the β-subunits of Na^+/K^+-ATPase in single *Xenopus* oocytes following alternate microinjection of synthetic mRNAs derived from specific cDNA clones. The crucial point of this experiment is whether an antisense oligonucleotide complementary to the mRNA first injected abolishes the translation of the mRNA

TABLE I

Na^+/K^+-ATPase and ouabain-binding activities in mRNA-injected oocytes

For samples 1 and 2, oocytes were injected with mRNAs and antisense oligonucleotides in the order as indicated in the left column. Sample 3 is a positive control in which both mRNAs were injected simultaneously, and sample 4 is used to assess the endogenous activities in oocytes. The concentrations of materials and time schedule for expression were the same as indicated in Fig. 3a. The values are the means of three independent experiments. The activities were assayed as described in the Materials and Methods. mRNAα and mRNAβ represent the mRNA specific for the α- and β-subunit, respectively. Anti.oligo.(α) and anti.oligo.(β) denote the antisense oligonucleotides specific for the α- and β-subunit, respectively.

Sample	ATPase activity (μmol P_i/ mg per h)	Ouabain-binding activity (fmol/oocyte)
1 mRNAα → anti.oligo.(α) → mRNAβ	2.65 ± 0.35	19.1 ± 3.7
2 mRNAβ → anti.oligo.(β) → mRNAα	3.71 ± 0.84	25.8 ± 3.1
3 mRNAα + mRNAβ	4.65 ± 0.09	40.5 ± 12.1
4 non-injected	2.28 ± 0.19	17.2 ± 3.4

while the second mRNA is translated. The following observations support the conclusion that the first-injected mRNA has ceased working when the second mRNA is injected: (1) The mRNA that had been injected into oocytes was essentially completely degraded at 12 h after the injection of a specific antisense oligonucleotide (Fig. 1), which is the time at which we routinely injected the second mRNA. (2) As shown in Figs. 2 and 3, hardly any translation products of the α- and β-subunit detected when the antisense oligonucleotide complementary to the respective mRNA was injected. (3) Sumikawa and Miledi [19] have reported that a small synthetic oligonucleotide designed to hybridize to the coding sequence of nicotinic acetylcholine receptor α-subunit almost completely abolished the appearance of a functional acetylcholine receptor when the oligonucleotide was injected together with the mRNAs of the receptor into *Xenopus* oocytes.

These observations strongly indicate that the first-injected mRNA did not co-exist in oocytes with the mRNA injected next as long as the latter mRNA injection was performed no earlier than 12 h after injection of the antisense oligonucleotide specific for the first mRNA. Instead, the translation products of the first-injected mRNA co-exist with mRNA injected second and hence with the nascent or newly synthesized polypeptides translated from the second mRNA.

When the β-subunit was expressed first, the resulting β-subunit could assemble with the α-subunit expressed later within single oocytes. Moreover, the αβ complex thus formed showed both Na^+/K^+-ATPase and ouabain-binding activities. In addition, the amount of the α-subunit found in these oocytes was much larger than that in oocytes injected with the α-subunit specific mRNA alone and nearly equal to that in oocytes injected with both mRNAs simultaneously. These results suggest that the β-subunit, probably remaining within endoplasmic reticulum, can assemble with and stabilize the newly synthesized α-subunit, leading to the formation of the active enzyme complex.

On the other hand, when oocytes injected with the mRNA for the α-subunit first, then with the antisense oligonucleotide and finally with the mRNA for the β subunit, the formation of the αβ complex was hardly supported by immunoprecipitation. However, the possibility that the amount of the αβ complex, even if formed in this case, may be less than the detection limit of our analytical methods cannot be excluded, since the α-subunit accumulated while the oocytes were incubated in the presence of mRNA for the α-subunit alone may be too small (Fig. 3b, lane 1) to form a detectable amount of the complex with the nascent β-subunit. A slight increase in Na^+/K^+-ATPase and ouabain-binding activities observed in these oocytes (Table I) may be due to this 'undetectable' αβ complex, but this is unlikely. Noguchi et al. have observed that when mRNA for the β-subunit is injected alone, the oocytes exhibit a slight increase in Na^+/K^+-ATPase activity probably due to the induction of the α-subunit of the host cell (Noguchi et al., unpublished data). Hence, it seems plausible to consider that the hybrid Na^+/K^+-ATPase between *Xenopus* α- and *Torpedo* β-subunit could explain the small increase in ATPase and ouabain-binding activities detected in the oocytes injected first with mRNA for the α-subunit and subsequently with mRNA for the β-subunit.

It is tempting to assume that, although the α-subunit alone is inserted in membrane [20], the conformation of the α-subunit thus inserted may be somehow different from that of the α-subunit in the complex with the β-subunit and that the correct conformation of the α-subunit may be obtained only when nascent polypeptides of the α-subunit are assembled with the β-subunit; i.e., the β-subunit is a receptor for the nascent α-subunit, as has been claimed by Sabatini et al. [21] and by Hiatt et al. [8].

Recently, Zamofing et al. [22] have claimed that the α-subunit is stabilized in the membrane through the assembly to the β-subunit and otherwise is rapidly degraded, which is consistent with the idea that the β-subunit is a receptor for the α-subunit. Our results emphasize the possible role of the β-subunit in the integration of the α-subunit into the membrane.

Acknowledgements

We thank Ms. Atsuko Sugino very much for her typing the manuscript. This work was supported by Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan.

References

1 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.
2 Shull, G.E., Schwartz, A. and Lingrel, J.B. (1985) Nature 316, 691–695.
3 Ovchinnikov, Yu.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.
4 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.
5 Kawakami, K., Nojima, H., Ohta, T. and Nagano, K. (1986) Nucleic Acids Res. 14, 2833–2844.
6 Shull, G.E., Lane, L.K. and Lingrel, J.B. (1986) Nature 321, 429–431.
7 Jørgensen, P.L. and Andersen, J.P. (1988) J. Membr. Biol. 103, 93–120.
8 Hiatt, A., McDonough, A.A. and Edelman, I.S. (1984) J. Biol. Chem. 259, 2629–2635.

9 Tamkun, M.M. and Fambrough, D.M. (1986) J. Biol. Chem. 261, 1009–1019.
10 Noguchi, S., Mishina, M., Kawamura, M. and Numa, S. (1987) FEBS Lett. 225, 27–32.
11 Gurdon, T.B. (1974) The Control of Gene Expression in Animal Development, Clarendon, Oxford.
12 Dash, P., Lotan, I., Knapp, M., Kandel, E.R. and Goelet, P. (1987) Proc. Natl. Acad. Sci. USA 84, 7896–7900.
13 Laemmli, U.K. (1970) Nature 227, 680–685.
14 Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold spring Harbor Laboratory, Cold spring Harbor, New York.
15 Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201–5205.
16 Skou, J.C. (1974) Biochim. Biophys. Acta 339, 234–245.
17 Colman, A. (1984) In Transcription and Translation. A practical Approach (Hames, B.D. and Higgins, S.J., eds.), pp 271–302, IRL Press, Oxford.
18 Takeda, K., Noguchi, S., Sugino, A. and Kawamura, M. (1988) FEBS Lett. 238, 201–204.
19 Sumikawa, K. and Miledi, R. (1988) Proc. Natl. Acad. Sci. USA 85, 1302–1306.
20 Geering, K., Meyer, D.I., Paccolat, M-P., Kraehenbuhl, J-P. and Rossier, B.C. (1985) J. Biol. Chem. 260, 5154–5160.
21 Sabatini, D., Colman, D., Sabban, E., Sherman, J., Morimoto, T., Kreibich, G. and Adesnik, M. (1981) Cold Spring Harbor Symp. Quant. Biol. 46, 807–818.
22 Zamofing, D., Rossier, B.C. and Geering, K. (1988) J. Membr. Biol. 104, 69–79.