Volume 306, number 2,3, 213–218 FEBS 11315 July 1992

Deduced amino acid sequence and E_1–E_2 equilibrium of the sarcoplasmic reticulum Ca^{2+}-ATPase of frog skeletal muscle

Comparison with the Ca^{2+}-ATPase of rabbit fast twitch muscle

Bente Vilsen and Jens Peter Andersen

Danish Biomembrane Research Centre, Institute of Physiology, University of Aarhus, DK-8000 Aarhus C, Denmark

Received 21 May 1992

The cDNA encoding a Ca^{2+}-transport ATPase of frog (*Rana esculenta*) skeletal muscle was isolated and characterized. The deduced amino acid sequence, consisting of 994 residues, showed 89% identity to the fast twitch muscle sarcoplasmic reticulum Ca^{2+}-ATPases of chicken and rabbit. Northern blot analysis using a fragment of this cDNA as probe detected a 5.0 kb message in frog skeletal muscle but did not detect any mRNA encoding sarcoplasmic reticulum Ca^{2+}-ATPase in frog cardiac muscle. The enzymatic properties of the amphibian skeletal muscle Ca^{2+}-ATPase were compared with those of the rabbit fast twitch muscle Ca^{2+}-ATPase by functional expression of the cDNAs in COS-1 cells. The amphibian Ca^{2+}-ATPase displayed a reduced apparent affinity for Ca^{2+} and an increased apparent affinity for the inhibitors, vanadate and thapsigargin, relative to the mammalian enzyme. This may be explained by a mechanism in which relatively more of the E_2 conformation accumulated in the frog Ca^{2+}-ATPase than in the mammalian enzyme.

Ca^{2+}-ATPase; Sarcoplasmic reticulum; Amino acid sequence comparison; Frog muscle; Ca^{2+} affinity

1. INTRODUCTION

In most mammalian cells, and in striated muscle in particular, the maintenance of a low intracellular Ca^{2+} concentration depends on the sequestering of Ca^{2+} in internal stores in the endoplasmic/sarcoplasmic reticulum [1]. The active transport of Ca^{2+} into these organelles is mediated by a Ca^{2+}-activated ATPase that belongs to a family of ATP-driven cation pumps for which phosphorylation of an aspartic acid residue at the active site is an obligatory step in the transport mechanism ('P-type ATPases'). Much is known about the reaction kinetics [2,3], structure [4,5] and primary sequence [6] of the Ca^{2+}-ATPase of rabbit fast twitch muscle sarcoplasmic reticulum. To further develop our understanding of the relationship between structure and function of the ATPase molecule, it is interesting to compare the functional properties of related molecules, such as isoenzymes, that differ at several defined residue positions. So far, however, no conspicuous functional differences have been revealed between the three known isoforms of the sarco(endo)plasmic reticulum Ca^{2+}-ATPases of the same species expressed under identical conditions in the absence of phospholamban [7–9], and detailed functional comparisons of Ca^{2+}-ATPases from different species have not been reported. In the present study we have cloned a cDNA encoding the Ca^{2+}-ATPase of frog skeletal muscle sarcoplasmic reticulum and expressed this cDNA functionally in COS-1 cells under the same conditions as applied for the rabbit fast twitch muscle Ca^{2+}-ATPase cDNA. This approach has permitted us to relate functional differences between the amphibian and mammalian Ca^{2+}-ATPases directly to differences in the deduced amino acid sequences. We have found that the Ca^{2+}-ATPases of frog and rabbit muscle differ with respect to their apparent affinities for Ca^{2+} and for the inhibitors, vanadate and thapsigargin, in a way suggesting that in the amphibian Ca^{2+}-ATPase the conformational equilibrium is poised more towards the form possessing low Ca^{2+} affinity and high reactivity towards vanadate (E_2), than in the rabbit enzyme.

Abbreviations: bp, base pair(s); Ca^{2+}-ATPase, Ca^{2+}-activated adenosine triphosphatase; EGTA, [ethylenebis(oxyethylene-nitrilo)]-tetraacetic acid; ER, endoplasmic reticulum; kb, kilo bases; MOPS, 3-(*N*-morpholino)propanesulfonic acid; oligo(dT), oligodeoxythymidine; poly(A^+), polyadenosine; TES, *N*-tris[-hydroxymethyl]methyl-2-aminoethanesulfonic acid; SDS, sodium dodecyl sulfate; SERCA, sarco(endo)plasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum.

Correspondence address: B. Vilsen, Institute of Physiology, University of Aarhus, Ole Worms Allé 160, DK-8000 Aarhus C, Denmark.

2. MATERIALS AND METHODS

2.1. *RNA and DNA manipulation*

Total RNA was extracted from frog (*Rana esculenta*) tissues (brain, heart, liver and thigh skeletal muscle) by homogenization of the tissue in a guanidine isothiocyanate-containing solution, followed by centrif-

ugation through a 5.7 M caesium chloride cushion [10]. Pure poly(A$^+$) RNA was isolated by oligo-(dT)-cellulose spun-column chromatography. A plasmid and a phage cDNA library were constructed from the mRNA isolated from frog thigh muscle. First- and second-strand cDNA synthesis were carried out according to the Pharmacia manual with two exceptions: (i) 1 U RNasin (Promega) was added to the first-strand reaction mixture in order to inhibit extraneous RNAse activity in this step; (ii) The synthetic oligonucleotide defined by the sequence 5'AGTCTTGTCAGAGCAGAT3' was added with the poly(dT) primer to prime the cDNA synthesis from poly(A$^+$) RNA, thereby extending the cDNA synthesis from a position corresponding to the phosphorylation site region in the rabbit fast twitch muscle Ca^{2+}-ATPase. The cDNA was size selected by agarose gel electrophoresis and converted to blunt-ended cDNA by a fill-in reaction using the Klenow fragment of DNA polymerase I. *Eco*RI/ *Not*I adaptors were ligated to blunt-ended cDNA with T4-ligase followed by purification of the cDNA from excess- and dimerised adaptors by spun-column chromatography, addition of terminal phosphates to the *Eco*RI-terminated cDNAs by polynucleotide kinase, and ligation of the cDNA to the Bluescript or lambda ZAP II cloning vectors (Stratagene, La Jolla, CA). Gigapack Gold packaging extracts (Stratagene) were used for packaging of the lambda ZAP II phage library. DH5α cells (Library efficiency, Bethesda Research Laboratories) were transformed with the Bluescript plasmid library cDNA.

2.2. *Screening of the cDNA libraries*

The Bluescript plasmid library was screened with an end-^{32}P-labeled probe derived from the above mentioned synthetic oligonucleotide corresponding to the phosphorylation site region. The filters were washed under low stringency conditions at 46°C in 5 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M trisodium citrate) for 4 × 15 min. One 3.4 kb cDNA clone (VCa.1) that hybridized strongly to the probe was isolated. Nucleotide sequencing demonstrated that the 5'-end of this clone was highly homologous to the cDNA encoding mammalian SR Ca^{2+}-ATPases, starting at the codon corresponding to Ile64 and extending about 600 bp beyond the COOH-terminus. The lambda ZAP II phage library was screened with a ^{32}P-labeled cDNA restriction fragment derived from the 5'-terminus of the VCa.1 clone. The filters were washed for 2 × 1 h at 60°C in 1 × SSCP (0.12 M NaCl, 0.015 M trisodium citrate, 0.013 M KH_2PO_4, 1 mM EDTA). Out of the 87 positive plaques isolated, 40 were further purified, and 2 of these, V34.10 of 1.3 kb and V35.10 of 2.5 kb, contained a sequence encoding the NH_2-terminal part of the Ca^{2+}-ATPase. The 3'-ends of V34.10 and V35.10 were 100% identical to the 5'-end of VCa.1 in the 860 bp overlaps. A 3.8 kb cDNA encoding the full-length sequence of the frog SR Ca^{2+}-ATPase was conveniently constructed by joining the partial sequences corresponding to clones VCa.1 and V34.10 at the *Kpn*I(636) restriction site. The nucleotide sequence of this cDNA construct has been submitted to the EMBL/GenBank Data Bank with accession number X63009.

2.3. *Northern blot analysis*

Samples containing total RNA (25 μg) were denatured with deionized formamide, electrophoresed in 0.8% agarose gels containing 0.6 M formaldehyde and 0.02 M MOPS buffer [10], and then blotted onto nylon membranes by vacuum transfer. The fixed RNA was probed with the *Bam*HI(1,801)–*Sal*I(2,352) restriction fragment of the full-length cDNA clone. Prehybridization (for 0.5 h) and hybridization (for 16 h) were performed in 50% (w/v) deionized formamide, 0.25 M Na_2HPO_4, pH 7.2, 0.25 M NaCl, 7% (w/v) SDS, 1 mM EDTA at 42°C. The filters were washed at low stringency in 2 × SSC and 0.1% SDS at 42°C.

2.4. *DNA sequence analysis*

Selected overlapping restriction fragments were subcloned in the Bluescript vector and sequenced in both directions by the dideoxynucleotide chain termination method [11] with modifications as described in [12]. To solve compressions in (G+C)-rich regions, the dITP analogue of dGTP was used.

2.5. *Expression in COS-1 cells*

The full-length frog muscle Ca^{2+}-ATPase cDNA, carrying *Not*I/ *Eco*RI adaptors, and the rabbit fast twitch muscle Ca^{2+}-ATPase cDNA [13] were cloned into the *Eco*RI site of the expression vector pMT2 [14], either in the right or in the reverse orientation. The plasmid DNA was transfected into COS-1 cells, and the microsomal fraction containing the expressed Ca^{2+}-ATPase was harvested by differential centrifugation 48–72 h later [15].

2.6. *Functional analysis*

The isolated COS-1 cell microsomes containing Ca^{2+}-ATPase of frog or rabbit were assayed for Ca^{2+}-activated ATP-driven Ca^{2+} transport by Millipore filtration [12]. Ca^{2+}-activated ATPase activity was measured at 37°C in the presence of 50 mM TES, pH 7.5, 0.1 M K^+, 1 mM Mg^{2+}, 5 mM MgATP, 0.1 mM Ca^{2+} [15]. The inhibitors, vanadate and thapsigargin, were added to the microsomes following the addition of ATP. To avoid contamination with decavanadate the stock solution of vanadate was prepared by dissolving $NaVO_3$ in excess NaOH. Phosphorylation from ATP or P_i was measured as previously [16,17] with modifications as described in the figure legends. All phosphorylation experiments were performed at least three times with closely agreeing results. After acid precipitation the phosphorylated microsomal protein was washed and subjected to SDS-PAGE at pH 6.0 followed by autoradiography of the dried gels and quantitation by densitometric analysis using a LKB 2202 Ultroscan Laser Densitometer.

3. RESULTS AND DISCUSSION

The nucleotide sequence of the cloned cDNA was used to search for homologues in the EMBL/GenBank database. We found the highest correlation to the nucleotide sequences encoding the fast twitch muscle SR Ca^{2+}-ATPases of rabbit [6,18] and chicken [19], for which the identity between the coding sequences was 77.7% (frog/rabbit) and 76.6% (frog/chicken). The amino acid sequence deduced from the open reading frame of the frog cDNA clone (Fig. 1) shows 88.8% identity with the fast twitch muscle SR Ca^{2+}-ATPase of rabbit and 89.2% identity with that of chicken. Less overall homology (84% identity) was found in comparison of the amino acid sequence of the amphibian Ca^{2+}-ATPase with that of the cardiac/slow twitch muscle isoforms of rabbit and chicken. Moreover, the deduced frog muscle Ca^{2+}-ATPase sequence clearly matches that of the fast twitch muscle isoforms at two characteristic points of deviation between the slow and the fast twitch isoforms of the rabbit enzyme. One of these is the COOH-terminus, the other is a stretch of 5 residues just COOH-terminal to Arg-505. Based on these considerations we suggest, following Burk et al. [7], that the nomenclature used for the Ca^{2+}-ATPase encoded by the cloned cDNA be SERCA1a. It should, however, be noted that there is a considerable overlap between species- and isoform-specific differences. Hence, out of the 111 amino acid differences between the amphibian sequence and the sequence of the rabbit fast twitch muscle isoform, 74 occur at the same position as a difference between the fast and slow twitch isoforms of the rabbit enzyme, and at 40 of these positions the frog Ca^{2+}-

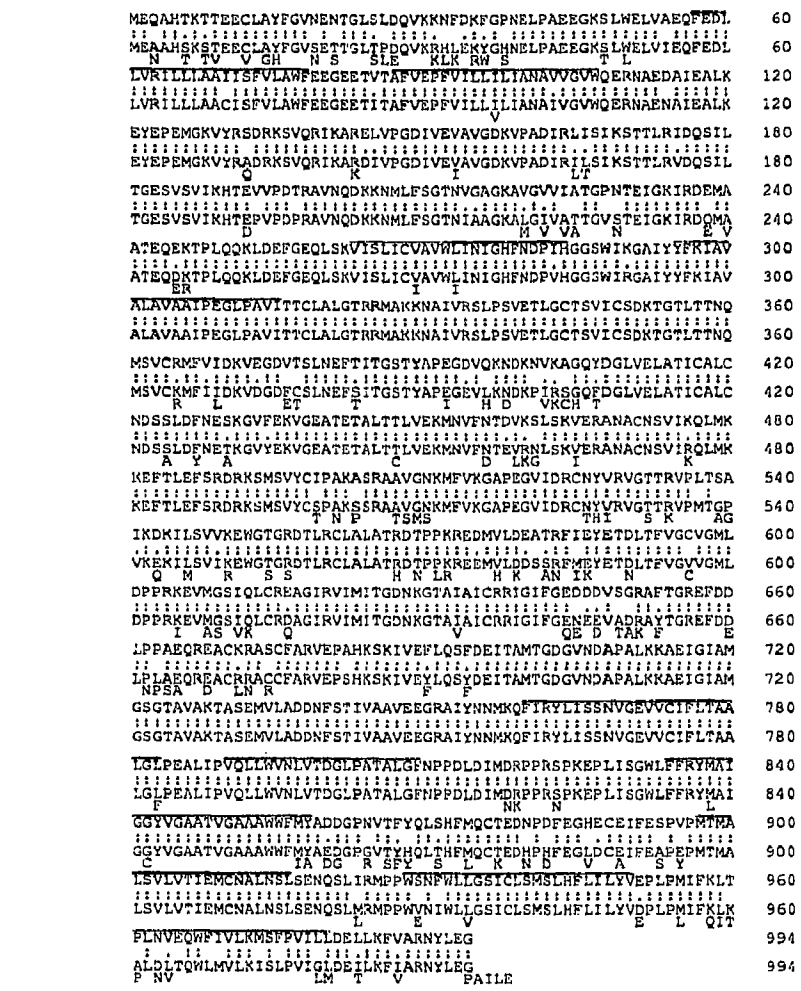

Fig. 1. Comparison of the deduced amino acid sequence of the frog SR Ca^{2+}-ATPase with those of SERCA1a and SERCA2a of rabbit. The frog sequence is shown above the SERCA1a sequence of rabbit. The presence of identical amino acids at homologous positions is indicated by two dots, whereas a conservative replacement is indicated by a single dot. The SERCA2a sequence of rabbit is indicated below the SERCA1a sequence of rabbit, with only the residues differing between the two isoforms shown. The putative transmembrane segments identified by the lines above the frog sequence are in accordance with the proposal by Green [34]. The nucleotide sequence from which the amino acid sequence reported in this figure was deduced is available form the authors or from the EMBL/GenBank Data Bank (accession number X63009).

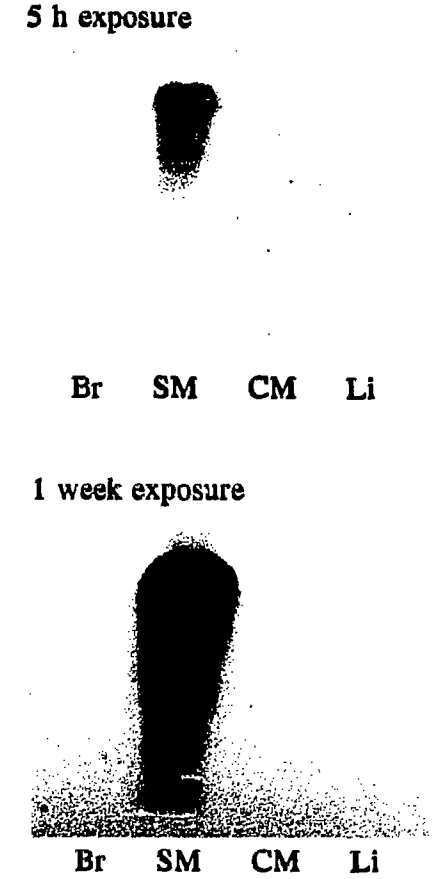

Fig. 2. Northern blot analysis of total RNA isolated from various frog tissues. Total RNA (25 μg in each lane) extracted from brain (Br), liver (Li), skeletal muscle (SM), and cardiac muscle (CM) of frogs (*Rana esculenta*) was electrophoresed and blotted onto nylon membranes. The *Bam*HI (1,801)–*Sau*I(2,352) cDNA fragment of the frog muscle Ca^{2+}-ATPase cDNA clone was used as probe under low stringency wash conditions. The films were exposed to the blot for 5 h or 1 week.

ATPase contains exactly the same residue as the slow isoform (see Fig. 1).

To determine the tissue distribution of sarco(endo)plasmic reticulum Ca^{2+}-ATPases in frog, samples of total RNA extracted from frog thigh muscle, heart, brain, and liver were analyzed by Northern blot hybridization using a 551 base *Bam*HI(1,801)–*Sau*I(2,352) cDNA probe derived from the highly conserved 'hinge domain' region. Under the low stringency wash conditions used, this probe should detect transcripts corresponding to either fast twitch or slow twitch/cardiac muscle isoforms [6,20]. As seen in Fig. 2, there was one major transcript size (5.0 kb) present in frog skeletal muscle. No hybridization could be observed to the RNA from the other tissues, including cardiac muscle. This finding supports the notion that the absence of 'staircase' and post-extrasystolic potentiation relations in frog cardiac muscle is ascribable to the lack of a significant amount of sarcoplasmic reticulum and Ca^{2+}-ATPases creating an internal Ca^{2+} pool similar to that contained in the sarcoplasmic reticulum of mammalian cardiac muscle [21–25].

The specific Ca^{2+} transport activity measured in the presence of 5 mM MgATP and 10 μM Ca^{2+} was 50–100-fold higher in the microsomal fraction harvested from COS-1 cells transfected with the full-length frog muscle Ca^{2+}-ATPase cDNA inserted in the right orientation in the pMT2 expression vector, relative to the control microsomes from cells transfected with the cDNA in the reverse orientation. Fig. 3 shows the ATP- and Ca^{2+} concentration dependencies measured with the ex-

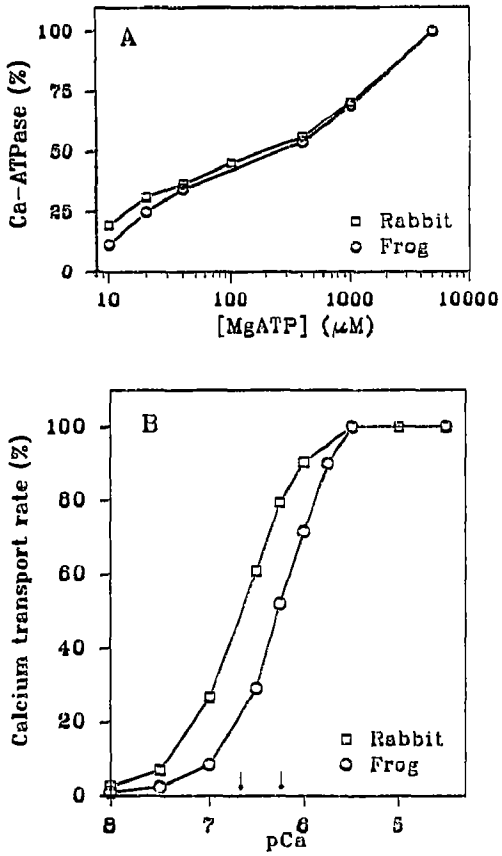

Fig. 3. Comparison of the ATP- and Ca^{2+} concentration dependencies of turnover of the frog muscle Ca^{2+}-ATPase and the rabbit fast twitch muscle Ca^{2+}-ATPase expressed in COS-1 cells. Measurements were carried out on the microsomal fractions isolated from COS-1 cells transfected with cDNA encoding either the frog muscle Ca^{2+}-ATPase or the rabbit fast twitch muscle Ca^{2+}-ATPase. The specific activities are shown as percentages of their maximal values, measured at saturating ATP and Ca^{2+} concentrations. (A) Ca^{2+}-activated ATP hydrolysis catalyzed by the microsomes was measured spectrophotometrically at 37°C in the presence of 50 mM TES, pH 7.5, 0.1 M K^+, 1 mM Mg^{2+}, 0.1 mM Ca^{2+} and the indicated concentrations of MgATP, with phosphoenolpyruvate (1 mM), lactate dehydrogenase (10 U/ml), pyruvate kinase (10 U/ml), and Ca^{2+}-ionophore A23187 (2 μM) added. (B) Oxalate-supported uptake of $^{45}Ca^{2+}$ in the microsomes was measured by Millipore filtration after 10 min incubation at 27°C in the presence of 5 mM MgATP, 0.1 M K^+, 5 mM oxalate, 20 mM MOPS, pH 6.8, and various concentrations of Ca^{2+} set with EGTA. The arrows indicate the Ca^{2+} concentration at which the transport rate was half maximal.

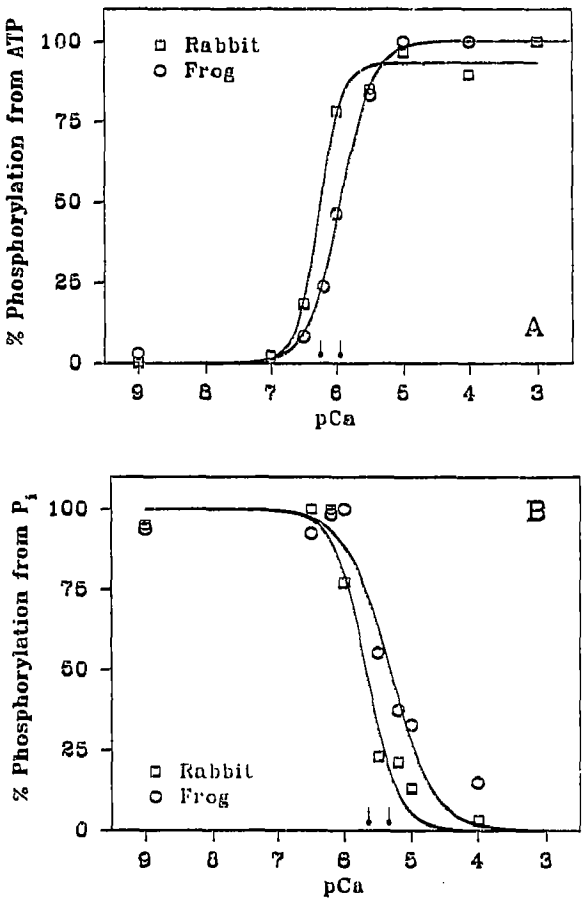

Fig. 4. Ca^{2+}-dependency of phosphorylation from ATP and P_i of the frog muscle Ca^{2+}-ATPase and the rabbit fast twitch muscle Ca^{2+}-ATPase expressed in COS-1 cells. Measurements were carried out on the microsomal fractions isolated from COS-1 cells transfected with cDNA encoding either the frog muscle Ca^{2+}-ATPase or the rabbit fast twitch muscle Ca^{2+}-ATPase. The acid-quenched ^{32}P-phosphorylated samples were subjected to SDS-PAGE at pH 6.0, and the autoradiograms of the dried gels were quantitated by densitometry. The specific phosphorylations are shown as percentages of their maximal values. The arrows indicate the Ca^{2+} concentration at which the phosphorylation was half maximal. (A) Phosphorylation from ATP was carried out at 0°C for 15 s in the presence of 50 mM MOPS buffer (pH 7.0), 80 mM K^+, 5 mM Mg^{2+}, 2 μM [γ-^{32}P]ATP, and the indicated Ca^{2+} concentrations. (B) Phosphorylation from P_i was carried out for 10 min at 25°C in the presence of 500 μM $^{32}P_i$, 100 mM TES/Tris buffer (pH 7.0), 10 mM $MgCl_2$, 20% (v/v) dimethylsulfoxide and the indicated Ca^{2+} concentrations.

pressed frog muscle Ca^{2+}-ATPase. For comparison, data obtained by parallel measurements on COS-1 cell microsomes containing expressed rabbit fast twitch muscle Ca^{2+}-ATPase are also shown. The Ca^{2+}-ATPase of frog muscle displayed a biphasic ATP concentration dependency very similar to that of the rabbit enzyme with a characteristic secondary low affinity activation that may be ascribed either to rebinding of ATP with low affinity at the catalytic site of the phosphorylated enzyme or to the existence of an independent modulatory ATP-binding site (for a review, see [5]). As seen in Fig. 3b, the enzymes of frog and rabbit differed with respect to the Ca^{2+} dependency of Ca^{2+} transport, with the apparent Ca^{2+} affinity of the frog Ca^{2+}-ATPase reduced relative to that of the rabbit enzyme by a factor of approximately 2.5 (corresponding to 0.4 pCa units). The difference between the Ca^{2+} affinities of the amphibian and mammalian enzymes was further substantiated by measurement of phosphorylation from ATP and inorganic phosphate (Fig. 4).

Fig. 5a shows for the frog and rabbit enzymes the dependence of the Ca^{2+}-ATPase activity on the concentration of vanadate. This inhibitor binds to the E_2 conformational state acting as an analogue of inorganic phosphate. In our assay, vanadate was added during ATP hydrolysis, so that the degree of inhibition observed at steady state reflected the concentration of the E_2 form accumulated as a result of the turnover of the enzyme. It is seen that the concentration of vanadate giving half-maximum inhibition in the frog Ca^{2+}-ATPase was about 1/50 of that required with the rabbit enzyme. As the vanadate-reactive E_2 form is supposed to be a state of low Ca^{2+} affinity, the results obtained by titration with vanadate and Ca^{2+} may be explained by a common mechanism in which relatively more E_2 accumulated in the frog Ca^{2+}-ATPase than in the rabbit enzyme.

The plant sesquiterpene lactone, thapsigargin [26], forms a 1:1 stoichiometric 'dead-end' complex with a Ca^{2+}-free conformation (probably E_2) of the SR/ER Ca^{2+}-ATPases [27]. Fig. 5b describes the results of experiments in which we compared the thapsigargin affinities of the amphibian and mammalian Ca^{2+}-ATPases, expressed in COS-1 cells. The $K_{0.5}$ for the thapsigargin interaction with the frog Ca^{2+}-ATPase measured during enzyme turnover was reduced 2.3-fold relative to that of the Ca^{2+}-ATPase of rabbit fast twitch muscle, in accordance with the accumulation of E_2 during steady state in the frog enzyme.

The putative transmembrane segments, M4–M8, which contain the proposed oxygen ligands for Ca^{2+} [28], are highly conserved between the Ca^{2+}-ATPases of frog and rabbit (Fig. 1). The structural basis for the functional difference between the enzymes of the two species must, therefore, be sought elsewhere, among the multiple amino acid differences near the NH_2- and COOH-termini and in central parts of the largest cytoplasmic loop. Only a few of the amino acid differences between the Ca^{2+}-ATPases of rabbit and frog are unique in the sense that none of the known sarcoplasmic reticulum Ca^{2+}-ATPase sequences contain the same or a homologous replacement [6,7,29–31], but since the present study is the first in which a detailed comparison of sarcoplasmic reticulum Ca^{2+}-ATPases of two different species has been carried out by expression of the cDNAs under identical conditions, it is premature to judge whether the unique substitutions were responsible for the funcitonal difference between the enzymes of frog and rabbit, or substitutions common in the family of Ca^{2+}-ATPases were more important.

In golden hamsters the Ca^{2+} affinity is shifted towards a lower value similar to that of frog muscle Ca^{2+}-ATPase, during their adaptation to cold climate [32], and it is tempting to speculate that the difference between frog and rabbit Ca^{2+}-ATPases, observed here, reflects the adaptation of the amphibia to a life at temperatures below 37°C. It should, however, be noted that

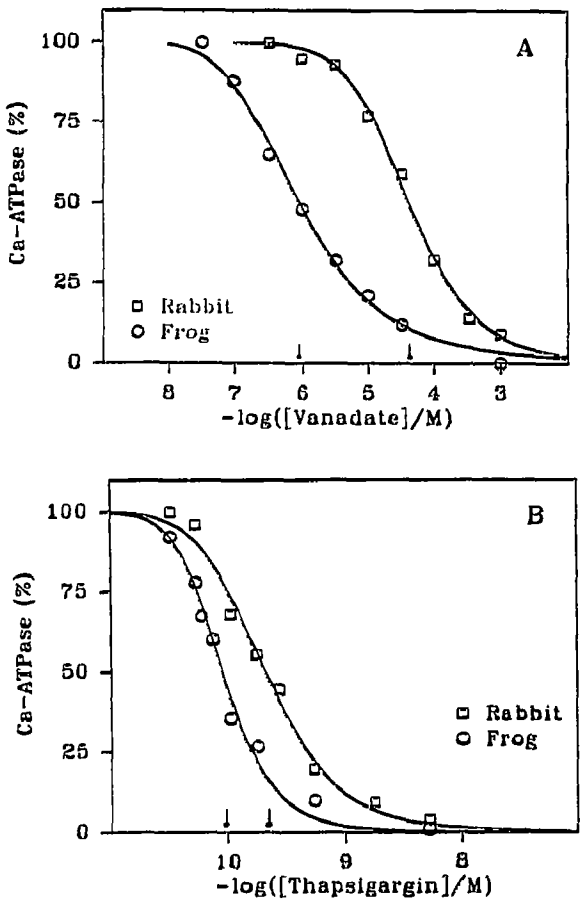

Fig. 5. Titration of the inhibition by vanadate and thapsigargin of the Ca^{2+}-activated ATPase activity of the frog muscle Ca^{2+}-ATPase and the rabbit fast twitch muscle Ca^{2+}-ATPase expressed in COS-1 cells. The Ca^{2+}-activated ATPase activity was measured on the microsomal fractions isolated from COS-1 cells transfected with cDNA encoding either the frog muscle Ca^{2+}-ATPase or the rabbit fast twitch muscle Ca^{2+}-ATPase. The measurements were carried out as described for Fig. 4a, at 37°C in the presence of 0.1 mM Ca^{2+} and 5 mM MgATP. The inhibitor was added to the microsomes after the addition of MgATP and the oxidation of NADH was recorded continuously. The activities shown were determined at steady state after the effect of the inhibitor had been fully expressed. The arrows indicate the inhibitor concentration at which the Ca^{2+}-ATPase activity was half maximal. (A) Effect of vanadate. A monovanadate solution kept at alkaline pH to avoid formation of decavanadate was used as stock. (B) Effect of thapsigargin. The abscissa shows the total concentration of thapsigargin present. At the lower thapsigargin concentrations most of the inhibitor is bound to the Ca^{2+}-ATPase [27], and in order to be able to compare the affinities of thapsigargin for the enzymes of frog and rabbit, identical enzyme concentrations of the two species (in U/ml) were assayed.

a previous comparison of the Ca^{2+}-ATPases in the native sarcoplasmic reticulum vesicles isolated from frog and rabbit fast twitch muscle failed to reveal any significant difference between their Ca^{2+} affinities [33]. It is, therefore, possible that the native membrane environments of the enzymes of frog and rabbit sarcoplasmic

reticulum differ, necessitating an evolutionary compensation by a change in protein structure.

Acknowledgements: We would like to thank Jytte Jørgensen, Karin Kracht, and Janne Petersen for their expert and invaluable technical assistance, Dr. F. Zorzato for his collaboration on the construction of the cDNA libraries, Dr. D.H. MacLennan for the rabbit cDNA clone, and Dr. R.J. Kaufman, Genetics Institute, Boston, for the gift of the expression vectors p91023 (B) and pMT2. This research was supported by grants from the Danish Biomembrane Research Centre, the Danish Medical Research Council, the NOVO Foundation, and the Nordic Insulin Foundation (Denmark).

REFERENCES

[1] Krause, K.-H. (1991) FEBS Lett. 285, 225–229.
[2] De Meis, L. (1981) Transport in the Life Sciences, vol. 2 (Bittar, E., Ed.), Wiley, New York.
[3] Inesi, G. (1985) Annu. Rev. Physiol. 47, 573–601.
[4] Taylor, K.A., Dux, L. and Martonosi, A. (1986) J. Mol. Biol. 187, 417–427.
[5] Andersen, J.P. (1989) Biochim. Biophys. Acta 988, 47–72.
[6] Brandl, C.J., Green, N.M., Korczak, B. and MacLennan, D.H. (1986) Cell 44, 597–607.
[7] Burk, S.E., Lytton, J., MacLennan, D.H. and Shull, G.E. (1989) J. Biol. Chem. 264, 18561–18568.
[8] Fujii, J., Maruyama, K., Tada, M. and MacLennan, D.H. (1990) FEBS Lett. 273, 232–234.
[9] Campbell, A.M., Kessler, P.D., Sagara, Y., Inesi, G. and Fambrough, D.M. (1991) J. Biol. Chem. 266, 16050–16055.
[10] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[11] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[12] Vilsen, B., Andersen, J.P., Clarke, D.M. and MacLennan, D.H. (1989) J. Biol. Chem. 264, 21024–21030.
[13] Maruyama, K. and MacLennan, D.H. (1988) Proc. Natl. Acad. Sci. USA 85, 3314–3318.
[14] Kaufman, R.J., Davies, M.V., Pathak, V.K. and Hershey, J.W.B. (1989) Mol. Cell Biol. 9, 946–958.
[15] Vilsen, B., Andersen, J.P. and MacLennan, D.H. (1991) J. Biol. Chem. 266, 16157–16164.
[16] Andersen, J.P., Vilsen, B., Leberer, E. and MacLennan, D.H. (1989) J. Biol. Chem. 264, 21018–21023.
[17] Andersen, J.P., Vilsen, B. and MacLennan, D.H. (1992) J. Biol. Chem. 267, 2767–2774.
[18] Brandl, C.J., DeLeon, S., Martin, D.R. and MacLennan, D.H. (1987) J. Biol. Chem. 262, 3768–3774.
[19] Karin, N.J., Kaprielian, Z. and Fambrough, D.M. (1989) Mol. Cell Biol. 9, 1978–1986.
[20] Simonides, W.S., van der Linden, G.C. and Hardeveld, C. (1990) FEBS Lett. 274, 73–76.
[21] Anderson, T.W., Hirsch, C. and Kavaler, F. (1977) Circ. Res. 41, 472–480.
[22] Klitzner, T. and Morad, M. (1983) Pflüger's Arch. 398, 274–283.
[23] Weiss, R.E. and Morad, M. (1983) J. Gen. Physiol. 82, 79×117.
[24] Anderson, M.E., Fox, I.J., Swayze, C.R. and Donaldson, S.K. (1989) Am. J. Physiol. 25, H1432–H1439.
[25] McLeod, A.G., Shen, A.C.-Y., Campbell, K.P., Michalak, M. and Jorgensen, A.O. (1991) Circ. Res. 69, 344–359.
[26] Thastrup, O., Cullen, P.J., Drøbak, B.K., Hanley, M.R. and Dawson, A.P. (1990) Proc. Natl. Acad. Sci. USA 87, 2466–2470.
[27] Sagara, Y., Wade, J.B. and Inesi, G. (1992) J. Biol. Chem. 267, 1286–1292.
[28] Clarke, D.M., Loo, T.W., Inesi, G. and MacLennan, D.H. (1989) Nature 339, 476–478.
[29] Palmero, I. and Sastre, L. (1989) J. Mol. Biol. 210, 737–748.
[30] Magyar, A. and Váradi, A. (1990) Biochem. Biophys. Res. Commun. 173, 872–877.
[31] Murakami, K., Tanabe, K. and Takada, S. (1990) J. Cell. Sci. 97, 487–495.
[32] Agostini, B., De Martino, L. and Hasselbach, W. (1990) Z. Naturforsch. 45c, 671–675.
[33] Hasselbach, W. (1966) Ann. N.Y. Acad. Sci. 137, 1041–1048.
[34] Green, N.M. (1989) Biochem. Soc. Trans. 17, 972–974.