The functional importance of the extreme C-terminal tail in the gene 2 organellar Ca^{2+}-transport ATPase (SERCA2a/b)

Hilde VERBOOMEN,* Frank WUYTACK, Ludo VAN DEN BOSCH, Luc MERTENS and Rik CASTEELS
Laboratorium voor Fysiologie, Katholieke Universiteit Leuven, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

Ca^{2+}-uptake experiments in microsomal fractions from transfected COS-1 cells have revealed a functional difference between the non-muscle SERCA2b Ca^{2+} pump and its muscle-specific SERCA2a splice variant. Structurally, the two pumps differ only in their C-terminal tail. The last four amino acids of SERCA2a are replaced in SERCA2b by a 49-residue-long peptide chain containing a very hydrophobic stretch which could be an additional transmembrane segment. The functionally important subdomains in the SERCA2b tail were analysed by constructing three SERCA2b deletion mutants lacking 12, 31 or 49 amino acids. The mutants and the parental SERCA2 pumps were expressed in COS-1 cells and analysed for functional difference. SERCA2b had a twofold higher Ca^{2+} affinity, a twofold lower turnover rate and a 10-fold lower vanadate-sensitivity than SERCA2a and the mutants. Since each of the three truncated versions of SERCA2b acquire the characteristic properties of SERCA2a, it is concluded that the stretch of the last 12 residues of SERCA2b is of critical importance.

INTRODUCTION

Ca^{2+} accumulation into non-mitochondrial intracellular stores is mediated by the SERCAs [sarco/endoplasmic-reticulum (SR/ER) Ca^{2+}-ATPases]. These Ca^{2+}-ATPases belong to a superfamily of P-type ion pumps that are characterized by the formation of a phosphorylated intermediate as part of their catalytic cycle [1]. In the catalytic cycle two major enzyme conformations, E1 and E2, can be distinguished. In E1 the Ca^{2+}-binding sites are oriented towards the cytoplasm and are of high affinity. E2 is the conformation with a low affinity for Ca^{2+} in which the Ca^{2+}-binding sites are oriented luminally. Ca^{2+} binding to the ATPase in the E1 conformation permits an ATP-dependent phosphorylation of an aspartic acid residue in the enzyme [phosphoenzyme intermediate (EP) formation]. This elicits a number of conformational changes (E1 → E2) affecting the configuration and affinity of the Ca^{2+}-binding sites. Site-directed mutagenesis has allocated the Ca^{2+}-binding sites near the middle of membrane-spanning helices M4, M5, M6 and M8 [2–4].

Thus far, three SERCA genes have been described: *SERCA1*, *SERCA2* and *SERCA3* [5–7]. The *SERCA1* primary gene transcript is alternatively spliced, thereby generating two Ca^{2+}-pump isoforms, SERCA1a (adult isoform) and SERCA1b (neonatal isoform), which are expressed in fast skeletal muscle in a developmentally regulated way [8]. Like for *SERCA1*, alternative processing of the *SERCA2* gene transcripts gives rise to two distinct protein isoforms, SERCA2a and SERCA2b, which also differ only in the C-terminal part [9–12]. SERCA2a, the muscle isoform, is expressed in slow skeletal muscle, in cardiac muscle and, to a limited extent, also in smooth muscle. SERCA2b, the non-muscle isoform, is expressed in smooth muscle and in a range of non-muscle tissues, hence it is also referred to as the 'housekeeping' isoform [13]. It is known that SERCA3 is expressed in blood platelets, but the overall picture of the tissue distribution is rather confusing [7,14].

As a result of alternative splicing, the last four amino acids of SERCA2a are replaced by a variant tail of 49 amino acids in SERCA2b, but up to amino acid-993 both SERCA2 pumps have the same primary sequence. The extended tail in SERCA2b contains a hydrophobic stretch which is suggested to be a possible eleventh transmembrane segment [9–12,15]. This characteristic structural SERCA2 duality and the underlying processing patterns have been discovered in mammals, in birds [12,15] and even in invertebrates (the crustacean *Artemia franciscana*) [16]. Thus far, the full physiological meaning for these evolutionary highly conserved isoforms is not understood.

A previous study showed that Ca^{2+} uptake in microsomes from COS-1 cells transfected with pig stomach SERCA2a or SERCA2b cDNA revealed a higher Ca^{2+} affinity for SERCA2b ($K_{0.5} = 0.17 \pm 0.01\ \mu M$) compared with SERCA2a ($K_{0.5} = 0.31 \pm 0.02\ \mu M$) [17]. In the present study we further explore the functionally important subdomains in the SERCA2b tail. Three SERCA2b deletion mutants, together with the parental SERCA2a and SERCA2b isoforms, were expressed in COS-1 cells and analysed for Ca^{2+} affinity, vanadate-sensitivity and turnover rate of EP. It was concluded that the functional difference between SERCA2a and SERCA2b can be ascribed to the presence of the last 12 amino acids in SERCA2b.

MATERIALS AND METHODS

Construction of the mutants and expression in COS-1 cells

Nucleic acid triplets encoding the SERCA2b amino acids Gly-994, Asp-1012 and Ser-1031 were mutated (Altered Sites *in vitro* Mutagenesis System; Promega, Madison, WI, U.S.A.) into a stop codon, generating mutants I, II and III respectively (Figure 1 below). The mutant cDNAs were ligated into the unique *Eco*RI site of the mammalian expression vector pSV57 [18,19]. For transfection of COS-1 cells, 15 μg of CsCl-purifed DNA was used/10 cm-diameter Petri dish. Transient DEAE-dextran transfection of COS-1 cells and preparation of the microsomes were done as described previously [17]. To check for expression,

Abbreviations used: SERCA, **s**arco/**e**ndoplasmic-**r**eticulum **C**a^{2+}-**A**TPase; ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; PMCA, **p**lasma-**m**embrane **C**a^{2+}-**A**TPase; EP, phosphoenzyme intermediate; $C_{12}E_8$, octaethylene glycol monododecyl ether; TBS, Tris-buffered saline.

* To whom correspondence should be addressed.

10–15 μg of microsomal proteins were separated on SDS-containing Laemmli slab gels (7.5% acrylamide; 0.75 mm thick) and semi-dry-immunoblotted on to Immobilon-P membranes (Millipore, Bedford, MA, U.S.A.). The blots were quenched overnight in Tris-buffered saline (TBS)/Tween [150 mM NaCl/10 mM Tris/HCl (pH 7.5)/0.05% Tween 20] and immunostained as described previously [14]. Primary antibodies were a monoclonal non-discriminating SERCA2 antibody IID8 kindly provided by Dr. K. P. Campbell (Howard Hughes Medical Institute, University of Iowa, Iowa City, IA, U.S.A.), or a SERCA2b-specific polyclonal antibody, raised against a peptide corresponding to the last 12 amino acids of the SERCA2b C-terminus [20].

Ca^{2+}-transport assay

Ca^{2+} uptake was measured as described previously [17], but with Mops (20 mM, pH 6.8) instead of imidazole (30 mM, pH 6.8) as buffer system and at 27 °C instead of 37 °C. For the determination of the vanadate-sensitivity, the microsomes were preincubated at room temperature for 15 min in the Ca^{2+}-uptake medium without ATP (pCa 5.5), containing different orthovanadate concentrations. Ca^{2+} uptake was initiated by adding ATP.

ATP-hydrolysis rate

ATP-hydrolysis rate was determined by a coupled enzyme ATPase assay at 37 °C [21]. Approx. 40 μg of microsomes were preincubated in 1 ml of reaction medium, containing 1 mM EGTA, 5 mM NaN_3, 5 mM disodium ATP, 5.7 mM $MgCl_2$, 20 mM Mops (pH 6.8), 100 mM KCl, 100 μM ouabain, 10 μM A23187 (Ca^{2+} ionophore), 36 i.u./ml lactate dehydrogenase, 40 i.u./ml pyruvate kinase (both enzymes from rabbit muscle; Boehringer Mannheim, Mannheim, Germany), 1.5 mM phosphoenolpyruvate and 0.42 mM disodium NADH. After 8 min, calculated small volumes of a 50 mM $CaCl_2$ stock solution were added at 5 min intervals, generating progressively increasing free-Ca^{2+} concentrations. Free-Ca^{2+} concentrations were calculated according to the dissociation constants published by Fabiato and Fabiato [22]. Corrections were made for the background Mg^{2+}-ATPase activity by subtracting the ATPase activity recorded without added $CaCl_2$. Volumes of water equal to the calculated volumes of $CaCl_2$ stock were therefore added in a parallel cuvette. The decrease in absorbance at 340 nm was recorded with a Beckman DU-7 spectrophotometer.

When measuring the ATPase activity on solubilized membranes, microsomal proteins were preincubated (1 h, 0 °C) in octaethylene glycol monododecyl ether ($C_{12}E_8$)/asolectin in a ratio of 2:1, with the ratio of total protein to $C_{12}E_8$ being 1:5.

Phosphorylation from ATP

The phosphorylation was done on ice. Microsomes were preincubated for 2–3 min in a buffer containing (in mM): 20 Mops, pH 7.0, 80 KCl, 5 $MgCl_2$ and 0.1 $CaCl_2$. The reaction was started by adding 2 μM [γ-^{32}P]ATP (2.5 μCi/μmol; Amersham International) and stopped after 15 s by adding 7% trichloracetic acid. The samples were precipitated on ice for 20 min, pelleted for 15 min at 15000 g and washed once with the stop solution. The EP was separated on an acid gel, pH 6.3 [23], and the intensity of the signal was quantified by means of a PhosphorImager (model 425; Molecular Dynamics, Sunnyvale, CA, U.S.A.).

RESULTS

Expression in COS-1 cells

COS-1 cells were transfected with vector constructs encoding SERCA2a, mutants I, II and III and SERCA2b (Figure 1). Microsomal proteins were separated on SDS-containing Laemmli slab gels, semi-dry-immunoblotted and probed using a monoclonal non-discriminating SERCA2 antibody IID8 or a polyclonal SERCA2b-specific antibody, raised against a peptide corresponding to the last 12 amino acids of the SERCA2b C-terminus [20] (Figure 2). The absolute expression levels varied in different transfection experiments ($n = 6$), but a similar relative expression pattern was observed. All pumps were expressed equally well, except for mutant II, which consistently showed an expression level of 40–60% compared with the other ATPases. As expected, due to the absence of the corresponding epitope neither of the mutants could be detected with the SERCA2b-specific antibody.

SERCA2a, SERCA2b and the three deletion mutants were phosphorylated with ATP as a substrate at pCa 5.5. Phosphoprotein intermediates were electrophoresed on acid gels (pH 6.3), revealed (Figure 3) and quantified by means of a PhosphorImager. All pumps were phosphorylated to a similar level: roughly 10-fold over that for the endogenous pump expressed in non-transfected COS-1 cells. An exception was mutant II which was phosphorylated only 4- to 6-fold higher than the background.

As can be seen on immunoblots and on PhosphorImages, SERCA2b, mutant III and mutant II show some tendency to form dimers on SDS-containing gels, in contrast with SERCA2a

SERCA2a **NYLEP/AILE*977**

SERCA2b **NYLEP/GKECVQPATKSCSFSACTDGISWPFVLLIMPLVIWVYSTDTNFSDMFWS*1042**

Mutant I **NYLEP*993**

Mutant II **NYLEP/GKECVQPATKSCSFSACT*1011**

Mutant III **NYLEP/GKECVQPATKSCSFSACTDGISWPFVLLIMPLVIWVY*1030**

Figure 1 Pump C-termini of SERCA2a, SERCA2b and the truncated SERCA2b mutants

The hydrophobic amino acids are printed in **bold**, the putative eleventh transmembrane domain is underlined.

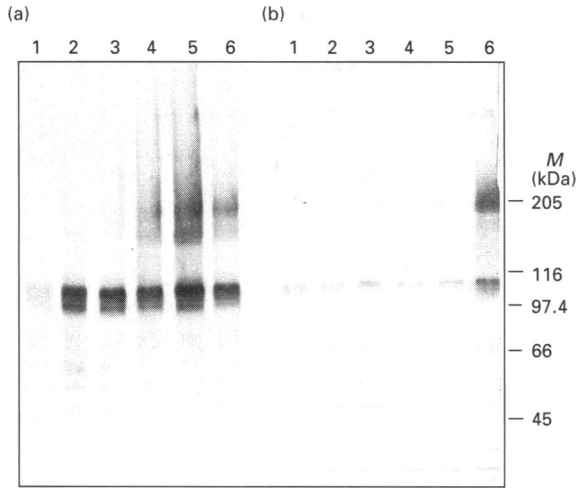

Figure 2 Semi-dry blotting and immunostaining of the expressed ATPases

Samples of 20 μg of microsomal proteins prepared from non-transfected control or transfected COS-1 cells were electrophoresed on 3–12% gradient Laemmli gels, semi-dry-blotted and immunostained as described in the the Materials and methods section. The primary antibody for immunodetection was a monoclonal non-discriminating IID8 antibody (**a**) or a polyclonal, SERCA2b-specific antibody, raised against the 12 C-terminal amino acids of SERCA2b (**b**). Control microsomes were prepared from COS-1 cells which were treated similarly to the transfected cells, but did not receive transfecting DNA. Molecular-mass (*M*) markers are indicated on the right. Lane 1, control; lane 2, SERCA2a; lane 3, mutant I; lane 4, mutant II; lane 5, mutant III; lane 6, SERCA2b.

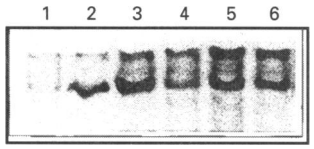

Figure 3 Phosphorimage of the phosphorylated ATPases

Samples of 5–10 μg of microsomal proteins from control and transfected cells were phosphorylated using ATP as a substrate and electrophoresed as described in the Materials and methods section. Revelation and quantification was done by means of a PhosphorImager. Lane 1, control; lane 2, SERCA2a; lane 3, mutant I; lane 4, mutant II; lane 5, mutant III; lane 6, SERCA2b.

and mutant I. The percentage of dimerization has been estimated to be 20 ± 3, 22 ± 4, 59 ± 4, 52 ± 2 and 53 ± 2 respectively for SERCA2a, mutant I, II, III and SERCA2b, based on the quantification of the intensity of the signal for the dimers and monomers on PhosphorImages. The results are the means for three to six experiments performed with independent membrane preparations. This suggests that the SERCA2b tail is involved in dimerization of the pump.

Ca^{2+}-uptake and turnover rate

For the membranes used in the experiment shown in Figure 4, the Ca^{2+}-uptake rate of SERCA2a and mutant III exceeded that of the non-transfected control about 13-fold. An exceptionally high uptake rate (22-fold over that for the control) was observed for mutant I. For mutant II and SERCA2b, however, uptake rates were only 5–6-fold higher than for the control. The lower activity of SERCA2b can be ascribed to its known lower turnover rate of EP [24]. The turnover rate of EP (v/EP) is defined as the

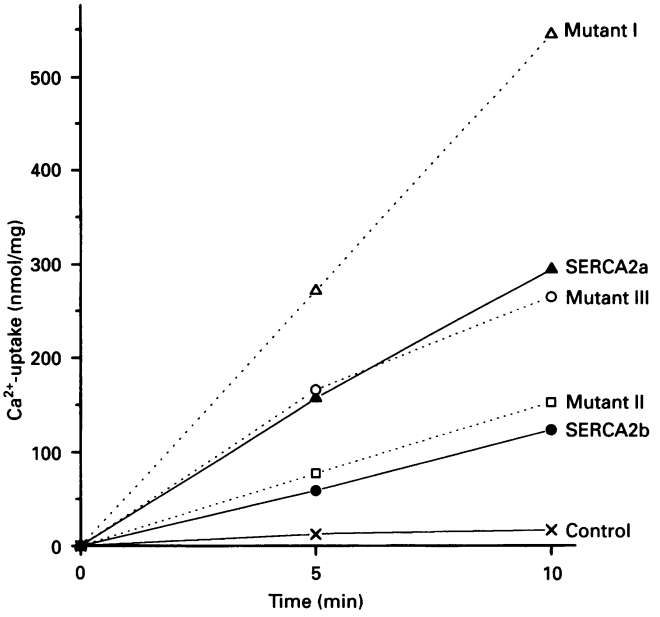

Figure 4 Time-dependent Ca^{2+}-uptake

Oxalate-stimulated Ca^{2+} uptake was measured at pCa 5.5 and stopped after 5 and 10 min as described in the Materials and methods section. Samples of 25–45 μg of microsomal proteins were used/ml of uptake medium. For this experiment, Ca^{2+}-uptake rates are 29, 52, 13, 31 and 12 nmol/min per mg for SERCA2a, mutant I, mutant II, mutant III and SERCA2b respectively. ·····, Mutant ATPases; ——, control and parental Ca^{2+} ATPases. ▲, SERCA2a; △, mutant I; □, mutant II; ○, mutant III; ●, SERCA2b.

initial rate of Ca^{2+} uptake divided by the steady-state level of EP which is used as a measure for the level of active Ca^{2+}-pump protein. From phosphorylation experiments we concluded that, for each of the truncated Ca^{2+} ATPases, v/EP was higher than for SERCA2b and reached a level similar to that of SERCA2a [in relative units: 0.87 ± 0.22, 0.74 ± 0.08, 0.82 ± 0.17 and 1 respectively for mutant I, II, III and SERCA2a (values normalized to SERCA2a), compared with 0.41 ± 0.11 for SERCA2b]. The final results are the means for three to six measurements performed with independent membrane preparations. When determining the v/EP, both mono- and di-mers were taken into account.

Ca^{2+}-dependency

For all three mutants, the Ca^{2+}-dependency of both Ca^{2+}-uptake activity and ATP-hydrolysis rate was similar to that of SERCA2a: $K_{0.5} = 0.47\pm0.03$ μM Ca^{2+} versus 0.24 ± 0.01 μM Ca^{2+} for SERCA2b (Figures 5a and 5b). The difference in absolute value with formerly reported values for $K_{0.5}$ (0.31 μM and 0.17 μM respectively) must be ascribed to different Ca^{2+}-uptake conditions. Previous uptake experiments were done at 37 °C with imidazole (30 mM, pH 6.8) as buffer system, whereas these experiments were performed at 27 °C with Mops (20 mM, pH 6.8) as buffer. In both conditions however, SERCA2a has a twofold lower affinity for Ca^{2+} than SERCA2b.

Vanadate-sensitivity

Since the apparent higher Ca^{2+} affinity of SERCA2b could be due to a difference in kinetics, with the E1 ↔ E2 equilibrium being shifted more towards E1 than for the SERCA2a isoform,

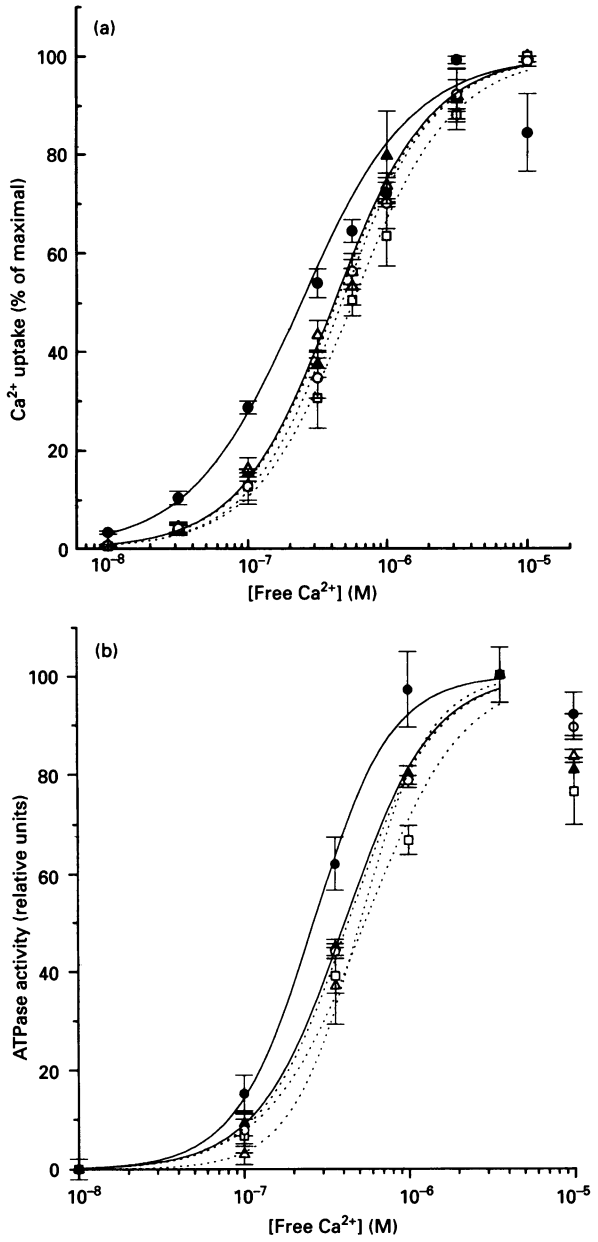

Figure 5 Ca^{2+}-dependence of Ca^{2+} uptake and ATPase activity

(**a**) Oxalate-stimulated Ca^{2+} uptake was measured at different Ca^{2+} concentrations as described in the Materials and methods section. Ca^{2+} uptake was stopped after 5 min. Samples of 25–30 μg of microsomal proteins prepared from control and transfected cells were used/ml of uptake medium. Values obtained for Ca^{2+} uptake in the control microsomes were subtracted from uptake values for microsomes from transfected cells. The results are the means of four measurements on independent membrane preparations; error bars represent S.E.M. Curve-fitting revealed the following $K_{0.5}$ values; 0.41 ± 0.02, 0.43 ± 0.02, 0.56 ± 0.04, 0.47 ± 0.03 and 0.24 ± 0.01 μM for SERCA2a, mutant I, mutant II, mutant III and SERCA2b respectively. (**b**) ATPase activity was measured at different Ca^{2+} concentrations as described in the Materials and methods section. Samples of 25–50 μg of microsomal proteins from transfected cells were used/ml of ATP-hydrolysis medium. The results are the means of four measurements on independent membrane preparations; error bars represent S.E.M. ·····, mutant ATPases; ——, parental Ca^{2+}-ATPases. ▲, SERCA2a; △, mutant I; □, mutant II; ○, mutant III; ●, SERCA2b.

we measured the vanadate-sensitivity. Vanadate (VO_4^{3-}) is a structural analogue of P_i (PO_4^{3-}), which binds to the pump in the E2 conformation, but does not allow the catalytic cycle to go on

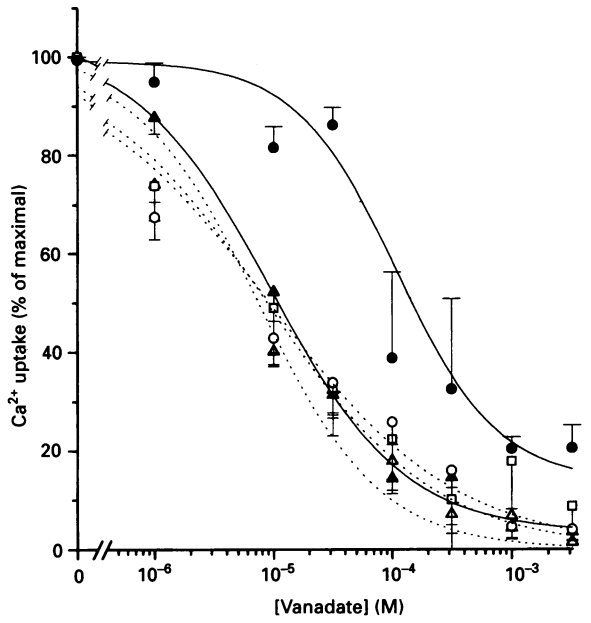

Figure 6 Vanadate-sensitivity

Oxalate-stimulated Ca^{2+} uptake was measured in the presence of different vanadate concentrations as described in the Materials and methods section. Ca^{2+} uptake was stopped after 5 min. Samples of 25–30 μg of microsomal proteins prepared from control and transfected cells were used/ml of Ca^{2+}-uptake medium. Values obtained for Ca^{2+} uptake in the control microsomes were subtracted from uptake values for microsomes from transfected cells. The results are the means of three measurements on independent membrane preparations; error bars represent S.E.M. Curve-fitting revealed the following $K_{0.5}$ values: 9.5 ± 1.8 μM, 7.5 ± 0.8 μM, 9.2 ± 3.3 μM, 8.8 ± 1.9 μM and 0.11 ± 0.04 mM for SERCA2a, mutant I, mutant II, mutant III and SERCA2b respectively. ·····, mutant ATPases; ——, parental Ca^{2+} ATPases. ▲, SERCA2a; △, mutant I; □, mutant II; ○ mutant III; ●, SERCA2b.

[25]. Also in this respect, mutants I, II and III resembled SERCA2a ($K_{0.5} = 0.01$ mM) and differed from SERCA2b ($K_{0.5} = 0.1$ mM) (Figure 6). This suggests that a larger part of the pump population is in the E1 conformation for SERCA2b than is the case for SERCA2a and the mutants, providing SERCA2b with a better protection against vanadate inhibition.

Restoration experiments

We tested whether re-addition of a peptide representing the 12-residue-long C-terminus of SERCA2b to the truncated variants or to SERCA2a could re-establish the wild-type properties of SERCA2b. ATPase activity was measured in the presence of a roughly 1000-fold molar excess of the peptide, both in intact microsomes and in solubilized ATPase from cardiac SR (expressing SERCA2a) and from COS-1 cells transfected with mutant III. However, in none of these conditions could a difference in Ca^{2+}-sensitivity or in the ATP-hydrolysis rate be detected (results not shown).

DISCUSSION

Structural differences

Alternative splicing of the *SERCA2*-gene transcript generates two Ca^{2+}-pump isoforms (SERCA2a and SERCA2b), which differ only in their C-terminal end. The last four amino acids of SERCA2a are replaced by 49 amino acids in SERCA2b, containing a very hydrophobic stretch which is suggested to be an eleventh transmembrane domain [9–12]. According to immuno-

cytochemical studies done by Campbell et al. [15], the extreme C-termini of SERCA2a and SERCA2b are localized on opposite sites of the ER membrane (with the SERCA2b tail protruding in the lumen). The RNA processing patterns underlying this structural duality are evolutionary highly conserved [12,16], but thus far the full extent of its physiological meaning remains unknown.

Functional difference

Despite the topological differences, Campbell et al. [12] have not been able to measure any functional difference between SERCA2a and SERCA2b expressed in COS-1 cells. On the other hand, a difference in Ca^{2+} affinity (SERCA2a < SERCA2b) and a difference in turnover rate of EP (SERCA2a > SERCA2b) was observed by Lytton et al. [24]. Our previous observations on both isoforms expressed in COS-1 cells are in the line with Lytton's [24] observations. They revealed a difference in Ca^{2+} affinity, but not in phospholamban- or thapsigargin-sensitivity [17].

To define further the functionally important subdomains in the SERCA2b C-terminus, three deletion mutants were constructed: mutants I, II, and III (Figure 1). SERCA2a, SERCA2b and the truncated mutants were expressed in COS-1 cells and analysed for functional differences. Remarkably, mutant II (which lacks the hydrophobic stretch, but retains an 18-residue-long tail beyond the point of divergence with SERCA2a) was consistently expressed at a lower level compared with the other pumps, but its catalytic properties did not differ from the other truncated variants nor from those of SERCA2a. Therefore a major defect in protein folding or insertion into the membrane seems unlikely. A decreased stability of the translated protein could be a possible reason for the lower expression level.

The tendency to form dimers in electrophoresis is equal for mutant II, mutant III and SERCA2b, in contrast with mutant I and SERCA2a. This suggests (1) that the whole extended SERCA2b tail, rather than just the hydrophobic stretch, is responsible for dimerization, and (2) that the dimerization is not responsible for the difference in characteristics between SERCA2a and SERCA2b. Whether the dimerization observed in Laemmli gels also occurs *in vivo* or has a physiological meaning remains unknown.

Both the Ca^{2+}-dependent uptake activities and the corresponding ATPase activities (Figures 5a and 5b) revealed a lower Ca^{2+} affinity for SERCA2a and the SERCA2b deletion mutants compared with the native SERCA2b. The apparent higher Ca^{2+} affinity of SERCA2b can be alternatively explained either by a structural difference in the Ca^{2+}-binding sites or by a shift in the $E1 \leftrightarrow E2$ equilibrium towards the high-affinity E1 state. Considering the first possibility, it should be noted that the residues directly involved in Ca^{2+} binding are localized in the transmembrane segments M4, M5, M6 and M8 [2–4] and are conserved in all ATPases used in this study. Still, a potential lateral interaction between the putative eleventh transmembrane segment in SERCA2b and the Ca^{2+} ligand-bearing helices cannot be excluded. This type of interaction has been described between the transmembrane segment of phospholamban and the SERCA pumps, with an effect on the Ca^{2+} affinity as a consequence [26]. However, such an interaction is unlikely in our observations, because the removal of the putative membrane segment (difference between mutant II and mutant III) does not affect the Ca^{2+} affinity. Our experiments support the second hypothesis, which supposes that a potential interaction between the SERCA2b tail and an as-yet-undefined more-upstream-localized region of the ATPase shifts the $E1 \leftrightarrow E2$ equilibrium more towards E1. This is confirmed by the observation that SERCA2b has a lower sensitivity towards vanadate than SERCA2a and the mutants (Figure 6). Vanadate binds selectively to the E2 conformation of the ATPase [25].

The turnover rate for SERCA2b was lower compared with that of SERCA2a and that of the mutants. Hence, an interaction of the last 12 residues of the SERCA2b tail with another pump domain apparently decreases the pump's turnover rate.

In short, as soon as the last 12 amino acids of SERCA2b are removed, the values for Ca^{2+} affinity, vanadate-sensitivity and turnover rate shift towards those for SERCA2a. Therefore we now believe that the functional difference between SERCA2a and SERCA2b can be ascribed to the last 12 amino acids of SERCA2b.

Recently, related observations have been made for the plasma-membrane Ca^{2+} pump by Enyedi et al. [27]. Alternative splicing of the plasma-membrane Ca^{2+}-ATPase (PMCA) gene *PMCA4* creates two isoforms (PMCA4a and PMCA4b) with a structural difference in their calmodulin-binding domain. This results in a reduced calmodulin affinity for PMCA4a, with a corresponding reduced Ca^{2+} affinity as a consequence. Deletion mutants in their experiments have shown that a peptide stretch comprising the last nine residues of the calmodulin-binding domain was responsible for the difference in affinity.

For PMCA it has been shown that re-addition of a peptide representing the calmodulin-binding domain to truncated Ca^{2+} pumps which lacked this domain was successful in restoring the full-length pump activity [28]. However, in our experiments, re-addition of the 12-residue-long peptide to the truncated SERCA2b pump or to SERCA2a was not effective in mimicking the SERCA2b properties. This was also the case for the solubilized ATPase, thereby excluding the possibility that the peptide could not reach the luminal side of the ATPase.

Tissue expression pattern and possible physiological meaning

It has been documented that the SERCA2 expression in slow skeletal and cardiac muscle differs not only quantitatively but also qualitatively from that in smooth muscle [13]. First of all, cardiac muscle expresses 10–20-fold higher levels of SERCA2 than smooth muscle and, secondly, slow skeletal and cardiac muscle express only SERCA2a, whereas 70–80% of smooth-muscle SERCA2 is represented by SERCA2b; the remainder is SERCA2a. In this respect it is tempting to speculate that tissues which have to pump relatively larger amounts of Ca^{2+}, i.e. cardiac muscle, slow skeletal muscle and, to a lesser extent, also smooth muscle, can economize on the number of Ca^{2+}-pump molecules by expressing a SERCA2 variant with a roughly twofold higher turnover rate, despite the lower Ca^{2+} affinity. Non-muscle tissues, however, express a slower pump variant with a higher Ca^{2+} affinity.

The mechanisms controlling the alternative processing responsible for generating the SERCA2a and SERCA2b isoforms are currently under investigation [29].

We thank Anja Florizoone, Marina Crabbé, Lea Bauwens and Yves Parijs for their excellent technical assistance, and Dr. Humbert De Smedt for advice in cell culturing. L.V.D.B. is a Postdoctoral Researcher of the National Fund for Scientific Research (NFWO, Belgium). L.M. is a Research Assistant of the National Fund for Scientific Research (NFWO, Belgium).

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Received 9 May 1994/20 June 1994; accepted 27 June 1994