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The $E_1 \rightarrow E_2$ transition of Ca^{2+}-transporting ATPase in sarcoplasmic reticulum occurs without major changes in secondary structure

A circular-dichroism study

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C.d. spectroscopy was used to investigate the structures of Ca^{2+}-ATPase (Ca^{2+}-transporting ATPase) in the E_1 and E_2 states in native, in fluorescein isothiocyanate (FITC)-labelled and in solubilized sarcoplasmic reticulum (SR) preparations. The E_1 state was stabilized by 100 μM-Ca^{2+} and the E_2 state by 0.5 mM-Na_3VO_4 and 0.1 mM-EGTA. There were no significant differences detected in the c.d. spectra and the calculated secondary structures between the E_1 and E_2 states in any of the three types of preparations. The FITC-labelled SR did show the characteristic changes in FITC fluorescence on addition of Ca^{2+} or vanadate, indicating that the preparation was competent for $E_1 \rightarrow E_2$ transitions. Therefore the absence of changes in the c.d. spectra implies that the $E_1 \rightarrow E_2$ transition in the Ca^{2+}-ATPase does not involve a major net rearrangement of the polypeptide backbone conformation.

INTRODUCTION

The ATP-dependent transport of Ca^{2+} by the Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) involves cyclic transitions between two major conformations (Martonosi & Beeler, 1983; Inesi, 1985). The enzyme interacts with Ca^{2+} and ATP on the cytoplasmic side of the membrane in the E_1 conformation; this is followed by the phosphorylation of an aspartic acid residue in the active site and the occlusion of Ca^{2+} by the ADP-sensitive phosphorylated enzyme intermediate

$$E_1 \begin{matrix} \nearrow P \\ \searrow 2Ca \end{matrix}$$

into an ADP-insensitive form

$$E_2 \begin{matrix} \nearrow P \\ \searrow 2Ca \end{matrix}$$

with a decrease in its Ca^{2+} affinity and the subsequent release of Ca^{2+} into the SR lumen. The Mg^{2+}-catalysed cleavage of the acyl–phosphate bond leads to the release of P_i, followed by the isomerization of the enzyme from the E_2 into the E_1 form.

The E_1 and E_2 states are characterized by distinct patterns of proteolysis (Dux & Martonosi, 1983*a*; Dux *et al.*, 1985), differences in the quantum yield of tryptophan and FITC fluorescence (Dupont, 1976; Dupont *et al.*, 1982; Pick, 1982; Pick & Karlish, 1980, 1982; Andersen *et al.*, 1982), changes in the reactivity of functional groups (Ikemoto, 1982) and different affinities of Ca^{2+}, ATP and Mg^{2+} for the ATPase (Martonosi & Beeler, 1983; Inesi & De Meis, 1985).

Stabilization of the enzyme by Ca^{2+} or lanthanides in the E_1 conformation induces the formation of P1-type crystalline arrays, with Ca^{2+}-ATPase monomers as structural units (Dux *et al.*, 1985). Vanadate and P_i in a Ca^{2+}-free medium shift the equilibrium in favour of the E_2 conformation and promote the formation of P2-type Ca^{2+}-ATPase crystals, with ATPase dimers as structural units (Dux & Martonosi, 1983*b*; Taylor *et al.*, 1984). The two distinct crystal forms appear to be specifically related to the two major conformations of the Ca^{2+}-ATPase and could reflect either differences in the secondary structure of the protein or altered patterns of interaction between ATPase molecules under the two sharply different ionic conditions.

The purpose of the present study was to determine conclusively whether the transition between the E_1 and E_2 enzyme forms is accompanied by changes in the c.d. spectrum of the protein, which would correspond to changes in the polypeptide backbone structure.

A preliminary account of parts of this work has already appeared (Csermely *et al.*, 1986).

MATERIALS AND METHODS

SR vesicles were isolated from predominantly white skeletal muscles of rabbit as described previously (Nakamura *et al.*, 1976). For protein determination, the method of Lowry *et al.* (1951) was used, with BSA as the calibration standard. Analysis of protein composition by polyacrylamide-gel electrophoresis was performed on a 6–18% (w/v) gradient gel as described previously (Dux & Martonosi, 1984).

ATPase activities were measured in 0.1 M-KCl/10 mM-

Abbreviations used: Ca^{2+}-ATPase, Ca^{2+}-transporting ATPase; FITC, fluorescein 5'-isothiocyanate; OG, octyl glucoside (n-octyl β-D-glucopyranoside); NRMSD, normalized root-mean-square deviation; Na^+,K^+-ATPase, $(Na^+ + K^+)$-transporting ATPase; BSA, bovine serum albumin; SR, sarcoplasmic reticulum.

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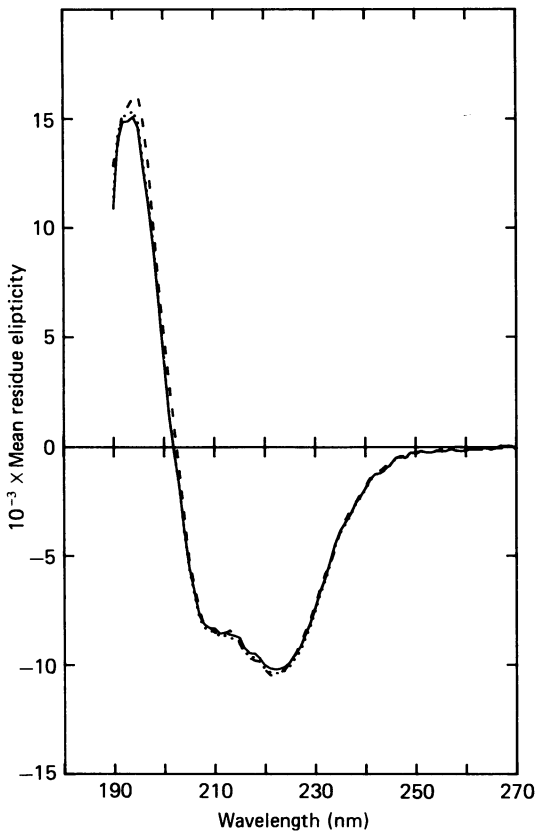

Fig. 1. C.d. spectra of SR vesicles (0.5 mg of protein/ml) in 0.05 M-KCl/3 mM-imidazole (pH 7.4)/5 mM-$MgCl_2$ (standard buffer solution) without further additions (——), or with 100 μM-Ca (– – – –) or 0.5 mM-vanadate plus 0.1 mM-EGTA (·····) added

These, and all other spectra were obtained under conditions for which differential light-scattering effects are minimal. The spectra are averages for at least six consecutive runs.

imidazole (pH 7.4)/5 mM-$MgCl_2$/0.5 mM-EGTA with or without 0.45 mM-$CaCl_2$ and 1 μM-Ca^{2+} ionophore A23187, at a temperature of 25 °C. The liberated P_i was analysed as described by Fiske & SubbaRow (1925).

Solubilization of SR with octyl glucoside (OG) was carried out at a protein concentration of 0.56–0.85 mg/ml in 0.05 M-KCl/3 mM-imidazole (pH 7.5)/5 mM-$MgCl_2$; the OG concentrations ranged from 7.5 to 10 mg/ml, where maximal ATPase activity was obtained. At OG concentrations in excess of 15 mg/ml, sharp inhibition of ATPase activity occurred.

Ultrasonic dispersion

To examine the effects of decrease in particle size on light-scattering, selected samples were sonicated in a circular bath sonicator (Laboratory Supplies, Hicksville, NY 11801, U.S.A.), for 10 min at room temperature. After sonication the samples were centrifuged (6000 *g*) in an Eppendorf centrifuge for 2 min. The clear supernatant contained vesicles of about 25 nm (250 Å) diameter.

For electron microscopy the specimens were negatively stained with 1% uranyl acetate and viewed in a JEOL–JEM or in a Siemens Elmiskop I electron microscope at an accelerating voltage of 60 or 80 kV. The average vesicle diameters were determined on randomly taken electron micrographs without corrections for the effects of flattening and distortion by negative stain. The proportion of vesicles originating from the SR was determined as described previously (Dux & Martonosi, 1984).

C.d. measurements

Parallel experiments were performed on pairs of samples containing 0.5 mg of protein/ml in 50 mM-KCl/5 mM-$MgCl_2$/3 mM-imidazole, pH 7.4 (standard buffer solution) with either 100 μM-$CaCl_2$ or with 0.5 mM-vanadate plus 0.1 mM-EGTA added. The ligands were introduced by adding 200 μl of 10 × concentrated stock solution to 2 ml of a sample. In control samples without added ligand, 200 μl aliquots of standard buffer solutions were substituted for the Ca^{2+} or vanadate + EGTA stock solutions. Corresponding baselines were obtained using protein-free standard buffer solution with $CaCl_2$, vanadate, EGTA, OG and/or FITC added as appropriate.

C.d. spectra were recorded on an Aviv 60DS spectropolarimeter, fitted with a variable position detector (Schneider & Harmatz, 1976) that allowed variation of the acceptance angle from 2° to 90°. Most spectra were taken with the phototube placed directly adjacent to the sample cell to minimize detection of differential light-scattering. To estimate the extent of differential scattering, some spectra were also obtained with a detector angle of 2° (Wallace & Mao, 1984).

The instrument was calibrated with *d*-camphorsulphonic acid at 192.5 and 290 nm. Measurements were made at 25 ± 2 °C with a 0.2-mm-pathlength cell. At least six spectra were averaged for each sample and the S.D. values between spectra were calculated. Three independent repeats of each experiment were run on a single SR preparation. In addition, two different preparations of SR were examined. All gave very similar (±1–2%) results.

Analysis of c.d. data

After averaging and subtraction of the baseline, data points at 1 nm intervals between 190 and 240 nm were fitted to a reference data set by a normalized linear least-squares procedure (Mao *et al.*, 1982) that did not require the fractions to be positive nor the sum of the fractions to equal unity. The former restraint was not necessary for these samples, as all calculated secondary structures were non-negative, suggesting a good correspondence between the structures in the sample and in the reference data set. The second restraint was not applied because it would require a knowledge of the absolute protein concentration. Since the Lowry *et al.* (1951) assay with a BSA standard does not give an accurate absolute value, application of such a restraint in the data analysis would distort significantly the values obtained. It is used as a measure of relative concentrations for scaling spectra. The fractions calculated were normalized to 100% by multiplication by 1/sum-of-the-fractions to obtain estimates of the relative proportions of secondary structures present. This procedure does not depend on accurate measurements of concentration.

The quality of each computer fit was evaluated by calculating NRMSD between calculated and observed spectra (Mao *et al.*, 1982). Values of $\leqslant 0.1$ are an indication of a close correspondence between the

Table 1. Calculated secondary structures

Samples were prepared and the measurements performed as described in the Materials and methods section. The vanadate samples also contained 0.1 M-EGTA to bind trace Ca^{2+}. For other details, see the text.

Sample	Ligand	Proportion of secondary structure attributable to				NRMSD
		α-Helix	β-Sheet	Turn	Random coil	
SR membranes	No addition	0.45	0.07	0.13	0.35	0.04
	0.1 mM-Ca^{2+}	0.45	0.07	0.13	0.35	0.04
	0.5 mM-Vanadate	0.47	0.09	0.12	0.32	0.05
SR membranes labelled with FITC	No addition	0.51	0.08	0.07	0.35	0.04
	0.1 mM-Ca^{2+}	0.49	0.06	0.10	0.33	0.05
	0.5 mM-Vanadate	0.49	0.12	0.10	0.30	0.05
SR solubilized with OG	No addition	0.42	0.26	0.03	0.29	0.05
	0.1 mM-Ca^{2+}	0.40	0.16	0.11	0.33	0.06
	0.5 mM-Vanadate	0.41	0.30	0.03	0.26	0.05
FITC-labelled SR solubilized with OG	No addition	0.39	0.32	0.00	0.29	0.06
	0.1 mM-Ca^{2+}	0.38	0.32	0.00	0.30	0.06
	0.5 mM-Vanadate	0.38	0.35	0.00	0.27	0.06

Table 2. Percentage standard deviations or differences from mean at selected wavelengths

For details, see the text.

Data set	S.D or difference	Wavelength (nm) . . .	S.D. or difference (%)				
			192	209	216	223	230
No additions	S.D.		3.2	2.3	1.1	1.0	1.1
Ca^{2+} (0.1 mM)	S.D.		2.9	1.8	1.7	1.4	1.3
Vanadate (0.5 mM)	S.D.		3.8	2.0	1.1	1.0	1.5
None versus Ca^{2+}	Difference		3.6	1.9	0.5	0.7	1.6
None versus vanadate	Difference		3.5	0.8	2.4	2.0	0.0
Ca^{2+} versus vanadate	Difference		0.2	1.1	2.8	1.4	1.6

calculated and actual secondary structures of a protein (Brahms & Brahms, 1980); for these samples the values of 0.04–0.06 suggest a strong correlation with the calculated structures.

Mean residue ellipticities were calculated based on a mean residue relative molecular mass of 110.

Fluorescence measurements

The SR was labelled with FITC (10–15 nmol/mg of protein) for 1 h at 25 °C in 0.3 M-sucrose/50 mM-Tris/maleate (pH 7.4)/5 mM-$MgCl_2$. After centrifugation at 80000 *g* for 30 min the sedimented microsomes were suspended in 0.05 M-KCl/3 mM-imidazole (pH 7.4)/5 mM-$MgCl_2$ and used within 8 h for c.d. measurements. The fluorescence of covalently bound FITC was measured on a Perkin–Elmer MPF 2A spectrofluorimeter at excitation and emission wavelengths of 495 nm and 525 nm respectively, with a 5 nm slit width.

RESULTS

C.d. spectra of native SR vesicles

SR preparations isolated from white skeletal muscles of rabbit by standard procedures consist of spherical or elongated vesicles with average diameters in the range 70–130 nm (700–1300 Å). On the basis of the potential of such samples to form Ca^{2+}-ATPase crystals, about 80–90% of the vesicle population is estimated to be derived from SR (Dux & Martonosi, 1984), and the Ca^{2+}-ATPase constitutes 70–80% of the total protein content. Among other proteins that are present in minor amounts are calsequestrin, high-affinity Ca^{2+}-binding protein, a 56 kDa glycoprotein and a group of proteolipids (Michalak, 1985).

C.d. spectra obtained on native SR vesicles suspended in 0.05 M-KCl/3 mM-imidazole (pH 7.4)/5 mM-$MgCl_2$ (Fig. 1) show a maximum at 192 nm and a double-lobed negative peak with a minimum at 223 nm. Linear least-squares analysis gave the following average composition: 45% α-helix, 7% β-sheet, 13% turn and 35% random coil (Table 1).

To examine whether the structural transition between the E_1 and E_2 states involves a change in the secondary structure of the Ca^{2+}-ATPase, the effects of Ca^{2+} and of vanadate on the c.d. spectrum of SR were analysed. The E_1 conformation of Ca^{2+}-ATPase is stabilized by Ca^{2+}, whereas vanadate in a Ca^{2+}-free medium shifts the equilibrium in favour of the E_2 form (Andersen *et al.*, 1982; Pick & Karlish, 1982; Dux *et al.*, 1985). Neither Ca^{2+} nor vanadate produced significant changes in the

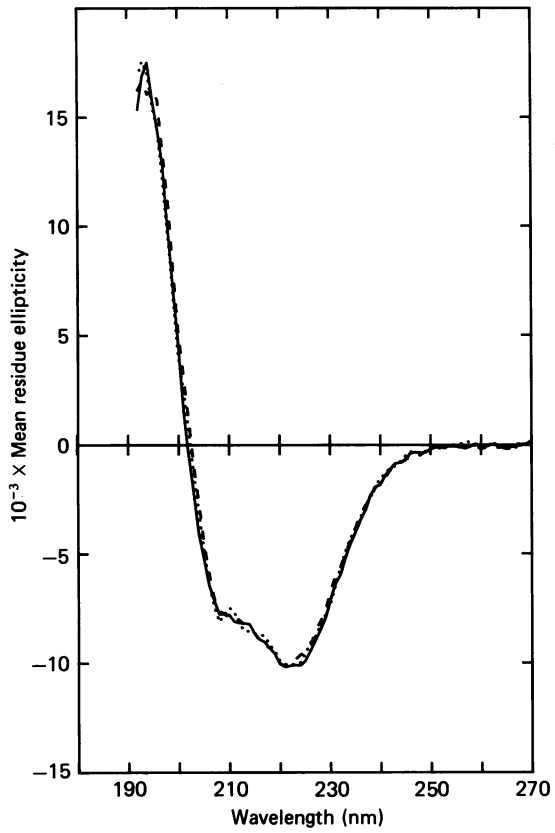

Fig. 2. C.d. spectra of FITC-labelled SR vesicles (0.5 mg of protein/ml) in 0.05 M-KCl/3 mM-imidazole (pH 7.4)/ 5 mM-$MgCl_2$ solution without further additions (——) or with 100 μM-Ca^{2+} (– – – –) or 0.5 mM-vanadate plus 0.1 mM-EGTA (·····) added

c.d. spectrum (Fig. 1), or in the calculated proportions of secondary structures (Table 1).

Such comparisons are meaningful only if the error levels of the measurements are very low. Systematic errors were minimized by comparing the native, the Ca^{2+}-treated and the vanadate-treated samples under identical conditions, using the same SR preparation and buffer solution in parallel experiments. In this way, the S.D. between repeated measurements was $\leqslant 4\%$ at all wavelengths used in the analyses. The differences between the spectra obtained for native, Ca^{2+}-treated, or vanadate-treated samples did not exceed the S.D. between measurements on the same sample (Table 2). Consequently the calculated secondary structures for all samples differed by $\leqslant \pm 2\%$ (Table 1). Identical results were obtained on two distinct SR preparations tested at three different enzyme concentrations.

Because these experiments have examined proteins in intact membranous vesicles, it was important to demonstrate that the results were not influenced by the optical effects of differential light-scattering and absorption flattening in the various samples (Wallace & Mao, 1984). The absence of differential scattering effects was shown in two ways: first, variation of the detector angle from 2° to 90° did not change the spectrum, indicating that most of the differential scattering was in the near forward direction (Mao & Wallace, 1984). Second, sonication did not influence the C.d. spectrum, although it diminished the average vesicle diameter from 100 nm

Table 3. Changes in the fluorescence of FITC-labelled SR connected with transition between the E_1 and E_2 states

The labelling of SR vesicles with FITC and the measurement of fluorescence were carried out as described in the Materials and methods section. The measurements were performed within 1–2 h after labelling in 0.05 M-KCl/3 mM-imidazole(pH 7.4)/5 mM-$MgCl_2$, with additions as indicated. Results are means (±S.D.) for five or six measurements.

	Relative fluorescence intensity	
Addition	Without detergent	Solubilized with OG (15 mg/mg of protein)
0.1 M-$CaCl_2$	46.00 (±0.55)	45.08 (±3.27)
0.1 mM-EGTA + 0.5 mM-Na_3VO_4	51.62 (±2.55)	50.83 (±3.82)

(1000 Å) to about 25 nm (250 Å). Because the purpose of these experiments was to examine changes between E_1 and E_2 states, all experiments were done in a parallel manner so any differential absorption flattening effects would be identical in all samples and could not account for any spectral differences that might be found between them. Finally, since no spectral changes were seen between different samples, concern that the optical effects were the source of any differences was unnecessary.

C.d. and fluorescence measurements on FITC-labelled SR

Since no differences were observed between c.d. spectra measured under conditions expected to stabilize either the E_1 or the E_2 conformation of the Ca^{2+}-ATPase, it was essential to prove that, in addition to having normal ATPase and Ca^{2+}-transport activities, the preparations were competent to carry out the $E_1 \rightarrow E_2$ transition. To demonstrate this, FITC was used as a conformational probe of the Ca^{2+}-ATPase. FITC covalently labels the Ca^{2+}-ATPase at a single site with inhibition of ATPase activity (Pick & Karlish, 1980; Mitchinson *et al.*, 1982). Pick & Karlish (1982) observed that, in FITC-labelled SR preparations, the FITC fluorescence intensity is lower in the presence of Ca^{2+} and increases after addition of EGTA and vanadate. Similar results were obtained for the FITC-labelled preparations used in the present study (Table 3).

The c.d. spectrum of FITC-labelled SR (Fig. 2, Table 1) was similar to that of native SR (Fig. 1, Table 1). Ca^{2+} and vanadate were without effect on the c.d. spectrum of FITC-labelled microsomes (microsomal fraction), although they did produce the characteristic changes in fluorescence intensity (Table 3). These observations constitute strong evidence, obtained on a single specimen, that transition between the E_1 and E_2 states occurs without detectable changes in the c.d. spectrum.

C.d. spectra of SR solubilized by OG

Solubilization of SR vesicles by OG (15–20 mg of detergent per mg of protein) stimulates 2–3-fold the Ca^{2+}-activated ATP hydrolysis and increases the magnitude of the 192 nm peak and the 209/224 ratio in c.d. spectra (Fig. 3*a*). These changes correspond to an

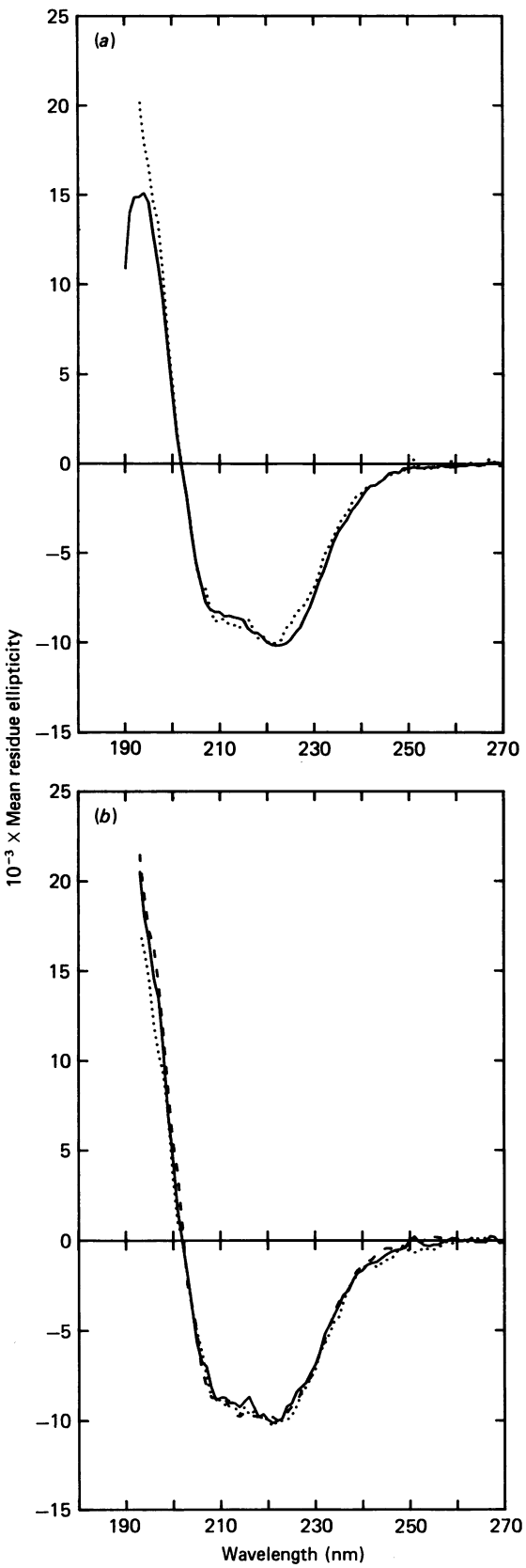

Fig. 3. C.d. spectra of native and OG-solubilized SR vesicles

(*a*) C.d. spectra of native (——) and OG-solubilized (·····) SR vesicles (0.5 mg of protein/ml) in 0.05 M-KCl/3 mM-imidazole(pH 7.4)/5 mM-$MgCl_2$ in the absence of added ligands. For solubilization of SR proteins, OG was added to a final concentration of 7.5 mg/ml 5–10 min before the measurements were made. (*b*) C.d. spectra of OG-solubilized SR vesicles prepared as described above, without further additions (——), or with 100 mM-Ca^{2+} (– – – –) or 0.5 mM-vanadate plus 0.1 mM-EGTA (·····) added. The ATPase activities of control and OG-solubilized samples were 0.54 and 1.65 μmol of P_i/min per mg of protein/min respectively, assayed in the presence of Ca^{2+} as described in the Materials and methods section.

average 6% decrease in α-helix and a 15% increase in β-sheet content on solubilization (Table 1).

Addition of Ca^{2+} or vanadate to the OG-solubilized SR had no statistically significant effects on the c.d. spectra (Fig. 3*b*). Ca^{2+} and vanadate were also without effect on the c.d. spectra and the calculated secondary structure of solubilized FITC-labelled SR (Table 1), although the two ligands produced the expected changes in FITC fluorescence, indicating changes in the conformation of the solubilized ATPase (Table 3).

DISCUSSION

Analysis of the c.d. data on SR yielded a secondary structure of 46% α-helix, 7% β-sheet, 12% turn and 35% random coil. This reflects primarily the structure of Ca^{2+}-ATPase, since Ca^{2+}-ATPase constitutes at least 70–80% of the protein content of the SR preparation. The interpretation of c.d. data on the total secondary structure of SR proteins must, however, take into account that the calculated secondary structure is a linear sum derived from all molecules present, each weighted by its abundance in the sample. The helix content present would be sufficient to form 19 helical segments each consisting of 25 amino acids, long enough to span the bilayer. On the basis of the primary amino acid sequence of Ca^{2+}-ATPase, MacLennan *et al.* (1985) proposed a folding model that envisages ten transmembrane helices, with an additional five helices of similar length in the stalk region and further helical elements distributed in the three major cytoplasmic domains. These predictions are consistent with the present c.d. data.

The observations presented here indicate that the transition between the E_1 and E_2 conformations of the Ca^{2+}-ATPase in native, in FITC-labelled and in solubilized SR preparations is not accompanied by detectable changes in the enzyme secondary structure. Given the error levels of the c.d. measurements, any net secondary-structural changes connected with the $E_1 \rightarrow E_2$ transition must involve less than 15 amino acids. Thus the previously reported changes in the susceptibility of the Ca^{2+}-ATPase to proteolysis (Dux & Martonosi, 1983*a*; Dux *et al.*, 1985) in the tryptophan and FITC fluorescence (Andersen *et al.*, 1982; Pick & Karlish, 1982) and in the kinetic parameters (Martonosi & Beeler, 1983) associated with the $E_1 \leftrightarrows E_2$ transition suggest local rearrangement of domains by a hinge-type or relative sliding motions within the ATPase molecule, rather than a refolding of the polypeptide backbone. The absence of detectable differences between the density contours of the ATPase molecules in low-resolution projection maps of E_1- and E_2-type Ca^{2+}-ATPase crystals (Dux *et al.*, 1985) is consistent with this conclusion. A similar explanation could apply to the neutron-diffraction studies by Blasie *et al.* (1985) that demonstrated relatively small changes in the mass distribution of

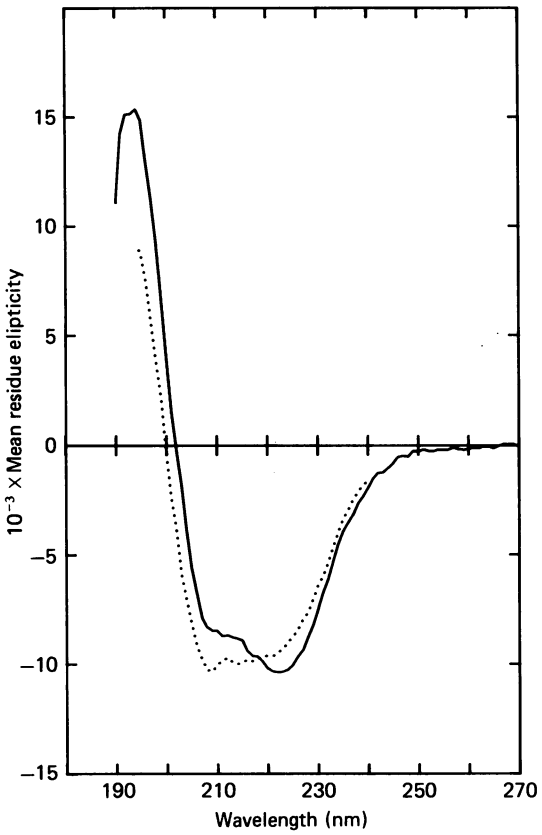

Fig. 4. Comparison of c.d. spectra of highly purified Na^+, K^+-ATPase from guinea-pig kidneys (——) (from Gresalfi & Wallace, 1984), and Ca^{2+}-ATPase in rabbit muscle SR (·····), both in the E_1 conformation

Ca^{2+}-ATPase with respect to the bilayer in different phases of the Ca^{2+}-transport cycle.

Essential to this argument is that the SR preparation used in the c.d. experiments is competent to undergo Ca^{2+}- or vanadate-induced state changes. This was demonstrated by changes in FITC fluorescence that occurred without any detectable c.d. changes. A recent study on Na^+,K^+-ATPase (Chetverin & Brazhnikov, 1985) also did not detect a change in secondary structure for that enzyme, whereas conformation changes were seen by Gresalfi & Wallace (1984). Such a difference between the two cation-transport ATPases would be unexpected, considering the similarities in their reaction mechanisms. Further studies are required to determine the contribution of the β-subunit of the Na^+,K^+-ATPase or calsequestrin and the high-affinity Ca^{2+}-binding proteins in SR to the c.d. spectra under the conditions used to stabilize the E_1 and E_2 conformations respectively. On the basis of preliminary calculations it appears unlikely that Ca^{2+}-induced changes in the helix content of calsequestrin (MacLennan *et al.*, 1983) would be sufficient to mask major changes in the secondary structure of the Ca^{2+}-ATPase, in view of the relatively small calsequestrin content of the SR used in these studies (less than 5% of the total protein).

The labelling of SR by FITC does not significantly alter the structure of Ca^{2+}-ATPase, since (1) the c.d. spectra are virtually the same as in unlabelled SR; (2) the fluorescence responses of tryptophan to Ca^{2+} and vanadate are identical in native and FITC-labelled SR vesicles (Andersen *et al.*, 1982; Jona & Martonosi, 1986); (3) FITC has no influence on the crystallization of Ca^{2+}-ATPase by vanadate (Csermely *et al.*, 1985).

The absence of significant changes in the c.d. spectra associated with E_1 and E_2 transition is consistent with previous observations (Mommaerts, 1967; Nakamoto & Inesi, 1986) and supports the prediction by Tanford (1982, 1984) that the conformational rearrangement connected with Ca^{2+} transport is not likely to involve significant changes in protein secondary structure.

In the light of the proposed homologies based on sequence comparisons between the Ca^{2+}-ATPase (MacLennan *et al.*, 1985) and the Na^+,K^+-ATPase (Shull *et al.*, 1985), it is interesting to compare the secondary structures of purified Na^+,K^+-ATPase (Gresalfi & Wallace, 1984) with that of the Ca^{2+}-ATPase in SR vesicles. The c.d. spectra of these two preparations are distinctly different (Fig. 4), and the different calculated secondary structure derived from them cannot be accounted for solely by the presence of an additional β-subunit in the Na^+,K^+-ATPase. However, since the Ca^{2+}-ATPase used in these studies is not entirely pure, we cannot absolutely exclude the presence of other protein components with particularly high helix contents as the source of part of this discrepancy.

OG and other detergents have been successfully employed for the purification of Ca^{2+}-ATPase, yielding preparations of high catalytic activity (Banerjee *et al.*, 1979). Yet solubilization of SR by OG causes large changes in the c.d. spectrum, similar to earlier observations of changes in the ratios of the ellipticities at 209 and 224 nm on solubilization with SDS (Mommaerts, 1967; Masotti *et al.*, 1972), deoxycholate (Hardwicke & Green, 1974; le Maire *et al.*, 1976) and the detergent $C_{12}E_8$ [dodecyl octa(ethylene glycol) monoether] (Dean & Tanford, 1978). Calculations on the present data show that these c.d. changes correspond to a decrease in α-helix and an increase in β-sheet content during the solubilization. Similar changes were also seen previously for Na^+,K^+-ATPase (Gresalfi & Wallace, 1984). The decrease in α-helix caused by OG could be due to a rearrangement of helical transmembrane segments of the ATPase upon transfer from the phospholipid bilayer into the OG micelles. The relatively major change in the secondary structure that accompanied solubilization in detergents, should dictate caution in the interpretation of kinetic data on the mechanism of Ca^{2+} transport obtained in solubilized systems.

In summary, parallel c.d. and fluorescence studies have demonstrated that the $E_1 \rightarrow E_2$ transition in Ca^{2+}-ATPase does not involve a major refolding of the polypeptide chain, but rather is consistent with a relative motion of domains.

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