Biochimica et Biophysica Acta, 1023 (1990) 107–118
Elsevier

BBAMEM 74754

Pressure effects on sarcoplasmic reticulum: a Fourier transform infrared spectroscopic study

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(Received 19 September 1989)

Key words: Sarcoplasmic reticulum; FTIR; Protein secondary structure; ATPase, Ca^{2+}-; Hydrogen–deuterium ion exchange; Protein denaturation

The Ca^{2+}-ATPase of sarcoplasmic reticulum is irreversibly inactivated by exposure to 1.5–2.0 kbar pressure for 30–60 min in a Ca^{2+}-free medium; mono- or decavanadate (5 mM) or to a lesser extent Ca^{2+} (2–20 mM) protect against inactivation (Varga et al. (1986) J. Biol. Chem. 261, 13943–13956). The structural basis of these effects was analyzed by FTIR spectroscopy of sarcoplasmic reticulum in ^2H_2O medium. The inactivation of the Ca^{2+}-ATPase at 1.5–2.0 kbar pressure in a Ca^{2+}-free medium was accompanied by changes in the Amide II region of the spectrum (1550 cm^{-1}), that are consistent with increased hydrogen-deuterium (H-^{2}H) exchange, and by the enhancement of a band at 1630 cm^{-1} in the Amide I region, that is attributed to an increase in β sheet. The frequency of the peak of the Amide I band shifted from about 1648 cm^{-1} at atmospheric pressure to 1642 cm^{-1} at $\cong$ 12.5 kbar pressure, suggesting a decrease in α helix, and an increase in β and/or random coil structures. Upon releasing the pressure, the shift of the Amide I band was partially reversed. Vanadate (5 mM), and to a lesser extent Ca^{2+} (2–20 mM), protected the Ca^{2+}-ATPase against pressure-induced changes both in the Amide I and Amide II regions of the spectrum, together with protection of ATPase activity. These observations establish a correlation between the conformation of the Ca^{2+}-ATPase and its sensitivity to pressure. The involvement of the ATP binding domain of the Ca^{2+}-ATPase in the pressure-induced structural changes is suggested by the decreased polarization of fluorescence of fluorescein 5′-isothiocyanate covalently attached to the enzyme.

Introduction

The hydrolysis of ATP and *p*-nitrophenyl phosphate by the Ca^{2+}-ATPase was reversibly inhibited during exposure to 0.5–1.0 kbar pressure [1–3]. The loss of enzymatic activity became irreversible after pressure treatment at 1.5–2.0 kbar in a Ca^{2+} free medium, but the irreversible inactivation of the enzyme could be prevented by inclusion of vanadate (5 mM) or Ca^{2+} (2–20 mM) in the incubation medium [4]. Some protection against pressure-induced inactivation was also provided by sucrose, glycerol, ethylene glycol and KCl at relatively high concentrations [4,5]. These observations suggested that the Ca^{2+}-ATPase is better protected against pressure-induced inactivation in the E_1 state stabilized by Ca^{2+} or in the E_2 state stabilized by vanadate, than in the conformational equilibrium that exists in a calcium-free medium in the absence of vanadate [4].

In this report FTIR and fluorescence data are presented on the structural changes of sarcoplasmic reticulum associated with the pressure-induced inactivation of Ca^{2+}-ATPase, and on the protection provided against these structural changes by vanadate and by Ca^{2+}. Depending on the ionic composition of the medium, the application of high pressure (1–13 kbar) brought about readily observable, conformationally specific changes in the secondary structure of the Ca^{2+}-ATPase, that are presumed to be related to the more subtle structural

Abbreviations: Ca^{2+}-ATPase, Ca^{2+}-transporting ATPase (EC 3.6.1.38); CD, circular dichroism; DMSO, dimethylsulfoxide; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; FITC, fluorescein 5′-isothiocyanate; FTIR, Fourier transform infrared; SR, sarcoplasmic reticulum.

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differences between the E_1 and E_2 states of the enzyme at atmospheric pressures.

Experimental procedures

Materials

Adenosine 5′-triphosphate, EGTA, imidazole, NADH, magnesium chloride, lactate dehydrogenase (rabbit muscle), pyruvate kinase (rabbit muscle), phospho*enol*pyruvate, and Trizma base were obtained from Sigma Chemical Co., St. Louis, MO 63178. Sodium vanadate was the product of Fisher Scientific Co., Fairlawn, NJ 07410. Calcium chloride was obtained from Baker Chemical Co., Phillipsburg, NJ 08854; A23187 from Behring Diagnostics, La Jolla, CA 92037; FITC from Molecular Probes, Inc., Eugene, OR 97402; ^2H_2O (99.9% ^2H) from MSD Isotope, Division of Merck Frosst, Canada, Inc., Montreal, Canada. All chemicals were of analytical grade.

Isolation of sarcoplasmic reticulum

Sarcoplasmic reticulum vesicles were isolated from rabbit muscles according to Nakamura et al. [6]. The preparations were frozen in liquid nitrogen and stored before use in a medium of 0.3 M sucrose, 10 mM Tris-maleate buffer (pH 7.0) at a protein concentration of 30–40 mg/ml in polyethylene containers at −70 °C.

Preparation of sarcoplasmic reticulum vesicles for FTIR measurements

Before pressure treatment, 4-ml aliquots of microsome suspensions were thawed, diluted six times with ^2H_2O medium containing 0.1 M KCl, 1 mM $MgCl_2$ and 10 mM imidazole, p^{2}H 7.4 (pH 7.0, according to Glasoe and Long [7]). The vesicles were centrifuged for 30 min at 92000 × *g*; the pellets were resuspended in the same medium and centrifugations were repeated twice more. The final sediments were taken up in ^2H_2O medium to a final protein concentration of 40–65 mg/ml. A similar procedure was used for the preparations of microsomes in H_2O medium. Protein concentration was determined according to Lowry et al. [8]. Additions of Ca^{2+}, EGTA and vanadate were made from ^2H_2O or H_2O-containing stock solutions to final concentrations indicated in the legends.

Since the Ca^{2+}-ATPase accounts for about 80% of the protein content of sarcoplasmic reticulum, we assume that the FTIR spectra represent primarily features of the Ca^{2+}-ATPase. The use of purified ATPase preparations is precluded by the regular presence of denatured material and residual detergents.

Measurement of the irreversible effects of 1–2 kbar pressure on the structure and ATPase activity of sarcoplasmic reticulum

For pressure treatment, glass vials containing 300 μl microsome suspensions were capped with three layers of parafilm and were placed in an SLM-Aminco French pressure cell (Model FA-030, 1-inch diameter piston, 35 ml capacity, rated at 2.72 kbar). Pressure was applied in the range of 1 to 2 kbar in the SLM-Aminco French press (Model FA-078) at 2–4 °C for periods ranging from 1 to 2 h. Aliquots of the same solutions were kept at atmospheric pressure as controls. The FTIR and ATPase activity measurements were performed after return to atmospheric pressure.

(a) FTIR measurements. Immediately after pressure treatment, 10-μl aliquots were taken from the control and pressure treated samples for parallel measurements of FTIR spectra in a Mattson Cygnus 100 spectrometer equipped with barium fluoride windows and 50 μm spacers for samples in ^2H_2O medium and 12 μm spacers for samples in H_2O medium. Temperature controlled cells were used (Model TFC-M25, Harrick Scientific Corp., Ossining, NY 10562) connected with a circulating water bath (Brinkmann model RM6), and maintained at 20 °C. The temperature was recorded with a thermocouple placed close to the cell windows. Routinely 256 scans were collected, coadded, zero filled once, and apodized with triangular function. The resolution was 2 cm^{-1}.

(b) ATPase activity measurements. ATPase activities were measured by a coupled enzyme system of pyruvate kinase and lactate dehydrogenase [4,9]. Medium I contained 0.1 M KCl, 0.02 M Tris-HCl (pH 7.5), 0.9 mM $CaCl_2$, 5 mM $MgCl_2$, 0.42 mM phospho*enol*pyruvate, 0.2 mM NADH, 7.5 IU/ml of pyruvate kinase, 18 IU/ml of lactate dehydrogenase and 2 μM A23187. Medium II contained 0.1 M KCl, 0.02 M Tris-HCl (pH 7.5), 1 mM EGTA, 5 mM $MgCl_2$, 10 mM ATP, 0.42 mM phospho*enol*pyruvate, 0.2 mM NADH, 7.5 IU/ml of pyruvate kinase and 18 IU/ml of lactate dehydrogenase.

Immediately after pressure treatment, 10-μl aliquots of control or pressure treated samples were diluted with H_2O medium to a final protein concentration of 1 mg/ml. Then 10-μl aliquots of the diluted samples were added to 1 ml medium I (microsomal protein concentration, 10 μg/ml), and the reaction was started after incubation for 5 min at 25 °C by addition of 1 ml of medium II. The ATPase activity was measured in an Aminco DW-2 spectrometer at 340 nm at 25 °C. For measurements of the Ca^{2+}-insensitive ATP hydrolysis, Ca^{2+} was omitted from the medium. The ATPase activity data were corrected for the Ca^{2+}-insensitive hydrolysis rate.

(c) Polarization of fluorescence of FITC-labeled sarcoplasmic reticulum. Sarcoplasmic reticulum vesicles (2 mg protein/ml) were labeled either before or after the pressure treatment with FITC at a concentration of 5 nmol/mg protein, essentially as described earlier [10]. The polarization of FITC fluorescence was measured at a protein concentration of 50 μg/ml, at 25 °C in an

SLM 4800 fluorescence spectrometer equipped with Glan-Thompson polarizers, at excitation and emission wavelengths of 490 and 525 nm, respectively.

Measurement of the FTIR spectra of sarcoplasmic reticulum in a diamond anvil cell at 0.5–13 kbar pressures

Sarcoplasmic reticulum suspension was placed at room temperature in a 0.37 mm diameter hole on a 0.23 mm thick stainless steel gasket mounted on a diamond anvil cell. Powdered α-quartz was used as internal pressure calibrant [11]. Spectra were acquired on a Digilab FTS-60 Fourier transform spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride detector. For each spectrum 512 interferograms were co-added and Fourier transformed to yield spectra with a resolution of 4 cm^{-1}. The pressures on the sample were calculated from the pressure dependence of the frequency shift of the 695 cm^{-1} absorption band of α-quartz according to the expression $P = a_1\Delta\nu + a_2\Delta\nu^2$, where $a_1 = 1.2062$ and $a_2 = 0.015164$ and $\Delta\nu$ is the measured frequency shift with respect to the frequency measured at atmospheric pressures.

Results

Pressure-induced changes in the FTIR spectra and enzymatic activity of the Ca^{2+}-ATPase in the E_1 state

Fourier transform infrared spectroscopy (FTIR) is a powerful method for analyzing structural changes in membrane proteins [12–17] and phospholipids [18–22], including sarcoplasmic reticulum.

A typical FTIR spectrum of sarcoplasmic reticulum in a ^2H_2O medium containing 0.1 M KCl, 10 mM imidazole, 1 mM $MgCl_2$ and about 10^{-5} M Ca^{2+} is shown in Fig. 1A. Under these conditions the Ca^{2+}-ATPase is assumed to be largely in the E_1 conformation. The band centered at 1728 cm^{-1} corresponds to the carbonyl stretching vibration of phospholipids. The intense asymmetric band centered at 1648 cm^{-1} is the Amide I mode of the proteins, while the two smaller bands at 1575 and 1548 cm^{-1} are attributed to side chain vibrations and to the Amide II mode of the protein, respectively.

The Amide I band in the frequency region between 1600 and 1700 cm^{-1} is due to in-plane C=O stretching vibration weakly coupled with C-N stretching and in-plane N-H bending [23]. The Amide II band located at 1565–1535 cm^{-1} is a mixture of N-H in-plane deformation and C-N stretching modes. This band shifts to $\cong$ 1457 cm^{-1} after exchange of the labile protons on the amide groups with deuterium, where it overlaps with the ^{2}H-O-H bending band centered at 1450 cm^{-1}. The presence of the Amide II band at 1548 cm^{-1} after extensive washing and overnight incubation of sarcoplasmic reticulum in a ^2H_2O medium at 2° C implies that a significant portion of the amide groups of the

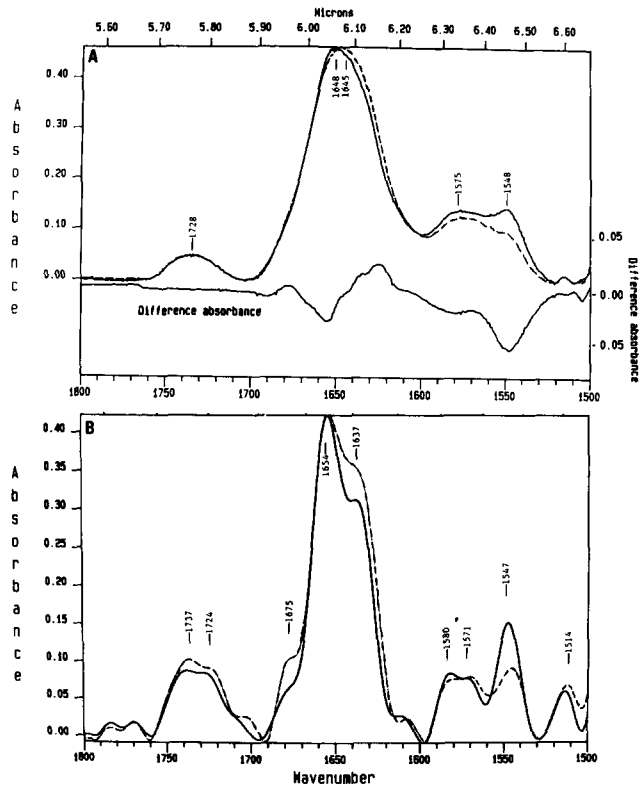

Fig. 1. FTIR spectra of sarcoplasmic reticulum in ^2H_2O medium containing $\cong 10^{-5}$ M Ca^{2+}. A (top panel). Infrared spectrum of SR vesicles (35 mg/ml protein) in ^2H_2O medium containing 0.1 M KCl, 10 mM imidazole (p^2H 7.4), 1 mM $MgCl_2$, and $\cong 10^{-5}$ M Ca^{2+}. Samples kept at 1 bar at 2° C (full line); samples after pressure treatment at 2 kbar for 2 h at 2° C (dashed line). The spectra have not been corrected for ^2H_2O medium but water vapors were subtracted and baseline correction was applied. The path length was 50 μm and windows were BaF_2. Bottom trace: difference spectrum of the two samples shown in A after baseline correction. Similar results were obtained after pressure treatment at 28° C. B (bottom panel). Deconvolved spectra after baseline correction. A 25 cm^{-1} Lorentzian bandwidth and a resolution factor of 1.8 have been used for deconvolution.

Ca^{2+}-ATPase is not accessible to exchange with medium ^2H_2O in the native sarcoplasmic reticulum at atmospheric pressure (Fig. 1A).

After exposure of the sarcoplasmic reticulum to 2 kbar for 2 h at 2° C, significant changes are observed in the Amide II and Amide I regions of the spectrum (Fig. 1A). The decrease in the intensity of the Amide II band (1548 cm^{-1}) indicates faster H-^{2}H exchange under high pressure. Similar H-^{2}H exchange was observed in sarcoplasmic reticulum during thermal denaturation [14,17], in the presence of DMSO (> 50%), or at alkaline pH [24]. Interestingly, the residual Amide II band of the pressure treated samples does not show continued H-^{2}H exchange during several days of incubation at 2° C after return to atmospheric pressure.

The maximum of the intense Amide I peak shifted after pressure treatment from 1648 cm^{-1} to 1645 cm^{-1} coupled with an increase in the intensity of the shoulder

at $\cong 1630\ cm^{-1}$ (Fig. 1A). The phospholipid headgroup band at $\cong 1728\ cm^{-1}$ was not affected significantly by pressure.

The Amide I band of proteins is usually broad and asymmetric because the various secondary substructures present in the molecule give peaks at different frequencies. The components of the Amide I band of sarcoplasmic reticulum were analyzed by deconvolution [25–27] using a resolution enhancement factor of 1.8 and 25 cm^{-1} for the width of the Lorentzian band [14]. The Amide I envelope was resolved by deconvolution into three component bands located at 1675, 1654, and 1637 cm^{-1}, respectively (Fig. 1B). The 1675 and 1637 cm^{-1} components are assigned to β structures, and the 1654 cm^{-1} band to α helix. Random coil and other structures also contribute. The increased intensity of the 1637 cm^{-1} band after pressure treatment (Fig. 1B) suggests an increase in β structure, while the decrease in the intensity at 1547 cm^{-1} band is attributed largely to H-^{2}H exchange. Similar changes are seen in the difference absorbance (Fig. 1A) obtained by subtraction of the original spectrum of control sarcoplasmic reticulum kept at atmospheric pressure from the spectrum obtained from the same preparation after treatment at 2 kbar pressure for 2 h.

The structural changes observed after exposure to 2 kbar for 2 h are accompanied by a decrease in Ca^{2+}-stimulated ATPase activity from 4.40 ± 0.5 to 0.28 ± 0.05 μmol/mg per min, in agreement with earlier data [4].

The protection of Ca^{2+}-ATPase by vanadate against pressure-induced changes in structure and enzymatic activity

Vanadate (5 mM) in the presence of sufficient EGTA to lower the free Ca^{2+} concentration of the medium below 10^{-8} M stabilizes the Ca^{2+}-ATPase in the E_2-V conformation and induces the formation of P2 type

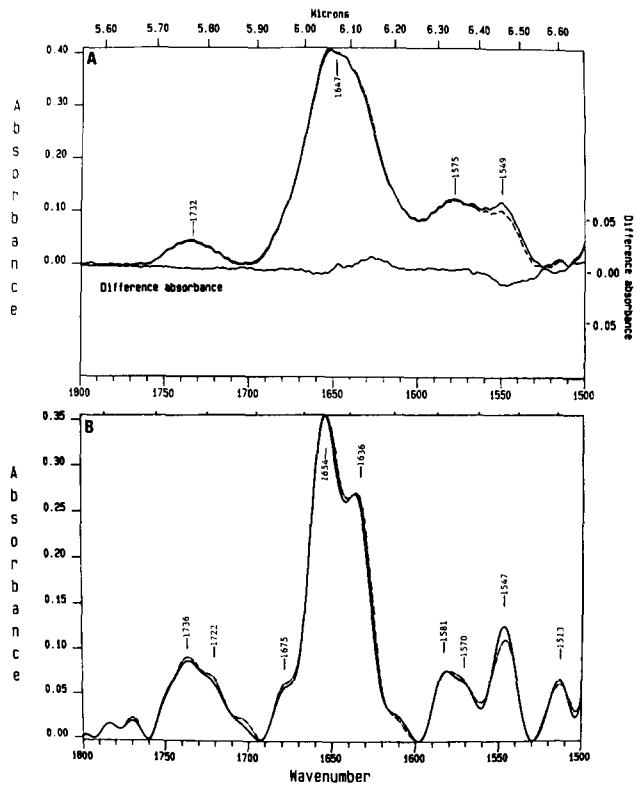

Fig. 2. FTIR spectra of sarcoplasmic reticulum in ^2H_2O medium containing EGTA and vanadate. A (top panel). Infrared spectrum of SR vesicles (33 mg/ml protein) in ^2H_2O medium containing 0.1 M KCl; 10 mM imidazole (p^{2}H 7.4), 1 mM $MgCl_2$, 1 mM EGTA and 5 mM vanadate. Sample kept at 1 bar at 2° C (full line); sample after pressure treatment at 2 kbar for 2 h at 2° C (dashed line). Other details as in Fig. 1. Bottom trace: difference spectrum of the samples shown in A. B (bottom panel). Same spectra after deconvolution, as described in the Legend to Fig. 1B.

Ca^{2+}-ATPase membrane crystals [28–35]. Saturation of the high affinity Ca^{2+} sites of the Ca^{2+}-ATPase by Ca^{2+} stabilizes the E_1 conformation with the formation of P1 type crystals of the Ca^{2+}-ATPase [36]. The transi-

TABLE I

Effects of ions on the pressure sensitivity of Ca^{2+}-ATPase, and on the fluorescence of covalently attached FITC

SR vesicles (protein concentration 20–50 mg/ml) were suspended in ^2H_2O medium containing 0.1 M KCl, 10 mM imidazole (p^{2}H 7.4), and 1 mM $MgCl_2$ with additions as indicated. After exposure to 1.5 kbar pressure for 1 h the samples were diluted and ATPase activities were measured as described under Methods. The activities are expressed as means ± standard deviation. The numbers in brackets correspond to the number of independent measurements. The sarcoplasmic reticulum vesicles were labeled with FITC before and after pressure treatment at a protein concentration of 2 mg/ml, as described earlier [10]. The polarization of FITC fluorescence was measured as described under Methods; for each polarization value an average of 400 data points were collected with a standard error ranging between 0.0004 and 0.001.

Additions	ATPase activities (μmol ATP·mg^{-1}·min^{-1})		Polarization of FITC fluorescence	
	control (1 bar)	pressure-treated (1.5 kbar, 1 h)	control (1 bar)	pressure-treated (1.5 kbar, 1 h)
None	4.40 ± 0.50 (9)	1.49 ± 0.44 (6)	0.3551	0.3278
2 mM Ca^{2+}	4.32 ± 0.65 (5)	1.71 ± 0.24 (6)	0.3647	0.3299
20 mM Ca^{2+}	4.10 ± 0.56 (7)	2.27 ± 0.40 (9)	0.3681	0.3335
200 mM Ca^{2+}	3.86 ± 0.68 (5)	1.13 ± 0.52 (6)	0.3587	0.3280
1 mM EGTA	4.47 ± 0.53 (6)	0.59 ± 0.15 (6)	0.3635	0.3066
1 mM EGTA and 5 mM vanadate	3.71 ± 0.56 (4)	3.29 ± 0.35 (5)	0.3653	0.3607

tion from the E_1 into the E_2-V state occurred with the appearance of a faint band in the FTIR spectrum at 1650 cm^{-1} that was attributed to a new α-helical substructure, while the β sheet content remains essentially unchanged [37]. In contrast to earlier observations [37], in our experiments the C=O stretching vibrations at 1760–1700 cm^{-1}, and the N-H bending vibrations at 1565–1535 cm^{-1} were unaltered by the $E_1 \rightarrow E_2$ transition at atmospheric pressure.

Stabilization of the Ca^{2+}-ATPase by vanadate in the E_2-V state provides striking protection against pressure-induced inactivation at 1.5 kbar (Table I). Correspondingly, the FTIR spectra show only a slight change in the Amide I region after exposure of sarcoplasmic reticulum to 1.5–2.0 kbar pressure, in the presence of 1 mM EGTA and 5 mM vanadate (Fig. 2A). The protection afforded by vanadate against pressure-induced structural changes is best seen by comparing the deconvolved spectra shown in Fig. 1B (no vanadate) with those in Fig. 2B (with vanadate). There was only slight change in the intensity of the shoulder at 1636 cm^{-1} in the deconvolved spectra shown in Fig. 2B, indicating that vanadate nearly completely prevented the pressure-induced formation of β structure. A slight decrease in the intensity of the Amide II band at 1548 cm^{-1} is still observed after pressure treatment in the presence of EGTA and vanadate (Fig. 2B), indicating that a H-^{2}H exchange due to exposure of protein amide groups to ^2H_2O during pressure treatment continued even in the E_2-V state, but its rate was much lower. Therefore the inhibition of ATPase activity observed after exposure of SR to 1.5–2 kbar pressure in the absence of vanadate (Table I) is correlated with the formation of β structure indicated by the increased intensity of the 1636 cm^{-1} band, and with the increased H-^{2}H exchange shown by the decrease in intensity at 1550 cm^{-1}.

The effect of Ca^{2+} on the pressure sensitivity of the Ca^{2+}-ATPase

Protection of ATPase activity against inactivation at 1.5 kbar pressure was also observed in the presence of 20 mM Ca^{2+} (Table I). An increase in Ca^{2+} concentration to 200 mM or a decrease to 10^{-5} M or below abolished this protection (Table I). Ca^{2+} (20 mM) also moderated the pressure-induced increase in the intensity of the 1630 cm^{-1} shoulder in the Amide I region of the spectrum (Fig. 3), and slightly reduced the pressure-induced changes in the Amide II region connected with H-^{2}H exchange (Fig. 3). The protection of Ca^{2+}-ATPase against pressure-induced inactivation shows a similar dependence on Ca^{2+} concentration as the formation of membrane crystals of Ca^{2+}-ATPase [38].

Circular dichroism data

The circular dichroism spectra of sarcoplasmic reticulum vesicles measured before and after exposure to

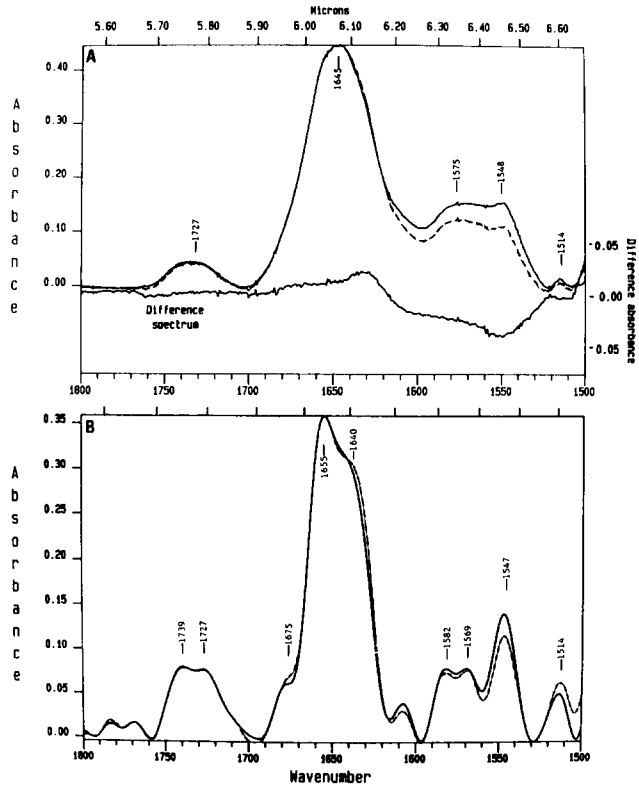

Fig. 3. Effect of Ca^{2+} on the pressure sensitivity of Ca^{2+}-ATPase. A (top panel). Infrared spectrum of SR vesicles (35 mg/ml protein) in ^2H_2O medium containing 0.1 M KCl, 10 mM imidazole (p^2H 7.4), 1 mM $MgCl_2$, and 20 mM Ca^{2+}. Full line: sample kept at atmospheric pressure, dashed line: sample after pressure treatment at 1.5 kbar for 1 h. Bottom trace: difference spectrum of the two samples shown in A after baseline correction. B (bottom panel). Same spectra after deconvolution, as described in the legend to Fig. 1B.

1.5 kbar for 1 h in the presence of 1 mM EGTA (Fig. 4A) or 1 mM EGTA and 5 mM Na_3VO_4 (Fig. 4B) indicate no significant change in the secondary structure of the sarcoplasmic reticulum proteins, although the Ca^{2+}-stimulated ATPase activity decreased from 3.57 ± 0.12 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ in the control sample (1 bar) to 0.23 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ after pressure treatment at 1.5 kbar for 1 h in the presence of 1 mM EGTA. The corresponding values in the presence of 1 mM EGTA and 5 mM Na_3VO_4 were 3.67 ± 0.22 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ in the control (1 bar) and 3.59 ± 0.11 $\mu mol \cdot mg^{-1} \cdot min^{-1}$ in the pressure treated sample, indicating full protection of ATPase activity. The absence of a significant change in the CD spectrum after pressure treatment that causes irreversible loss of ATPase activity (Fig. 4A) indicates that the inhibition of ATPase occurs without major rearrangement of the secondary structure. We estimate that changes of less than 5% would be difficult to detect reliably by circular dichroism spectroscopy, while with FTIR the detection limit is of the order of 1%. The difference in sensitivity between the two techniques may partly explain why the pressure-induced changes seen by FTIR are not ob-

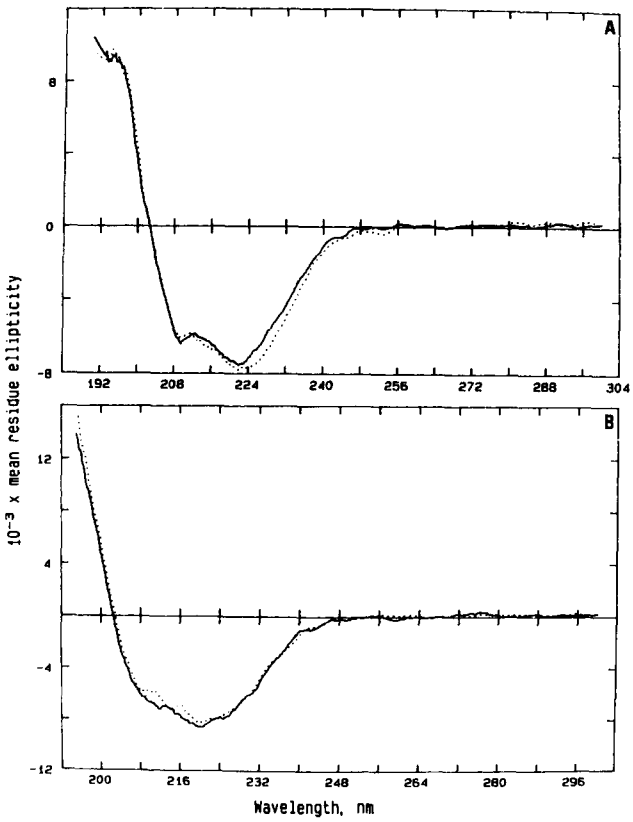

Fig. 4. Circular dichroism spectra of sarcoplasmic reticulum before and after pressure treatment. Sarcoplasmic reticulum vesicles (20 mg protein/ml) suspended in 0.1 M KCl, 10 mM imidazole (p^2H 7.4), 1 mM $MgCl_2$ and 1 mM EGTA (Fig. 4A), or 1 mM EGTA and 5 mM Na_3VO_4 (Fig. 4B) were exposed to 1 bar (broken line) or 1.5 kbar (solid line) for 1 h. After pressure treatment the samples were diluted with the same medium to a protein concentration of 3 mg/ml for measurement of the CD spectra on an Aviv 60DS spectropolarimeter as described earlier [58]. ATPase activities of the same samples were determined as described in Methods. The slight difference between the two spectra in Fig. 4A was not reproducible and for this reason is not considered significant.

served by CD. FTIR also detects side chain vibrations near the Amide I and Amide II regions of the spectra [39]; the possibility of pressure-induced changes in side chain vibrations cannot be excluded.

Reversible and irreversible pressure-induced changes in the FTIR spectra of sarcoplasmic reticulum between 0 and 12.5 kbar

The FTIR spectra shown in Figs. 1–3 record the irreversible structural changes caused by exposure of sarcoplasmic reticulum to 1.5–2.0 kbar pressure after return to atmospheric pressure. The diamond anvil cell allows measurement of the FTIR spectra of the sample as it experiences high pressure. Reversible effects can thus be observed, in addition to the irreversible changes described above. A second advantage of the diamond anvil cell is the wider pressure range accessible.

The FTIR spectra of SR membranes in a medium containing 1 mM EGTA obtained during a stepwise increase of pressure from 0.09 to 12.8 kbar are shown in Fig. 5C. Upon compression, there is a gradual decrease of the frequency of the Amide I band maximum from 1648 cm^{-1} at atmospheric pressure to approx. 1642 cm^{-1} at 12.8 kbar (Fig. 6). This frequency shift and the accompanying modification of the band shape is caused by an intensity redistribution among the various conformers present in the protein. Resolution enhancement using self-deconvolution (Fig. 5D) reveals dramatic changes in the Amide I region. The comparison between spectra taken at increasing pressure (solid line) and during the following pressure release (dashed line) in the three bottom traces confirm the irreversible changes described in Fig. 1, that is an increase in β-sheet structure giving rise to an increase in intensity at approx. 1630 cm^{-1} and 1675 cm^{-1}, and a concomitant decrease in α helix responsible for the decrease in absorbance at 1653 cm^{-1}. The band maximum on the deconvolved spectrum in Fig. 5D at the end of the experiment, 1649 cm^{-1}, falls close to the range normally found for random coil, but the overlap of random coil Amide I band with α-helix and β-sheet peaks precludes any conclusion relative to the effect of pressure on its intensity. As the pressure is raised above 4 kbar, the Amide I band shape is further modified. The rather good coincidence of spectra at increasing and decreasing pressure indicates that these changes are reversible. They consist mainly of band broadening and a relative increase of the low frequency component at 1635 cm^{-1}, which suggest a further increase of β sheet. Fig. 5D also shows a decrease in the intensity at 1550 cm^{-1} with increasing pressure, reflecting the course of H-^{2}H exchange. This latter is essentially complete at approx. 4 kbar and the residual intensity is attributed to side chain vibrations [39,40].

In the presence of 1 mM EGTA and 5 mM vanadate (Fig. 5A,B), there is also an increase in the intensity at approx. 1630 cm^{-1} and at 1675 cm^{-1}, and a decrease of the 1653 cm^{-1} component. In addition to these irreversible changes, some band broadening is observed at pressures above 4 kbar and the maximum of the undeconvolved Amide I band shifts to lower frequency (Fig. 6).

Even though the effects of high pressure seen in Fig. 5 are qualitatively similar with and without vanadate ions, the extent of the changes is markedly different. The decrease in the frequency of the Amide I peak with increasing pressure is slightly less in the vanadate-containing than in the vanadate-free samples (Fig. 6). The superposition of deconvolved spectra with vanadate (Fig. 5B) and without vanadate (Fig. 5D) reveals that both the increase in β sheet and the decrease in α helix are reduced in the presence of vanadate ions. As a consequence of this protective effect of vanadate, the spectral

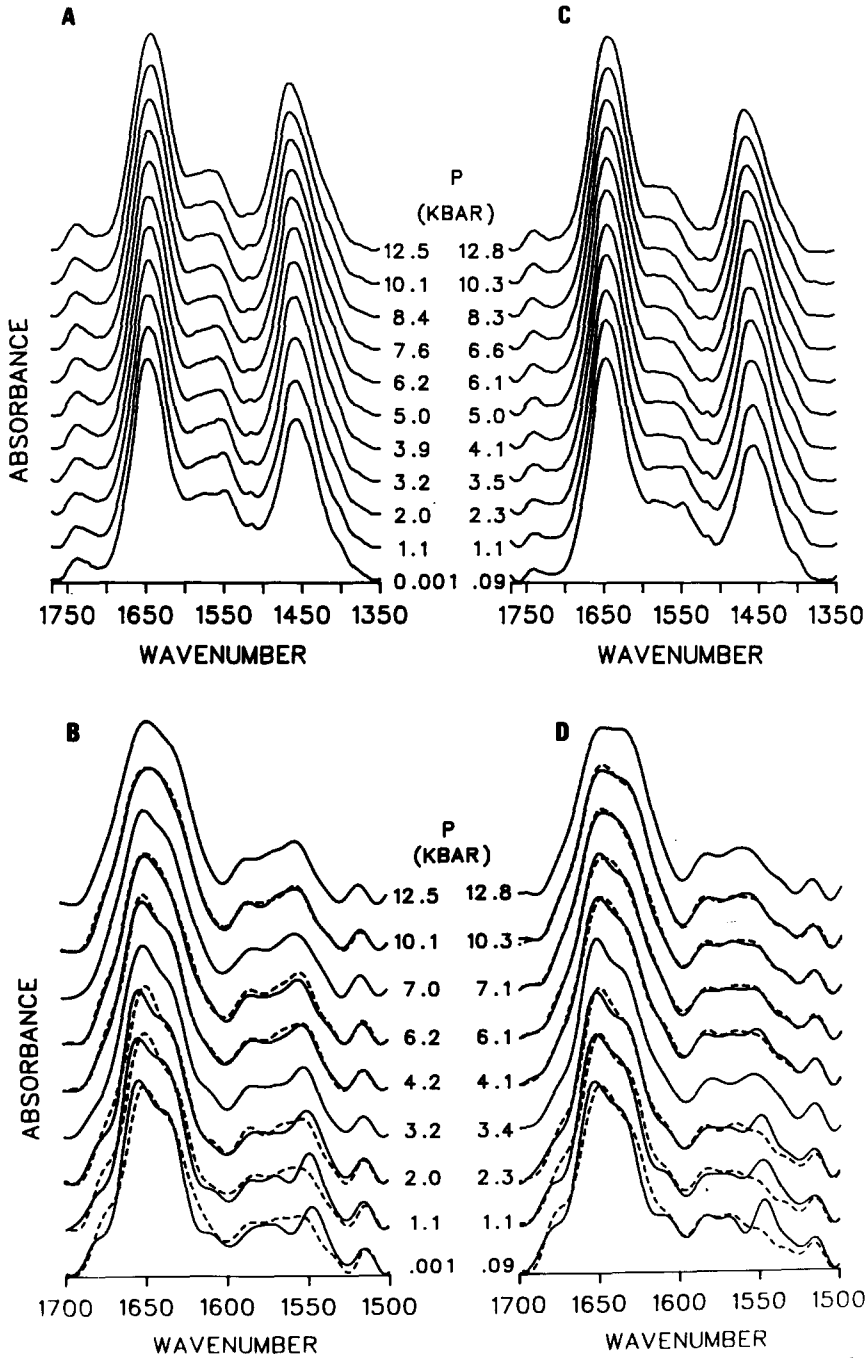

Fig. 5. FTIR spectra of sarcoplasmic reticulum under high pressure. Infrared spectra of SR vesicles (54 mg/ml protein) in ^2H_2O medium containing 0.1 M KCl, 10 mM imidazole (p^2H 7.4), 1 mM $MgCl_2$ and 1 mM EGTA with 5 mM vanadate (A, B, left panels) or without vanadate (C, D, right panels). Top panels A and C show the original spectra recorded at increasing pressure, in a diamond anvil cell. Bottom panels B and D show the same spectra after deconvolution using Lorentzian lines of 25 cm^{-1} full width at half-height and a resolution enhancement factor of 2.2. Full lines in panels B and D correspond to spectra recorded at increasing pressure and dashed lines to spectra recorded at decreasing pressure.

differences in the Amide I region are dramatically enhanced at 12.5 kbar. Although high pressure promotes the H-^{2}H exchange in the presence of vanadate ions, as evidenced by the decrease in the intensity of the Amide II band at approx. 1550 cm^{-1} (Fig. 5A,B), the persistence of an Amide II band even at the highest pressures indicates that vanadate ions impede the completion of the pressure-induced H-^{2}H exchange. This inaccessibility of some regions of the protein to solvent molecules is thus a second aspect of the protection by vanadate.

Upon pressure release, the frequency of the maximum of the Amide I band increases back more or less linearly, both in the presence and absence of vanadate (Fig. 6), reaching a maximum of only approx. 1646 cm^{-1} at atmospheric pressure. The absence of any

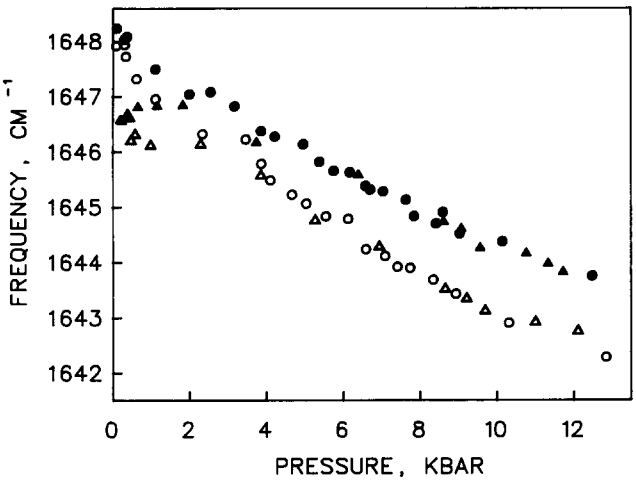

Fig. 6. Pressure dependence of the frequency of the Amide I band of sarcoplasmic reticulum. Infrared spectra of SR vesicles with vanadate (filled symbols) and without vanadate (open symbols) were recorded as described in Figs. 5A and 5C. Circles correspond to compression and triangles to decompression.

significant hysteresis between compression and decompression in the range of 4–13 kbar reflects the reversibility of the pressure-induced changes above 4 kbar. Below 4 kbar, the profiles at increasing and decreasing pressures differ markedly owing to the irreversible changes or conformational drift [41] that have occurred in the structure of Ca^{2+}-ATPase. So far, no reactivation of the pressure denatured protein has been observed after incubation for several days at 2°C, but additional studies at higher temperatures must be done, since the energy required to convert the 'drifted' aggregates into native, enzymatically active ATPase aligomers may be greater than that available at 2°C.

The effect of pressure treatment on the polarization of fluorescence of FITC covalently bound to the Ca^{2+}-ATPase

FITC selectively reacts with lysine 515 in the vicinity of the ATP binding site of the Ca^{2+}-ATPase [42–49]. The high polarization of fluorescence of covalently bound FITC indicates significant restriction of the mobility of FITC in the native ATPase molecule [50]. The polarization of FITC fluorescence decreased from a control value of 0.363 at atmospheric pressure to 0.306 after pressure treatment at 1.5 kbar for 1 h in the presence of 1 mM EGTA (Table I). The decrease in polarization indicates either a 'loosening' of the ATP binding domain of Ca^{2+}-ATPase, with increased segmental mobility of the FITC binding site or increased association of the denatured ATPase molecules that leads to energy transfer depolarization [50]. Irreversible inhibition of ATPase activity accompanied this change (Table I). The presence of 1 mM EGTA and 5 mM vanadate during pressure treatment nearly completely prevented the change in the polarization of FITC fluorescence, with preservation of ATPase activity (Table I). Ca^{2+} (20 mM) provided partial protection against the pressure-induced changes in enzymatic activity and fluorescence polarization, while 200 mM Ca^{2+} was less effective (Table I). The pressure-induced changes in the polarization of FITC fluorescence and the protective effects of vanadate and Ca^{2+} suggest that the structural changes may involve the putative ATP binding site labeled by FITC.

The emission and excitation spectrum of protein tryptophan remained unaltered after pressure treatment at 2 kbar for 2 h (not shown), indicating no significant changes in the average environment of tryptophan residues.

The rate of H-^{2}H exchange

The sarcoplasmic reticulum membranes used in these studies were exposed to the ^2H_2O medium before the FTIR measurements for several hours at 2°C, permitting some H-^{2}H exchange to take place. The remaining Amide II band corresponds to the NH groups that are not deuterated and assumed to be poorly accessible to the solvent. The approximate rate of H-^{2}H exchange was determined from the decrease in the intensity of the

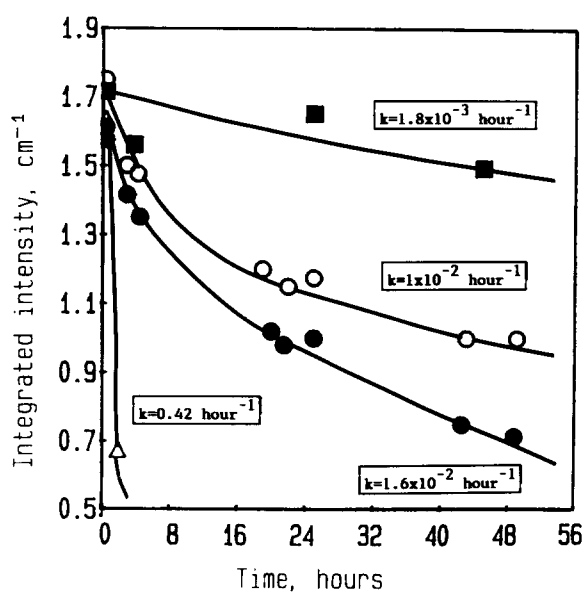

Fig. 7. Changes in the intensity of the Amide II band (1570–1520 cm^{-1}) due to H-^{2}H exchange reaction during incubation of sarcoplasmic reticulum in ^2H_2O medium at 2 and 20°C. Sarcoplasmic reticulum vesicles (35 mg protein/ml) were suspended in 0.1 M KCl, 10 mM imidazole (p^2H 7.4), 1 mM $MgCl_2$ and 1 mM EGTA (■, ○, △), or 1 mM EGTA and 5 mM vanadate (●) and incubated at 2°C (△, ■) or at 20°C (○, ●) for times indicated on the abscissa. One of the samples (△) was exposed to 2 kbar pressure at 2°C before the incubation began, the others were kept at 1 bar (○, ●, △) throughout. The intensity of the Amide II band was measured between 1570 and 1520 cm^{-1} and after a linear baseline correction between these limits, the integrated intensity was determined from the area under the Amide II peak. The error in the measurement of the intensity of the Amide II band is 0.1 cm^{-1}.

remaining Amide II band as a function of time during continued incubation in ^2H_2O medium at 2 and 20 °C (Fig. 7). The rate constant of exchange was calculated according to the following equation:

$$I(t) = I(0)\, e^{-kt}$$

where $I(t)$ is the integrated intensity of the Amide II band (measured between 1570 and 1520 cm^{-1}) as function of time, t, and k is the rate constant. This equation is an approximation, since it does not take into account the heterogeneous kinetics of the process [12,51,52].

There is only a slight decrease in the intensity of the Amide II band in the samples incubated at 2 °C for 2 days in the presence of 1 mM EGTA (Fig. 7). On raising the temperature to 20 °C, the rate of H-^{2}H exchange increased about 5–6-fold (Fig. 7). Addition of 5 mM vanadate did not alter markedly the rate of H-^{2}H exchange at 20 °C and atmospheric pressure (Fig. 7). This is in contrast to the protection provided by vanadate against the pressure-induced increase in the rate of H-^{2}H exchange (Figs. 1 and 2). The H-^{2}H exchange rate was at least 230-times greater after pressure treatment at 2 kbar for 2 h at 2 °C than in the control sample incubated at 2 °C at 1 bar (Fig. 7). These are minimum estimates, since the exchange was determined 1–2 h after the release of high pressure, but there was no indication of fast H-^{2}H exchange after return of the pressure treated samples to 1 bar.

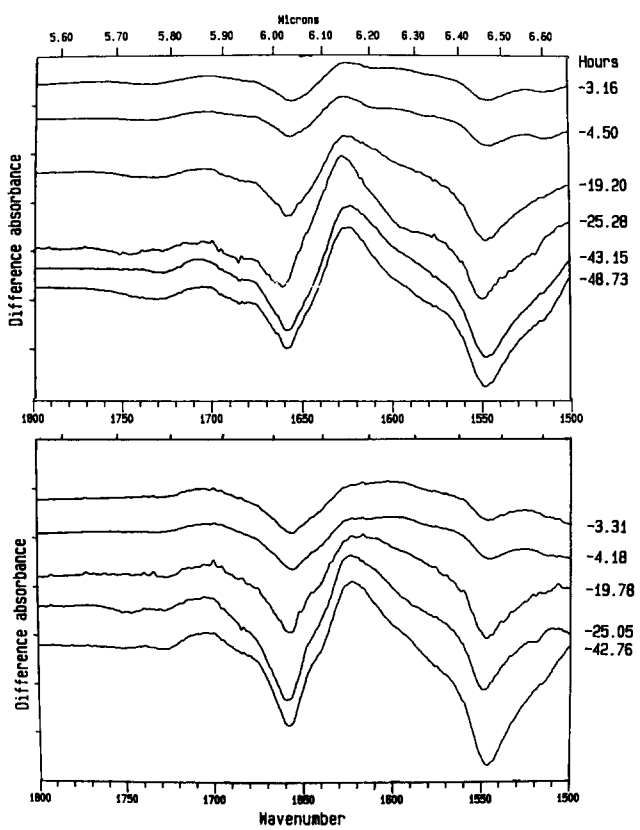

Fig. 8. Difference FTIR spectra of sarcoplasmic reticulum during incubation in ^2H_2O medium during the H-^{2}H exchange reaction. Sarcoplasmic reticulum vesicles (35 mg/ml) were suspended at 2 °C in a ^2H_2O medium containing 0.1 M KCl, 10 mM imidazole (p^{2}H 7.4), 1 mM $MgCl_2$ and 1 mM EGTA (top panel) or 1 mM EGTA and 5 mM vanadate (bottom panel). After incubation at 2 °C for 8–10 h the samples were transferred to 20 °C and incubated for 3–49 h at that temperature. The difference spectra represent the difference absorbance between spectra taken at the time the samples were transferred to 20 °C (zero time) and the incubation times indicated in the figure.

Proteolysis of the Ca^{2+}-ATPase during pressure treatment at 1–2 kbar for 1–2 h is not likely to contribute to the increased H-^{2}H exchange since there was no significant change in the protein pattern of sarcoplasmic reticulum determined by polyacrylamide gel electrophoresis ([4]; confirmed in this study).

Incubation of SR for several hours at 20 °C also induces changes in the Amide I region spectrum (Fig. 8) that are qualitatively similar to those observed during and after pressure treatment and probably reflect an increase in β or random structures and a decrease in α helices.

These structural changes are likely to contribute to the loss of Ca^{2+} transport activity in sarcoplasmic reticulum vesicles after prolonged incubation at room temperature.

Discussion

The molecular description of the Ca^{2+} transport process requires the integration of the available kinetic information with data on the structure of the Ca^{2+}-ATPase. The primary sequences of the Ca^{2+}-ATPase isoenzymes are established [47–49,53], and reconstructions of Ca^{2+}-ATPase crystals are beginning to yield information on the overall shape of the Ca^{2+}-ATPase [33–36,54,55]. Relatively little is known about the secondary, tertiary and quaternary structure of the ATPase molecule [56,57]. Circular dichroism studies of sarcoplasmic reticulum yielded a secondary structure of 46% α helix, 7% β sheet, 12% turn, and 35% random coil [58]. Similar estimates were derived from Raman spectroscopy [59], and from secondary structure prediction based on amino acid sequence [47]. The predicted secondary structure of Ca^{2+}-ATPase was arranged into hypothetical functional domains [47] that show some consistency with the general shape of the ATPase molecule [34,35], and with the intramolecular distances calculated from energy transfer measurements [60,61], but the model requires corroboration by other physical techniques.

The transition of the Ca^{2+}-ATPase between the E_1 conformation stabilized by 0.1 mM Ca^{2+} and the E_2 conformation stabilized by 0.1 mM EGTA and 0.5 mM vanadate was not accompanied by significant changes in the circular dichroism spectra [58], leading to the conclusion that the $E_1 \rightarrow E_2$ transition, and by implica-

tion the transport of Ca^{2+}, involves primarily a rearrangement of domains within the ATPase molecule by hinge-type or relative sliding motions, rather than a refolding of the polypeptide backbone. Although there are slight differences between the FTIR spectra of sarcoplasmic reticulum in the E_1 and E_2 states [37], these are near the limit of detection by present instrumentation and difficult to apply as a routine test of the conformational transition between the E_1 and E_2 states.

The application of pressure to sarcoplasmic reticulum in media that favor different conformations of the Ca^{2+}-ATPase brings about highly significant, readily reproducible changes in the structure and ATPase activity of the SR that are defined by the preexisting conformation of the Ca^{2+}-ATPase at atmospheric pressures.

Pressure treatment of SR at 0.8 kbar for 1 h in the presence of 0.5 mM EGTA decreased the ATPase activity by $\cong 20\%$ without significant change in the Amide I region of the FTIR spectrum; some increase in H-^{2}H exchange is shown by a slight decrease in intensity at 1550 cm^{-1}. In the presence of 5 mM vanadate these changes were prevented. After exposure of sarcoplasmic reticulum to 1.5–2.0 kbar pressure for 1–2 h in a medium of 0.1 M KCl, 10 mM imidazole (p^2H 7.4), 1 mM $MgCl_2$ and $\cong 10^{-8}$–10^{-5} M $CaCl_2$ there was a nearly complete irreversible loss of ATPase activity, an increase in the intensity of a band at 1630 cm^{-1}, signaling an increase in β structures, and a decrease in the intensity of the Amide II band at 1548 cm^{-1}, due to increased rate of H-^{2}H exchange.

The irreversible inactivation of the enzyme occurred at similar pressures where Verjovski-Almeida et al. [62] observed the dissociation of Ca^{2+}-ATPase dimers into monomers. Therefore the inactivation of the enzyme may follow the pressure-induced dissociation of ATPase oligomers into unstable monomers. It is interesting that this dissociation occurred even at the very high ATPase concentration ($\cong 5$ mM) that exists in the membrane of sarcoplasmic reticulum.

The volume change calculated for the pressure-induced inactivation of Ca^{2+}-ATPase in the presence of $> 10^{-5}$ M Ca^{2+} is $\cong 200$–250 ml/mol [4], i.e., greater than the volume change ($\cong 160$ ml/mole) calculated for the pressure-induced dissociation of Ca^{2+}-ATPase oligomers into monomers in detergent solutions [62]. The larger partial specific volume of the ATPase oligomers that exist in low-Ca^{2+} medium can be explained by poor atomic packing at the interfaces between the ATPase molecules, leaving voids between the subunits; these would disappear upon dissociation due to improved packing of solvent molecules against the interfaces of the dissociated monomers [41,62,63]. Charge fluctuations that affect carboxyl groups involved in salt linkages at the intersubunit or interdomain interfaces may also play a role [41]. The increased ionization of carboxyl groups with pressure [64] supports this possibility. Based on this interpretation, the stabilization of Ca^{2+}-ATPase against pressure by vanadate and by Ca^{2+} may be related to a decrease in the molecular volume of Ca^{2+}-ATPase during the formation of crystalline arrays by improved packing at the protein/protein or protein/lipid interfaces.

The increased deuterium exchange during pressure treatment is consistent with increased solvation of the pressure-treated membrane, and with the replacement of nonpolar interactions between ATPase molecules by dipole-induced dipole interactions in which water and hydrophobic protein residues participate [65]. These changes are likely to affect both the cytoplasmic and the membrane embedded domains of the Ca^{2+}-ATPase. There are suggestions that the depth of immersion of Ca^{2+}-ATPase into the bilayer may be different in the E_2 from that in the E_1 state [66,67]. Although the excitation and emission spectrum of protein tryptophan remained unchanged after pressure treatment, localized changes in the polarity of the environment of tryptophan residues under pressure due to water penetration into the protein cannot be excluded.

The H-^{2}H exchange rate for the backbone and side chain amide groups located on the protein surface is much faster than for the amide groups that are buried in the protein interior or are involved in internal hydrogen bonding within elements of the secondary structure [68–71]. Therefore the residual Amide II band observed at $\cong 1550$ cm^{-1} after several hours of incubation of sarcoplasmic reticulum in ^2H_2O medium is attributable to N-H groups in the protein interior that are inaccessible to solvent. At atmospheric pressure the residual Amide II band of sarcoplasmic reticulum is extremely stable and shows little exchange, even after several days of incubation at 2° C. Upon application of greater than $\cong 1$ kbar pressure the rate of H-^{2}H exchange dramatically increases, and at about 4–6 kbar pressure, essentially complete deuteration of the Amide II band is achieved within a few hours. The remaining intensity at $\cong 1570$ cm^{-1} is attributed to the bending mode of the ionized carboxyl groups [40]. Similar effects of pressure were observed on chymotrypsinogen [72], and on other proteins [73]. Release of pressure before the deuteration of the amide groups is completed immediately stops further exchange; therefore the acceleration of H-^{2}H exchange takes place only under the influence of pressure.

Pressure is known to enhance hydrogen bonding and restrict molecular fluctuations [18–20]. Therefore it is difficult to explain the increased rate of H-^{2}H exchange at high pressures by local or regional unfolding of the protein due to breaking of the hydrogen bonds, or by increased penetration of solvent into the protein interior due to fluctuations and mobile defects in the protein structure. We suggest that in addition to these mechanisms, exposure of interfaces between protein domains

to the solvent due to their rearrangement by sliding or hinge-like motions under the influence of pressure may provide a significant contribution to H-^{2}H exchange. We attribute the protection by vanadate against the pressure-induced increase in H-^{2}H exchange rate to the stabilization of the interactions between domains within the ATPase molecules and to increased association of ATPase molecules into crystalline arrays.

Such rearrangement of protein domains during $E_1 \rightarrow E_2$ transition was also invoked as the explanation for the relatively small differences in secondary structures between the E_1 and E_2 conformations of the Ca^{2+}-ATPase [37,58], that is in striking contrast with the large differences in their crystal structures [36,56,57], tryptic digestion profiles [36], and differential sensitivity to high pressure.

Some pressure-induced change in the secondary structure of Ca^{2+}-ATPase is suggested by the shift of the peak of the Amide I band to lower frequencies and by the increased intensity of the 1635 cm^{-1} and 1675 cm^{-1} bands at high pressure. These changes are consistent with the conversion of α helix into β structure. The changes are partially reversed upon return to atmospheric pressures, but irreversible structural change associated with irreversible loss of ATPase activity remains. The shift of the Amide I peak to lower frequency at high pressure is not a general property of proteins, but apparently depends on their unique structure. A shift of the Amide I peak with increasing pressure to higher frequencies was observed in chymotrypsinogen [72] and attributed to the conversion of α helix and β sheet into random coil.

The stabilization of Ca^{2+}-ATPase against pressure by vanadate can be attributed with reasonable confidence to the stabilization of the E_2 state and the induction of P2 type crystals of Ca^{2+}-ATPase [56,57,74]. The conformational basis of the protective effect of 20 mM Ca^{2+} is less obvious. Based on kinetic studies, the Ca^{2+}-ATPase has high-affinity Ca^{2+} binding sites in the E_1 state with a dissociation constant of about 0.1 μM, and low-affinity Ca^{2+} binding sites in the E_2 conformation with apparent dissociation constant of $\cong 1$ mM [75,76]. Therefore Ca^{2+} at millimolar concentrations may be expected to shift the conformational equilibrium in favor of the E_2 form. However, based on the fluorescence responses of tryptophan and FITC, removal of Ca^{2+} by EGTA was also suggested to produce the E_2 state [45,74]. Yet EGTA in the absence of vanadate, not only did not stabilize the enzyme, but promoted the pressure-induced inactivation. Therefore the description of the system in terms of two distinct conformations (E_1 and E_2) is an oversimplification. Several conformational substates of the Ca^{2+}-ATPase must exist in the presence of different substrates and ions, that differ in their interactions, molecular volumes and sensitivity to pressure.

The Ca^{2+}-transport and ATPase activities of sarcoplasmic reticulum are dependent on membrane phospholipids [77–79], suggesting an intimate relationship of the ATPase with the lipid bilayer [75]. Therefore the pressure-induced modification of the structural and dynamic properties of the lipid phase [20] may also contribute indirectly to the structural changes induced by pressure in the proteins of sarcoplasmic reticulum. While detailed information is available on pressure effects on the properties of model membranes of well-defined phospholipid composition [20], the effects of compression on the structure of sarcoplasmic reticulum lipids remains to be investigated.

Acknowledgments

This study was supported by research grants from the NIH (AR 26545), NSF (84-03679 and Int. 86-17848) and the Muscular Dystrophy Association.

Our thanks are due to Dr. Bonnie A. Wallace of the Department of Chemistry and Center for Biophysics, Rensselaer Polytechnic Institute, Troy, NY, for permitting us to use her facilities for circular dichroism measurements and for providing help in the analysis and interpretation of the data.

References

1 Penniston, J.T. (1971) Arch. Biochem. Biophys. 142, 322–330.
2 Heremans, K. and Wuytack, F. (1980) FEBS Lett. 117, 161–163.
3 Konig, K.G. and Hasselbach, W. (1984) Z. Naturforsch. 39c, 282–288.
4 Varga, S., Mullner, N., Pikula, S., Papp, S., Varga, K. and Martonosi, A. (1986) J. Biol. Chem. 261, 13943–13956.
5 Champeil, P., Büschlen, S. and Guillain, F. (1981) Biochemistry 20, 1520–1524.
6 Nakamura, H., Jilka, R.L., Boland, R. and Martonosi, A. (1976) J. Biol. Chem. 251, 5414–5423.
7 Glasoe, P.K. and Long, F.A. (1960) J. Phys. Chem. 188–191.
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–276.
9 Dean, W.L. and Tanford, C. (1977) J. Biol. Chem. 252, 3551–3553.
10 Papp, S., Pikula, S. and Martonosi, A. (1987) Biophys. J. 51, 205–220.
11 Wong, P.T.T., Moffatt, D.J. and Baudais, F.L. (1985) Appl. Spectrosc. 39, 733–735.
12 Kirino, Y., Anzai, K., Shimizu, H., Ohta, S., Nakanishi, M. and Tsuboi, M. (1977) J. Biochem. 82, 1181–1184.
13 Cortijo, M., Alonso, A., Gomez Fernandez, J.C. and Chapman, D. (1982) J. Mol. Biol. 157, 597–618.
14 Mendelsohn, R., Anderle, G., Jaworski, M., Mantsch, H.H. and Dluhy, R.A. (1984) Biochim. Biophys. Acta 775, 215–224.
15 Jaworsky, M. and Mendelsohn, R. (1985) Biochemistry 24, 3422–3428.
16 Jaworsky, M. and Mendelsohn, R. (1987) Biophys. J .52, 241–248.
17 Jaworsky, M., Brauner, J.W. and Mendelsohn, R. (1986) Spectrochim. Acta, Part A, Mol. Spectrosc. 42, 191–198.
18 Wong, P.T.T. (1984) Annu. Rev. Biophys. Bioeng. 13, 1–24.
19 Wong, P.T.T. (1987) in Vibrational Spectra and Structures. A Series of Advances, Vol. 16 (Durig, J.R., ed.), pp. 357–445, Elsevier, New York.

20 Wong, P.T.T. (1987) in High Pressure Chemistry and Biochemistry (Van Eldik, R. and Jonas, J., eds.), pp. 381–400, Reidel, Dordrecht.
21 Anderle, G. and Mendelsohn, T. (1986) Biochemistry 25, 2174–2179.
22 Wong, P.T.T., Siminovitch, D.J. and Mantsch, H.H. (1988) Biochim. Biophys. Acta 947, 139–171.
23 Susi, H. (1969) in Structure and Stability of Biological Macromolecules (Timasheff, S.N. and Fasman, G.D., eds.), pp. 575–663, Marcel Dekker, New York.
24 Buchet, R., Jona, I. and Martonosi, A. (1989) Biochim. Biophys. Acta 983, 167–178.
25 Kauppinen, J.K., Moffat, D.J., Mantsch, H.H. and Cameron, D.G. (1981) Appl. Spectrosc. 35, 271–276.
26 Cameron, D.G., Kauppinen, J.K., Moffat, D.J. and Mantsch, H.H. (1982) Appl. Spectrosc. 36, 245–249.
27 Surewicz, W.K. and Mantsch, H.H. (1988) Biochim. Biophys. Acta 952, 115–130.
28 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 2599–2603.
29 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 10111–10115.
30 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 11896–11902.
31 Dux, L. and Martonosi, A. (1983) J. Biol. Chem. 258, 11903–11907
32 Dux, L. and Martonosi, A. (1984) Eur. J. Biochem. 151, 43–49.
33 Taylor, K.A., Dux, L. and Martonosi, A. (1984) J. Mol. Biol. 174, 193–204.
34 Taylor, K.A., Dux, L. and Martonosi, A. (1986) J. Mol. Biol. 187, 417–427.
35 Taylor, K.A., Ho, M.-H. and Martonosi, A. (1986) Ann. NY Acad. Sci. 483, 31–43.
36 Dux, L., Taylor, K.A., Ting-Beall, H.P. and Martonosi, A. (1985) J. Biol. Chem. 260, 11730–11743.
37 Arrondo, J.L.R., Mantsch, H.H., Mullner, N., Pikula, S. and Martonosi, A. (1987) J. Biol. Chem. 262, 9037–9043.
38 Dux, L., Pikula, S., Mullner, N. and Martonosi, A. (1987) J. Biol. Chem. 262, 6439–6442.
39 Chirgadze, Y.N., Fedorov, O.V. and Trushina, N.P. (1975) Biopolymers 14, 679–694.
40 Willumsen, L. (1968) Compt. Rend. Trav. Lab. Carlsberg 36, 327–345.
41 Weber, G. (1987) in High Pressure Chemistry and Biochemistry (Van Eldik, R. and Jonas, J., eds.), pp. 401–420, Reidel, Dordrecht.
42 Pick, U. (1982) J. Biol. Chem. 257, 6111–6119.
43 Pick, U. and Bassilian, S. (1981) FEBS Lett. 123, 127–130.
44 Pick, U. and Karlish, S.J.D. (1980) Biochim. Biophys. Acta 626, 255–261.
45 Pick, U. and Karlish, S.J.D. (1982) J. Biol. Chem. 257, 6120–6126.
46 Mitchinson, C., Wilderspin, A.F., Trinnaman, B.J. and Green, N.M. (1982) FEBS Lett. 146, 87–92.
47 Brandl, C.J., Green, N.M., Korczak, B. and MacLennan, D.H. (1986) Cell 44, 597–607.
48 Green, N.M., Taylor, W.R., Brandl, C., Korczak, B. and MacLennan, D.H. (1986) Calcium and the Cell, CIBA Fdn. Symp. 122, pp. 93–107, Wiley, New York.
49 Green, N.M., Taylor, W.R. and MacLennan, D.H. (1988) in The Ion Pumps. Structure and Function (Stein, W.D., ed.), pp. 15–24, A.R. Liss, New York.
50 Highsmith, S. and Cohen, J. (1987) Biochemistry 26, 154–161.
51 Anzai, K., Kirino, Y. and Shimizu, H. (1978) J. Biochem. (Tokyo) 84, 815–821.
52 Anzai, K., Kirino, Y. and Shimizu, H. (1981) J. Biochem. (Tokyo) 90, 349–354.
53 MacLennan, D.H., Brandl, C.J., Korczak, B. and Green, N.M. (1985) Nature 316, 696–700.
54 Taylor, K.A., Mullner, N., Pikula, S., Dux, L., Peracchia, C., Varga, S. and Martonosi, A. (1988) J. Biol. Chem. 263, 5287–5294.
55 Taylor, K.A., Dux, L., Varga, S., Ting-Beall, H.P. and Martonosi, A. (1988) Methods Enzymol. 157, 271–289.
56 Martonosi, A., Dux, L., Taylor, K.A., Ting-Beall, H.P., Varga, S., Csermely, P., Mullner, N., Papp, S. and Jona, I. (1987) in Proteins and Excitable Membranes (Fambrough, D. and Hille, B., eds.), pp. 257–286, Wiley, New York.
57 Martonosi, A., Taylor, K.A., Varga, S. and Ting-Beall, H.P. (1987) in Electron Microscopy of Proteins, Vol. 6, Membranous Structures (Harris, J.R. and Horne, R.W., eds.), pp. 255–376, Academic Press, London.
58 Csermely, P., Katopis, C., Wallace, B.A. and Martonosi, A. (1987) Biochem. J. 241, 663–669.
59 Williams, R.W., McIntyre, J.O., Gaber, B.P. and Fleischer, S. (1986) J. Biol. Chem. 261, 14520–14524.
60 Scott, T.L. (1985) J. Biol. Chem. 260, 14421–14423.
61 Squier, T.C., Bigelow, D.J., De Ancos, J.G. and Inesi, G. (1987) J. Biol. Chem. 262, 4748–4754.
62 Verjovski-Almeida, S., Kurtenbach, E., Amorim, A.F. and Weber, G. (1986) J. Biol. Chem. 261, 9872–9878.
63 Weber, G. and Drickamer, H.G. (1983) Q. Rev. Biophys. 16, 89–112.
64 Bradley, R.S. and Munro, D.C. (1965) High Pressure Chemistry, p. 96, Pergamon, Oxford.
65 Thompson, R. and Lakowicz, J. (1984) Biochemistry 23, 3411–3417.
66 Peracchia, C., Dux, L. and Martonosi, A. (1984) J. Muscle Res. Cell Motil. 5, 431–442.
67 Blasie, J.K., Herbette, L.G., Pascolini, D., Skita, V., Pierce, D.H. and Scarpa, A. (1985) Biophys. J. 48, 9–18.
68 Woodward, C.K. and Hilton, B.D. (1979) Annu. Rev. Biophys. Bioeng. 8, 99–127.
69 Barksdale, A.D. and Rosenberg, A. (1982) Methods Biochem. Anal. 28, 1–113.
70 Englander, S.W. and Kallenbach, N.R. (1984) Q. Rev. Biophys. 16, 521–655.
71 Gregory, R.B. and Rosenberg, A. (1986) Methods Enzymol. 131, 448–508.
72 Wong, P.T.T. and Heremans, K. (1988) Biochim. Biophys. Acta 956, 1–9.
73 Carter, J.V., Knox, D.G. and Rosenberg, A. (1978) J. Biol. Chem. 253, 1947–1953.
74 Jona, I. and Martonosi, A. (1986) Biochem. J. 234, 363–371.
75 Martonosi, A. and Beeler, T.J. (1983) in Handbook of Physiology, Section 10. Skeletal Muscle (Peachey, L.D., Adrian, R.H. and Geiger, S.R., eds.), pp. 417–485, American Physiological Society, Bethesda.
76 Inesi, G., and De Meis, L. (1985) in The Enzymes of Biological Membranes, 2nd edn., Vol. 3 (Martonosi, A., ed.), pp. 157–191, Plenum, New York.
77 Martonosi, A. (1963) Biochem. Biophys. Res. Commun. 13, 273–278.
78 Martonosi, A. (1964) Fed. Proc. 23, 913–921.
79 Martonosi, A., Donley, J. and Halpin, R.A. (1968) J. Biol. Chem. 243, 61–70.