Conformational Differences between the E_1 and E_2 States of the Calcium Adenosinetriphosphatase of the Erythrocyte Plasma Membrane As Revealed by Circular Dichroism and Fluorescence Spectroscopy†

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ABSTRACT: Different conformational states of the purified plasma membrane Ca^{2+}-ATPase from pig erythrocytes have been detected by circular dichroism (CD) and fluorescence spectroscopy. The helical content of the enzyme decreased by about 10% in the transition from the Ca^{2+} high-affinity form (10 μM free Ca^{2+} = E_1 state) to the VO_4^{3-}-inhibited state (20 μM VO_4^{3-} = E_2 state). The changes in the CD spectra did not show full reversibility upon reversing the E_1–E_2 transition, whereas those in the fluorescence spectra did. A temperature-dependent loss of α-helical content in the presence of Ca^{2+} was also observed. Intrinsic fluorescence measurements revealed an increase in fluorescence intensity upon addition of Ca^{2+}. The change was fully reversed by ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid. The increase in fluorescence intensity was partly reversed by adding ATP, an effect which is suggested to correspond to the "Ca^{2+}-occluded" form of the ATPase. The steady-state level of the fluorescence intensity was stable for several minutes in the presence of 100 μM ATP. By contrast, the decrease of fluorescence intensity induced by limiting concentrations of ATP (=1 μM) was only transient, indicating the decomposition of the phosphorylated intermediate of the ATPase and the reestablishment of the Ca^{2+} high-affinity form of the enzyme.

The plasma membrane Ca^{2+}-ATPase is one of the two systems responsible for ejecting Ca^{2+} from the cell [for recent reviews, see Inesi (1985), Penniston (1984), Schatzmann (1982), and Carafoli and Zurini (1982)]. It has been first described by Schatzmann and his co-workers (Schatzmann, 1966; Schatzmann & Vincenzi, 1969) and has been purified to apparent homogeneity from erythrocyte (Niggli et al., 1979, 1981a) and several other plasma membranes (Caroni & Carafoli, 1981; Hakim et al., 1982; Wuytack et al., 1981; Michalak et al., 1984).

The enzyme is a single polypeptide of M_r 138 000 (Graf et al., 1982). It belongs to the E_1–E_2 class of ion-motive ATPases; i.e., it forms an acyl phosphate intermediate during the reaction cycle (Knauf et al., 1974; Niggli et al., 1979) and is inhibited by low concentrations of vanadate [Niggli et al., 1981a; e.g., also see Schatzmann (1982)]. A series of studies have shown that the erythrocyte Ca^{2+}-ATPase can be activated not only by calmodulin (CaM)[1] (Niggli et al., 1981a), or calmodulin tryptic fragments (Guerini et al., 1984), but also by acidic phospholipids or polyunsaturated fatty acids (Ronner et al., 1977; Niggli et al., 1981b) and by controlled proteolysis either in the native membrane (Taverna & Hanahan, 1980; Sarkadi et al., 1980) or in the purified state (Niggli et al., 1981b; Stieger & Schatzmann, 1981; Caroni et al., 1982; Zurini et al., 1984; Benaim et al., 1984). The proteolysis studies have provided evidence for conformational changes of the purified erythrocyte enzyme induced by different effectors since striking differences in the digestion pattern were observed if the purified enzyme was submitted to proteolysis in different functional states.

Fluorescence spectroscopy and circular dichroism studies on other ATPases of the E_1–E_2 type, e.g., the (Na^+,K^+)-ATPase or the Ca^{2+}-ATPase of sarcoplasmic reticulum, have also provided evidence for the existence of at least two reaction cycle linked conformational states. Karlish and Yates (1978) have reported increased intrinsic tryptophan fluorescence in the E_2 form of the (Na^+,K^+)-ATPase as compared to the E_1 form, whereas the intrinsic fluorescence of the SR Ca^{2+}-ATPase decreases in the E_1 to E_2 transition induced by complexing Ca^{2+} with EGTA (Dupont, 1976; Inesi et al., 1980; Jona & Martonosi 1986).

The major difficulty in using CD spectroscopy to monitor conformational changes of membrane proteins is linked to the light-scattering and absorption-flattening effects due to the special environment of these proteins. As a result, the number of studies on conformational transitions of E_1–E_2-type ATPases using CD spectroscopy is still limited (Mommaerts, 1967; Gresalfi & Wallace, 1984; Le Maire et al., 1978; Fronticelli et al., 1984; Nakamoto & Inesi, 1986). Therefore, in this paper a combination of intrinsic tryptophan fluorescence and an improved CD spectroscopic technique have been used to overcome the problems mentioned above. The results have shown that very evident structural changes take place in the protein during the E_1–E_2 transition. Preliminary accounts of

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[1] Abbreviations: SR, sarcoplasmic reticulum; CaM, calmodulin; CD, circular dichroism; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; $C_{12}E_8$, dodecyl octaoxyethylene glycol monoether; [θ], mean residue ellipticity in degrees centimeter squared per decimole; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; hbw, half-bandwidth.

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some of the data have been given elsewhere (Krebs et al., 1986).

Experimental Procedures

Materials

All reagents were of the highest purity grade available. Calmodulin was isolated from bovine brain as described previously (Guerini et al., 1984) and coupled to CNBr–Sepharose (Pharmacia, Uppsala, Sweden) as described by Niggli et al. (1979). Dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$) was purchased from Tokyo Nakei Kogyo, Japan. It was 2 times recrystallized from isooctane before use. Phospholipids (grade I) were obtained from Lipid Products, Nutfield Ridge, Sussex, England.

Methods

Preparation of the Ca^{2+}-ATPase. The Ca^{2+}-ATPase was purified from pig red cell membranes essentially as described for human red cell membranes by Niggli et al. (1981a) with some minor modifications of the procedure. The solubilization of the membrane and the purification of the enzyme were carried out by using dodecyl octaoxyethylene glycol monoether ($C_{12}E_8$) instead of Triton X-100 as the detergent. Tris-HCl (pH 7.4 at 4 °C) instead of Hepes was used as the buffer throughout the purification. The buffer eluting the Ca^{2+}-ATPase from the calmodulin–Sepharose column had the following composition: 20 mM Tris-HCl, pH 7.4 (at 4 °C), 30 mM KCl, 1 mM $MgCl_2$, 2 mM EDTA, 0.05% $C_{12}E_8$, 0.05% phosphatidylcholine, and 5% glycerol. The purification and the elution of the enzyme were constantly monitored at 280 nm. The fractions of the protein peak eluted by the EDTA buffer were checked for Ca^{2+}-ATPase activity by using the spectrophotometric-coupled enzyme assay described by Niggli et al. (1981a). They were pooled; $MgCl_2$ and $CaCl_2$ were added up to 2 mM and 50 μM final concentrations, respectively, and concentrated to 250–300 μg/mL by using the Centricon microconcentrators of Amicon (Centricon 30, with a YM-membrane). The concentration was achieved by centrifuging at 5000g; 400-μL aliquots of the protein were stored at −80 °C. The protein concentration was determined by a modification of the method of Lowry et al. (1951) as described by Zurini et al. (1984) using bovine serum albumin as standard. The isolated protein was better than 90% pure as determined by gel electrophoresis (Laemmli, 1970) and was stimulated by calmodulin at least 4–5-fold. The results of the fluorescence and CD measurements presented in this paper were obtained on samples from the same enzyme purification batch.

CD Measurements. The CD spectra were recorded on a Jasco J-500 spectropolarimeter utilizing a far-UV jacketed quartz cell with an effective light path length of 0.1 cm. The cuvette temperature was maintained at the values given in the legends to the figures. Spectra were run at a scanning speed of 20 (Figure 1) or 50 nm/min (Figures 2 and 3) and a time constant of 1 or 0.5 s, respectively, over the wavelength range of 200–300 nm. The absorbance of the samples in the spectral region of interest, i.e., 200–250 nm, was of the order of 0.25 ODU. Usually, 16 spectra were time averaged and normalized, the base line of the spectra containing the corresponding buffer was subtracted, and the spectrum was smoothed by digital filtering according to the instructions of the manufacturer. UV absorption spectra, run from 200 to 400 nm, were obtained either with a Cary 15 recording spectrophotometer or with a Shimadzu double-wavelength spectrophotometer (Model UV-3000). UV–visible spectra recorded before and after the CD measurements were identical, indicating that no changes in the aggregation state of the protein occurred during the measurement. Furthermore, the activity of the enzyme was determined in the presence of Ca^{2+} before and after the CD measurements. No significant differences could be detected.

Uncorrected mean residue ellipticities were calculated by using M_r 128 as a mean residue weight, as obtained from the amino acid analysis of the purified protein (Graf et al., 1982). In view of the noise level, several independent samples of enzyme preparations were examined, and the results did not differ by more than 4%. The results presented in this paper are from the same enzyme preparation.

Fluorescence Measurements. Changes of the intrinsic fluorescence of the purified Ca^{2+}-ATPase were measured with an instrument designed and built by the Biomedical Instrumentation Group, University of Pennsylvania. The fluorometer consisted essentially of a stabilized 75-W xenon lamp, a 290-nm excitation filter (12-nm hbw), a 50–50 bifurcated fully randomized quartz optical fiber (Welch-Allyn, Boston, MA) which carries both excitation and emission lights to and from the cuvette in an area of 4-mm diameter, a cuvette holder for a minicuvette thermostated at 25 °C and equipped with continuous magnetic stirring, a photomultiplier with a 330-nm (7-nm hbw) interference filter (Omega Optical Co., Brattleboro, VT), a direct current amplifier equipped with scale expansion, zero offset, and filtering, and a strip-chart recorder.

The reaction media contained in 0.5 mL final volume of 20 mM Hepes, pH 7.2, 130 mM KCl, 2 mM $MgCl_2$, 0.5 μM calmodulin, 7–12 μg of pig erythrocyte Ca^{2+}-ATPase, and the concentrations of Ca^{2+}, EGTA, ATP, and vanadate as described in each experiment. Proper controls were carried out to verify and minimize dilution artifacts. To this end, each addition of the reactants was made in volumes smaller than 1 μL.

Results

Circular Dichroism. Since the purified enzyme was usually obtained at rather low concentrations, spectra had to be accumulated and time averaged. Figure 1 demonstrates that the raw spectral data (Figure 1, dashed lines) and the smoothed curves (Figure 1, solid lines) of the Ca^{2+}-ATPase are in excellent agreement. In addition, it provides evidence that significant differences between different conformations of the enzyme also become apparent from the raw spectral data. Thus, smoothed curves have been used here as reasonable approximations to the real data.

The spectra presented in Figure 1 compare the putative E_1 and E_2 conformations of the purified $C_{12}E_8$-solubilized pig erythrocyte Ca^{2+}-ATPase. The E_1 conformation was induced by adding low concentrations of Ca^{2+} (free Ca^{2+} concentration 10–20 μM, Ca^{2+} high-affinity form), whereas the E_2 conformation was stabilized by low concentrations of vanadate (20 μM), an analogue of inorganic orthophosphate (Pick, 1982).

The ellipticity change of the protein during the E_1–E_2 transition is clearly visible on comparing the spectra obtained under the two conditions. Calculations based on $[\theta]$ at 222 nm, which is often used as a representative value for the α-helical content, indicate a loss of α-helix, corresponding quantitatively to about 10% (see Table I). Even if precise quantitative estimates of secondary structural changes, e.g., by using the method developed by Provencher and Gloeckner (1981), are made difficult under these conditions by the lack of data below 200 nm (due to the low protein concentrations and to the strong background absorption), the spectral profile of Figure 1a obviously represented a CD spectrum typical of proteins with a high degree of α-helical content (e.g., myo-

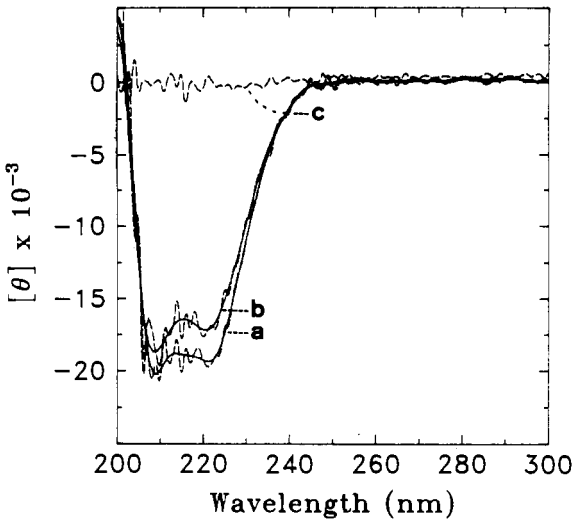

FIGURE 1: CD spectra of the erythrocyte Ca^{2+}-ATPase in the presence of 10 μM free Ca^{2+} (a) and 20 μM VO_4^{3-} (b) at 4 °C using a scanning speed of 20 nm/min and a time constant of 1 s. Full details on the experimental conditions are described under Experimental Procedures. The dashed lines represent the raw data (nine accumulations) whereas the solid lines are the corresponding smoothed curves. The CD spectra of the Ca^{2+}-ATPase (200 μg/mL) were obtained in a buffer containing 20 mM Tris-HCl, pH 7.5, 25 mM KCl, 2 mM $MgCl_2$, 0.05% $C_{12}E_8$, 0.05% phosphatidylcholine, and 5% glycerol (base line, indicated as c), using a cuvette of 0.1-cm path length (volume = 200 μL). Additions were made from concentrated stock solutions to keep dilution effects at a minimum.

Table I: α-Helical Content of Ca^{2+}-ATPase under Different Experimental Conditions[a]

Ca-ATPase	temp (°C)	α-helix content (%)
$+Ca^{2+}$ (=E_1)	4	55 ± 1.5 (n = 6)
	37	46 ± 1.5 (n = 2)
$+VO_4$ (=E_2)	4	48 ± 1.8 (n = 4)
+EGTA	4	46 ± 1.8 (n = 3)
$+Ca^{2+}$ [b]	4	49 ± 2.0 (n = 3)

[a] The values are based on $[\theta]_{222}$ and have been obtained by using the calculations of Chen et al. (1972) on the data presented in Figures 1–3. The data are expressed as the standard error. The number of experiments is given in parentheses. [b] These values have been obtained after adding EGTA to reverse the E_1–E_2 transition (see Figure 2).

globin), since it showed two pronounced minima at 208 and 222 nm of almost equal magnitude. On the other hand, the decrease of the molar ellipticity at 222 nm, inferred from the spectrum of Figure 1b, indicated a higher content of β-pleated sheet structure. In addition, the magnitude of the minimum at 222 nm was markedly reduced as compared to that at 208 nm. It is worth mentioning that even though data below 200 nm were not available calculation of the spectra based on the method of Provencher and Gloeckner (1981) revealed 56% α-helix, 10% β-sheet, and 35% remainder for the E_1 form and 51% α-helix, 13% β-sheet, and 36% remainder for the E_2 form, respectively. These calculations support, at least qualitatively, the interpretation offered before. It is interesting that virtually no changes in the CD spectrum could be observed upon addition of ATP to the Ca^{2+} high-affinity form of the Ca^{2+}-ATPase to induce the phenomenon of "Ca^{2+} occlusion" (data not shown). This is at variance with the fluorescence data presented below.

Spectral changes similar to those shown in Figure 1 could be observed by removing Ca^{2+} from the high-affinity site with EGTA to transform the enzyme from the E_1 to the E_2 state (Figure 2). The estimated decrease of α-helical content was comparable to that obtained in the presence of vanadate, but not identical (see Table I). To assess the reversibility of the

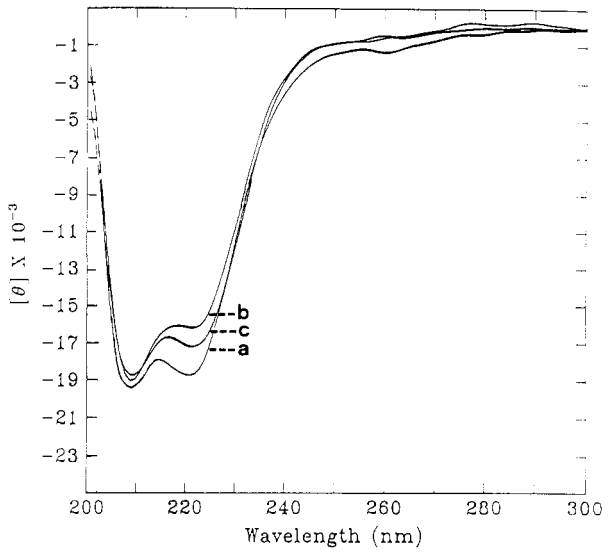

FIGURE 2: CD spectra of the Ca^{2+}-ATPase at 4 °C in the presence of 10 μM free Ca^{2+} (a) or 500 μM EGTA (b) and after adding 600 μM Ca^{2+} (=20 μM free Ca^{2+}) to reverse the E_1–E_2 transition (c). Other experimental conditions are as described in the legend.

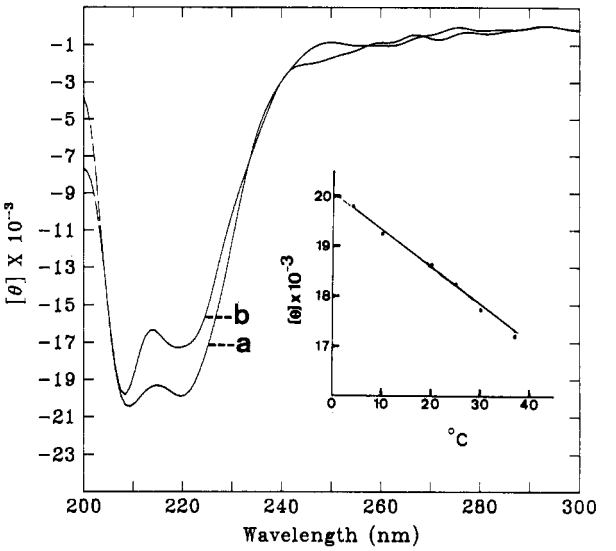

FIGURE 3: Temperature dependence of the CD spectra of the Ca^{2+}-ATPase. The CD spectra of the enzyme were obtained at 4 (a) and 37 °C (b) in the presence of 10 μM free Ca^{2+} under the experimental conditions described in the legend of Figure 1. The inset of the figure relates the decrease of $[\theta]_{222}$ to increasing temperatures.

E_1–E_2 transition, excess Ca^{2+} was added to the EGTA-treated enzyme to return it to the E_1 state. As illustrated in Figure 2 (trace c), this transition was not fully reversible. Under these conditions, the enzyme experienced an approximate 20% loss of specific activity, indicating the lability of the ATPase in the E_2 conformation. However, single spectra obtained immediately after the addition(s) indicated complete reversibility, and preservation of full enzyme activity was observed. Since the signal/noise ratio under these conditions did not permit firm conclusions, multiple spectra had to be accumulated, resulting in only partial reversibility of the spectra.

Pronounced conformational differences of the purified Ca^{2+}-ATPase have been evidenced by controlled proteolysis experiments carried out at different temperatures (Zurini et al., 1984; Benaim et al., 1984). CD measurements were thus carried out at different temperatures. Figure 3 shows that the $[\theta]_{222}$ decreased constantly as the medium temperature was increased (see inset of Figure 3), indicating a less constrained structure of the enzyme at higher temperatures, i.e., a pro-

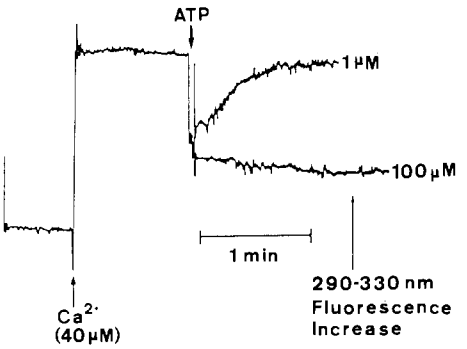

FIGURE 4: Effects of Ca^{2+} and ATP on the intrinsic fluorescence of the purified Ca^{2+}-ATPase. The reaction mixture contained the following in a final volume of 0.5 mL: 20 mM Hepes, pH 7.2, 130 mM KCl, 5 mM $MgCl_2$, 9 μg of ATPase, 8 μM EGTA, and 0.5 μM calmodulin. The concentrations of the reagents added are shown in the figure.

gressive decrease of α-helical content.

Fluorescence Spectroscopy. The intrinsic fluorescence of the Ca^{2+}-ATPase was measured as originally described by Dupont and Leigh (1978) (see Experimental Procedures). The enzyme was added to a medium containing 0.85 μM free Ca^{2+} as measured by a Ca^{2+} electrode in the reaction cuvette. When 40 μM $CaCl_2$ was added to the reaction mixture to raise the concentration of free Ca^{2+} to 31 μM, the ATPase exhibited an increase in intrinsic fluorescence (Figure 4) which reached steady-state levels within the mixing time. This effect was fully reversed when the free Ca^{2+} concentration in the medium was lowered below 1 μM by adding EGTA (not shown). The addition of 100 μM ATP caused a decrease in fluorescence which was completed during the mixing time and remained at steady-state levels for several minutes thereafter. In a second experiment under identical conditions (superimposed upper trace), the addition of 1 μM ATP caused a smaller decrease in protein fluorescence followed by a time-resolved increase in fluorescence intensity which reached values similar to those seen before the additions of ATP within 30 s.

Figure 5 shows the results of an experiment in which the ATPase was incubated in a reaction mixture similar to that of Figure 4. The addition of 2 μL of H_2O induced a small decrease in fluorescence intensity due to dilution effects. The addition of 30 μM $CaCl_2$ to raise the free Ca^{2+} concentration in the medium from 0.7 to 23.5 μM resulted in the expected increase in fluorescence intensity (see Figure 4). At this point, the addition of 10 μM vanadate was followed by a decrease in intrinsic fluorescence which was completed during the mixing time and remained at steady-state levels thereafter. The following addition of ATP resulted only in a negligible decrease of fluorescence intensity, consistent with a dilution effect.

Discussion

The results presented in this paper demonstrate significant structural differences between two functional states of the red blood cell Ca^{2+}-ATPase. They most likely correspond to the E_1 and E_2 states, frequently described for ion-motive ATPases of this type. Three major observations have been made:

(1) The putative E_1 state (Ca^{2+} high-affinity form) of the solubilized ATPase has a significantly higher α-helical content (based on $[\theta]$ at 222 nm) than the E_2 state (Ca^{2+} low-affinity form, VO_4^{3-} inhibited). (2) The intrinsic fluorescence increased during the transition of the enzyme from the Ca^{2+} low-affinity form to the Ca^{2+} high-affinity form. (3) The increase in the fluorescence intensity due to the transition of the enzyme into the Ca^{2+} high-affinity state was partially

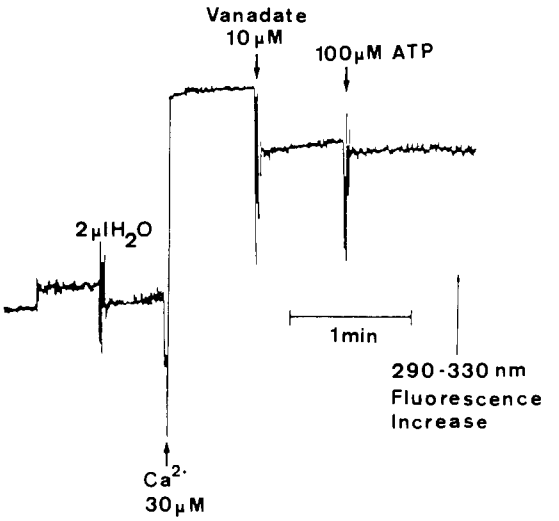

FIGURE 5: Effects of Ca^{2+}, vanadate, and ATP on the intrinsic fluorescence of the purified Ca^{2+}-ATPase. The reaction mixture was identical with that of Figure 4, except for the concentration of the purified ATPase which was 11 μg.

reversed by the addition of ATP.

Dupont and Leigh (1978) have observed Ca^{2+}-dependent fluorescence intensity changes of the Ca^{2+}-ATPase of sarcoplasmic reticulum vesicles upon addition of ATP. The changes were attributed to the occlusion of Ca^{2+} within the enzyme, as supported by the observation that in the presence of excess ATP the occluded Ca^{2+} could not be removed by EGTA. On the other hand, excess ADP reversed the putative occlusion process and made Ca^{2+} fully accessible to EGTA (Dupont, 1980). The data presented in Figure 4 can be conveniently interpreted in a similar way. The finding that in the presence of low amounts of ATP (i.e., when only a limited number of reaction cycles is permitted) Ca^{2+} was apparently slowly released from the occluded site due to the breakdown of the acyl phosphate intermediate (Figure 4, upper trace) corroborates this interpretation. Under these conditions, the enzyme returned to the original Ca^{2+} high-affinity state within 1 min. A similar time course was reported by Dupont and Leigh (1978) for the Ca^{2+}-ATPase in the sarcoplasmic reticulum membrane. It is important that in the latter case the transient decrease of the fluorescence intensity in the presence of ATP was only observed below 0 °C. Above 0 °C, the dephosphorylation of the E–P intermediate evidently became much slower than the rebinding of Ca^{2+}, resulting in a much weaker fluorescence change (Dupont & Leigh 1978). By contrast, the data reported here have been obtained at 25 °C. This indicates that the erythrocyte enzyme in micellar form, i.e., in the presence of detergent and phospholipids, is apparently dephosphorylated at a rate which is sufficiently fast to permit the detection of the transient change of fluorescence intensity.

The environment of the enzyme, i.e., a membrane bilayer or a detergent micellar form, may influence the time scale of the conversion from the low- to the high-affinity Ca^{2+} form. This is indicated by a study of Kosk-Kosicka and Inesi (1985), who have observed that the Ca^{2+}-induced rise in intrinsic fluorescence of the red blood cell membrane Ca^{2+}-ATPase solubilized in $C_{12}E_8$ was completed within 50 ms, whereas the time course for the SR Ca^{2+}-ATPase in the vesicular membrane was about 20 time longer. Analogous observations have been made by the same authors (Kosk-Kosicka et al., 1983) on comparing directly the solubilized and the membrane-bound forms of the SR Ca^{2+}-ATPase. The rapid increase in fluorescence intensity upon addition of Ca^{2+} observed by Kosk-Kosicka and Inesi (1985) for the solubilized erythrocyte

Ca^{2+}-ATPase is in excellent agreement with the observations reported here; i.e., the fluorescence intensity increased to the steady-state level within the mixing time after the addition of Ca^{2+} (see Figures 4 and 5).

The reversal of the fluorescence change resulting from the putative occlusion of Ca^{2+} induced by the addition of ATP probably reflects limited conformational changes of the enzyme around the high-affinity Ca^{2+} binding site since corresponding differences in the CD spectra, which are suggested here to indicate overall conformational changes of the protein, were not observed. If, as suggested for the SR Ca^{2+}-ATPase [see MacLennan et al. (1985) and Brandl et al. (1986)], the high-affinity Ca^{2+} binding site and the ATP binding site are remote from each other also in the erythrocyte ATPase, the conformational change induced by the binding of ATP in the microenvironment of the Ca^{2+} binding site could be transmitted through rearrangement of helical segments involved in Ca^{2+} binding without affecting the net content of secondary structural elements.

The observation that the Ca^{2+}-induced increase in fluorescence intensity was fully reversed by EGTA [data not shown; see also Kosk-Kosicka and Inesi (1985)], whereas this could not be demonstrated convincingly in the CD spectra, was probably due to the difference in time scale between the two types of experiments. The fluorescence spectra were obtained within a few minutes, whereas the accumulation of CD spectra during one experiment usually required 45–50 min. Since the erythrocyte Ca^{2+}-ATPase is rather labile in the presence of EGTA, the loss of spectral intensity in the course of the experiment could in principle be due to the irreversible denaturation of a portion of the enzyme. This would be in agreement with the observation that a 10–20% decrease in enzyme activity was routinely observed on comparing the ATPase before and after the complete CD measurements in the presence of EGTA. However, no decrease in activity was detected after the completion of one single spectrum (ca. 3 min). Similar observations have been made on the solubilized SR Ca^{2+}-ATPase (Fronticelli et al., 1984; Nakamoto & Inesi, 1986).

Previous investigations on conformational states of other ion-motive ATPases using CD spectroscopy (Gresalfi & Wallace, 1984; Le Maire et al., 1978; Andersen et al., 1980; Fronticelli et al., 1984; Nakamoto & Inesi, 1986) have indicated significant differences in secondary structure induced by the E_1–E_2 transition. Most of these measurements have been performed with solubilized enzymes, and it is interesting that whenever differences in the CD spectra were observed, the E_2 state had a lower α-helical content than the E_1. The case of the (Na,K)-ATPase (Gresalfi & Wallace, 1984) is of special interest since in this case the study was performed on the enzyme in situ; a 7% loss of α-helix and a 10% increase of β-sheets were detected when the environment of the enzyme was changed from Na^+ (E_1) to K^+ (E_2). These findings are similar to those reported here for the solubilized erythrocyte Ca^{2+}-ATPase (see Figure 1) where a decrease of about 8% α-helix content, in parallel with a possible increase of β-pleated sheet structures (see above), was observed for the transition from the putative E_1 to the putative E_2 state. The models proposed for the SR Ca^{2+}-ATPase and the catalytic subunit of the (Na,K)-ATPase (Brandl et al., 1986; Shull et al., 1985), based on amino acid sequence homologies, are remarkably similar. It will be of interest to establish whether the significant α-helix to β-sheet transition proposed for the functional cycle of the (Na,K)-ATPase (Gresalfi & Wallace, 1984) can be extended to the plasma membrane Ca^{2+}-ATPase, once the primary structure of the latter enzyme becomes known. This would also be important for the temperature-dependent decrease of α-helical content shown in Figure 3 (insert), which indicates a less constrained structure: temperature-dependent differences in the proteolytic fragmentation pattern of the erythrocyte Ca^{2+}-ATPase in its various functional states have been observed (Benaim et al., 1984).

Nakamoto and Inesi (1986) have recently concluded that the reaction cycle of the SR Ca^{2+}-ATPase is not reflected in differences in secondary structure, provided the ATPase is in the membrane-bound form. This is in contrast to the previously mentioned observations by Gresalfi and Wallace (1984) on the (Na,K)-ATPase. The resolution of the spectra presented by Nakamoto and Inesi (1986), however, is rather low, probably due to the particulate form of the protein embedded in the bilayer. This results in light-scattering effects which are more pronounced at lower wavelengths (Gresalfi & Wallace, 1984). Measurements on the plasma membrane Ca^{2+}-ATPase in the original membrane environment are unfortunately not possible due to its extreme paucity within the membrane (about 0.1% of the total membrane protein). Thus, a comparison of the solubilized and the membrane-bound form of the plasma membrane enzyme will have to wait for experiments on reconstituted artificial membranes.

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