Volume 277, number 1,2, 147-150 FEBS 09278 December 1990

Molecular changes in the sarcoplasmic reticulum calcium ATPase during catalytic activity

A Fourier transform infrared (FTIR) study using photolysis of caged ATP to trigger the reaction cycle

Andreas Barth, Werner Kreutz and Werner Mäntele

Institut für Biophysik und Strahlenbiologie der Universität Freiburg, Albertstraße 23, D-7800 Freiburg, Germany

Received 29 October 1990

Fourier transform infrared spectroscopy was used to study ligand binding and conformational changes in the Ca^{2+}-ATPase of sarcoplasmic reticulum. Novel in infrared difference spectroscopy, the catalytic cycle in the IR sample was started by photolytic release of ATP from an inactive, photolabile ATP-derivative (caged ATP). Small, but characteristic infrared absorbance changes were observed upon ATP release. On the basis of model spectra, the absorbance changes corresponding to the trigger and substrate reactions, i.e. to photolysis of caged ATP and hydrolysis of ATP, were separated from the absorbance changes due to the active ATPase reflecting formation of the phosphorylated Ca_2E_1P enzyme form. A major rearrangement of ATPase conformation as the result of catalysis can be excluded.

Ca^{2+}-ATPase; Sarcoplasmic reticulum; Protein conformation; Fourier transform infrared spectroscopy; Caged ATP

1. INTRODUCTION

The active transport of Ca^{2+} from the cytoplasm of muscle cells into the sarcoplasmic reticulum (SR) is performed by the Ca^{2+}-ATPase, an intrinsic membrane protein of about 110 kDa molecular mass, through hydrolysis of ATP (for reviews see [1-4]). A reaction scheme has been proposed by de Meis and Vianna [4], including a change from a high Ca^{2+} affinity, ATP-phosphorylated, enzyme form E_1 to a low Ca^{2+} affinity, P_i-phosphorylated, E_2 form [5]. Details of the transport mechanism, especially the coupling between ATP hydrolysis and active calcium transport, are still unknown.

To elucidate this point, it should help to investigate changes of the enzyme conformation, especially of the E_1P-E_2P conversion, which affects Ca^{2+}-binding affinity and the reactivity towards ADP in the catalytically active ATP-ase. Fluorescence studies have indicated conformational changes in connection with the E_1P-E_2P transition [6-11]. However, recent analysis of the ATPase infrared (IR) absorption spectrum did not unambiguously detect differences between the E_1 and E_2 conformation [12-14]. While these approaches used comparisons of two enzyme samples prepared in different states, we use in the present paper the photolytic release of ATP from caged ATP to start the reaction cycle. Caged ATP is an inactive, photolabile ATP-derivative that releases ATP upon ultraviolet illumination [15,16]. This 'hands-off' photochemically triggered difference spectroscopy avoids uncertainties due to buffer subtraction or different sample concentration and thus allows a comparison of different enzyme states on the level of individual bonds, as has been demonstrated before for photobiological [17,18] or redox reactions [19,20].

Correspondence address: W. Mäntele, Institut für Biophysik und Strahlenbiologie der Universität Freiburg, Albertstrabe 23, D-7800 Freiburg, FRG

Abbreviations: SR, sarcoplasmic reticulum; Ca^{2+}-ATPase, Ca^{2+}-transporting ATPase (EC 3.6.1.38); caged ATP, P-1-(2-nitro)phenylethyl adenosine 5'-triphosphate; (FT)IR, (Fourier transform) infrared; EGTA, [ethyleneglycobis(oxyethylenenitrilo)]tetraacetic acid

2. MATERIALS AND METHODS

Ca^{2+}-ATPase prepared according to [21] was a generous gift of W. Hasselbach (Heidelberg). Samples for IR spectroscopy were prepared according to [22] by partial drying of a SR vesicle suspension on a CaF_2 IR window and sealing of the sample with a second window separated by a 6 μm spacer. 'Normal' ATPase samples contained 100-150 μg protein, 300 nmol 4-morpholinopropanesulphonic acid (Mops)/Tris (pH 6.8), 150 nmol KCl, 12 nmol $MgCl_2$, 0.12 nmol $CaCl_2$ in addition to the Ca^{2+} bound by SR, 15 nmol caged ATP, 0.1 nmol Ca^{2+}-ionophore A23187 and 10 nmol glutathione. Ca^{2+}-transport activity of these samples was tested as in [22]. Photolysis of caged ATP was triggered with a xenon flash. Caged ATP model samples were of 'normal' composition, but without the ATPase. Model spectra for the absorbance differences due to ATP hydrolysis were obtained from aqueous solutions of 100 mM ATP

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

and 100 mM (ADP + P_i) at pH 6.8 recorded in a thin-layer cell. Details of these procedures will be reported elsewhere [23].

FTIR spectra at 4 cm^{-1} resolution were obtained with a Bruker IFS 25 instrument equipped with a HgCdTe detector. Single-beam spectra were recorded before and every 15 s after the photolysis flash. Difference spectra calculated from spectra recorded before the flash were used for the control of sample stability; those from spectra recorded after the flash were used to follow the kinetics of hydrolysis and enzymatic activity.

3. RESULTS AND DISCUSSION

Flash-induced difference spectra of an active ATPase IR sample containing caged ATP exhibit characteristic and highly reproducible changes of IR absorbance with different kinetic properties in the region from 1800 cm^{-1} to 950 cm^{-1}. Fig. 1a shows the flash-induced difference spectra at about 8 s (full line) and 2 min (dashed line) after ATP release. On the basis of their kinetic properties, we distinguish (i) permanent signals that appear in the first spectrum within 8 s after ATP release and are still present 2 min later (for example at 1524 cm^{-1}, 1342 cm^{-1}, 1270–950 cm^{-1}), (ii) signals between 1300 cm^{-1} and 1000 cm^{-1} that rise slower on the time scale from several seconds to minutes, and (iii) transient changes between 1750 cm^{-1} and 1520 cm^{-1}, appearing immediately after ATP release and decreasing when the slow change comes to its end. A baseline control spectrum which was recorded before ATP release (Fig. 1b) indicates the level of confidence for the flash-induced signals.

As a working hypothesis, we assume that the different types of absorbance changes reflect the different reactions that take place in the sample upon flash excitation, i.e. caged ATP photolysis (i), ATP hydrolysis (ii), and conformational dynamics of the ATPase (iii) associated with its catalytic activity. In order to test this hypothesis, caged ATP photolysis and ATP hydrolysis were investigated separately.

Fig. 1c shows the flash-induced difference spectrum obtained from an IR sample containing caged ATP but no SR vesicles. This spectrum, which appears instantly and does not change with time as does the ATPase spectrum (Fig. 1a), was scaled to fit with the 1524 cm^{-1} and 1342 cm^{-1} peaks of the ATPase difference spectrum observed 2 min after ATP release (dashed line in Fig. 1a). The modification of several chemical groups of the caged ATP molecule [18] upon photolysis (inset in Fig. 1c) reflects in this difference spectrum between caged ATP and its photolysis products. For example, the minima at 1524 cm^{-1} and 1342 cm^{-1} can be assigned to the asymmetrical and symmetrical stretching vibrations of the nitro group of caged ATP, which disappears during photolysis. The bonds below 1270 cm^{-1} can be attributed to a diminution of P-O double bond character in the γ-phosphate, and presumably to the breaking of a C–O bond. No remarkable absorbance changes appear in the region between 1800 cm^{-1} and 1550 cm^{-1} in the caged ATP model sample.

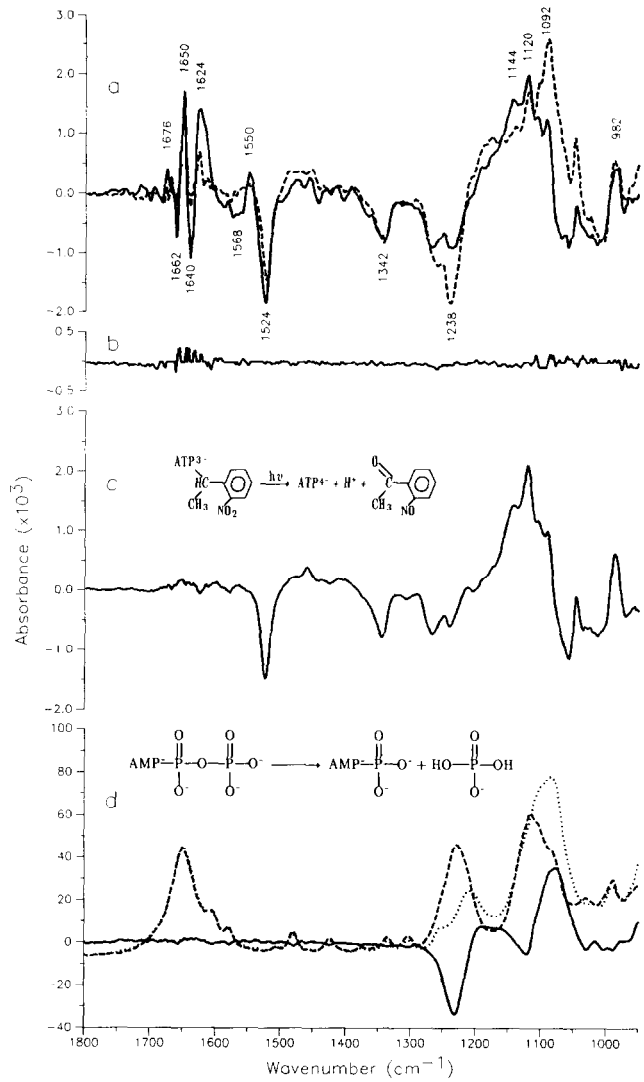

Fig. 1. (a) Flash-induced IR difference spectra due to ATP release from caged ATP in an active SR ATPase sample, calculated from a single beam spectrum recorded before the photolysis flash and single beam spectra recorded within the first 8 s (full line) and at 2 min (dashed line) after release of approx. 6.5 nmol ATP. Conditions: 20 interferometer scans, T = 0°C. (b) Control spectrum from two single-beam spectra recorded before the photolysis flash. (c) IR difference spectrum due to the release of approx. 19 nmol ATP from caged ATP in a sample *without* ATPase. The difference spectrum was scaled to fit with the 1524 cm^{-1} and 1342 cm^{-1} peaks of Fig. 1a. Inset: structural formula of caged ATP and its photolysis products. (d) IR absorbance spectra of 100 mM ATP (dashed line) and 100 mM ADP + P_i (dotted line) at pH 6.8 in a thin-layer cell (approx. 6 μm optical pathlength). Full line: difference (ADP + P_i) minus (ATP). Inset: Structural formula of ATP and (ADP + P_i).

Fig. 1d shows the absorbance spectra of ATP (dashed line) and {ADP + P_i} (dotted line). The difference spectrum {ADP + P_i} minus {ATP} (full line) shows a minimum at 1230 cm^{-1} and two maxima at 1170 cm^{-1} and 1080 cm^{-1}. The main features in this difference spectrum arise from the difference in P-O bond electron density. *No* absorbance difference is observed in the region from 1800 cm^{-1} to 1300 cm^{-1}.

Comparing the absorbance changes due to caged ATP photolysis (Fig. 1c) and to ATP hydrolysis (full line in Fig. 1d) with the ATPase difference spectra in Fig. 1a, it is evident that the *permanent* absorbance changes (i) of the ATPase sample (bands at 1524 cm^{-1}, 1342 cm^{-1}, 1270–950 cm^{-1} in Fig. 1a) can be attributed to photolysis of caged ATP. In addition, the slow absorbance changes (ii) between 1300 cm^{-1} and 1000 cm^{-1} of the ATPase sample are predominantly due to hydrolysis of ATP.

The absorbance change at 1238 cm^{-1} (ATP P-O vibration) due to ADP hydrolysis provides an elegant in situ assay for the activity of the ATPase IR sample, in addition to the assay for Ca^{2+} transport described in [22]. Typical sample activities were about 0.25 $\mu mol \cdot min^{-1} \cdot (mg\ protein)^{-1}$ for the first 20s at 5°C. It is important to note that careful drying and rehydration does not affect the functional properties of the ATPase.

The transient absorbance changes (iii) in the 1800 cm^{-1}–1520 cm^{-1} region (Fig. 1a) cannot be explained by the reactions of caged ATP photolysis and ATP hydrolysis. We attribute these changes to ligand binding and conformational changes in the ATPase during the catalysis of Ca^{2+}-transport. This is supported by FTIR difference spectra of ATPase samples with caged ADP instead of caged ATP and of EGTA-inhibited [24] samples (data not shown). The transient absorbance changes of the 'normal' ATPase sample (Fig. 1a) are not observed in these samples. However, very small bands just above the noise level in the 1700 cm^{-1}–1600 cm^{-1} spectral region are observed, which may correspond to nucleotide binding to the ATPase without resulting catalytic activity [23].

On the basis of a recent FTIR analysis of the Ca^{2+}-ATPase in the Ca_2E_1 state and an E_2P-like state, Arrondo et al. [14] have claimed an additional α-helical structure, a missing β-sheet or turn as well as a modified lipid–protein interaction for the E_2 state with respect to the Ca_2E_1 state. However, these differences could not be confirmed by other groups [12,13]. The method described here to start the reaction cycle by release of ATP from caged ATP appears to be considerably more sensitive to detect changes of IR absorbance during catalytic activity than the comparison of absorbance spectra from different samples. In fact, the low level of noise as indicated in Fig. 1b enables us to resolve absorbance changes as small as 0.1% of total protein absorbance in the amide I region (approx. 5×10^{-4} AU of 0.5 AU). This sensitivity corresponds to the absorbance of single peptide C=O groups.

The changes in IR absorption observed under steady state activity (Fig. 1a) are very small compared to the total protein absorbance. Under conditions close to ours [25–27], the ATPase was found to accumulate to 75–90% in the phosphorylated intermediate E_1P. We thus conclude that the $Ca_2E_1 \rightarrow Ca_2E_1P$ transition mainly contributing to the difference spectrum in Fig. 1a is *not* accompanied by major conformational changes. This view seems to be supported by only very small changes in the amide II region (1540 cm^{-1}–1550 cm^{-1}). The small size of the difference bands in the amide I region as well as the highly structured band features favour an interpretation in terms of localized structural modifications at around the phosphorylation and the calcium binding site. In the spectral region above 1700 cm^{-1}, absorption from the phospholipid C=O groups might contribute around 1730 cm^{-1}–1740 cm^{-1}. From the almost complete absence of difference bands in this spectral region (Fig. 1a), we conclude that the lipid–protein interaction is not perturbed upon accumulation in the active state that leads to the difference spectrum in Fig. 1a.

At present, the molecular interpretation of the difference spectra is far from being complete. Preliminary deuteration experiments (data not shown) have indicated that the main bands in Fig. 1a at 1662 cm^{-1}, 1650 cm^{-1}, 1640 cm^{-1} and 1624 cm^{-1} remain almost unchanged in position. However, additional bands in the region above 1700 cm^{-1} indicate that ^1H-^2H substitution, in addition to the mass effect on IR vibrational absorption, causes a different equilibrium of E states in the reaction cycle to be accumulated, presumably by a kinetic isotope effect.

In summary, the photolysis of caged ATP represents an elegant trigger for the IR difference spectroscopy of ATPases and permits a sensitive probing of the molecular processes during catalytic activity. Since the photolysis reaction and hydrolysis of ATP do not produce changes in infrared absorbance in the amide I and amide II region of protein absorbance, the molecular processes in ATPases can be studied on a level of sensitivity, which has before only been reached for proteins performing light-induced or redox-induced reactions. A further assignment of bands will make use of chemical modification of amino acid side chain groups as well as of site-directed mutagenesis. In addition, the conformational dynamics of other steps of the reaction cycle can be studied using different sample conditions and further photolabile 'caged' substrate analogues.

Acknowledgements: The authors would like to thank Prof. W. Hasselbach and Dr R.S. Goody, MPI Heidelberg, for generous gifts of Ca^{2+}-ATPase and caged ADP.

REFERENCES

[1] Inesi, G. and De Meis, L. (1985) in: The Enzymes of Biological Membranes, vol. 3 (Martonosi, A. ed.) pp. 157–191, Plenum Press, New York.
[2] Hasselbach, W. (1974) in: The Enzymes (Boyer, P.D. ed.) pp. 431–467, Academic Press, New York, London.
[3] Andersen, J.P. (1989) Biochim. Biophys. Acta 988, 47–72.
[4] De Meis, L. and Vianna, A. (1979) Annu. Rev. Biochem. 48, 275–292.
[5] Carvalho, M., de Souza, D. and De Meis, L. (1976) J. Biol. Chem. 251, 3629–3636.

[6] Champeil, P., Le Maire, M., Möller, J.V., Riollet, S., Guillain, F. and Green, N.M. (1986) FEBS Lett. 206, 93–98.
[7] Dupont, Y. and Leigh, J.B. (1978) Nature 273, 396–398.
[8] Fernandez-Belda, F., Kurzmack, M. and Inesi, G. (1984) J. Biol. Chem. 259, 9687–9698.
[9] Andersen, J.P., Lassen, K. and Möller, J.V. (1985) J. Biol. Chem. 260, 371–380.
[10] Obara, M., Suzuki, H. and Kanazawa, T. (1988) J. Biol. Chem. 263, 3690–3697.
[11] Andersen, J.P., Jörgensen, P.L. and Möller, J.V. (1985) Proc. Natl. Acad. Sci. USA 82, 4573–4577.
[12] Villalain, J., Gomez-Fernandez, J.C., Jackson, M. and Chapman, D. (1989) Biochim. Biophys. Acta 978, 305–312.
[13] Buchet, R., Carrier, D., Wong, P.T.T., Jona, I. and Martonosi, A. (1990) Biochim. Biophys. Acta Bio-Membr. 1023, 107–118.
[14] Arrondo, L.R., Mantsch, H.H., Mullner, N., Pikula, S. and Martonosi, A. (1987) J. Biol. Chem. 262, 9037–9043.
[15] Kaplan, J.H., Forbush III, B. and Hoffman, J.F. (1978) Biochemistry 17, 1929–1935.
[16] McCray, J.A., Herbette, L., Kihara, T. and Trentham, D.R. (1980) Proc. Natl. Acad. Sci. USA 77, 7237–7241.
[17] Siebert, F., Mäntele, W. and Kreutz, W. (1982) FEBS Lett. 141, 82.
[18] Mäntele, W., Nabedryk, E., Tavitian, B.A., Kreutz, W. and Breton, J. (1985) FEBS Lett. 187, 227–232.
[19] Moss, D.A., Nabedryk, E., Breton, J. and Mäntele, W. (1990) Eur. J. Biochem. 187, 565–572.
[20] Mäntele, W., Leonhard, M., Bauscher, M., Nabedryk, E., Breton, J. and Moss, D.A. (1990) in: Reaction Centers of Photosynthetic Bacteria, Structure and Dynamics (Michel-Beyerle, M.E. ed.) Springer Series in Biophysics (in press).
[21] De Meis, L. and Hasselbach, W. (1971) J. Biol. Chem. 246, 4759–4763.
[22] Barth, A., Mäntele, W. and Kreutz, W. (1989) in: Spectroscopy of Biological Molecules - State of the Art (Bertoluzza, A., Fagnano, C. and Monti, P. eds) pp. 245–246, Societa Editrice Esculapio, Bologna.
[23] Barth, A., Mäntele, W. and Kreutz, W. (1990) Biochim. Biophys. Acta (submitted).
[24] Petithory, J.R. and Jencks, W.P. (1988) Biochemistry 27, 5553–5564.
[25] Nakamura, Y. and Tonomura, Y. (1982) J. Biochem. 91, 449–461.
[26] Shigekawa, M., Wakabayashi, S. and Nakamura, H. (1983) J. Biol. Chem. 258, 14157–14161.
[27] Shigekawa, M. and Dougherty, J.P. (1978) J. Biol. Chem. 253, 1451–1457.