Journal of Bioenergetics and Biomembranes, Vol. 34, No. 4, August 2002 (© 2002)

Phosphorylation of a Peptide Related to Subunit *c* of the F_0F_1-ATPase/ATP Synthase and Relationship to Permeability Transition Pore Opening in Mitochondria

Tamara S. Azarashvili,[1,3,4] Jaana Tyynelä,[3] Irina V. Odinokova,[1] Pavel A. Grigorjev,[2,4] Marc Baumann,[3] Yuri V. Evtodienko,[1] and Nils-Erik L. Saris[4,5]

Received October 31, 2001; accepted May 1, 2002

A phosphorylated polypeptide (ScIRP) from the inner membrane of rat liver mitochondria with an apparent molecular mass of 3.5 kDa was found to be immunoreactive with specific antibodies against subunit *c* of F_0F_1-ATPase/ATP synthase (Azarashvily, T. S., Tyynelä, J., Baumann, M., Evtodienko, Yu. V., and Saris, N.-E. L. (2000). *Biochem. Biophys. Res. Commun.* **270**, 741–744. In the present paper we show that the dephosphorylation of ScIRP was promoted by the Ca^{2+}-induced mitochondrial permeability transition (MPT) and prevented by cyclosporin A. Preincubation of ScIRP isolated in its dephosphorylated form with the mitochondrial suspension decreased the membrane potential ($\Delta\Psi_M$) and the Ca^{2+}-uptake capacity by promoting MPT. Incorporation of ScIRP into black-lipid membranes increased the membrane conductivity by inducing channel formation that was also suppressed by antibodies to subunit *c*. These data indicate that the phosphorylation level of ScIRP is influenced by the MPT pore state, presumably by stimulation of calcineurin phosphatase by the Ca^{2+} used to induce MPT. The possibility of ScIRP being part of the MPT pore assembly is discussed in view of its capability to induced channel activity.

KEY WORDS: Ca^{2+}; calcineurin; F_0F_1-ATPase; mitochondria; permeability transition; phosphorylation; protein phosphatase; subunit *c*.

Key to abbreviations: $\Delta\Psi_M$, mitochondrial transmembrane potential; CCCP, *m*-chlorocarbonylcyanide phenylhydrazone; CsA, cyclosporin A; DCCD, dicyclohexylcarbodiimide; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N′*-(2-ethanesulfonic acid); MPT, the mitochondrial permeability transition; NET, 150 mM NaCl, 1 mM EDTA, 10 mM Tris–Cl, pH 8.0; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecylsulfate polyacrylamide electrophoresis; ScIRP, F_0F_1-ATPase/ATP synthase subunit *c*-immunoreactive polypeptide, 3.5 kDa peptide; TLC, thin-layer chromatography.

[1]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, Russia.

[2]Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, Russia.

[3]Institute of Biomedicine, Biomedicum, Viikki Biocenter 1, University of Helsinki, Helsinki, Finland.

[4]Department of Applied Chemistry and Microbiology, Viikki Biocenter 1, University of Helsinki, Helsinki, Finland.

[5]To whom correspondence should be addressed at Department of Applied Chemistry and Microbiology, Viikki Biocenter 1, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland; e-mail: nils-erik.saris@helsinki.fi.

INTRODUCTION

Phosphorylation and dephosphorylation of target proteins is common in the regulation of intracellular metabolism by signal transduction pathways. In mitochondria, the regulation by phosphorylation and dephosphorylation of the activities of pyruvate dehydrogenase and branched-chain α-oxoacid dehydrogenase is well known (Bradford and Yedman, 1986; Sarrouille and Boudry, 1996). Various cAMP-dependent and independent protein kinases have been found in the inner membrane and in the intermembrane space of mitochondria (Henrikson and Gergil, 1979; Kitagawa and Racker, 1982; Schwoch *et al.*, 1990; Technikova-Dobrova *et al.*, 2001; Verdanis, 1977), and phosphorylation of several membrane-bound proteins, 6.5, 18, 29 kDa, has been shown (Papa *et al.*, 1999; Sardanelli *et al.*, 1996; Technikova-Dobrova *et al.*, 2001). The 18-kDa species has been identified as the

0145-479X/02/0800-0279/0 © 2002 Plenum Publishing Corporation

AQDQ subunit of complex I of the respiratory chain (Sardanelli *et al*., 1995). Recently we reported that a low-molecular mass peptide (apparent M_R 3.5 kD) in the inner membrane of rat liver mitochondria (RLM)[1] was phosphorylated, presumably by the cAMP-dependent protein kinase (PKA) (Azarashvily *et al.*, 1999). The peptide was found to react with a specific antiserum to subunit *c* of F_0F_1-ATPase (Azarashvily *et al.*, 2000), which confirmed a similar study where another antiserum against subunit *c* was used (Hagopian, 1999). This authors considered the low kDa in view of the molecular mass of subunit *c*, 7602, which is close to the 6.5 kDa found for the phosphorylated protein reported by the Papa group, see above, and for subunit *c* (DCCD-binding proteolipid) by another group (Glaser *et al*., 1981) . The variation in apparent kDa may be due to variations in the electrophoretic technique and its calibration, or it may reflect a property of subunit *c* or otherwise be related to it. Phosphorylation and dephosphorylation of this subunit *c*-immunoreactive peptide (ScIRP) is modulated by physiological concentrations of Ca^{2+}, and correlates with ATPase activity (Evtodienko *et al.*, 2000).

It is widely accepted that subunit *c* of mitochondrial or bacterial F_0F_1-ATPase is subjected to proton-motive force-driven rotation in the inner mitochondrial membrane, and takes part in H^+ transport through the membrane (Fillingame *et al.*, 2000). The role of protonation and deprotonation of Glu58 or Asp61 in the H^+-transporting pathway has been discussed (Kluge and Dimroth, 1993). Direct involvement of subunit *c* of F_0F_1-ATPase in transmembrane ion transport has not been shown yet.

It is known that subunit *c* homologs are present in the plasma and intracellular membranes and perform multiple functions. The subunits *c* of the vacuolar, V type H^+-ATPases are able to form a H^+-conducting pathway (Finbow and Harrison, 1997; Stevens, 1997) and another subunit *c*-like protein in gap-junction structures may form large channels permeable for small molecules (Dunlop *et al.*, 1995; Levy, 1996). Recently it was shown that subunit *c* homologs in neuronal plasma membrane (McGeoch and Guiotti, 1997) are able to form transmembrane ion channels, and in artificial membranes subunit *c* and the channel-forming activity was strongly modified by Ca^{2+}, cGMP, and dicyclocarbodiimide (DCCD) (McGeoch *et al.*, 2000).

In the mitochondrial permeability transition (MPT) the permeability is dramatically increased by the opening of a large pore (Bernardi, 1992; Bernardi *et al*., 1994). The relation between MPT and subunit *c* of mitochondrial F_0F_1-ATPase and its phosphorylation has not been studied. The data presented in this study demonstrate a strong decrease in the phosphorylation level of ScIRP during Ca^{2+}-induced pore opening, which was prevented by CsA, the inhibitor of MPT. An increase in the conductivity of BLM and mitochondrial inner membrane modified by dephosphorylated ScIRP was also demonstrated.

MATERIAL AND METHODS

Liver mitochondria were isolated from male Wistar rats (150–200 g) by an established procedure (Johnson and Lardy, 1967) using a buffer containing 210 mM mannitol, 20 mM sucrose, 5 mM EGTA, and 5 mM HEPES (pH 7.5). The mitochondria were washed and resuspended in the same buffer without EGTA. Samples of ScIRP were purified as described earlier (Azarashvily *et al.*, 2000) from mitochondria that had undergone Ca^{2+}-induced MPT using chloroform/methanol extraction and preparative electrophoresis.

Changes in the mitochondrial membrane potential ($\Delta\Psi_M$) were followed with the aid of a tetraphenylphosphonium (TPP^+)-sensitive electrode (Kamo *et al*., 1979). The medium contained 100 mM KCl, 50 mM sucrose, 5 mM succinate, 5 mM HEPES, pH 7.5, rotenone (2 μg/mL) and oligomycin (4 μg/mL). The mitochondrial protein concentration was 3 mg/mL. The same conditions were used for the phosphorylation of mitochondrial proteins with addition of $\gamma[^{32}P]$-ATP, $MgCl_2$, and cold ATP to aliquots of the mitochondrial suspension to give a final concentration equal to 1 mM Mg and 300 μM ATP, containing 5–7 μCi $\gamma[^{32}P]$-ATP in the samples. Mitochondria were incubated in the presence of $\gamma[^{2}P]$-ATP for 3 min and the protein phosphorylation was terminated by adding Laemmli buffer and boiling for 3 min.

SDS-PAGE was performed according to Laemmli (1970) using 15% gels for preparative electrophoresis and 17% gels for analytical study and blotting. Proteins on gels were visualized by Coomassie Brilliant Blue or Silver staining, and protein phosphorylation was detected by autoradiography using Kodak X-omat AR-5 film. Absorbance of the $\gamma[^{32}P]$-labeled bands was measured using the densitometer SD-1M (SCB-Pushchino). For immunoblotting, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes and stained for subunit *c* detection using a polyclonal antiserum (a kind gift of Dr. D. N. Palmer, Lincoln University, New Zealand (Palmer *et al.*, 1995), peroxidase-conjugated species-specific secondary antibodies, and chemiluminescence.

Black-lipid membranes (BLM) were formed in a circular hole in a teflon cylinder placed in a rectangular glass cuvette (25 mL). A mixture (1:3) of L-phosphatidylcholine (PC 32:0) and L-phosphatidylserine (PS 32:0) (20 mg/mL) dissolved in heptane was used for formation of unmodified BLM. The BLM was modified by ScIRP, corresponding

to 20–30 μg of mitochondrial protein, first dried under nitrogen and then dissolved in heptane. The electrolyte solutions surrounded BLM were prepared according to McGeoch and Guiotti, 1997. The membrane voltage applied was 50 mV. The area of BLM was estimated from the heights of the capacitive current spikes generated by applying to BLM voltage of 20 mV. The voltage-clamp method was used for measurement of membrane current as described by Mikkola *et al.*, 1999.

RESULTS

Dephosphorylation of ScIRP and MPT Pore Opening

In order to study the effect of a threshold Ca^{2+} overload—that induces MPT—on $\Delta\Psi_M$, RLM were incubated under different conditions and $\Delta\Psi_M$ constantly monitored (Fig. 1, panel A). Aliquots of the mitochondrial suspension at the points marked in Fig. 1(A) were taken for further incubation in the presence of $\gamma[^{32}P]$-ATP in order to assess the phosphorylation level of ScIRP. As shown in Fig. 1 (panel A), at the threshold Ca^{2+} load, a dissipation of $\Delta\Psi_M$ took place and CsA—a potent inhibitor of MPT pore opening—was able to prevent Ca^{2+}-induced loss of $\Delta\Psi_M$. Samples were taken before and after the addition of the threshold Ca^{2+} load (points 1 and 2), before and after the same Ca^{2+} load in the presence of CsA, when there was no MPT pore opening (points 3 and 4), and finally after the $\Delta\Psi$ drop induced by addition of the uncoupling agent *m*-chlorocarbonylcyanide phenylhydrazone (CCCP) (point 5). Figure 1 (panel B) shows typical autoradiograms of labeled ScIRP and Fig. 1 (panel C), the mean data, characterizing the level of ^{32}P incorporation into ScIRP in the samples 1–5. These data show that after the Ca^{2+}-induced MPT pore opening, ^{32}P-labeling of ScIRP was strongly decreased (compare samples 1 and 2). On the contrary, after the same Ca^{2+} load in the presence of CsA the labeling of ScIRP was increased (compare samples 3 and 4). Very low ^{32}P incorporation into ScIRP was observed after CCCP-induced collapse of $\Delta\Psi_M$ (sample 5).

Effect of ScIRP on Permeability of BLM and Mitochondrial Membranes

The possible effect of ScIRP on the permeability of mitochondrial membranes was studied using ScIRP obtained from RLM by preparative electrophoresis. The purity of the used ScIRP was checked by Western blotting (Fig. 2, panel C), and samples of pure subunit *c* (panel A) and RLM (panel B) were used as controls.

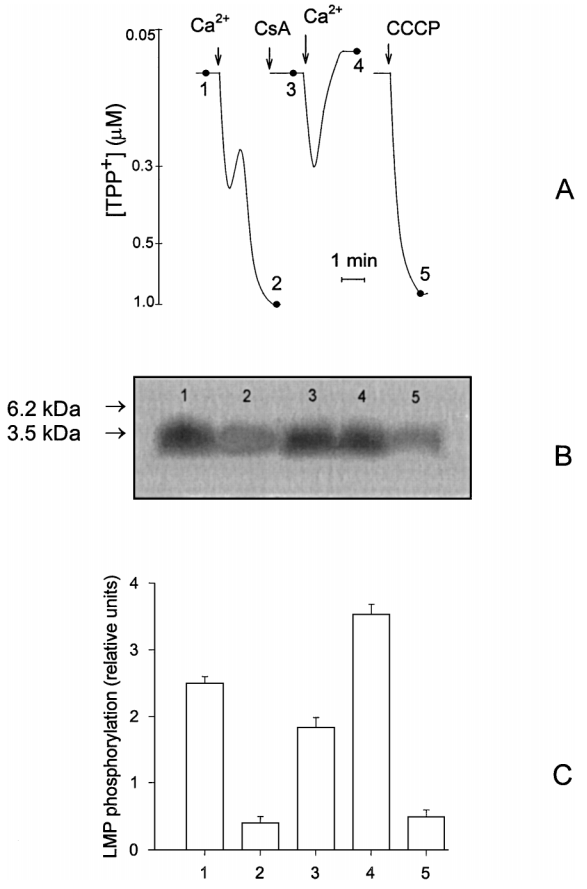

Fig. 1. The level of ScIRP phosphorylation during MPT pore opening/closing in mitochondria. Mitochondria (3 mg protein/mL) were preincubated for 2 min in incubation medium and threshold amounts of Ca^{2+} was added to mitochondria suspension to induce mPTP opening. $\Delta\Psi_M$ was measured using TPP^+ electrode (see Material and Methods). Aliquots of the mitochondrial suspension was taken (before and after Ca^{2+}-induced MPT pore opening, and also in the presence CsA or CCCP) at the points 1–5, mixed with $\gamma[^{32}P]$ATP containing ATP and incubated for 3 min for protein phosphorylation (samples 1–5). The reaction was terminated by addition of Laemmli sample buffer. Panel A, changes in the $\Delta\Psi_M$ under Ca^{2+} load or after CCCP addition. Ca^{2+} (200 μM), CsA (1 μM), and CCCP (0.5 nM) were added to mitochondria at points indicated by arrows. Panel B, autoradiogram of phosphorylated ScIRP in samples 1–5; Panel C, average levels of ScIRP phosphorylation in samples 1–5.

Mitochondria were preincubated in the absence and in the presence of ScIRP or antibodies to subunit *c* for 1.5 h at 4°C. The mitochondria were then transferred to the incubation medium and $\Delta\Psi_M$ was measured under conditions of addition of Ca^{2+} load. Mitochondrial samples were taken at the points shown (points 1–6; Fig. 3, panel A) and levels of ScIRP phosphorylation were examined (Fig. 3, panel B) as described in Materials and Methods. As shown in Fig. 3, panel A, $\Delta\Psi_M$ dissipation was observed after the second Ca^{2+} pulse that lead

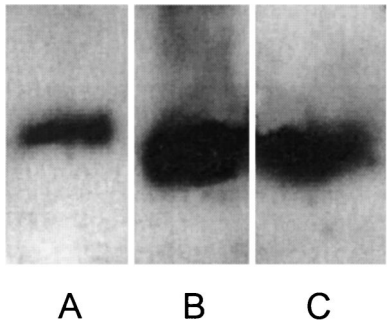

Fig. 2. Immunoblot stained for subunit *c* of F_0F_1-ATPase/ATP synthase of RLM. Lane A, subunit *c* (control); Lane B, RLM proteins; Lane C, ScIRP purified from RLM. Experimental conditions, see Material and Methods.

to ScIRP dephosphorylation (compare samples 1 and 2). Preincubation of ScIRP with the mitochondrial suspension induced an irreversible drop in $\Delta\Psi_M$ after the first Ca^{2+} addition, indicating enhanced MPT pore opening, which was again associated with strong decrease in ScIRP phosphorylation (compare samples 3 and 4, panel B). In the presence of subunit *c* antibodies, some resistance of MPT pore opening under Ca^{2+} load was seen and the level of ScIRP phosphorylation was increased (samples 5 and 6, panel B). In these experiments ScIRP was present mainly in the dephosphorylated form. The effect of the phosphorylated form of ScIRP on MPT was smaller. However, this effect was poorly reproducible, perhaps as a result of variation in the phosphorylation level

The channel-forming properties of ScIRP were tested using the BLM technique. Phospholipids were mixed with ScIRP and BLM were formed. Single channel opening and closing was observed (Fig. 4). Single channel conductance was in the range of 0.4–0.5 nS. The specific conductivity of unmodified BLM was about 10 nS/cm^2 and no channels were seen in BLM. With a 20× increased amount of ScIRP incorporated in BLM, the conductivity was increased up to 0.5–1 μS/cm^2. When subunit *c* antibodies were added to the electrolyte solution, the conductivity was reduced by 70–80%.

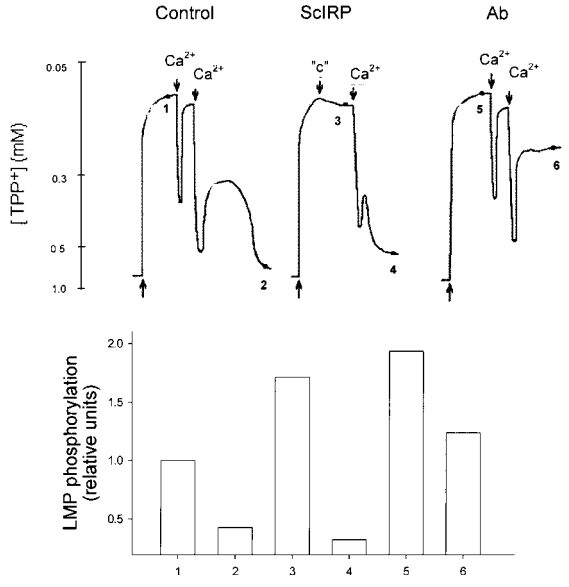

Fig. 3. Effect of ScIRP and antibodies against subunit *c* on the MPT pore opening/closing. RLM were preincubated for 1.5 h at +4°C in the absence of added ScIRP (control mitochondria), in the presence of added ScIRP (ScIRP-treated mitochondria) and in the presence of subunit *c* antiserum (Ab-treated mitochondria). The amount of added ScIRP corresponded to 80 mg mitochondrial protein, and antiserum was used in 1:500 dilution. Then the mitochondria were incubated at +25°C and $\Delta\Psi_M$ and ScIRP phosphorylation were measured as in Fig. 1. Mitochondrial samples for analyzing ScIRP phosphorylation were taken at points 1–6. Panel A, changes in $\Delta\Psi_M$ under threshold Ca^{2+} load; Panel B, average levels of ScIRP phosphorylation in samples 1–6.

DISCUSSION

As shown in the present study, the level of phosphorylation of ScIRP was strongly decreased during MPT pore opening induced by a Ca^{2+} overload, and CsA was able to prevent this. The rates of both phosphorylation and dephosphorylation are at a minimum at [Ca^{2+}] of 1 μM and slightly below (Evtodienko *et al.*, 2000). The observed drop in ScIRP phosphorylation level associated with MPT pore opening is likely to be mainly due to increased rate of dephosphorylation at the higher [Ca^{2+}]. MPT may also influence these processes indirectly by changing the conformation of inner membrane components and inducing mitochondrial swelling. The higher level of phosphorylation when MPT is blocked by CsA (Fig. 1) is in favor of such a mechanism.

The nature of the protein phosphatase catalyzing the dephosphorylation of ScIRP is not known with certainty. In mitochondria there is a serine/threonine specific phosphatase of the PP2C type has been identified in dephosphorylation of the 18 kDa protein (Signorile *et al.*, 2002). It is however inhibited by Ca^{2+}. For the subunit *c* dephosphorylation one should look for a Ca^{2+}-activated phosphatase. A likely candidate is a type PP2B phosphatase, the calcineurin phosphatase activated by a complex between cytosolic cyclophilin, calmodulin and Ca^{2+} (Rusnak and Mertz, 2000). Under certain conditions, calcineurin has been shown to be tightly bound with mitochondrial membranes in the presence of Bcl-2 protein family members (Catell *et al.*, 1971; Hemenway and Heitman, 1999). In addition, the presence of several other calmodulin-binding

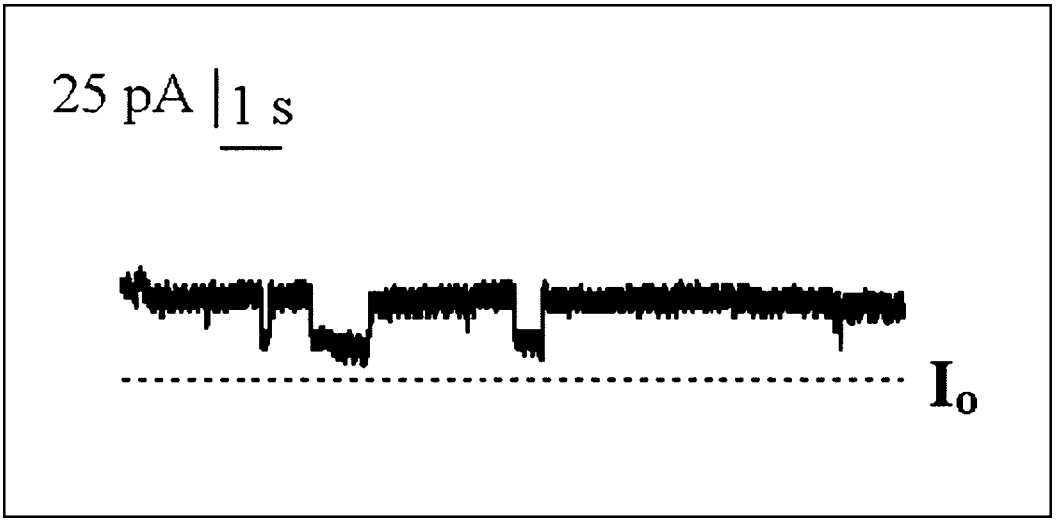

Fig. 4. Channel-forming ability of ScIRP in BLM. Experimental conditions, see Material and Methods.

proteins in highly purified mitochondria has been reported (Gazotti *et al.*, 1984). CsA blocks MPT pore opening by binding to mitochondrial cyclophilin whereby it is removed from the pore assembly (Bernardi *et al.*, 1994; Halestrap *et al.*, 1998; Nicolli *et al.*, 1996). CsA also binds to the cytosolic cyclophilin D, creating a composite surface able to make high affinity contacts with calcineurin and specifically inhibit its phosphatase activity (Papageorgiu *et al.*, 1994). Cyclophilin D also binds to the mitochondrial MPT pore complex under certain conditions (Halestrap *et al.*, 1998). Our data indicate that Ca^{2+}-induced and CsA-sensitive dephosphorylation of ScIRP takes place during MPT pore opening, and these reactions are supposed to be connected with cyclophilin and possibly regulated by calcineurin.

Incorporation of ScIRP into BLM increased the permeability by forming single channels (Fig. 4). The properties of these channels were similar to those of the channels composed of subunit *c* isolated from neuronal plasma membranes (McGeoch *et al.*, 2000). Subunit *c* of the F_0F_1-ATPase belongs to a highly conserved family of polypeptides localized in various biomembranes and implicated in diverse transport processes (Dunlop *et al.*, 1995; Holzenburg *et al.*, 1993; Levy, 1996). One is a 16-kDa proteolipid, the ductin or subunit c of the vacuolar V type H^+-ATPase, involved in H^+ transport (Finbow and Harrison, 1997; Harvey and Nelson, 1992); another is the mediatophore-phospholipid complex in synaptosomes that participate in neuromediator transport (Birman *et al.*, 1990); and finally, there is the 16-kDa polypeptide of gap-junction like structures that form channels for small molecules (Finbow and Harrison, 1997). The channel-forming capability of these proteins is dependent on their topological orientation in the membrane (Dunlop *et al.*, 1995; Levy, 1996), on interaction with other proteins in multiprotein structures (Harrison *et al.*, 1994), and can be regulated by Ca^{2+} (Israel *et al.*, 1986; McGeoch *et al.*, 2000; Peters *et al.*, 2001).

As shown in Fig. 3, addition of ScIRP promoted MPT pore opening, which was prevented by antibodies against subunit *c*. Several mechanisms for the interaction between ScIRP and the MPT pore could be thought of. Binding of the hydrophobic ScIRP to the pore assembly could change its conformation, inducing pore opening. An interesting model is formation of CsA-sensitive pores by aggregated mis-folded integral proteins, which thus could become part of the pore assembly. Added ScIRP could well act in this way. The channel activity (Fig. 4) could be involved, making possible an access of ions to pore components that promote pore opening. The channel activity would also affect the $\Delta\Psi_M$, a lowering of which promotes pore opening (Bernardi, 1992). The correlation between the MPT pore state and the ScIRP phosphorylation further supports a close connection between the pore assembly and ScIRP. The dephosphorylated form of ScIRP may thus be involved in the MPT pore formation or it may independently increase membrane permeability during MPT.

It was early shown that Mg^{2+} counteracts the Ca^{2+}-induced Ca^{2+} release in mitochondria (Saris, 1963) that we now know is due to MPT. Increase in $[Mg^{2+}]$ was recently shown to increase ScIRP phosphorylation (Saris *et al.*, 2001). The effect of CsA in preventing the dephosphorylation of ScIRP and MPT opening indicates a

possible involvement of cyclophilin and calcineurin in the regulation of the extent of ScIRP phosphorylation. Further studies are in progress on this question and on the effect of phosphorylation/dephosphorylation on the function of ScRP.

ACKNOWLEDGMENTS

This study has been supported by grants from the Russian Foundation for Fundamental Investigations (RFFI N 02-04-49701), by travel grants for Russian scientists from the Academy of Finland, and research grants to Dr Saris from the Finnish Society of Sciences and Letters, the Magnus Ehrnrooth's Foundation, The Sigrid Jusélius Foundation, and the Medical Society of Finland.

REFERENCES

Azarashvili, T. S., Odinokova, I. V., and Evtodienko, Yu. V. (1999). *Biochemistry* (*Moscow*) **64**, 668–673.

Azarashvily, T. S., Tyynela, J., Baumann, M., Evtodienko, Yu. V., and Saris, N.-E. L. (2000). *Biochem. Biophys. Res. Commun*. **270**, 741–744.

Bernardi, P. (1992). *J. Biol. Chem.* **267**, 8834–8839.

Bernardi, P., Broekemeier, K. M., and Pfeiffer, D. R. (1994). *J. Bioenerg. Biomembr.* **26**, 509–517.

Birman, S., Meunier, F.-M., Lesbats, B., Le Caer, J.-P., Rossier, J., and Israel, M. (1990). *FEBS Lett.* **261**, 303–306.

Bradford, A. P., and Yedman, S. J. (1986). In *Advances in Protein Phosphatase, Vol.* 3, (Merlvede, W., and Di Salvo, J., eds.), Leun University Press, Belgium, pp. 73–106.

Catell, K. J., Lindop, C. R., Knight, I. G., and Beechey, R. B. (1971). *Biochem. J.* **125**, 169–177.

Dunlop, J., Jones, P. C., and Finbow, M. E. (1995). *EMBO J*. **14**, 3609–3616.

Evtodienko, Yu. V., Azarashvili, T. S., Teplova, V. V., Odinokova, I. V., and Saris, N.-E. (2000). *Biochemistry* (*Moscow*) **65**, 1023–1026.

Fillingame, R. H., Jiang, W., and Dmitriev, O. Y. (2000). *J. Exp. Biol*. **203**, 9–17.

Finbow, M. E., and Harrison, M. A. (1997). *Biochem. J*. **324**, 697–712.

Gazotti, P., Gloor, M., and Carafoli, E. (1984). *Biochem. Biophys. Res. Commun*. **119**, 341–351.

Glaser, E., Norling, B., and Ernster, L. (1981). *Eur. J. Biochem*. **115**, 189–196.

Hagopian, K. (1999). *Anal. Biochem*. **273**, 240–251.

Halestrap, A. P., Kerr, P. M., Javadov, S., and Woodfield, K.-Y. (1998). *Biochim. Biophys. Acta* **1366**, 79–94.

Harrison, M. A., Jones, P. C., Kim Yu.-I., Finbow, M. E., and Findlay, J. B. C. (1994). *Eur. J. Biochem*. **221**, 111–120.

Harvey, W. R., and Nelson, N. (1992). *J. Exp. Biol*. **172**, 1–485.

He, L., and Lemasters, J. J. (2002). *FEBS Lett*. **512**, 1–7.

Hemenway, C. S., and Heitman, J. (1999). *Cell Biochem. Biophys*. **30**, 115–151.

Henrikson, T. H., and Gergil, B. (1979). *Biochem. Biophys. Acta* **588**, 380–381.

Holzenburg, A., Jones, P. C., Franklin, T., Pali, T., Heimburg, T., Marsh, D., Findlay, J. B. C., and Finbow, M. E. (1993). *Eur. J. Biochem*. **213**, 21–30.

Israel, M., Morel, N., Lesbats, B., Birman, S., and Manaranche, R. (1986). *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9226–9230.

Johnson, D., and Lardy, H. A. (1967). *Methods Enzymol*. **10**, 94–96.

Kamo, N., Muratsugu, M., Hongoh, R., and Kobatke, Y. (1979). *J. Membr. Biol*. **49**, 105–121.

Kitagawa, Y., and Racker, E. (1982). *J. Biol. Chem.* **257**, 4547–4551.

Kluge, C., and Dimroth, P. (1993). *Biochemistry* **32**, 10378–10386.

Laemmli, U. K. (1970). *Nature* **227**, 680–685.

Levy, D. (1996). *Essays Biochem.* **31**, 49–60.

McGeoch, J. E. M., and Guiotti, G. (1997). *Brain Res.* **766**, 188–194.

McGeoch, J. E. M., McGeoch, M. W., Mao, R., and Guiotti, G. (2000). *Biochem. Biophys. Res*. *Commun.* **277**, 835–840.

Mikkola, R., Saris, N.-E. L., Grigoriev, P. A., Andersson, W. A., and Salkinoja-Salonen, M. S. (1999). *Eur. J. Biochem*. **263**, 112–117.

Nicolli, A., Basso, E., Petronilli, V., Wenger, R. M., and Bernardi, P. (1996). *J. Biol. Chem*. **271**, 2185–2192.

Palmer, D. N., Bayliss, S., and Westlake, V. J. (1995). *Am. J. Med. Genet.* **57**, 260–265.

Papa, S., Sardanelli, A. M., Scacco, S., and Technikova-Dobrova, Z. (1999). *FEBS Lett.* **444**, 245–249.

Papageorgiu, C., Borer, H., and French, R. R. (1994). *Bioorg. Med. Chem. Lett.* **4**, 267–272.

Peters, C., Bayer, M. J., Buhler, S., Andersen, J. S., Mann, M., and Mayer, A. (2001). *Nature* **409**, 581–588.

Rusnak, F., and Mertz, P. (2000). *Physiol. Rev.* **80**, 1483–1521.

Sardanelli, A. M., Technikova-Dobrova, Z., Scacco, S. C., Speranza, F., and Papa, S. (1995). *FEBS Lett*. **377**, 470–474.

Sardanelli, A. M., Technikova-Dobrova, Z., Speranza, F., Mazzocca, A., Scacco, S., and Papa, S. (1996). *FEBS Lett*. **396**, 276–278.

Saris, N.-E. (1963). *Proc. Sci. Fenn.; Comment. Phys.-Math.* **28**(11), 1–77.

Saris, N.-E. L., Krestinina, O. V., Azarashvili, T. S., Odinokova, I. V., Tyynelä, J., and Evtodienko, Yu. V. (2001). In *Advances in Magnesium Research: Nutrition and Health* (Rayssiguier, Y., Mazur, A., and Durlach, J., eds.), John Libbey & Co, London, pp. 101–106.

Sarrouile, D., and Boudry, M. (1996). *Cell. Mol. Biol*. **42**, 189–197.

Schwoch, G., Trinczek, B., and Bode, C. H. (1990). *Biochem. J.* **270**, 181–188.

Signorile, A., Sardanelli, A. M., Nuzzi, R., and Papa, S. (2002). *FEBS Lett.* **512**, 91–94.

Stevens, T. H. (1997). *Annu. Rev. Cell. Dev. Biol*. **13**, 779–808.

Technikova-Dobrova, Z., Sardanelli, A.-M., Speranza, F., Scacco, S., Signorile, A., Lorusso, V., and Papa, S. (2001). *Biochemistry* **40**, 13941–13947.

Verdanis, A. (1977). *J. Biol. Chem.* **252**, 807–813.