Free radical-induced inactivation of creatine kinase: influence on the octameric and dimeric states of the mitochondrial enzyme (Mi_b-CK)

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Free radicals of X-ray-induced water radiolysis, either directly or indirectly via their reaction products, reduce the activity of both dimeric cytoplasmic muscle-type creatine kinase (MM-CK) and octameric mitochondrial creatine kinase (Mi-CK) to virtually zero. Similarly values of the characteristic D₃₇-dose of enzyme inactivation (dose required to reduce enzyme activity to 37%) were found for the two isoenzymes of CK under identical conditions. Octamer stability was not significantly affected within the dose range considered. However, both the dissociation of octamers into dimers by a transition-state analogue complex (TSAC), and the reassociation of the dimers into octamers, showed dose-dependent reduction. Binding of the TSAC to the active centre was found to protect the enzyme against inactivation

by free radicals. No protection was observed for the radiation-induced decrease of the endogenous tryptophan fluorescence. The experimental results are in line with the following interpretation: (i) the reduction of Mi_b-CK dimer association is due to free radical-induced modification of Trp-264, situated at the dimer/dimer interface; (ii) the active-site Trp-223 is not a prime target for free radicals and is not involved in the inactivation of the enzyme; (iii) the inhibition of TSAC-induced dissociation of Mi_b-CK, like enzyme inactivation, is primarily due to a modification of the active-site Cys-278.

Key words: creatine kinase, free radical, tryptophan, transition-state analogue complex.

INTRODUCTION

Creatine kinase (CK, EC 2.7.3.2) catalyses the reversible transfer of the high-energy N-phosphoryl group from phosphocreatine to ADP. Three cytosolic and two mitochondrial isoforms (ubiquitous Mi_a-CK and sarcomeric Mi_b-CK) of CK are known (for reviews see [1] and [2]). Part of the cytosolic isoenzyme activity associates with ATPases at subcellular compartments such as the sarcoplasmic reticulum or the myofibrillar M-band, where CK is functionally coupled to the Ca²⁺ pump [3,4] and the acto-myosin ATPase [5,6], respectively. The mitochondrial isoenzymes (Mi-CK) are located in the mitochondrial intermembrane space [7] and are associated with the outer side of the inner membrane [8,9]. A functional interplay between mitochondrial and cytosolic isoforms of CK is thought to be important for the regulation of cellular energy homeostasis. The cytosolic enzymes re-phosphorylate the locally produced free ADP and increase creatine globally. The mitochondrial enzyme catalyses the reaction of creatine to phosphocreatine at the expense of mitochondrial ATP [1,2,10–12].

Mi-CK differs from its cytosolic counterparts by the ability to form a highly ordered octamer from four dimers as the stable building blocks [13], whereas the cytosolic enzymes have a strictly dimeric structure. The Mi-CK octamers display a cube-like structure with identical top and bottom faces that appear appropriate to interact with the two mitochondrial boundary membranes [14]. This structural consideration is supported by a series of experimental findings. Rojo et al. [15] and Stachowiak et al. [16] demonstrated *in vitro* that the octameric form of Mi-CK was able to connect two artificial membranes. Kottke et al. [17] observed that mainly the octameric form of the enzyme was

present in isolated contact site fractions of brain mitochondria. In electron microscopical studies, the enzyme was found between adhering outer membrane vesicles and the inner envelope membrane as well as between the cristae membranes [18]. Recently, complexes of CK with other mitochondrial constituents, the adenylate translocator from the inner mitochondrial membrane and porin from the outer membrane, could be isolated from washed kidney and brain membrane fractions by mild detergent extraction [19,20]. Formation of these complexes of approx. 400 kDa might be an important function of the octameric structure [21,22].

Since the specific activity of octamers and dimers is very similar [23], the function of the dimer/octamer transition seems to involve structural aspects of mitochondrial energy metabolism. Destabilization of the Mi-CK octamer was found to impair Mi-CK functions in stimulation of oxidative phosphorylation, as demonstrated recently [24]. The octameric state of Mi-CK is also essential for its protective effect on the opening of the mitochondrial permeability transition pore [22]. This effect of Mi-CK disappears if octamers are dissociated by the components of the transition-state analogue complex, TSAC [25]. Summarizing, the Mi-CK octamer–dimer transition could play a crucial role in the regulation of mitochondrial energy metabolism as well as in cellular apoptosis, although this role has not been established *in vivo* so far.

The present study aims at a clarification of the oxidative damage of CK isoenzymes. These enzymes have been considered as sensitive targets of reactive species such as H₂O₂, and peroxynitrite [26–29]. Mi_b-CK may contribute to the damage of mitochondrial energy metabolism generated by reactive oxygen species (ROS) during phases of ischaemia and reperfusion.

Abbreviations used: CK, creatine kinase; MM-CK, cytosolic muscle creatine kinase; Mi_b-CK, sarcomeric mitochondrial creatine kinase; 2-ME, 2-mercaptoethanol; ROS, reactive oxygen species; D₃₇, dose required to reduce the enzyme activity to 37%; TSAC, transition-state analogue complex.

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Oxidative damage is also produced by free radicals of water radiolysis, the method applied throughout our study, which concentrates on the comparison between the cytosolic and the mitochondrial isoenzymes as well as on Mi_b -CK octamer dissociation and Mi_b -CK dimer reassociation.

EXPERIMENTAL

Materials

Chicken sarcomeric mitochondrial creatine kinase (Mi_b -CK) was prepared as described elsewhere [28] and was stored at -70°C (or up to 14 days at 4°C) at a concentration of 3 mg/ml in a buffer containing about 150 mM NaCl, 25 mM sodium phosphate, 0.2 mM EDTA and 2 mM 2-mercaptoethanol (2-ME) (pH 7.2). Cytosolic muscle CK (MM-CK) (from rabbit muscle), nucleotides and pyruvate kinase/lactate dehydrogenase were obtained from Boehringer Mannheim. Salts were from Riedel de Haën (Seelze). All other reagents were obtained from Sigma.

Methods

Free radicals of water radiolysis were generated by placing polycarbonate vessels (containing solution volumes of 100 μl) below an X-ray-tube (Philips-Müller RT 100, Philips, Hamburg, Germany). The tube was mounted in a Faraday cage with lead shielding and was operated at 80 kV. X-rays were filtered by 0.3 mm Al. The applied dose rate was 98 Gy/min in most cases (measured via Fricke dosimetry and via ionization chamber PTW DL 4, Physikalisch-Technische Werkstätten Dr. Pychlau, Freiburg, Germany).

Irradiation of oxygen containing aqueous solutions primarily generates the radical species $\text{OH}^\bullet$ and $\text{O}_2^{\bullet-}/\text{HO}_2^\bullet$. The latter form a conjugated acid-base pair and originate from reactions of the primary radicals $\text{H}^\bullet$ and $\text{e}_{\text{aq}}^{\bullet-}$ with molecular oxygen. The primary radicals are converted into different secondary radicals which depend on the composition of the aqueous solution [30,31]. In the presence of Cl^- in water, $\text{OH}^\bullet$ radicals generate $\text{Cl}_2^{\bullet-}$. The latter radical species is of lower chemical reactivity and shows an increased selectivity of reactions compared with the rather non-selective $\text{OH}^\bullet$ radical. The same is true for the nitrogen dioxide radical $\text{NO}_2^\bullet$, which is formed by reaction of $\text{e}_{\text{aq}}^{\bullet-}$ with NO_3^- (at the expense of the formation of $\text{O}_2^{\bullet-}$) [32].

The standard irradiation medium (PBS) was 150 mM NaCl and 25 mM sodium phosphate buffer, pH 7.0. Irradiation was performed at room temperature ($20 \pm 2^\circ\text{C}$). Gel permeation chromatography was performed using a Biosil (SEC 400-5) 300 mm $\times$ 7.8 mm gel permeation column operated with a flow rate of 1 ml/min. The running buffer consisted of standard irradiation medium plus 1 mM 2-ME and 2 mM NaN_3 . Peak areas were integrated using the D-6000 HPLC-Manager software by Merck-Hitachi.

Dissociation of octamer Mi_b -CK was induced by incubation in a TSAC mixture [33]. Removal of the TSAC to achieve reoctamerization was performed by dialysis in a high-speed tube (Spectra/Por Biotech Membranes, 15000 Da cut-off) 1:1000 against TSAC-free medium for 3 h at room temperature and additionally 1:1000 overnight in the same medium at 4°C .

Measurement of the enzyme activity was performed via the pyruvate kinase/lactate dehydrogenase assay ([34], see [35] for details). TSAC is known to inhibit the enzyme activity [36]. Inhibition can, however, be neglected under the conditions chosen for activity measurements, since, after dilution of the enzyme, the remaining ADP was allowed to react with the ingredients of the pyruvate kinase/lactate dehydrogenase assay. Thus the for-

mation of the TSAC with Mi_b -cK was avoided before the start of the enzyme test.

RESULTS AND DISCUSSION

Mitochondrial (Mi_b -CK) and cytosolic (MM-CK) isoforms of creatine kinase in many respects show similar modifications in the presence of radiation-induced free radicals. This holds for the D_{37} -dose of enzyme inactivation, for the repair of the enzyme activity by 2-ME and for the decay of the tryptophan fluorescence (Figure 1). There is an exponential decay of the enzyme activity which is characterized by the dose, D_{37} , necessary to reduce the activity to $1/e$ (e = Euler's number), i.e. to about 37% of the initial activity.

A similar behaviour of the two isoenzymes was also observed throughout the investigation of enzyme protection by a TSAC. TSAC is formed by the binding of creatine, NO_3^- , ADP, and Mg^{2+} to the active centre of the enzyme. The D_{37} -dose was increased by factors of about 3 (MM-CK) and 10 (Mi_b -CK) in the presence of the TSAC (Figure 2). As a control, ADP was exchanged against AMP. Alternatively, MgCl_2 was omitted. This is known to prevent TSAC formation, since the Mg-nucleotide is needed for proper binding of this substrate [36]. Protection by TSAC-binding is believed to reflect the shielding of sensitive sites towards free radical attack. This may occur by direct (steric) limitation by the TSAC of the access of free radicals to the sites considered. Alternatively, protection may occur indirectly, via a conformational change of the protein resulting in a reduced accessibility of sensitive target site(s) different from the binding site of the TSAC.

Under the experimental conditions of Figure 2 (i.e. in the presence of NO_3^-), Cys-282 of MM-CK modified by $\text{NO}_2^\bullet$ radicals has been found to be responsible for enzyme inactivation

Figure 1 Comparison of Mi_b -CK and MM-CK activities before and after reactivation by 2-ME and tryptophan fluorescence as a function of the relative radiation dose, r

r is defined as the ratio of the dose D and the characteristic D_{37} dose. The values of the latter refer to the activity immediately after irradiation ($\square$). Irradiation was performed in PBS containing additionally 1 mM 2-ME (and in the case of Mi_b -CK 0.2 mM EDTA) at a protein concentration of 250 $\mu\text{g}/\text{ml}$. The characteristic dose values obtained are D_{37} (Mi_b -CK) = 388 Gy, D_{37} (MM-CK) = 395 Gy. Reactivation (indicated by arrows) of the enzyme activity was induced by incubation after irradiation for at least 1 h with 10 mM 2-ME ($\circ$). Endogenous Trp-fluorescence measurements ($\triangle$) were performed at an excitation and an emission wavelength of 295 nm and of 330 nm, respectively.

Figure 2 Protection of the enzyme activity by TSAC

Aqueous solutions of either 50 $\mu\text{g/ml}$ $\text{Mi}_b\text{-CK}$ or MM-CK were exposed to 80 kV X-rays. The solutions in addition contained standard irradiation medium plus the ingredients for TSAC formation (50 mM KNO_3 , 20 mM creatine, 5 mM MgCl_2 , 4 mM ADP). In the case of $\text{Mi}_b\text{-CK}$, 16 μM 2-ME and 3 μM EDTA were present. In the Figure, inactivation curves in the presence of TSAC ($\square$) are compared with those in the absence ($\triangle$). In the latter case 4 mM ADP was exchanged against 4 mM AMP. The following D_{37} -values were obtained: $\text{Mi}_b\text{-CK}$ 50.8 Gy and 5 Gy; MM-CK 10.4 Gy and 3.2 Gy.

(P. Koufen and G. Stark, unpublished work; see also [35]). The same should be valid for Cys-278 in the case of $\text{Mi}_b\text{-CK}$. Since Cys-278 is not directly involved in TSAC binding [37], indirect protection via conformational changes has to be considered. This idea is supported by the observation of structural changes of CK upon binding of Mg-nucleotide (or TSAC) [38]. The data shown in Figure 2 are in line with previous reports about the protection of the active-site cysteine by TSAC against iodoacetamide-induced modification [36].

Protection of the enzyme activity was also observed in the absence of $\text{NO}_2^{\cdot}$ radicals. In this case, enzyme inactivation proceeds by means of a different mechanism, since blockage of cysteine has no effect on the D_{37} -dose (P. Koufen and G. Stark, unpublished work). The experiments shown in Figure 3 were performed in the presence of a modified TSAC. In order to exclude $\text{NO}_2^{\cdot}$ formation, KNO_3 was omitted. A dead end complex (with Cl^- instead of NO_3^-) is formed in this case [36]. The targets modified under the applied conditions are unknown so far. Trp-223 is an important aromatic amino acid residue of the active centre which is crucial for the catalytic efficiency of the enzyme [39]. In view of the well-known reactivity of aromatic amino acid residues towards free radicals, Trp-223 might be a good candidate to explain enzyme inactivation. Indeed a decay of tryptophan fluorescence was observed with increasing dose, which is most pronounced in standard irradiation medium (i.e. the absence of nitrate, compare Figures 1 and 3). The decay of the tryptophan fluorescence was, however, identical in the absence and the presence of the dead end complex (in spite of its protecting action on the enzyme activity). This is thought to indicate that Trp-223, although strongly contributing to the total fluorescence of the enzyme [39], is not involved in the decrease of the enzyme activity. Otherwise, in view of the importance of Trp-223 for the TSAC binding [40], a direct protection effect of TSAC would be expected. As a consequence, the decay of the fluorescence must be due to modification of tryptophan residues of the enzyme other than Trp-223.

The binding of the TSAC has been found to destabilize $\text{Mi}_b\text{-CK}$ octamers finally leading to dissociation into dimers [33]. We have been using this method in order to study the dissociation of

Figure 3 Comparison of TSAC effect on fluorescence and enzyme activity

An aqueous solution of 250 $\mu\text{g/ml}$ MM-CK was exposed to 80 kV X-rays. In addition, the solution contained standard irradiation medium plus ingredients for TSAC formation (20 mM creatine, 5 mM MgCl_2 , 4 mM ADP), however, in the absence of KNO_3 . In the Figure, inactivation curves of the enzyme activity ($\square$, $\blacksquare$) are compared with the Trp-fluorescence decay ($\circ$, $\bullet$) in the presence (solid symbols) and absence (open symbols) of TSAC. To prevent TSAC formation, MgCl_2 was omitted. The following D_{37} values (mean values of three different experiments) were obtained: 106 Gy (without MgCl_2), 210 Gy (presence of MgCl_2).

octamers and the reassociation of dimers in the presence of free radicals. In the absence of the TSAC, the stability of $\text{Mi}_b\text{-CK}$ octamers was found to be virtually unaffected by a radiation dose sufficient to inactivate 99 % of the enzyme activity (Figure 4). In the native state, octamers readily dissociate into dimers after TSAC binding to the catalytic sites of the enzyme. If TSAC was added after irradiation of the enzyme, a dose-dependent

Figure 4 Stability of the octameric state of $\text{Mi}_b\text{-CK}$ during free radical-induced enzyme inactivation

A solution of 250 $\mu\text{g/ml}$ $\text{Mi}_b\text{-CK}$ in standard medium additionally containing 1 mM 2-ME and 0.2 mM EDTA was exposed to a dose of 1960 Gy, leading to an inactivation of the enzyme with a residual activity of 1 %. Subsequently, gel permeation chromatography was performed (see the Experimental section). The Figure shows gel filtration profiles, followed via the absorbance at 280 nm, of the native enzyme (1) and of the inactivated enzyme (2).

Figure 5 Effect of irradiation on the efficiency of TSAC to destabilize octameric Mi_b -CK

A solution of 250 μ g/ml Mi_b -CK in standard medium additionally containing 1 mM 2-ME and 0.2 mM EDTA was exposed to irradiation. Subsequently, the ingredients for TSAC formation (50 mM KNO_3 , 20 mM creatine, 5 mM $MgCl_2$, 4 mM ADP) in standard medium containing in addition 2 mM 2-ME were added. After incubation over night at 4 °C, gel permeation chromatography was performed (see the Experimental section). The Figure shows the enzyme activity after TSAC incubation (●), and the resulting dimer peak areas of the corresponding gel permeation profiles (□).

Figure 6 Gel filtration profiles of Mi_b -CK irradiated in the presence of TSAC

A solution of 250 μ g/ml octameric Mi_b -CK was incubated for 2 h at room temperature in standard medium additionally containing 1 mM 2-ME, 0.2 mM EDTA plus the ingredients for TSAC formation (50 mM KNO_3 , 20 mM creatine, 5 mM $MgCl_2$, 4 mM ADP). Traces 1 and 2 show the profiles, followed via the absorbance at 280 nm, obtained before (1) and after (2) irradiation with 2940 Gy. Subsequently, TSAC was removed by dialysis in order to study the reoctamerization of the dissociated Mi_b -CK. Traces 3 and 4 refer to the doses 0 Gy and 2940 Gy, respectively.

reduction of the dimer peak area was found (Figure 5). The radiation effect occurred in the same dose range, where inactivation of the enzyme activity was observed under the conditions applied (i.e. in the presence of TSAC and at high concentrations of 2-ME). This is an indication that the reduction of octamer dissociation is due to modified catalytic sites of the enzyme preventing correct binding of TSAC. A reduced

Figure 7 Quantitative analysis of the profiles of Figure 6

Percentage of dimer peak areas before TSAC-removal (□), after TSAC-removal (■) and residual enzyme activity (○) as a function of radiation dose.

dissociation effect of TSAC was also found upon peroxyxynitrite-induced modification of the enzyme [28].

TSAC-induced dissociation of octamers and the subsequent reassociation of the dimers (after TSAC removal by dialysis) is shown by quantitative analysis of gel filtration profiles of Mi_b -CK (Figures 6 and 7). Traces 1 and 3 of Figure 6 show the normal behaviour of the enzyme with a high percentage of dissociation (induced by the TSAC) and a high percentage of the subsequent reoctamerization (after TSAC removal). This is in line with previous studies [33]. Irradiation of the enzyme in the presence of TSAC (traces 2 and 4) gave rise to a small reduction of the amount of octamers dissociated and to a substantial reduction of the percentage of dimers capable of reassociation (i.e. to an increase of the dimer peak of trace 4 as compared with that of trace 3). Figure 7 shows the dose dependence of the phenomena, which is compared with that of the residual enzyme activity. No evidence for crosslinking of dimers was obtained. Incubation for 3.5 h of irradiated octamers or dimers (in the presence of TSAC) with 4.8 M guanidine hydrochloride yielded 100% dimers in a subsequent HPLC analysis (results not shown).

The data may be explained as follows: Mi_b -CK octamers are fairly resistant structures in the presence of free radicals of water radiolysis (see Figure 4), as they are towards a number of proteolytic enzymes [41]. Upon dissociation of octamers into dimers, however, a series of amino acid residues, normally buried inside the protein, become accessible to free radical attack. Their modification hinders the reassociation of the dimers. Trp-264 should be mentioned in this context, since it was shown to stabilize (as a part of a hydrophobic patch) dimer-dimer interactions of Mi_b -CK [39]. The decay of the dimer peak area observed before TSAC removal (open squares in Figure 7) may be understood as a result of two opposing effects on the dynamic octamer/dimer equilibrium: a reduced mean rate of TSAC-induced dissociation (by modification of the catalytic site of Mi_b -CK), and a reduced mean rate of the back reaction via weakened dimer-dimer interactions (presumably induced by Trp-264 modification). At zero dose, removal of the TSAC shifts the equilibrium between octamers and dimers to the side of octamers. At large doses, however, there is hardly an effect of TSAC removal on the association/dissociation equilibrium of Mi_b -CK dimers. This is indicated by the roughly identical dimer peak areas observed in the presence of the TSAC and after its removal at large radiation doses (Figure 7).

Summarizing, the experiments support the conclusion that both, the active-site residue Trp-223 as well as Trp-264 do not represent sensitive targets for free radical attack in the octameric state of the enzyme. However, as may be concluded from the X-ray structure [14], Trp-264 becomes readily accessible to free radicals after dissociation of Mi_b -CK octamers into dimers, since the dimer/dimer interface is fully exposed to the environment. The sensitivity of intact octamer Mi_b -CK against the attack of free radicals seems to be governed by modifications of the active centre and its environment including cysteine 278. This is similar to cytosolic MM-CK, where cysteine 282 (the analogue of Cys-278 of Mi_b -CK) has been found to represent the most important and exquisitely sensitive target of the enzyme (P. Koufen and G. Stark, unpublished work; see also [35]).

ROS are generated especially during phases of ischaemia and reperfusion in mitochondria due to the activity of mitochondrial nitric oxide synthase [42] and oxygen reduction in the respiratory chain. NO and other ROS were shown to irreversibly inactivate cytosolic MM-CK [27,29,35] and to decrease cardiac work [43] and contractile reserve [44]. Recently, exercise intolerance in patients with chronic heart failure was correlated with an increased expression of inducible nitric oxide synthase and a decrease in Mi -CK content [45]. Our results suggest that these cardiac defects in addition to MM-CK could result from free-radical-dependent inactivation of Mi -CK and from an inhibition of octamer formation by dimer association. In support of this assumption is the observation of a significant decrease of the octamer/dimer relation in rat hearts after ischaemia and reperfusion in infarcted areas [46]. The octamer decrease would certainly alter cardiac work, as octamers of Mi_b -CK are thought to be important for efficient energy channelling [21,24] as well as for stabilization of mitochondrial contacts [22].

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REFERENCES

- Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K. and Eppenberger, H. M. (1992) *Biochem. J.* **281**, 21–40
- Wyss, M., Smeitink, J., Wevers, R. A. and Wallimann, T. (1992) *Biochim. Biophys. Acta* **1102**, 119–166
- Rossi, A. M., Eppenberger, H. M., Volpe, P., Cotrufo, R. and Wallimann, T. (1990) *J. Biol. Chem.* **265**, 5258–5266
- Korge, P., Byrd, S. K. and Campbell, K. B. (1993) *Eur. J. Biochem.* **213**, 973–980
- Wallimann, T. and Eppenberger, H. M. (1985) in *Cell and Muscle Motility*, vol. 6, (Shay, I. W., ed.), pp. 239–285, Plenum Press, New York
- Krause, S. M. and Jacobus, W. E. (1992) *J. Biol. Chem.* **267**, 2480–2486
- Jacobs, H., Heldt, H. W. and Klingenberg, M. (1964) *Biochem. Biophys. Res. Commun.* **16**, 516–521
- Kaldis, P. (1994) Dissertation No. 10686, ETH Zürich, Switzerland
- Brooks, S. P. J. and Suelter, C. H. (1987) *Arch. Biochem. Biophys.* **257**, 144–153
- Bessman, S. P. and Carpenter, C. L. (1985) *Annu. Rev. Biochem.* **54**, 831–865
- Jacobus, W. E. (1985) *Annu. Rev. Physiol.* **47**, 707–725
- Saks, V. A., Rosenshtraukh, L. V., Smirnov, N. and Chazov, E. I. (1978) *Can. J. Physiol. Pharmacol.* **56**, 691–706
- Schlegel, J., Wyss, M., Eppenberger, H. M. and Wallimann, T. (1990) *J. Biol. Chem.* **265**, 9221–9227
- Fritz-Wolf, K., Schnyder, T., Wallimann, T. and Kapsch, W. (1996) *Nature (London)* **381**, 341–345
- Rojo, M., Hovius, R., Demel, R. A., Nicolay, K. and Wallimann, T. (1991) *J. Biol. Chem.* **266**, 20290–20295
- Stachowiak, O., Dolder, M. and Wallimann, T. (1996) *Biochemistry* **35**, 15522–15528
- Kottke, M., Adams, V., Wallimann, T., Nalam, V. K. and Brdiczka, D. (1991) *Biochim. Biophys. Acta* **1061**, 215–225
- Kottke, M., Wallimann, T. and Brdiczka, D. (1994) *Biochem. Med. Metab. Biol.* **51**, 105–117
- Beutner, G., Rück, A., Riede, B., Welte, W. and Brdiczka, D. (1996) *FEBS Lett.* **396**, 189–195
- Beutner, G., Rück, A., Riede, B. and Brdiczka, D. (1998) *Biochim. Biophys. Acta* **1368**, 7–18
- Schlattner, U., Forstner, M., Eder, M., Stachowiak, O., Fritz-Wolf, K. and Wallimann, T. (1998) *Mol. Cell. Biochem.* **184**, 125–140
- Brdiczka, D., Beutner, G., Rück, A., Dolder, M. and Wallimann, T. (1998) *BioFactors* **8**, 235–242
- Kaldis, P. and Wallimann, T. (1995) *Biochem. J.* **308**, 623–627
- Khuchua, Z. A., Qin, W., Boero, J., Cheng, J., Payne, R. M., Saks, V. A. and Strauss, A. W. (1998) *J. Biol. Chem.* **273**, 22990–22996
- O'Gorman, E., Beutner, G., Dolder, M., Koretsky, A. P., Brdiczka, D. and Wallimann, T. (1997) *FEBS Lett.* **414**, 253–257
- Yuan, G., Kaneko, M., Masuda, H., Hon, R. B., Kobayashi, A. Q. and Yamazaki, N. (1992) *Biochim. Biophys. Acta* **1140**, 78–84
- Mekhfi, H., Veksler, V., Mateo, P., Maupou, V., Rochette, L. and Ventura-Clapier, R. (1996) *Circ. Res.* **78**, 1016–1027
- Stachowiak, O., Dolder, M., Wallimann, T. and Richter, C. (1998) *J. Biol. Chem.* **273**, 16694–16699
- Konorev, E. A., Hogg, N. and Kalyanaram, B. (1998) *FEBS Lett.* **427**, 171–174
- v. Sonntag, C. (1987) *The Chemical Basis of Radiation Biology*, Taylor & Francis, London
- Stark, G. (1991) *Biochim. Biophys. Acta* **1071**, 103–122
- Neta, P., Huie, R. E. and Ross, A. B. (1988) *J. Phys. Chem. Ref. Data* **17**, 1027–1247
- Gross, M. and Wallimann, T. (1993) *Biochemistry* **32**, 13933–13940
- Tanzer, M. L. and Gilvary, C. J. (1959) *J. Biol. Chem.* **234**, 3201–3204
- Koufen, P. (1999) Dissertation, University of Konstanz, Konstanz, Germany
- Milner-White, E. J. and Watts, D. C. (1971) *Biochem. J.* **122**, 727–740
- Furter, R., Furter-Graves, E. M. and Wallimann, T. (1993) *Biochemistry* **32**, 7022–7029
- Forstner, M., Kriechbaum, M., Laggner, P. and Wallimann, T. (1998) *Biophys. J.* **75**, 1016–1023
- Gross, M., Furter-Graves, E. M., Wallimann, T., Eppenberger, H. M. and Furter, R. (1994) *Protein Sci.* **3**, 1058–1068
- Messmer, C. H. and Kägi, J. H. R. (1985) *Biochemistry* **24**, 7172–7178
- Wyss, M., James, P., Schlegel, J. and Wallimann, T. (1993) *Biochemistry* **32**, 10727–10735
- Ghafoorifar, P. and Richter, Ch. (1997) *FEBS Lett.* **418**, 291–296
- Schulz, R., Dodge, K. L., Lopaschuk, G. D. and Clanachan, A. S. (1997) *Am. J. Physiol.* **272**, H1212–H1219
- Gross, W. L., Bak, M. I., Ingwall, J. S., Arstall, M. A., Smith, T. W., Balligand, J. L. and Kelly, R. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5604–5609
- Hambrecht, R., Adams, V., Gielen, S., Linke, A., Möbius-Winkler, S., Yu, J., Niebauer, J., Jiang, H., Fiehn, D. and Schuler, G. (1999) *J. Am. Coll. Cardiol.* **33**, 174–179
- Soboll, S., Brdiczka, D., Jahnke, D., Schmidt, A., Schlattner, U., Wendt, S., Wyss, M. and Wallimann, T. (1999) *J. Mol. Cell. Cardiol.* **31**, 857–866

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