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Biochimica et Biophysica Acta 1658 (2004) 44–49

BIOCHIMICA ET BIOPHYSICA ACTA

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Review

Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols

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Received 18 February 2004; accepted 4 March 2004
Available online 15 June 2004

Abstract

NO or its derivatives (reactive nitrogen species, RNS) inhibit mitochondrial complex I by several different mechanisms that are not well characterised. There is an inactivation by NO, peroxynitrite and S-nitrosothiols that is reversible by light or reduced thiols, and therefore may be due to S-nitrosation or Fe-nitrosylation of the complex. There is also an irreversible inhibition by peroxynitrite, other oxidants and high levels of NO, which may be due to tyrosine nitration, oxidation of residues or damage of iron sulfur centres. Inactivation of complex I by NO or RNS is seen in cells or tissues expressing iNOS, and may be relevant to inflammatory pathologies, such as septic shock and Parkinson's disease.

Keywords: Nitric oxide; Mitochondria; Complex I; NADH-ubiquinone oxidoreductase; Parkinson's disease; Respiration

1. Complex I

Mitochondrial respiratory complex I (NADH:ubiquinone oxidoreductase) is a large and complex enzyme coupling electron transfer to pumping of protons across the mitochondrial inner membrane (reviewed in Refs. [1,2]). Bovine heart complex I has been found to contain 45–46 subunits, FMN, about eight iron–sulfur clusters and one or more ubiquinone binding sites. All cofactors are located on 14 ("core") subunits that are sufficient for catalysis of energy transduction by complex I. The remaining subunits are thought to be involved in assembly of the complex, in maintaining its stability or in regulation of the activity.

The main function of complex I is transfer of electrons from NADH to ubiquinone in a reaction that is coupled to translocation of protons across the mitochondrial inner membrane. However, recently it has been suggested that complex I might have some additional functions. The SDAP subunit of complex I has been identified as an acyl carrier protein [3] and might be involved in biosynthesis of lipoic acid. There are also three possible links between complex I functions and cell death. Complex I is an important source of production of reactive oxygen species (ROS) [4–6]. It has also been suggested to be involved in the regulation of mitochondrial permeability transition pore [7]. And finally, the 43rd subunit B16.6 of complex I has been found to be homologous to human GRIM-19 protein which is thought to be a part of the interferon-β- and retinoic acid-induced pathway of cell death [8].

Little is known about regulation of complex I functions. It has been shown that in vitro complex I undergoes a slow, reversible active/de-active transition and that this transition involves some (as yet non-identified) conformational change [9,10]. It has also been reported that complex I may be activated by mitochondrial cAMP-dependent protein kinase phosphorylating the 18-kDa subunit of complex I [11].

2. Nitric oxide biochemistry

NO is a small molecule, highly diffusible in water and through biological membranes, so NO can easily diffuse from one cell to the next. The main source of NO within the cells is synthesis by three isoforms of NO synthase: two

Abbreviations: eNOS, iNOS and nNOS, endothelial inducible and neuronal nitric oxide synthases; EPR, electron paramagnetic resonance; GSH, reduced glutathione; RNS, reactive nitrogen species; ROS, reactive oxygen species

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0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2004.03.016

constitutively expressed isoenzymes eNOS and nNOS and the third, iNOS, which is induced during inflammation and produces higher levels of NO for a longer period [12]. There may also be a mitochondrial NOS (mtNOS), whose origin and status is still unclear, but if mtNOS exists is probably derived from eNOS, iNOS or nNOS [13,14]. NO may also be produced nonenzymatically from nitrite at low pH (< pH 5), e.g. during ischaemia [15].

NO itself at physiological concentrations (unclear, but probably 0.1–100 nM) is relatively unreactive, and most of its physiological actions are mediated by NO binding to Fe^{2+} in the haem of soluble guanylate cyclase causing activation and cGMP production [12]. NO may also bind with high affinity to free Fe^{2+} or Fe^{2+} within any other haem protein with a free ligand position, such as cytochrome *c* oxidase or catalase. However, NO may be converted to a number of more reactive derivatives, known collectively as reactive nitrogen species (RNS) (Fig. 1). At high concentrations (or within membranes) NO reacts directly with oxygen to produce NO_2, which rapidly reacts with a further NO to give N_2O_3. NO_2 may oxidise or nitrate (add an NO_2^+ group to) a variety of molecules, while N_2O_3 can nitrosate (add an NO^+ group to) amines (to give N-nitrosoamines) or thiols (to give S-nitrosothiols). Nitrosation is often called nitrosylation, but the latter term should refer to addition of NO (rather than NO^+), e.g. to Fe^{2+}. NO reacts at the diffusion-limited rate with O_2^- to produce peroxynitrite ($ONOO^-$), which can oxidise or nitrate other molecules, or can decay producing other damaging species (possibly the hydroxyl radical $OH^\bullet$ and NO_2). NO may indirectly (possibly via N_2O_3 or via NO reaction with $RS^\cdot$) nitrosate thiols (e.g. in proteins or glutathione) to give S-nitrosothiols (RSNO, e.g. S-nitroso-glutathione and S-nitroso-albumin). The NO^+ group is directly transferable between different S-nitrosothiols, a process known as transnitrosation or transnitrosylation. S-nitrosated or tyrosine-nitrated proteins may have altered function. S-nitrosothiols can also release NO in the presence of either light or reductants such as reduced thiols.

3. NO effects on mitochondria

NO and RNS inhibit mitochondrial respiration by different means: (A) NO itself causes rapid, selective, potent, but reversible inhibition of cytochrome oxidase, and (B) RNS cause slow, nonselective, weak, but irreversible inhibition of many mitochondrial components [16–18]. The reversible NO inhibition of cytochrome oxidase occurs at nanomolar levels of NO and in competition with oxygen [19–21], so that NO is potentially a physiological regulator of respiration [22,23]. Peroxynitrite can inhibit complex I, complex II, cytochrome oxidase (complex IV), the ATP synthase, aconitase, Mn-SOD, creatine kinase, and probably many other proteins [16,18,24]. Peroxynitrite is a strong oxidant and can also induce lipid peroxidation and increase mito-

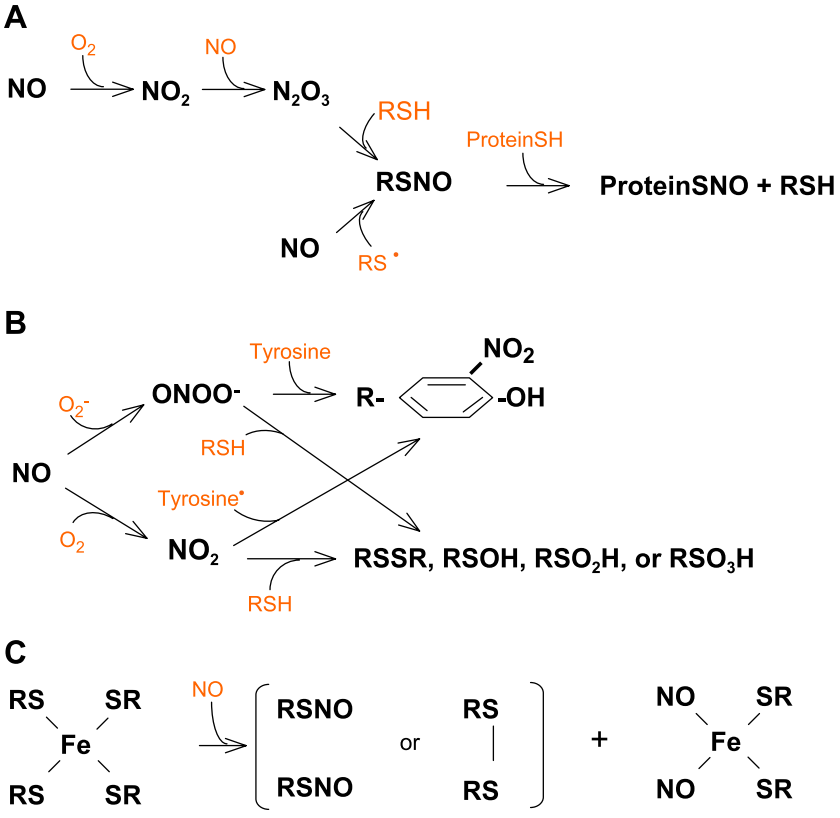

Fig. 1. Reactions of NO.

chondrial proton (and other ion) permeability (probably by lipid peroxidation or thiol cross-linking) [24]. Apart from inhibiting respiration, NO has two other relevant effects on mitochondria: (1) induction of ROS and RNS production from mitochondria [25], and (2) induction of mitochondrial permeability transition by RNS [26,27].

4. Mechanisms of NO inhibition of complex I

NO/RNS might inhibit complex I by several different mechanisms, some of which have supporting evidence and some are speculative: (i) release of iron from complex I iron–sulfur (FeS) centres, (ii) reversible modification of Fe, S or cysteine in FeS centres, (iii) S-nitrosation of cysteine residues, (iv) oxidation of cysteine residues, (v) glutathionylation of cysteine residues, (vi) N-nitrosation of secondary amines, e.g. in tryptophan residues, (vii) oxidation of tryptophan residues, (viii) nitration of tyrosine residues, (ix) modification of coenzymes NADH, ubiquinone or FMN, or (x) oxidation of phospholipids. Inhibition could be mediated by NO itself, any of the RNS: $ONOO^-$, NO_2, N_2O_3, N_2O_4, S-nitrosothiols or any of the ROS: O_2^-, H_2O_2 or $OH^\bullet$.

4.1. Discovery of macrophage NO and its role in cytostasis/cytotoxicity of tumour cells

Historically the first reported complex I inhibition attributable to NO predates the discovery of biological NO, and contributed to that discovery [28–31]. Inflammatory-activated macrophages were found to inhibit complex I (as well as complex II and aconitase, but not other complexes) within co-cultured tumour cells or cell lines or the macrophages themselves, and the inhibition depended on the presence of L-arginine (substrate of NO synthase) [28–31]. NO itself was subsequently found to cause complex I inhibition within these cells, leading to the conclusion that NO derived from the macrophages was inhibiting complex I within the tumour cells [30]. That the respiratory inhibition (by whatever mechanisms) mediated the killing and/or cytostasis of tumour cells was suggested by the finding that tumour cell death could be completely prevented by glucose acting presumably as substrate for glycolysis and thus an alternative source of ATP [31].

4.2. Damage to FeS centres

The inhibition of complex I, complex II and aconitase induced by NO from the macrophages was attributed to NO-induced damage to iron–sulfur centres and the release of iron from these centres [29,31]. Cells exposed to NO or NO from iNOS were subsequently found to exhibit a $g=2.04$ electron paramagnetic resonance (EPR) signal characteristic of dinitrosyl iron complexes $Fe(NO)_2(SR)_2$ [32], suggesting that the iron was removed from susceptible iron sulfur centres by NO reacting directly with the iron, displacing elemental sulfur or cysteine residues (Fig. 1C). However, it was never shown that the dinitrosyl iron complex appearing in NO-exposed cells was derived from mitochondrial iron–sulfur centres, and indeed on quantitative grounds this seemed unlikely.

Despite the lack of direct evidence that NO can remove iron from iron–sulfur centres in complex I, it is clear that NO can displace iron from other iron–sulfur centres, and understanding how this occurs mechanistically may be of some relevance to the actions of NO on complex I. High concentrations of NO can destroy iron–sulfur centres by binding and displacing the iron as dinitrosyl iron, and there is EPR evidence for such damage in complex II [33], but not so far in complex I. Reversible NO inactivation of *E. coli* exonuclease III, ferredoxin and other iron–sulfur proteins has been shown to be mediated by disruption of the iron–sulfur centre to form a dinitrosyl iron complex (Fig. 1C), which can be reconverted to the functional iron–sulfur centre by reduced thiols [34]. NO can cause inactivation of aconitase in some conditions that may involve catalytic S-nitrosation of the iron–sulfur centre [35]. Peroxynitrite can more rapidly inactivate aconitase via removal of one particular labile iron atom of the active site 4Fe4S iron–sulfur centre [36]. We might speculate that damage to complex I iron–sulfur centres may start with NO reacting with the iron (to form dinitrosyl iron), and/or the sulfide or cysteine residues that bind the iron (to form RSNO) (Fig. 1C). This initial phase of inhibition may be reversible by light or reduced thiols, but further reactions with the Fe or S may completely disassemble the iron–sulfur centres and thus irreversibly inhibit activity.

4.3. S-nitrosation of complex I

More recently, it was found that several cell types exposed to NO for several hours developed an irreversible inhibition of cellular respiration due specifically to inactivation of complex I [23,37]. However, this inactivation of complex I and respiration was completely reversed by either light or reduced thiols, suggesting that the inactivation was due to S-nitrosation of complex I (as S-nitrosation is generally reversed by these treatments). This was an important finding as it indicated: (a) that NO inhibition of cellular respiration was mainly due to complex I inhibition, and (b) the inhibition was reversible by specific agents indicating potential mechanisms. However, there is no direct evidence for S-nitrosation of complex I as yet, and reduced thiols and light might potentially reverse other forms of NO inhibition such as nitrosylation of iron.

The S-nitrosothiols: S-nitrosoglutathione (SNOG) and S-nitrosoacetylpenicillamine (SNAP) can rapidly inactivate complex I when added to isolated mitochondria, even in conditions when little or no NO is released, and such inhibition is reversed by light or reduced thiols (glutathione or dithiothreitol), suggesting that the inactivation is mediated by transnitrosation [38]. However, the inhibition

of complex I induced by peroxynitrite (which is regarded as a poor S-nitrosating agent) is also partly reversed by light and reduced thiols [38], suggesting that mechanisms other than nitrosation might be involved or that peroxynitrite may nitrosate. Recently we found that NO and calcium synergistically inhibited the complex I activity and oxygen consumption of isolated mitochondria [39]. This inhibition was prevented by superoxide dismutase or urate, suggesting that the inhibition was mediated by peroxynitrite. And the inhibition was reversed by reduced thiols or light suggesting that the inhibition (although mediated by peroxynitrite) may involve S-nitrosation or Fe-nitrosylation of complex I [39].

4.4. Tyrosine nitration by peroxynitrite

Long-term exposure of isolated mitochondria or submitochodrial particles was reported to result in relatively specific inhibition of complex I that was partially prevented by superoxide dismutase or urate (a superoxide and peroxynitrite scavenger) implicating peroxynitrite as mediating the inhibition [40]. The inhibition was not reversed by reduced glutathione, suggesting S-nitrosation was not involved, but was accompanied by tyrosine nitration of complex I subunits, suggesting that nitration may mediate the inhibition [40].

Others have also suggested that peroxynitrite inhibits complex I by tyrosine nitration [41,42]. Addition of 0.2–2.0 mM peroxynitrite to isolated mitochondria has been shown by several groups to result in inhibition of complex I, complex II and complex V (the ATP synthase) with relatively little affect on complexes III and IV [18,41,43,44]. Accompanying such inhibition there was tyrosine nitration of five complex I peptides: the 49-kDa subunit (NDUFS2), TYHY (NDUFS8), B17.2 (17.2-kDa differentiation associated protein), B15 (NDUFB4) and B14 (NDUFA6). However, the estimated level of nitration was low (a few percent) except on peptides B14 and B15, and these peptides are not thought to be involved in complex I respiratory activity [42]. So it is still unclear whether tyrosine nitration mediates peroxynitrite-induced inhibition of complex I.

Evidence of tyrosine nitration of mitochondrial complex I subunits was found in an untreated dopaminergic SH-SY5Y "neuronal" cell line using an antibody against protein-bound 3-nitro tyrosine [41]. $ONOO^-$ generating SIN-1 induced apoptosis in these cells with concomitant increase in tyrosine nitration and reduction in mitochondrial ATP synthesis. These results suggest that $ONOO^-$ may induce mitochondrial dysfunction and cell death in neurons through nitration of mitochondrial complex I subunits [41].

4.5. Other thiol modifications

Exposure of mitochondria or isolated complex I to SNAP or oxidised glutathione (GSSG) resulted in blockage of reduced thiols specifically on the 51-kDa and 75-kDa subunits of complex I, which might result from S-nitrosation, oxidation or glutathionylation of cysteine residues [45]. This thiol modification correlated in time with inhibition of complex I activity, but while thiol modification was reversed by dithiothreitol and GSH, the inhibition was not [45], suggesting either that the thiol modification is not involved in inhibition or that thiol modification proceeds from a reversible to an irreversible form.

Addition of peroxynitrite to mitochondria resulted in the oxidation of a methionine and a tryptophan residue in the B17.2 (DAP) subunit of complex I, although the extent of oxidation was not quantified, and it is unclear whether such modification in this subunit could inhibit complex activity [42].

5. Pathological relevance of NO inhibition of complex I

Whatever the mechanism, NO inhibition of complex I may be important in cell pathology and disease. A decrease in complex I activity and ATP levels has been observed in skeletal muscle biopsies from critically ill patients in septic shock; the decrease in complex I activity correlated with increased clinical severity and NO production (and decreased GSH levels), and was suggested to be causative in the pathology [46]. A rat model of septic shock reproduced these findings in both skeletal muscle and liver [47].

Parkinson's disease results from degeneration of dopaminergic neurons in the substantia nigra of the brain. Degeneration is accompanied by substantial inhibition of complex I activity in these cells, and complex I inhibitors can result in symptoms similar or identical to the disease, leading to the suggestion that complex I inhibition in the cells leads to their degeneration and hence the disease [48]. NO or RNS, derived from inflammatory activated glia, have been suggested as potential mediators of this complex I inhibition [48], however, there is no direct evidence for this. Inflammatory activation of a microglial (brain macrophage) cell line resulted in iNOS expression and inhibition of mitochondrial respiratory complexes I and II and aconitase, which was prevented by iNOS inhibition [49]. However, it was found that coculture of primary forebrain neurons with iNOS-expressing astrocytes did not cause complex I inactivation, unless cellular glutathione was depleted, suggesting that glutathione protects complex I from NO-induced inhibition [50]. Other authors found that dopamine synergised with NO to inhibit complex I in PC12 cells for reasons that are unclear, but this may indicate that dopaminergic neurons are more susceptible to NO inhibition of complex I [51].

Emotional stress also induced iNOS in rat brain, and caused substantial inhibition of the apparent brain activities of mitochondrial complexes I and II, which was prevented by an iNOS inhibitor, suggesting that NO from iNOS may inhibit mitochondrial complex activities in vivo [52].

6. Conclusions

At least three mechanisms have been invoked in the NO inhibition of complex I: S-nitrosation, tyrosine nitration and damage to FeS centres. None of these mechanisms has been definitively shown to mediate inhibition, although it is likely that all three mechanisms, as well as others, occur. In some conditions the NO inhibition of complex I is reversible by light or reduced thiols, and this may indicate S-nitrosation or Fe-nitrosylation. The inhibition of complex I appears to be one of the most important means of NO-induced inhibition of cellular respiration, at least in some conditions, although other mitochondrial components are also inhibited. iNOS-expressing cells and tissues exhibit an inhibition of complex I, which might contribute to inflammatory pathology for example in Parkinson's disease and septic shock.

Acknowledgements

Our own work in this field has been supported by the Royal Society, Wellcome Trust, Medical Research Council and British Heart Foundation.

References

[1] J. Carroll, R.J. Fearnley, J. Shannon, J.E. Hirst, J.E. Walker, Analysis of the subunit composition of complex I from bovine heart mitochondria, Mol. Cell. Proteomics 2 (2003) 117–126.
[2] J. Hirst, J. Carroll, I.M. Fearnley, R.J. Shannon, J.E. Walker, The nuclear encoded subunits of complex I from bovine heart mitochondria, Biochim. Biophys. Acta 1604 (2003) 135–150.
[3] M.J. Runswick, I.M. Fearnley, J.M. Skehel, J.E. Walker, Presence of an acyl carrier protein in NADH:ubiquinone oxidoreductase from bovine heart mitochondria, FEBS Lett. 286 (1991) 121–124.
[4] J.F. Turrens, A. Boveris, Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria, Biochem. J. 191 (1980) 421–427.
[5] J. St-Pierre, J.A. Buckingham, S.J. Roebuck, M.D. Brand, Topology of superoxide production from different sites in the mitochondrial electron transport chain, J. Biol. Chem. 277 (2002) 44784–44790.
[6] Y. Liu, G. Fiskum, D. Schubert, Generation of reactive oxygen species by the mitochondrial electron transport chain, J. Neurochem. 80 (2002) 780–787.
[7] C. Chauvin, F. De Oliveira, X. Ronot, M. Mousseau, X. Leverve, E. Fontain, Rotenone inhibits the mitochondrial permeability transition-induced cell death in U937 and KB cells, J. Biol. Chem. 276 (2001) 41394–41398.
[8] I.M. Fearnley, J. Carroll, R.J. Shannon, M.J. Runswick, J.E. Walker, J.E. Hirst, GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH:ubiquinone oxidoreductase (complex I), J. Biol. Chem. 276 (2001) 38345–38348.
[9] E.V. Gavrikova, A.D. Vinogradov, Active/de-active state transition of the mitochondrial complex I as revealed by specific sulfhydryl group labeling, FEBS Lett. 455 (1999) 36–40.
[10] A.D. Vinogradov, Catalytic properties of the mitochondrial NADH-ubiquinone oxidoreductase (complex I) and the pseudo-reversible active/inactive enzyme transition, Biochim. Biophys. Acta 1364 (1998) 169–185.
[11] S. Papa, A.M. Sardanelli, S. Scacco, V. Petruzzella, Z. Technikova-Dobrova, R. Vergari, A. Signorile, The NADH:ubiquinone oxidoreductase (complex I) of the mammalian respiratory chain and the cAMP cascade, J. Bioenerg. Biomembranes 34 (2002) 1–10.
[12] L.J. Ignarro, Nitric Oxide: Biology and Pathobiology, Academic Press, San Diego, 2000.
[13] C. Giulivi, J.J. Poderoso, A. Boveris, Production of NO by mitochondria, J. Biol. Chem. 273 (1998) 11038–11043.
[14] P. Ghafourifar, U. Schenk, S.D. Klein, C. Richter, Mitochondrial nitric-oxide synthase stimulation causes cytochrome *c* release from isolated mitochondria—evidence for intramitochondrial peroxynitrite formation, J. Biol. Chem. 274 (1999) 31185–31188.
[15] J.L. Zweier, A. Samouilov, P. Kuppusamy, Non-enzymatic nitric oxide synthesis in biological systems, Biochim. Biophys. Acta 1411 (1999) 250–262.
[16] G.C. Brown, Nitric oxide and mitochondrial respiration, Biochim. Biophys. Acta 1411 (1999) 351–369.
[17] G.C. Brown, Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome oxidase, Biochim. Biophys. Acta 1504 (2001) 46–57.
[18] A. Cassina, R. Radi, Different inhibitory actions of NO and peroxynitrite on mitochondrial electron transport, Arch. Biochem. Biophys. 328 (1996) 309–316.
[19] M.W.J. Cleeter, J.M. Cooper, V.M. Darley-Usmar, S. Moncada, A.H.V. Schapira, Reversible inhibition of cytochrome oxidase, the terminal enzyme of the mitochondrial respiratory chain by NO, FEBS Lett. 345 (1994) 50–54.
[20] G.C. Brown, C.E. Cooper, Nanomolar concentrations of NO reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase, FEBS Lett. 356 (1994) 295–298.
[21] M. Schweizer, C. Richter, NO potently and reversibly deenergizes mitochondria at low oxygen tension, Biochim. Biophys. Acta 204 (1994) 169–175.
[22] G.C. Brown, V. Borutaite, Nitric oxide, mitochondria, and cell death, IUBMB Life 52 (2001) 189–195.
[23] E. Clementi, G.C. Brown, M. Feelisch, S. Moncada, Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 7631–7636.
[24] F.R. Gadelha, L. Thomson, M.M. Fagian, A.D.T. Costa, R. Radi, A.E. Vercesi, Calcium-independent permeabilization of the inner mitochondrial membrane by peroxynitrite is mediated by membrane protein thiol cross-linking and lipid peroxidation, Arch. Biochem. Biophys. 345 (1997) 243–250.
[25] J.J. Poderoso, M.C. Carreras, C. Lisdero, N. Riobo, F. Schopfer, A. Boveris, NO inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles, Arch. Biochem. Biophys. 328 (1996) 85–92.
[26] M.A. Packer, M.P. Murphy, Peroxynitrite causes calcium efflux from mitochondria which is prevented by cyclosporin A, FEBS Lett. 345 (1994) 237–240.
[27] V. Borutaite, R. Morkuniene, G.C. Brown, Release of cytochrome *c* from heart mitochondria is induced by high calcium and peroxynitrite and is responsible for calcium-induced inhibition of substrate oxidation, Biochim. Biophys. Acta 1453 (1999) 41–48.
[28] D.L. Granger, A.L. Lehninger, Sites of inhibition of mitochondrial electron transport in macrophage-injured neoplastic cells, J. Cell Biol. 95 (1982) 527–535.
[29] J.C. Drapier, J.B. Hibbs Jr., Differentiation of murine macrophages to express non-specific cytotoxicity for tumour cells results in L-arginine-dependent inhibition of mitochondrial iron–sulfur in the macrophage effector cells, J. Immunol. 140 (1988) 2829–2838.
[30] D.J. Stuehr, C.F. Nathan, NO: a macrophage product responsible for cytostasis and respiratory inhibition in tumour target cells, J. Exp. Med. 169 (1989) 1543–1555.
[31] J.B. Hibbs Jr., R.R. Taintor, Z. Vavrin, E.M. Rachlin, Nitric oxide: a cytotoxic activated macrophage effector molecule, Biochem. Biophys. Res. Commun. 157 (1988) 87–94.

[32] J.R. Lancaster, J.B. Hibbs, EPR demonstration of iron–nitrosyl complex formation by cytotoxic activated macrophages, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1223–1227.
[33] R. Welter, L. Yu, C.A. Yu, The effects of NO on electron transport complexes, Arch. Biochem. Biophys. 331 (1996) 9–14.
[34] P.A. Rogers, L. Eide, A. Klungland, H. Ding, Reversible inactivation of *E. coli* endonuclease III via modification of its [4Fe–4S] cluster by nitric oxide, DNA Repair 2 (2003) 809–817.
[35] P.R. Gardner, G. Costantino, C. Szabo, A.L. Salzman, Nitric oxide sensitivity of the aconitases, J. Biol. Chem. 272 (1997) 25071–25076.
[36] L.A. Castro, R.L. Robalinho, A. Cayota, R. Meneghini, R. Radi, Nitric oxide and peroxynitrite-dependent aconitase inactivation and iron-regulatory protein-1 activation in mammalian fibroblasts, Arch. Biochem. Biophys. 359 (1998) 215–224.
[37] B. Beltran, A. Orsi, E. Clementi, S. Moncada, Oxidative stress and S-nitrosylation of proteins in cells, Br. J. Pharmacol. 129 (2000) 953–960.
[38] V. Borutaite, A. Budriunaite, G.C. Brown, Reversal of nitric oxide-, peroxynitrite-, and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols, Biochim. Biophys. Acta 1459 (2000) 405–412.
[39] A. Jekabsone, L. Ivanoviene, G.C. Brown, V. Borutaite, Nitric oxide and calcium together inactivate mitochondrial complex I and induce cytochrome *c* release, J. Mol. Cell. Cardiol. 35 (2003) 803–809.
[40] N.A. Riobo, E. Clementi, M. Melani, A. Boveris, E. Cadenas, S. Moncada, J.J. Poderoso, Nitric oxide inhibits mitochondrial NADH: ubiquinone reductase activity through peroxynitrite formation, Biochem. J. 359 (2001) 139–145.
[41] T. Yamamoto, W. Maruyama, Y. Kato, H. Yi, M. Shamoto-Nagai, M. Tanaka, Y. Sato, M. Naoi, Selective nitration of mitochondrial complex I by peroxynitrite: involvement in mitochondria dysfunction and cell death of dopaminergic SH-SY5Y cells, J. Neural Transm. 109 (2002) 1–13.
[42] J. Murray, S.W. Taylor, B. Zhang, S.S. Ghosh, R.A. Capaldi, Oxidative damage to mitochondrial complex I due to peroxynitrite. Identification of reactive tyrosines by mass spectrometry, J. Biol. Chem. 278 (2003) 37223–37230.
[43] R. Radi, M. Rodriguez, L. Castro, R. Telleri, Inhibition of mitochondrial electron transport by peroxynitrite, Arch. Biochem. Biophys. 308 (1994) 89–95.
[44] P.S. Brookes, J.M. Land, J.B. Clark, S.J. Heales, Peroxynitrite and brain mitochondria: evidence for increased proton leak, J. Neurochem. 70 (1998) 2195–2202.
[45] E.R. Taylor, F. Hurrell, R.J. Shannon, T.-K. Lin, J. Hirst, M.P. Murphy, Reversible glutathionylation of complex I increases mitochondrial superoxide formation, J. Biol. Chem. 278 (2003) 19603–19610.
[46] D. Brealey, M. Brand, I. Hargreaves, S. Heales, J. Land, R. Smolenski, N.A. Davies, C.E. Cooper, M. Singer, Association between mitochondrial dysfunction and severity and outcome of septic shock, Lancet 360 (2002) 219–223.
[47] D. Brealey, S. Karyampudi, T.S. Jaques, M. Novelli, R. Stidwill, V. Taylor, R.T. Smolenski, M. Singer, Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure, Am. J. Physiol. 286 (2004) R491–R497.
[48] M. Orth, A.H. Schapira, Mitochondrial involvement in Parkinson's disease, Neurochem. Int. 40 (2002) 533–541.
[49] B. Chenais, H. Morjani, J.C. Drapier, Impact of endogenous nitric oxide on microglial cell energy metabolism and labile iron pool, J. Neurochem. 81 (2002) 615–623.
[50] S.J. Heales, J.P. Bolanos, V.C. Stewart, P.S. Brookes, J.M. Land, J.B. Clark, Nitric oxide, mitochondria and neurological disease, Biochim. Biophys. Acta 1410 (1999) 215–228.
[51] F. Antunes, D. Han, D. Rettori, E. Cadenas, Mitochondrial damage by nitric oxide is potentiated by dopamine in PC12 cells, Biochim. Biophys. Acta 1556 (2002) 233–238.
[52] J.L. Madrigal, R. Olivenza, M.A. Moro, I. Lizasoain, P. Lorenzo, J. Rodrigo, J.C. Leza, Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain, Neuropsychopharmacology 24 (2001) 420–429.