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Effect of anoxia/reperfusion on the reversible active/de-active transition of NADH–ubiquinone oxidoreductase (complex I) in rat heart

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Abstract

The multi-subunit mammalian NADH–ubiquinone oxidoreductase (complex I) is part of the mitochondrial electron transport chain and physiologically serves to reduce ubiquinone with NADH as the electron donor. The three-dimensional structure of this enzyme complex remains to be elucidated and also little is known about the physiological regulation of complex I. The enzyme complex in vitro is known to exist as a mixture of active (A) and de-active (D) forms [Biochim. Biophys. Acta 1364 (1998) 169]. Studies are reported here examining the effect of anoxia and reperfusion on the A/D-equilibrium of complex I in rat hearts ex vivo. Complex I from the freshly isolated rat heart or after prolonged (1 h) normoxic perfusion exists in almost fully active form (87±2%). Either 30 min of nitrogen perfusion or global ischemia decreases the portion of active form of complex I to 40±2%. Upon re-oxygenation of cardiac tissue, complex I is converted back predominantly to the active form (80–85%). Abrupt alternation of anoxic and normoxic perfusion allows cycling between the two states of the enzyme. The possible role in the physiological regulation of complex I activity is discussed.

Keywords: NADH–ubiquinone oxidoreductase; Complex I; Ischemia; Mitochondrial respiration; Regulation

1. Introduction

NADH–ubiquinone oxidoreductase (complex I) catalyzes the first step in the mitochondrial electron transport chain, the reduction of ubiquinone by NADH, which is coupled to proton translocation across the inner mitochondrial membrane [1,2]. Complex I has a key role in supplying the mitochondrial respiratory chain with reducing equivalents since the majority of the electrons are provided by NADH generated via reactions of the Krebs cycle. Bovine complex I consists of at least 43 individual subunits [1,3]. The redox centers of the enzyme include one noncovalent FMN moiety, as many as eight iron sulfur clusters [2,4], and up to three detectable ubisemiquinone species [5]. Several quite different catalytic mechanisms have been proposed over the years for complex I [6,7]. Nevertheless, a full understanding of the catalytic mechanism of complex I remains to be elucidated.

In addition to its role in electron transport, recent experimental observations suggest the involvement of complex I or its subunits in other metabolic pathways. A small nuclear encoded subunit of complex I (10 kDa) has been identified as an acyl carrier protein [8]. Its removal makes yeast respiration defective [9] and the subunit may be involved in lipoic acid synthesis [10]. Complex I also may play a key role in regulation of the permeability transition pore of mitochondria [11] and is an important source of reactive oxygen species (ROS) [12,13]. Recently, complex I was linked to the apoptotic cell death pathway when GRIM-19 was shown to be the 43rd subunit of bovine NADH–ubiquinone oxidoreductase [3].

Abbreviations: SMP, submitochondrial particles; ROS, reactive oxygen species; A-form, active form of complex I; D-form, de-active form of complex I; A↔D, reversible active/de-active transition of complex I; BSA, bovine serum albumin; Q_1, homologue of natural ubiquinone; NEM, *N*-ethylmaleimide; SQR, succinate–ubiquinone reductase

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Although little is known about the regulation of complex I, it has been shown that the enzyme undergoes a slow active/de-active transition in vitro [14]. It was proposed that the transition between Active (A) and De-active (D) forms might play a functional role in mammalian cell metabolism [14,15]. The A-form catalyzes the physiologically relevant rotenone-sensitive NADH–ubiquinone reductase reaction. The D-form, by contrast, can be fully reduced by NADH and oxidized by artificial electron acceptors, but is unable to transfer electrons to ubiquinone [16]. The de-activation of complex I is reversible, although transitions between the A- and D-form require different conditions. De-activation, or the A to D transition, is strongly temperature-dependent (270 kJ/mol) and essentially does not occur at temperatures below 20 °C (i.e., the enzyme remains active). Under conditions where no enzyme turnover is permitted due to complete reduction of the membrane ubiquinone pool (regardless of whether the enzyme is reduced with NADH or not), complex I is found largely in the de-activated form [17]. Activation of the D-form of the enzyme is a result of slow activating turnover(s), which includes fast reduction by NADH and a slow (at least two orders of magnitude compared to catalytic turnover) oxidation by quinone [17]. The rate of activation of the enzyme is less dependent upon temperature (170 kJ/mol), and decreases at alkaline pH and in the presence of divalent cations [18]. The A-form also is not sensitive to sulfhydryl reagents, whereas the D-form is specifically modified and inactivated by *N*-ethylmaleimide (NEM) and other sulfhydryl agents [19].

The phenomenon of the reversible active/de-active (A↔D) transition described above has been shown for isolated bovine complex I and for the enzyme in submitochondrial particles (SMP) [16,20]. Therefore, it is of interest that recently, the transitions were also observed in isolated mitochondria permeabilized with the channel-forming antibiotic alamethicin to deplete the mitochondria of endogenous NADH [21]. However, it is still unknown whether complex I undergoes the A↔D transition in vivo. In cells operating in normoxic conditions, where complex I catalyzes steady-state NADH oxidation, it seems likely that the majority of the enzyme would be in the active form [21]. Under hypoxic conditions such as global ischemia, where lack of molecular oxygen leads to high levels of ubiquinol, it is expected that complex I would be de-activated. Cardiac function can be fully restored upon reperfusion if periods of ischemia due to coronary occlusion are relatively short, while longer periods of ischemia cause irreversible tissue injury, and this damage can be exacerbated by reoxygenation during reperfusion [22]. It has been shown in the Langendorff-perfused rat heart that complex I is very sensitive to this damage [23]. Anoxic perfusion had a protective effect on complex I activity and subsequent oxygen reperfusion did not cause further loss of its activity [24]. Thus, in order to study the A↔D transition of complex I in cardiac tissue, we subjected standard Langendorff-perfused rat hearts to global ischemia or anoxic perfusion followed by subsequent normoxic reperfusion. The data presented here show that in freshly isolated rat heart, as well as after 60 min of normoxic perfusion, complex I is present in an almost fully active state. Either global ischemia or anoxic perfusion converts the majority of complex I to the de-active state. Subsequent normoxic reperfusion returns complex I to the predominantly active form. Therefore, these results demonstrate that alternation of anoxic and normoxic conditions does cause the A↔D transition of complex I in a whole organ preparation.

2. Materials and methods

2.1. Langendorff-perfused rat hearts

Male Sprague–Dawley rats (250–300 g) were anaesthetized with sodium pentobarbital (60 mg/kg, IP) and anticoagulated with heparin (5000 USP units/kg, IP). The thorax was opened and the heart excised and rapidly transferred into an ice-cold solution of 120 mM NaCl, 30 mM KCl. The heart was then cannulated via the aorta and perfusion started within 1 min in the Langendorff apparatus with a constant pressure of 70 mm Hg and at a constant perfusate temperature of 37 °C as previously described [25]. Before any further treatment, the heart was equilibrated for 5 min by perfusion with a modified Krebs–Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$, 5.5 mM glucose, 5 mM sodium pyruvate, 0.5 mM Na_2EDTA, pH 7.4) that was vigorously bubbled with O_2/CO_2 (19:1, normoxia). Anoxia was induced by switching the perfusion to the same buffer saturated with N_2/CO_2 (19:1). Global ischemia was achieved by the cessation of coronary flow.

2.2. Isolation of rat heart mitochondria

Immediately after being removed from the perfusion apparatus, the heart was placed in isolation buffer (0.25 mM sucrose, 50 mM Tris–HCl (pH 7.8), 1 mM EDTA, 20 mM sodium azide, 20 mM succinate) that had been cooled to 0–2 °C in a salt/ice bath. All subsequent steps were performed at 0–2 °C, with all equipment and buffer precooled and stored on ice. Any fatty tissue present on the heart was removed and the heart was then minced into 2–3-mm pieces, isolation buffer added, and the tissue homogenized with a glass homogenizer with a motor-driven pestle. The homogenized preparation was then centrifuged at 600×*g* for 10 min. The supernatant containing the mitochondria was carefully decanted and then centrifuged at 8000×*g* for 10 min. The pellet was then suspended in the isolation buffer and washed two times by centrifugation at 8000×*g* for 10 min in the same buffer. After the final centrifugation the washed mitochondria were stored at −80 °C.

2.3. Preparation of SMP

All steps were performed at 0–4 °C. The mitochondria were resuspended in 4-ml of 0.125 mM sucrose, 20 mM sodium azide, 20 mM succinate and the pH adjusted to 9.6 with ammonium hydroxide [26]. The mixture was sonicated four times for 15 s with a cooling interval of 30 s and then centrifuged at 35,000×*g* for 15 min. The resulting supernatant was centrifuged for 1.5 h at 130,000×*g*. The pellet containing the SMP was resuspended in 0.25 mM sucrose and stored at −80 °C. Protein concentration was determined with the BCA protein assay (Pierce, Rockford, IL) using bovine serum albumin (BSA) as standard.

2.4. NADH–Q_1 reductase activity

The standard assay mixture contained 0.25 mM sucrose, 20 mM Tris–HCl (pH 8.4), 0.2 mM EDTA, 0.5 μg/ml gramicidin D, 1 mg/ml BSA in a 1-ml cuvette. The NADH–Q_1 reductase reaction was assayed at 25 °C with 40 μM Q_1 in the presence of 2 mM KCN after initiating the reaction by addition of 150 μM NADH (ε^{340}=6.22 mM^{-1} cm^{-1}). Since only the A-form of complex I is able to catalyze quinone reduction in the presence of divalent cations [18], 7 mM $MgCl_2$ was added to the assay to determine the fraction of the A-form of complex I. The fraction of the A-form was calculated as the ratio of NADH–Q_1 reductase activity of as isolated SMP to the activity of fully activated SMP. As isolated SMP were assayed in the presence of 7 mM $MgCl_2$. Full activation of SMP was achieved by oxidation of 5 μM NADH in a cuvette with Q_1 before $MgCl_2$ was added and then 150 μM NADH initiated the reaction.

2.5. NADH–hexaaminoruthenium reductase activity

Activity was determined as described [27] at 25 °C in 2 ml of the standard assay mixture described above and with 0.5 mM NADH (ε^{380}=1.23 mM^{-1} cm^{-1}) and 2 mM $Ru(NH_3)_6^{3+}$ (Strem Chemicals, Newburyport, MA) in the presence of 1 μM rotenone.

3. Results

We hypothesized that various complex I A/D equilibria should be established in cardiac tissue associated with different perfusion conditions. Thus, it was necessary to find suitable conditions to preserve the A/D ratios by preventing activation/de-activation of complex I throughout SMP isolation. Such conditions have been established for in vitro studies of bovine complex I [14] and initial isolation of SMP relied on these procedures. De-activation of complex I is completely prevented at low temperatures (0–2 °C) [16]. Therefore, as soon as perfusion ceased, the heart was immediately placed in isolation medium that was cooled to 0–2 °C in a salt/ice bath. Although the rate of the D-to-A transition does not proceed at a significant rate at this temperature [16], additional protection against redox-dependent activation was thought to be necessary. Divalent cations reversibly bind to the D-form and slow activation, while the NADH–quinone reductase reaction catalyzed by the A-form is not affected [18,20]. Therefore, 10 mM $MgCl_2$ was added to the mitochondrial isolation buffer in order to determine if this would block any residual activation. This concentration of Mg^{2+} had no effect on normoxically perfused hearts, however, when the hearts were subjected to 45 min of ischemia, complex I activity was permanently decreased. Additional incubation of SMP with EDTA did not restore the activity (data not shown). Another approach to prevent complex I activation is to maintain the quinone pool in a highly reduced state. Therefore, 20 mM succinate and 20 mM sodium azide were used throughout SMP isolation. Under these conditions, complex I activity of SMP from normoxic or ischemic hearts was not affected. Thus, sodium azide and succinate were used in the isolation medium for mitochondria and SMP (see Materials and methods).

To identify the A- and D-form of complex I, a simple kinetic assay is routinely used. The A-form of the enzyme is able to catalyze the NADH–quinone reductase reaction while transition of the D-form to the A-form is characterized by a pronounced lag-phase at alkaline pH in the presence of divalent cations. Fig. 1 shows complex I activity catalyzed by SMP isolated from Langendorff-perfused rat hearts subjected to different perfusion conditions. The addition of $MgCl_2$ to the assay medium, prior to initiation of the reaction with NADH, allows only the A-form to operate (Fig. 1; traces 1a and b). Full activation of complex I is achieved by oxidation of 5 μM NADH in the presence of

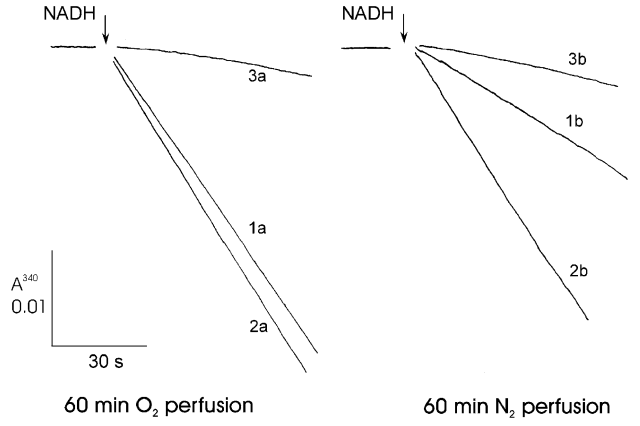

Fig. 1. Complex I activity of submitochondrial particles isolated from Langendorff perfused rat hearts. NADH–Q_1 reductase of SMP (5 μg/ml final concentration) was assayed as described in Materials and methods; 7 mM $MgCl_2$ was added into the cuvette prior to initiation of the reaction by 150 μM NADH (traces 1a and 1b). SMP (5 μg/ml) were fully activated by oxidation of 5 μM NADH before 7 mM $MgCl_2$ was added and the reaction started with 150 μM NADH (traces 2a and 2b). SMP (1 mg/ml) were thermally de-activated (15 min at 37 °C) and then assayed in the presence of 7 mM $MgCl_2$ (traces 3a and 3b).

quinone before the addition of Mg^{2+} (Fig. 1; traces 2a and b). The activation state of complex I from normoxically perfused hearts determined in Fig. 1A is 87% (ratio of activity from trace 1a to activity from trace 2a), which is very similar to that from freshly isolated rat hearts seen in Table 1. By contrast, prolonged anoxic perfusion with nitrogen markedly reduces the portion of A-form of complex I to only 40% (Fig. 1; ratio of activity from trace 1b to activity from trace 2b). The SMP isolated from either normoxic or hypoxic hearts can be fully de-activated by incubation for 15 min at 37 °C (Fig. 1; traces 3a and b). Such thermally deactivated SMP can be activated again by preincubation with 5 μM NADH and quinone as shown for traces 2a and b.

Another characteristic of de-active complex I is rapid and irreversible inhibition of the enzyme by NEM, while the active form is not sensitive to sulfhydryl agents under the same conditions [14]. Fig. 2 represents the data comparing the kinetic of SMP modification by NEM after prolonged normoxic and anoxic perfusion of the hearts. Normoxic SMP, as isolated (Fig. 1, trace 1a), were quite resistant to treatments with 1 mM NEM (Fig. 2; closed circles, ●). When SMP were incubated for 15 min at 37 °C to convert complex I into the de-active form (Fig. 1, trace 3a) prior to the modification, the enzyme was rapidly inactivated by NEM (Fig. 2; closed triangles, ▲). In contrast, the NEM modification of anoxic SMP (Fig. 1, trace1b) showed partial inhibition (Fig. 2; open diamonds, ◇) of NADH–Q_1 reductase that correlates to about 40% of complex I active form remaining in anoxic SMP.

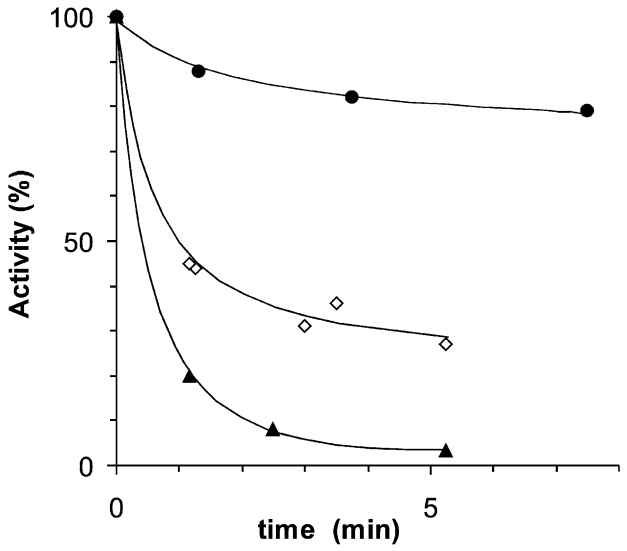

Fig. 2. Irreversible inhibition of complex I by NEM in submitochondrial particles isolated from perfused rat hearts after 60-min normoxic or 60-min anoxic perfusion. SMP (0.5 mg/ml) were incubated at 25 °C in the presence of 1 mM NEM in the assay buffer (pH 7.8) for the time indicated on the abscissa. The aliquots of the SMP suspension (5 μg) were withdrawn and NADH–Q_1 reductase was determined at 25 °C (pH 7.8) with 150 μM NADH and 40 μM Q_1: (◇) 60-min nitrogen perfusion; (●) 60-min normoxic perfusion; (▲) SMP from normoxic perfused heart subjected to 15 min incubation at 37 °C, then cooled and incubated with 1 mM NEM. One hundred percent activity corresponds to 1.4 μmol NADH/min/mg protein.

Table 1
Effect of anoxia/reperfusion on activities and activation state of complex I from rat hearts

	NADH–Ru^{3+}	NADH–Q_1 (+7 mM Mg^{2+})		Active-form complex I (%)[a]
		As isolated[b]	Pre-activated[c]	
	μmol/min/mg protein			
Freshly isolated heart	13.7±0.06	1.16±0.03	1.33±0.03	87±2
60 min normoxic perfusion	14.7±0.10	1.20±0.02	1.40±0.02	86±2
30 min global ischemia	12.7±0.02	0.43±0.02	1.14±0.03	38±2
+20 min reperfusion	13.0±0.03	0.75±0.02	1.00±0.03	75±2
45 min global ischemia	12.07±0.06	0.28±0.01	0.69±0.04	41±3
+20 min reperfusion	12.26±0.02	0.40±0.01	0.58±0.03	69±3
60 min nitrogen perfusion	14.1±0.16	0.52±0.01	1.34±0.04	39±2
+20 min reperfusion	13.6±0.08	0.97±0.03	1.29±0.03	75±4

Each point is a mean±S.E. of three independent experiments.
[a] Activation status as ratio of the as isolated to pre-activated enzyme.
[b] Initial rate of the reaction determined with 7 mM $MgCl_2$ started by addition of NADH.
[c] SMP were pre-incubated with 5 μM NADH in the reaction medium, then 7 mM $MgCl_2$ was added, and the reaction started by NADH.

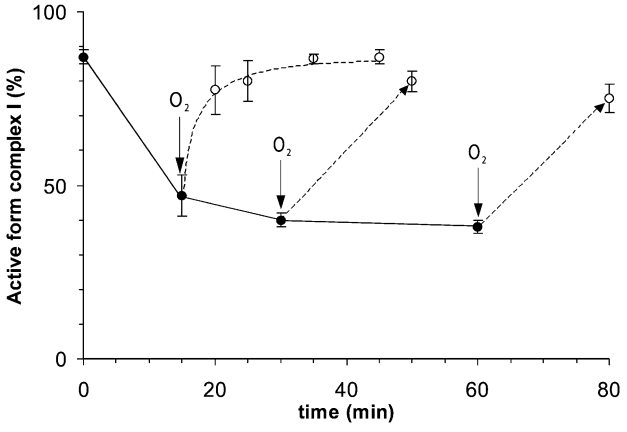

Fig. 3. Effect of nitrogen perfusion and oxygen reperfusion of Langendorff rat hearts on the activation state of complex I. Rat hearts were equilibrated with normoxic buffer for 5 min after that the perfusion line was switched to nitrogen saturated buffer (zero time) and the hearts were continuously perfused (closed symbols, ●). Dashed lines (open symbols, ○) indicate when normoxic reperfusion started after 15, 30, and 60 min of nitrogen perfusion. Submitochondrial particles were isolated from each heart and NADH–Q_1 reductase were assayed in the presence of 7 mM $MgCl_2$ as described in Materials and methods. Each point is a mean±S.E. of three independent experiments.

Fig. 3 represents the time dependence of the A/D equilibrium of SMP isolated from rat hearts upon anoxic perfusion (closed circles, ●). Although the apparent equilibrium of 38±2% A-form is established by 30 min of the perfusion, 15 min of anoxia already significantly reduces the portion of A-form to 47±6%. When oxygen is re-introduced to the anoxic cardiac tissue through normoxic reperfusion, the hearts resume beating and reach regular rhythmical contraction within 5–7 min of reperfusion (data not shown). Analysis of the activation state of complex I following reperfusion (Fig. 3, open circles, ○; dashed lines, --) shows that following 15 min of anoxia, substantial activation of complex I occurs within the first 5 min of reperfusion and the initial activation state is reached in 20 min. The data in Fig. 3 show that complex I can also be activated to approximately the same extent after 30 and 60 min of anoxia. Global ischemia (for 30 min) produces similar results compared to anoxic perfusion (Table 1) since both conditions eliminate oxygen supply from the tissue. The data in Fig. 3 and Table 1 indicate that complex I can alternate between the A-form and D-form depending upon the state of oxygenation of the cardiac tissue. However, in an agreement with previous studies [23], complex I can be inactivated by prolonged global ischemia. This may be due to different metabolic conditions established in ischemia and anoxic perfusion: (i) while ischemia leads to substrate deprivation and waste buildup; (ii) perfusion constantly provides substrates for glycolysis and prevents accumulation of its products.

In order to further investigate the ability of complex I to undergo the A↔D transition in ex vivo conditions, two perfusion cycles were performed. Each cycle consisted of 15 min of anoxic and 20 min of normoxic perfusion. As seen in Fig. 4, the cardiac tissue that had been through the first cycle responded to the second nitrogen perfusion by a decrease of the A-form fraction of complex I to a level similar to the first cycle of anoxia. The final normoxic reperfusion again caused significant activation of complex I. Thus, alternation of normoxic/anoxic perfusion in rat hearts causes reversible transition of complex I from highly active (normoxic) to predominantly de-active (anoxic) state.

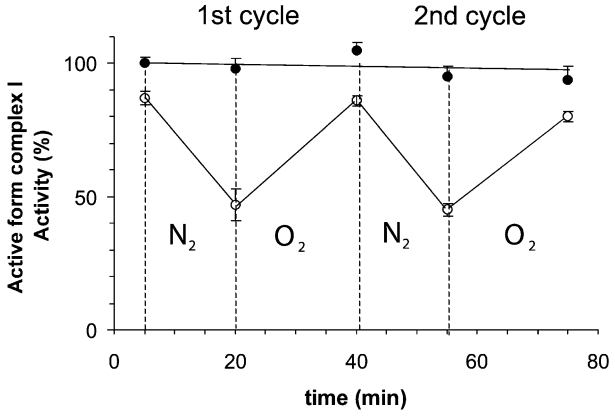

Fig. 4. Effect of alternation of anoxic and normoxic perfusion of Langendorff rat hearts on activity and activation state of complex I. Rat hearts were equilibrated with normoxic buffer for 5 min and then two cycles each consisting of 15-min nitrogen and 20-min oxygen perfusion were performed. Rotenone-sensitive NADH–Q reductase (●) and fraction of the active form of complex I (○) were analyzed in SMP obtained from the hearts. One hundred percent NADH–Q reductase activity corresponds to 1.4 μmol/min/mg protein. Each point is a mean±S.E. of three independent experiments.

4. Discussion

The data presented show that either nitrogen perfusion or global ischemia in isolated rat hearts significantly reduced the fraction of the active form of complex I compared to normoxic perfusion. The alternation between normoxic and anoxic perfusion in isolated beating rat hearts resulted in modulation of complex I between almost fully active (normoxic) and predominately de-active (anoxic) forms. In vitro data have shown that complex I within SMP can be thermally de-activated to only 2–8% of the A-form [14–16]. This communication reveals that SMP from nitrogen perfused rat hearts retain about 40% of the A-form. It thus becomes important to ascertain whether the higher activation levels of complex I found in SMP isolated from perfused hearts reflect the true A/D equilibria or if the higher activation levels observed might be due to the experimental conditions. To preserve the equilibrium between forms, isolation of SMP took into account properties of the A↔D transition known from in vitro experiments and was done in the presence of succinate and sodium azide to maintain the quinone pool in the reduced state.

Nevertheless, we cannot exclude the possibility that oxygen diffusion through the outer surface of the heart during anoxic perfusion may have contributed to the higher state of activation observed. Hearts were washed with eluted buffer from the perfusate, but they were not placed in an anoxic chamber. Since the conditions leading to de-activation of complex I produce high levels of mitochondrial NADH [28,29], traces of oxygen in cardiac tissue could provide slow turnovers of the respiratory chain which are able to shift the A/D equilibrium to a higher activation state. A simple experiment to test this suggestion was conducted by imposing 30 min of global ischemia on rat heart in the presence of 5 mM potassium cyanide in the perfusate buffer to block cytochrome c-oxidase. Indeed, the activation state of complex I determined in the presence of the respiratory inhibitor decreased to 32% (data not shown) similar to the state of activation seen for global ischemia alone (Table 1), which is consistent with residual oxygen in the tissue being responsible for the remaining active form of complex I. Another possibility for a slow turnover of complex I during anoxic conditions is the ability of succinate–ubiquinone reductase (SQR, complex II) to act reversibly and reduce fumarate by quinol. It has been suggested that anaerobic metabolic pathways involving complexes I and II can

account for maintenance of residual ΔμH during hypoxia [30].

There are a number of low molecular weight ligands that are known to affect the A/D equilibrium in vitro. It has been suggested that millimolar levels of free Mg^{2+} and variable concentrations of Ca^{2+} in the mitochondrial matrix should preserve the D-form of complex I formed during hypoxia even after re-oxygenation [21]. Indeed, as seen in Fig. 3, establishing a new A/D equilibrium after re-oxygenation is not instantaneous. It has been shown that the A- and D-form of complex I exist in different conformations based on their different affinities for sulfhydryl agents and on a change in affinity for quinone binding site inhibitors [31]. For example, rotenone preferentially binds to the A-form of the enzyme (K_i=1 nM, versus 80 nM for the D-form) and partially protects the complex I A-form from thermal deactivation [31]. In addition, it was shown that when deactivated SMP were incubated with rotenone, the A/D equilibrium increased from 2% up to 50% active form. A similar effect was demonstrated by incubating complex I with Triton X-100 which is known to inhibit the enzyme [32]. In agreement with the data above, when SMP had been pre-incubated with NADH (conditions which shift the equilibrium towards the A-form), photoaffinity labeling of complex I with Q-site inhibitors increased PSST subunit labeling, but decreased ND1 subunit labeling [33]. Arachidonic acid is a natural high affinity quinone site inhibitor of complex I [34] and it is known that hypoxia significantly increases the levels of this fatty acid in mitochondria [35]. Therefore, it is possible that arachidonic acid and/or other long chain fatty acids may affect the A/D equilibrium by binding differently to the A- and D-form of complex I.

In this work we have used an ex vivo experimental model for the study of the complex I A↔D transition where a perfused heart retains physiological properties attributed to live tissue. Thus, mitochondria maintain their integrity, homeostasis, transmembrane potential, and respond to metabolic changes in the cells. The observations reported here that complex I in ex vivo conditions undergoes changes in the A/D equilibria as a response to hypoxia/normoxia do not yet prove the physiological relevance of the phenomenon as a regulatory mechanism for NADH oxidation. Nevertheless, the nature of the phenomenon allows us to suggest at least two mechanisms for the regulation of complex I involving the A↔D transition. One is the dependence of A/D equilibria on a combination of factors such as temperature, oxygenation, mitochondrial Ca^{2+} and Mg^{2+} concentration, and natural complex I Q-site inhibitors. A second mechanism which should be considered is the selective covalent modification of the A- and D-form, as is clearly seen for the highly reactive cysteine residue present in the D-form [19]. Recently, cAMP-mediated intracellular signal transduction through serine phosphorylation of the 18-kDa subunit of complex I has been reported to affect enzyme activity [36]. Thus, triggering cell signaling pathways may affect the A/D equilibrium and study of such pathways may answer whether the A↔D transition controls mitochondrial respiration. Regulation of complex I activity may have important consequences for the production of ROS which might be formed as cardiac tissue is re-oxygenated following a coronary occlusion.

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