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Conformation-specific crosslinking of mitochondrial complex I

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ABSTRACT

Complex I is the only component of the eukaryotic respiratory chain of which no high-resolution structure is yet available. A notable feature of mitochondrial complex I is the so-called active/de-active conformational transition of the idle enzyme from the active (A) to the de-active, (D) form.

Using an amine- and sulfhydryl-reactive crosslinker of 6.8 Å length (SPDP) we found that in the D-form of complex I the ND3 subunit crosslinked to the 39 kDa (NDUFA9) subunit. These proteins could not be crosslinked in the A-form. Most likely, both subunits are closely located in the critical junction region connecting the peripheral hydrophilic domain to the membrane arm of the enzyme where the entrance path for substrate ubiquinone is and where energy transduction takes place.

Structured summary of protein interactions:
ND3 and **NDUFA9** physically interact by cross-linking study (View interaction)

1. Introduction

Mitochondrial complex I (EC 1.6.5.3, proton-translocating NADH:ubiquinone oxidoreductase) is responsible for oxidation of matrix NADH by membrane-bound ubiquinone and most likely, is also a major source of reactive oxygen species (ROS) generated by the mitochondrial respiratory chain [1–3]. This enzyme is the largest component of the mitochondrial and bacterial respiratory chains [4]. Structural information about mitochondrial complex I is still very limited, although recently the atomic structure of the 14 subunits of the prokaryotic homologue enzyme was determined at 4.5 Å resolution [5]. Only a low resolution structure is available for the eukaryotic enzyme [6]. Unlike the 14 subunit bacterial enzyme, the mammalian complex I is composed of at least 44 different subunits and the function of more than 30 of the so-called accessory subunits is not completely understood [7,8].

A notable feature of mitochondrial complex I is the so-called active/de-active conformational transition of the idle enzyme from the fully competent, "active" (A) to the dormant, "de-activated" D-form. These catalytically and structurally distinct forms of the enzyme have been identified in purified preparations in vitro, in cells, as well as in rat heart ex vivo during lack of oxygen [9–11] (see [12] for detailed review). Only the A-form catalyses rotenone-sensitive oxidation of NADH with a fast rate of 10^4 min^{-1}. Incubation of idle enzyme at 30–37 °C results in its transition to the D-form. In presence of NADH and ubiquinone the D-form of complex I can be converted back to the A-form during slow (4 min^{-1}) catalytic turnover(s). It can be seen as a lag phase during continuous assay of the NADH-oxidase reaction catalysed by the D-form. This lag phase represents a slow activation of the D-form of the enzyme during the time of the measurement [10]. Several hypotheses for the function of the D-form of complex I accumulated in tissues in conditions of lack of oxygen were suggested: prevention of proton leakage through the idle enzyme [13], fine tuning of mitochondrial activity [14,15], interaction with nitric oxide metabolites [16], alleviating respiratory burst during reperfusion [17] and potential Na^+/H^+ antiporter activity [18].

The molecular mechanism of A/D transition is not known at present. Sensitivity of NADH-oxidation by submitochondrial particles (SMP) to SH-reagents after incubation at 37 °C was observed many years ago [19]. Recently the thiol group exposed to the

Abbreviations: DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); DTT, dithiothreitol; HAR, hexaammineruthenium(III)-chloride; NEM, N-ethylmaleimide; nLC-ESI-MSMS, nano-HPLC electrospray ionization multistage tandem mass spectrometry; ROS, reactive oxygen species; SMP, submitochondrial particles; SPDP, N-succinimidyl 3-(2-pyridyldithio) propionate

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outside and rendering sensitivity to SH-reagent of the D-form was identified as cysteine-39 of mitochondrial ND3 subunit [20]. This is the only known structural difference between two forms of the enzyme. However, the high activation energy of the A–D transition (270 kJ/mol [9]) indicates that major conformational changes take place during the de-activation process and, therefore, other subunits are most likely involved. It has been suggested that more than one protein is involved in complex I A/D transitions in *Neurospora crassa* complex I and 29.9 kDa protein (B13 in bovine enzyme) may modulate the process of de-activation [21].

Here we explored subunit proximity around the cysteine-39 residue of ND3 subunit in order to gain further information on conformational differences between two forms. Treatment of the D-form of complex I by the amine- and sulfhydryl-reactive heterobifunctional crosslinker N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP, length 6.8 Å) resulted in covalent binding of ND3 to the 39 kDa (NDUFA9) subunit indicating that these subunits are very closely located in the enzyme molecule. These subunits could not be crosslinked in the A-form. The implications of this finding with regard to the changes in the complex I molecule upon deactivation are discussed.

2. Materials and methods

2.1. SMP and activity measurements

Bovine heart SMP were prepared according to the standard procedure [9] and stored in liquid nitrogen. In order to prepare SMP in which complex I is present almost entirely in the D-form, an aliquot of frozen membranes was thawed, diluted to 5 mg/ml with standard medium (comprising 0.25 M sucrose, 50 mM Tris–HCl (pH 7.5), 0.2 mM EDTA) and incubated at 35 °C for 1 h and 30 min. This treatment resulted in almost complete deactivation of complex I. For complex I activation, SMP (5 mg/ml) were incubated aerobically for 30 min at room temperature with 1% ethanol and 400 μM NADH and 0.1 mg/ml alcohol dehydrogenase from *Saccharomyces cerevisiae* (Sigma). During incubation with alcohol dehydrogenase, the sub-stoichiometric amounts of NADH produced by the regeneration system were oxidized by complex I; resulting in full activation of the enzyme. The diagnostic test for determination of the A/D ratio at the end of the procedure, based on the fact that in the presence of divalent cations and at alkaline pH the rate of re-activation of complex I is very slow was performed as previously described [11].

Oxidation of NADH was determined spectrophotometrically (Varian Cary 4000) as a decrease in absorption at 340 nm (ε_{340} - = 6.22 mM^{-1} cm^{-1}) with 165 μM NADH in 1 ml of standard assay medium (0.25 M sucrose, 50 mM Tris–HCl (pH 7.5), 0.2 mM EDTA) containing 10–50 μg protein/ml. Additional measurements of NADH:Q_1 or NADH:HAR reductase were assayed in the presence of 1 mM cyanide with the addition of 45 μM Q_1 or 1 mM HAR, respectively (Sigma). In all inhibition studies the D-form was treated with an effector and activity was assessed only after activation by 10 μM NADH.

2.2. SPDP crosslinker treatment

SMP containing either the A or the D complex I were diluted to 50 μg/ml with PBS (pH 8.0), 8 μM SPDP dissolved in DMF was added and samples were incubated on ice under constant stirring. A sample of the A-form of the enzyme treated with an equal volume of DMF was used as a control. Residual NADH-oxidase activity was checked, every 10 min for both samples and once it reached its final level the reaction was stopped by adding 50 mM Tris–HCl (pH 7.0).

2.3. Protein electrophoresis

Native mitochondrial respiratory complexes were separated using Blue native gel electrophoresis in accordance to published protocols [22]. SMP samples were solubilized by 3 mg of DDM per mg protein as described previously [20]. Due to the formation of large intercomplex oligomers, careful titration of SMP activity by SPDP was performed before upscaling of labelling experiments in order to isolate complex I by BN PAGE. The band containing complex I was cut out, treated with 1% SDS and placed on top of a 10% acrylamide gel containing 6 M urea and subjected to Tricine/SDS–PAGE for the first dimension. For the second dimension lane strips of 10% gels were cut out treated with DTT (20 mM, 20 min, 40 °C) and mounted on the top of a 16% acrylamide gel. Tricine–SDS–PAGE in the absence or in the presence of 20 mM DTT was performed as described before [20,23]. Gels were stained with silver using a mass spectrometry compatible protocol [23] or by Sypro Ruby fluorescent dye (Sigma).

2.4. Mass spectrometric analysis

The gel band was excised and cut into 1 mm cubes. These were then subjected to in-gel digestion, using a ProGest Investigator in-gel digestion robot (Genomic Solutions, Ann Arbor, MI) using standard protocols [24]. Briefly the gel cubes were destained by washing with a 1:1 mixture of 30 mM potassium ferricyanide and 100 mM sodium thiosulphate for the silver stained bands [25] and washing with acetonitrile for the Sypro Ruby stained bands and subjected to DTT reduction and alkylation by iodoacetamide before digestion with trypsin at 37 °C. The peptides were extracted with 10% formic acid and concentrated down to 20 μl using a SpeedVac (ThermoSavant).

The peptides were then separated using a nanoLC Ultra 2D plus loading pump and nanoLC AS2 autosampler equipped with a nanoflex cHiPLC chip-based chromatography system (Eksigent), using a 200 μm × 0.5 mm ChromXP C18-CL 3 μm 120 Å trap and 75 μm × 15 cm ChromXP C18-CL 3 μm 120 Å column (Eksigent). The peptides were eluted at 300 nl/min with a gradient of increasing acetonitrile, containing 0.1% formic acid (15–40% acetonitrile in 5 min, 40–95% in a further 1 min, followed by 95% acetonitrile for 3 min to clean the column, before reequilibration to 5% acetonitrile for 10 min). The eluent was sprayed into a TripleTOF 5600 electrospray tandem mass spectrometer (ABSciex, Foster City, CA) fitted with a NANOSpray® III ion source (ABSciex), running in positive ion mode, and analysed in Information Dependent Acquisition (IDA) mode, performing 250 ms of MS followed by 20 × 150 ms MS/MS analyses on the 20 most intense MS peaks with charge states +2 to +5 which exceed 150 cps. Target ions were subsequently excluded from analysis for 13 s. The MS ions tolerance was set at 50 mDa. LINAC® Collision Induced Dissociation (CID) was performed with a collision energy of 45 and spread of 15 at high sensitivity and unit resolution. The MS/MS data file generated was analysed using the ProteinPilot Beta 4.1.46 Paragon algorithm (ABSciex) against the UniProt/SwissProt database (accessed Nov 2011) in 'Thorough' mode with the 'Biological Modifications' ID focus specified. The search was performed with no species restriction, trypsin as the cleavage enzyme and 'Gel-Based ID' as a special factor. Specific, bespoke, modification parameterizations were created to accommodate both a SPDP [$C_5H_8O_2NS$] artifact (a covalently bound residual of crosslinking and DTT cleavage) within ProteinPilot's ParameterTranslation file with prior probabilities of 0.01.

All proteins were identified with >99% confidence (Prot Score > 2.0) with False Discovery Rates of: Local FDR < 5%, Global FDR < 1%.

In addition, identification was performed using an Orbitrap XL mass spectrometer (Thermo) (see Supplementary information).

All chemicals were purchased from Sigma, except SPDP, which was from ProteoChem. Protein content was determined with a BCA assay. Data are presented as the arithmetic means ± S.D. The experimental details are described in the legends to the figures.

3. Results

3.1. Effect of SPDP on activity of the A and the D-form of complex I

Fig. 1 demonstrates a difference in sensitivity of the A- and D-form of complex I towards the crosslinker SPDP. This is a heterobifunctional chemical crosslinker containing at one end N-succinimidyl ester, which reacts with primary amines, and 2-pyridyldithio group, which reacts with cysteine thiol, at the other end. As expected, only the D-form of the enzyme was inhibited by the treatment since only this form is sensitive to SH-specific reagents. The A-form was much less sensitive to the SPDP crosslinker. The inhibitory effect was similar for NADH-oxidase activity and NADH:Q_1 reductase activity, indicating a direct effect on complex I and not on downstream components of the respiratory chain. Addition of 50 mM Tris–HCl (pH 7.0) to stop the crosslinking followed by treatment with 2 mM DTT, did not recover the activity of the SPDP-inhibited D-form of the enzyme. Furthermore, the oxidation of NADH by the artificial acceptor hexaammineruthenium (HAR) was not affected by the treatment (not shown).

3.2. Identification of crosslinked subunits

To determine if some of the complex I subunits were crosslinked by SPDP, samples of SMP containing either the A or the D-form of complex I were treated with the crosslinker and subjected to Blue Native Page electrophoresis (BN-PAGE) and doubled SDS–PAGE as in [20]. The complex I band from the BN-PAGE was excised and subunits were separated by 10% Tricine/SDS–PAGE containing 6 M urea. Strips of the first dimension lanes were treated with DTT to cleave the possible crosslinked product and subunits were separated further by 16% Tricine/SDS–PAGE in the second dimension. Therefore, subunits involved in crosslinked product formation were expected to be found under the main diagonal and at the same vertical.

As shown in Fig. 2, SPDP treatment resulted in the formation of multiple crosslinked products most likely comprised of high-molecular weight subunits of the hydrophilic domain. This is evident from the appearance of horizontal smears at the left upper side of the second dimension gel. These horizontal spears were present in both forms of the enzyme. Two spots located exactly at the same vertical were found only in the D-form of complex I and not in the A-form. These were a low-molecular weight spot 1 (~15 kDa) and a high-molecular weight spot 2 (~40 kDa). These spots correspond to subunits of the crosslinked product, cleaved by DTT, which would have an apparent molecular mass of 55 kDa.

Then, we went on to identify the proteins that had been cleaved from the crosslinked product formed specifically in the D-form of the enzyme, by mass spectrometry. Spot 1 was positively identified as subunit ND3 (NU3M) and spot 2 as 39 kDa subunit (NDUFA9) (Table 1). The MS/MS spectrum of the peptide from the ND3 subunit, TSPYECGFDPMGSAR, is shown in Fig. 3. This peptide has a propionamide modification of the cysteine, arising from the exposure to acrylamide. Non-cleaved crosslinked product of these two subunits should have an apparent molecular mass of around 55 kDa. Due to the high density of other subunits in that region of the gel it was not possible to detect an appearance of a separate spot for the non-cleaved product in DTT untreated samples. However, specimens excised from the region of the probable location of the crosslinked product (spot 3) were analysed and peptides matching to 39 kDa were found (Table 1).

3.3. Effect of protection of protein thiols by DTNB on inhibition of the D-form of complex I by SPDP

The key cysteine residue rendering the sensitivity of the D-form of the enzyme to SH reagent was previously determined as Cys-39 of ND3 subunit [20]. It can be reversibly modified by other SH-reagents such as DTNB, so that the activity of the DTNB-inhibited enzyme can be restored by treatment with reducing agents [26]. We investigated whether DTNB-treatment of the D-form protects the enzyme from inhibition by SPDP by treating the D-form with DTNB and then with a crosslinker. As shown in Fig. 4, treatment of the D-form of complex I by DTNB resulted in full inhibition of the enzyme. Around 75% of activity can be restored after incubation of the DTNB-treated enzyme with excess of DTT. The enzyme reduced by such treatment can still be inhibited by SPDP. At the same time, treatment of the DTNB-blocked D-form of complex I by SPDP, followed by removal of the any thiol modification by DTT, did not result in significant decrease in activity. Therefore, DTNB-treatment protected the enzyme against irreversible inactivation by SPDP. Most likely it indicates that both DTNB and SPDP react with the same cysteine residue. The inhibitory effect of DTNB alone was reversed by treatment with DTT, whereas that of SPDP alone was not affected by the reduction by DTT (Fig. 1).

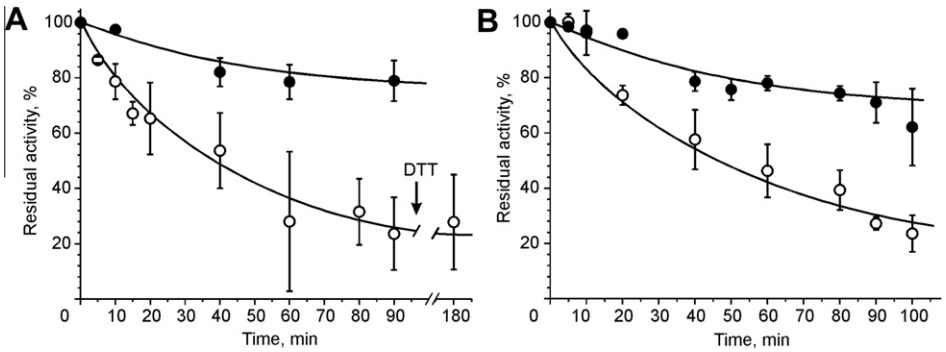

Fig. 1. Effect of crosslinker SPDP on NADH-oxidase (A) or NADH:Q1-reductase (B) activity of the A-form and the D-form of complex I in SMP (solid and empty circles, respectively). SMP (50 μg/ml) in PBS buffer (pH 8.0) incubated with 8 μM SPDP on ice. Addition of 50 mM Tris–HCl (pH 7.0) and 2 mM DTT to the D-sample is shown by arrow. Aliquots were taken during incubation and activity was assayed as described in Section 2. 100% corresponds to 1.1 and 0.7 μmol NADH·min^{-1} mg^{-1} protein for NADH-oxidase and NADH:Q_1 reductase reaction, respectively.

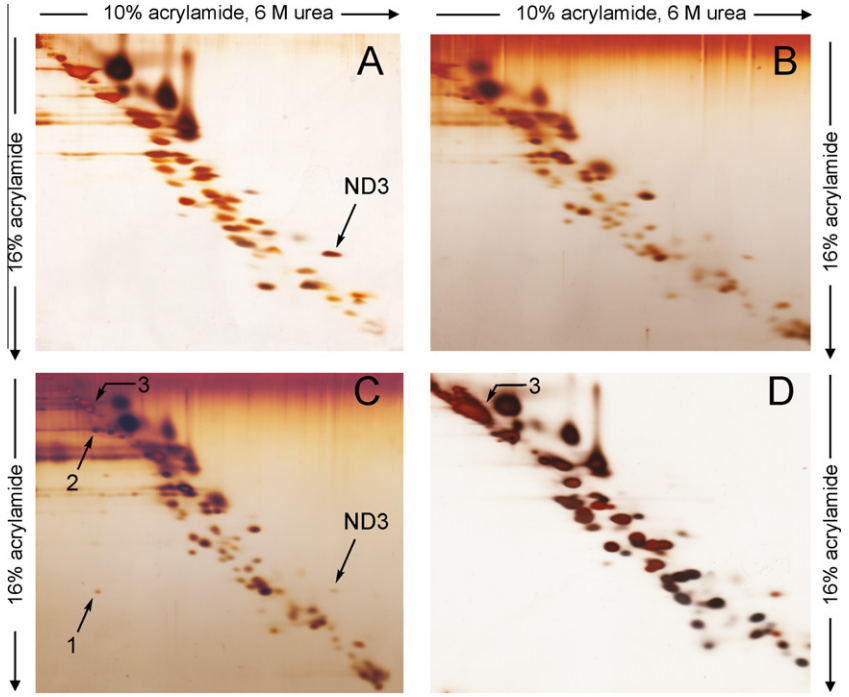

Fig. 2. Separation of the complex I crosslinked subunits by doubled SDS–PAGE. Second dimension 16% SDS–PAGE was performed using DTT-treated gel strips excised from the lanes of the 10% acrylamide with 6 M urea SDS-gel. Representative silver-stained gels of complex I from control (A), SPDP-treated A-form (B) and SPDP-treated D-form (C) SMP are shown. (D) Doubled SDS–PAGE of SPDP treated samples D without reduction by DTT. Two D-form specific subunits cleaved from SPDP-crosslinked product and possible location of the crosslinked product are shown by arrows.

Table 1
Peptides, identified in the tryptic digestion of spots 1–3 obtained from the doubled SDS–PAGE of the crosslinked D-form of mitochondrial complex I.

Spot[a]	Confidence (%)	Sequence	Modifications	Cleavages	Δmass (Da)	Precursor (MW, Da)	Precursor (*m*/*z*)	Charge state (*z*)
1	99	TSPYECGFDPMGSAR	Propionamide(C)@6		−0.0002	1687.6970	844.8558	2
2	99	GKTFAFVGPSR	Formyl@N-term	Missed K-T@2	−0.0026	1193.6167	597.8156	2
	99	IPQAIAQVSK			−0.0030	1053.6125	527.8149	2
	99	MGSQVIVPHR			−0.0042	1122.5927	562.3036	2
	99	QPVYIVDVTK	Gln->pyro-Glu@N-term		−0.0024	1143.6152	572.8149	2
	98.14	TFAFVGPSR			−0.0006	980.5074	491.261	2
	99	WLSSEIEDVQPAK			−0.0046	1500.7415	751.378	2
3	99	IPQAIAQVSK			−0.0008	1053.6125	527.8164	2
	99	TVKQPVYIVDVTK	Missed K-Q@3		0.0036	1488.8586	497.2923	3

[a] Spots were excised from the doubled SDS–PAGE of SPDP treated D-form of complex I shown on Fig. 2.

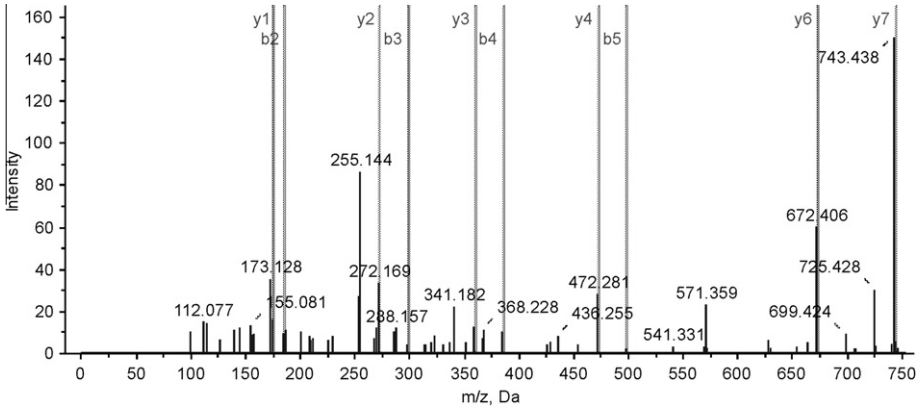

Fig. 3. MS/MS spectrum of the ND3 peptide TSPYECGFDPMGSAR obtained by nLC-ESI MSMS after tryptic digestion of spot 1 (Fig. 2).

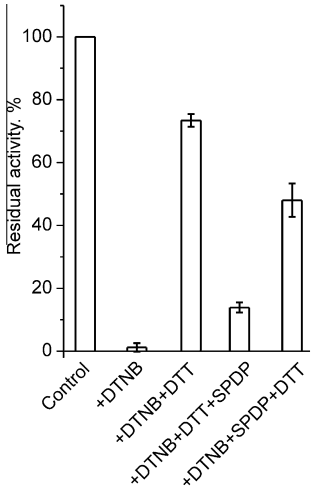

Fig. 4. Effect of protection of protein thiols by DTNB on inhibition of the D-form of complex I by SPDP. SMP containing the D-form of the enzyme (1 mg/ml) were treated by 0.2 mM DTNB for 20 min on ice and washed by centrifugation. The sample was resuspended in PBS buffer (pH 8.0) and divided into two parts. One was treated with 2 mM DTT and another with 8 μM SPDP for 40 min on ice. The second sample was washed again and resuspended in the same buffer containing 5 mM DTT to remove any thiol modification. NADH oxidase activity was assayed at each stage of treatment as described in Section 2. 100% corresponds to 1.1 μmol NADH min^{-1} mg^{-1} protein.

4. Discussion

Our crosslinking studies identified the 39 kDa (NDUFA9) subunit as a close neighbour of the mitochondrially encoded ND3 subunit in the complex I. Polypeptides forming a crosslinked product in the D-form were identified after resolving all enzyme subunits first by 10% acrylamide SDS–PAGE with urea, cleavage with DTT, and separation in a second dimension by 16% acrylamide SDS–PAGE electrophoresis. Two conformation specific spots observed only in the D-form were identified as ND3 and 39 kDa. These subunits were crosslinked in the D-form of the enzyme and not in the A-form, where the critical cysteine-39 of ND3 is not accessible for modification [20,27].

This cysteine-39 of mitochondrial ND3 subunit was recently identified in the D-form, where it is accessible from the outside, thus rendering the sensitivity of the D-form to SH-reagents [20]. At the same time the cysteine containing 29.9 kDa protein (B13 in bovine enzyme) was also shown to be involved in SH-reagents sensitivity of the D-form. Therefore, the observed spot 1 could correspond to subunit ND3 or B13. Tryptic digestion of subunit B13 (NDUFA5) generates detectable peptides and can be readily identified by mass spectrometry (H Heide, unpublished results). However, the single tryptic peptide TSPYECGFDPMGSAR belonging to the highly hydrophobic mitochondrially encoded subunit ND3 was identified in spot 1. As previously observed [20], due to the extremely high hydrophobicity of ND3, other peptides are unlikely to be detected. Indeed, there are only five potential tryptic fragments in the m/z range of 700–5000. Lower molecular weights are not detectable and large hydrophobic peptides are non-extractable from the 16% acrylamide gel. Therefore, the single identified peptide from the hydrophilic region between the transmembrane helices is the only detectable peptide from that subunit. No peptides from other complex I subunits or mitochondrial proteins were found in samples from spot 1. Additional support comes from the observation that the appearance of the low-molecular weight spot 1 was associated with a decrease in intensity of the spot corresponding to ND3 subunit at the same horizontal above the main diagonal.

Most likely, SPDP reacts with a lysine amino group in the 39 kDa subunit. After DTT cleavage, the lysine side chain involved in SPDP crosslinking would bear a 4-thiol-propanoyl group of SPDP, which would contribute +88.1 Da of additional mass and therefore might be expected to be detected by mass spectrometry. The 39 kDa subunit contains 20 lysine residues and one N-terminus amino group. Based on a peptide coverage obtained from spots 2 and 3 we could exclude only 8 lysines (Table 1). The rest of the amines that potentially could be involved into cross-linking were not detectable likely due to a number of possible reasons inherent in in-gel digestion and nLC-ESI-MSMS procedures, thus preventing us from identifying the exact site of modification among those 13 candidates.

Exposure of cysteine-39 of ND3 residue is the only conformational difference between the A- and the D-forms of the enzyme identified so far [20]. The D-form can be modified by the SH-reactive DTNB and the activity of the DTNB-inhibited enzyme can be restored by treatment with reducing agents [26]. Treatment of the D-form of complex I by DTNB followed by removal of the thiol blockage protected the enzyme against inactivation by SPDP. Firstly, this indicates that both DTNB and SPDP modify the same sulfhydryl group, namely, cysteine-39 of ND3. Secondly, taking into account that the activity of the SPDP-modified enzyme cannot be recovered by reduction with DTT, it is possible to conclude that SPDP first binds to the cysteine of ND3 and then reacts with an amino group in the vicinity. Alternatively, the presence of the 2-nitro-5-thiobenzoate group bound to the sulphur of the cysteine residue could result in steric or charge-based hindrance to SPDP binding within the local area between ND3 and the 39 kDa subunit. Since SPDP inhibits the A-form of complex I to a lesser extent, it means that the amino group of the 39 kDa subunit is susceptible for modification by SPDP only after the conformational changes in the enzyme complex. However the magnitude of these changes is very hard to predict, although it is unlikely, that ND3 and 39 kDa subunits significantly change their location within the enzyme complex.

The 39 kDa (NDUFA9) subunit is one of the nuclear encoded structural accessory subunits. It contains one hydrophobic segment of sufficient length to form a transmembrane helix [28]. It belongs to the family of short-chain dehydrogenase/reductases and contains a nucleotide binding Rossmann fold motive [29]. This subunit bears a non-covalently bound NADPH [30] and most likely plays an important role in assembly and stability of complex I [31,32]. It was also suggested, that the 39 kDa subunit together with the acyl carrier protein of complex I might be involved in the synthesis of a not yet identified lipid cofactor [33]. From assembly studies of complex I, the position of the 39 kDa subunit is at the crucial junction region connecting the peripheral hydrophilic domain and the membrane arm of the enzyme [34]. It corresponds exactly to the location of the ND3 subunit as seen in the structure of the enzyme from *Thermus thermophilus* (NqoA subunit) [35]. This is the region where two critical events take place: entrance of substrate ubiquinone towards its reduction site [6,36] and energy transduction from the redox reaction at the hydrophilic part of the enzyme to the membrane part, where endergonic proton-translocation occurs [37,38]. It would be tempting to speculate, that, by changing the location of the domain of this particular subunit, deactivation prevents the entrance of ubiquinone molecule through the narrow path from the membrane at the interface between the 49 kDa and PSST subunits to the terminal cluster N2 [6,35,36].

The functional role of the close association of the ND3 and 39 kDa subunits is not clear yet. Interestingly, it has been reported that T10191C mutation in the ND3 gene (substitution of serine-34 to proline) affects the stability of the 39 kDa subunit and impairs the formation of subcomplexes during enzyme assembly [39].

It is hard to say if the formation of the crosslink between ND3 and 39 kDa in the D-form is a result of changes in the position of both subunits or other subunits are involved in exerting or controllingA/D transition. Our study raises two important questions: (i) whether or not the 39 kDa subunit is directly involved in conformational changes during A/D transition (ii) and if the association of 39 kDa and ND3 is part of the enzyme mechanism responsible for execution of the A/D transition. The final answer to these questions awaits the high resolution structure of mammalian complex I.

Note added in proof

After the submission of this manuscript, an important paper on this subject was published. In this article (Baradaran R., Berrisford J.M., Minhas G.S., Sazanov L.A. (2013) Nature 494, 443–448) the crystal structure of the entire, intact complex I (from *Thermus thermophilus*) at 3.3Å resolution was reported. It was found that a critical hydrophilic loop region of the ND3 (NqoA) subunit provides a part of the seal for the quinone binding site. Therefore, most likely, movement of this loop upon de-activation of the mammalian enzyme affects interaction of the quinone headgroup with its binding site resulting in loss of activity in the D-form.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.02.039.

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