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Submitochondrial Fragments of Brain Mitochondria: General Characteristics and Catalytic Properties of NADH:ubiquinone Oxidoreductase (Complex I)

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Abstract—A number of genetic or drug-induced pathophysiological disorders, particularly neurodegenerative diseases, have been reported to correlate with catalytic impairments of NADH:ubiquinone oxidoreductase (mitochondrial complex I). The vast majority of the data on catalytic properties of this energy-transducing enzyme have been accumulated from studies on bovine heart complex I preparations of different degrees of resolution, whereas almost nothing is known about the functional activities of the enzyme in neuronal tissues. Here a procedure for preparation of coupled inside-out submitochondrial particles from brain is described and their NADH oxidase activity is characterized. The basic characteristics of brain complex I, particularly the parameters of A/D-transition are found to be essentially the same as those previously reported for heart enzyme. The results show that coupled submitochondrial particles prepared from either heart or brain can equally be used as a model system for *in vitro* studies aimed to delineate neurodegenerative-associated defects of complex I.

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Key words: NADH:ubiquinone oxidoreductase, complex I, mitochondria, brain

NADH:ubiquinone oxidoreductase (complex I) is the most complex component of the mitochondrial respiratory chain. The major function of the enzyme is oxidation of the intramitochondrial NADH by ubiquinone (finally by oxygen) thus maintaining the steady-state $NADH/NAD^+$ ratio, which determines intensity of aerobic oxidative metabolism. Mammalian, yeast, plant, and prokaryotic complex I (NDH-1 homolog) catalyze NADH:quinone oxidoreduction coupled with vectorial proton translocation, thus building up $\Delta\tilde{\mu}_{H^+}$ needed for ATP synthesis. Mammalian complex I (bovine heart) is composed of 45 different subunits (total molecular mass of about 10^6 Da) [1, 2] bearing at least 10 individual redox components (FMN [3, 4], seven iron–sulfur clusters [5, 6], two species of bound ubisemiquinones [7-10]), and a zinc atom [11, 12]. The prokaryotic enzymes (NDH-1) in *Escherichia coli* [13], *Rhodobacter capsulatus* [14], *Paracoccus denitrificans* [15], and *Thermus thermophilus* [16] contain almost the same set of the components, although their structures are much simpler: they are composed of only 13-14 polypeptides, and each is homologous to a corresponding subunit of mitochondrial complex I. The structure of mitochondrial [1] and prokaryotic [17] enzymes, their redox components [10], catalytic properties [18], and possible energy-coupling mechanism [19] have been extensively reviewed. Very recently atomic structures of a hydrophilic fragment of *T. thermophilus* [16, 20, 21], a hydrophobic part of *E. coli* and *T. thermophilus* [22], and *Yarrowia lipolytica* complex I [23] have been solved at different degrees of resolution.

With the assumption that prokaryotic NDH-1 catalyzes the same reactions, including vectorial proton translocation with $\vec{H}^+/2\bar{e}$ stoichiometry of 4 [24-26] as the mitochondrial complex I does, an obvious question arises: what is(are) the function(s) of the more than 30 additional subunits of the mitochondrial enzyme? No

Abbreviations: B-SMP, brain SMP; B_H-SMP, B-SMP prepared from "heavy" brain mitochondria; B_T-SMP, B-SMP prepared from "total" brain mitochondria; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); DTT, 1,4-dithiothreitol; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; FMN, flavin mononucleotide; HAR, hexaammineruthenium (III) chloride; H-SMP, heart SMP; $\Delta\tilde{\mu}_{H^+}$, difference of electrochemical potentials of protons across the coupling membrane; Q_1, 2,3-dimethoxy-5-methyl-6-(3-methyl-2-butenyl)-1,4-benzoquinone; SMP, submitochondrial particles; SOD, superoxide dismutase.

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experimentally testable hypotheses (besides general statements about possible regulation and structure stabilization) have been proposed. On the other hand, several subunits of complex I have been shown to be posttranslationally phosphorylated [27, 28], S-nitrosylated [29], glutathionylated [30], and acetylated/deacetylated [31] without significant effects on the catalytic activity of the enzyme.

Numerous studies have been reported in recent years on correlations between mitochondrial complex I activity and various diseases, particularly neurodegenerative ones, caused by either specific mutations or drug-induced (more than 50 more or less specific inhibitors of complex I have been described [32]). The neurodegenerative diseases are of particular attention. The vast majority of the data on the mammalian enzyme have been accumulated from studies of bovine heart complex I. It is not known whether tissues- or species-specificity exist for the mammalian complex I. We believe that inside-out coupled ($\Delta\tilde{\mu}_{H^+}$ generating) SMP are the most appropriate system for studies on catalytic properties of the respiratory complexes. The aim of this study was to establish a procedure for preparation of brain SMP. Here we describe such a procedure and the general catalytic properties of brain mitochondrial complex I.

MATERIALS AND METHODS

Chemicals. NADH, NADPH, Q_1, hexaammineruthenium (III) chloride, myxothiazol, rotenone, piericidin, oligomycin, antimycin A, DTNB, DTT, gramicidin D, FCCP, Tris, Hepes, EGTA, EDTA, BSA, bacterial subtilisin A (type VIII, from *Bacillus licheniformis*), malonate, and succinate were from Sigma-Aldrich (USA). Sucrose was from MP Biomedicals, Inc. (USA). NADH-OH, a specific complex I-directed high affinity inhibitor, was a kind gift of Dr. Alexander Kotlyar (Tel-Aviv University, Israel). Other chemicals of highest purity were obtained from local suppliers.

Pig brain mitochondria were prepared essentially as described by Lee et al. [33] as adapted for large-scale preparation. Pig brains were obtained from a local slaughter house and transported (50-60 min) in ice to the laboratory. Further operations were carried out in a cold room. Brain (500 g) was minced with scissors and suspended in 1 liter of buffer A composed of 0.15 M sucrose, 10 mM Hepes, 0.5 mM EGTA, pH 7.4. Subtilisin A (6 units per g wet weight tissue) was added and the suspension was stirred for 5-7 min. The mixture was homogenized in 200-250 ml portions in a glass–Teflon Potter–Elvehjem homogenizer (4-5 slow strokes). An equal volume of buffer A containing BSA (1 mg/ml) was added to each portion of homogenate, and the mixtures were centrifuged for 5 min at 2000*g*. The supernatants were decanted through cheesecloth and centrifuged for 10 min at 12,000*g*. The precipitated material was washed by repeated centrifugation (10 min, 12,000*g*) in buffer A (200 ml) without BSA. The precipitates were suspended in 200 ml of mixture composed of 0.25 M sucrose and 10 mM Tris-HCl, pH 7.8, and centrifuged for 10 min at 12,000*g*. The precipitated material appeared as separated into two layers, lower dense dark colored (heavy mitochondria) and upper, loose light colored (light mitochondria) and was collected separately. Three-to-four milliliters of 0.25 M sucrose was added to each centrifuge tube, and upper layers were separated by gentle shaking. This operation was repeated 2-3 times, and light mitochondria were suspended in ~15 ml of 0.25 M sucrose (40-50 mg protein per ml). The heavy mitochondria were suspended in ~20-25 ml of 0.25 M sucrose (~30 mg protein per ml), and both preparations were stored frozen at –20°C.

Submitochondrial particles (SMP) were prepared by the procedure routinely used in our laboratory for bovine heart SMP [34]. The detailed description of this procedure is given below. All operations except for those where a temperature is specified were carried out in a cold room. Heavy mitochondria (0.50-0.75 g of protein in 20-25 ml of 0.25 M sucrose) were thawed, diluted 2-fold with ice-cold water, and K^+-EDTA (1 mM final concentration) was added. The suspension was saturated with argon (with well stirring to avoid foaming) for about 20 min and pH was adjusted to 8.6 with 0.05 M NH_4OH). The mixture was sonicated in ice (under argon flow) five times for 0.5 min with 1-min interruptions (MSE Soniprep-150, maximal power output) and centrifuged for 15 min at 26,000*g*. Thorough decanted supernatant (avoiding contamination by precipitated material) was centrifuged for 1 h at 105,000*g* (6°C). The precipitated material was suspended in a small amount of buffer B composed of 75 mM sucrose, 0.25 M KCl, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, and applied on a column (40 × 1 cm) of Sephadex G-50 (coarse) equilibrated with the same buffer B at 25°C. The suspension was slowly (40-50 min) passed through the column at 25°C. The filtration was monitored visually, and SMP were collected in about 10-fold volume of that applied on the column. The collected suspension was centrifuged for 1 h at 105,000*g* at 18°C. The precipitated residues were washed in 0.25 M sucrose by repeated centrifugation for 1 h at 105,000*g* at 6°C, suspended in 0.25 M sucrose (10-20 mg protein/ml), and stored in small vials in liquid nitrogen.

Activation and coupling of SMP [7]. A sample of SMP was thawed and suspended (5 mg protein/ml) in the standard mixture composed of 0.25 M sucrose, 0.2 mM EDTA, 50 mM Tris-HCl, pH 8.0. Potassium malonate (1 mM final concentration, to activate succinate dehydrogenase by the displacement of tightly bound oxaloacetate [35]) and oligomycin (0.5 μg/mg protein, to block $F_O{\cdot}F_1$-mediated proton permeability [36, 37]) were added to well-stirred mixture, and the suspension was incubated for 30 min at 30°C. The mixture was diluted with the stan-

dard mixture to 0.5 mg protein/ml, 1 mM NADPH was added to activate complex I [7], and incubation was continued for 1 h at 25°C. The samples were chilled and centrifuged for 1 h at 26,000*g* at 4°C. The precipitated material was suspended in a small volume of 0.25 M sucrose containing 0.1 mM malonate and stored in small (0.2 ml) vials in liquid nitrogen. Before the experiments samples were thawed, diluted in the standard mixture (~4 mg protein/ml), and kept in ice.

Respiratory activity was assayed as oxygen consumption measured with a covered Clark-type electrode in reaction mixture composed of 0.25 M sucrose, 10 mM KCl, 0.2 mM EDTA, 5 mM potassium phosphate, pH 7.4, and the respiratory substrates—1 mM NADH or 10 mM potassium succinate. When succinate oxidase activity was assayed the samples were preincubated 5-10 min in the presence of 5 mM succinate and 5 μM rotenone.

Heme *a* content was determined in solubilized samples (5% potassium deoxycholate, pH 8.5, 30 min at 25°C). Equal volumes of the samples (0.25 ml) containing 10-15 mg protein were added to the standard spectrophotometer cuvettes (2 ml) filled with 0.1 M potassium phosphate, pH 7.0. A small amount of solid sodium dithionite was added to one cuvette, and the spectrum was recorded (510-630 nm) (Cintra 20, GBC spectrophotometer equipped with integrating sphere). Heme *a* content was calculated [33] using molar absorption coefficient ($\varepsilon_{605\text{-}630}$) of $24 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$.

Complex I content was determined as the piericidin or NADH-OH NADH oxidase inhibitory titers by measuring of the residual activities after preincubation of the samples (1 mg protein/ml) with the inhibitors for 15 min at 25°C.

NADH oxidase and NADH:acceptor reductase activities were assayed at 25°C photometrically as NADH concentration decrease ($\varepsilon_{340} = 6.22 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ or $\varepsilon_{380} = 1.25 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$) in the standard reaction mixture supplemented with NADH (100 μM), gramicidin D (0.2 μg/ml), and BSA (2 mg/ml). The reaction was initiated by introduction of SMP (20 μg/ml). NADH:Q_1 reductase was assayed in standard reaction mixture containing additionally Q_1 (80 μM) and myxothiazol (5 μM). NADH:HAR (III) reductase was assayed in the standard reaction mixture containing additionally HAR, NADH, and antimycin A (1 μg/ml) [38]. The maximal activities were calculated from linear anamorphoses of Michaelis curves obtained at constant HAR (5 mM) and variable NADH concentrations or at constant NADH (1.2 mM) and variable HAR concentrations.

Superoxide production was measured as SOD-sensitive (3 units/ml) acetylated cytochrome *c* (15 μM) reduction ($\varepsilon_{550} = 20 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ [39]) in the standard reaction mixture at 30°C.

Protein content was determined by the biuret procedure [40] using BSA as the calibrating standard.

RESULTS AND DISCUSSION

A number of methods for brain mitochondria preparation from small laboratory animals published in the literature were explored at the preliminary stage of this study. Since the specific aim of our work was to obtain substantial amounts of SMP suitable for long storage, the criteria of choice were rapidness and simplicity of the procedure for preparation of well coupled mitochondria. In our hands the best procedure to meet these criteria was that described by Lee et al. [33]. Before the large-scale procedure for pig brain mitochondria from slaughter house material was initiated, we isolated rat brain mitochondria according to Lee et al. [33]. The mitochondria thus obtained oxidized NAD^+-dependent substrates (glutamate + malate) and succinate in the presence of ADP (state 3 [41]) at the rate of 40 and 80 nmol of O_2 consumed per min per mg protein, respectively, and showed respiratory control ratio of about 8 and 6, respectively. Heme *a* content was 0.12 nmol per mg protein. No specific activities of pig brain mitochondria were measured during further development of the procedure (see "Materials and Methods" section).

Table 1 depicts catalytic activities of B-SMP as compared with those for bovine heart SMP and for classical Keilin–Hartree preparation [42] as assayed under slightly different conditions (25°C, phosphate buffer, pH 7.8).

Respiration with both NADH and succinate was decreased by oligomycin (specific inhibitor of $F_O \cdot F_1$-ATPase [36, 37]). The maximal specific uncoupled NADH oxidase activity of B_H-SMP and H-SMP were about the same (0.82 and 1.0 μmol/min per mg protein, respectively). The NADH oxidase activity (complete respiratory chain operation only) and rotenone-sensitive NADH:quinone (Q_1) reductase activity (complex I operation) of B-SMP were approximately the same. Thus, the limiting step of uncoupled NADH oxidase appears to be located at the iron–sulfur cluster N2–ubiquinone junction site and the respiration (oxidase activity) can be approximated as the catalytic activity of complex I. The rate of NADH oxidation by the artificial electron acceptor HAR was about 10-fold higher than NADH oxidase activity and was insensitive to rotenone and piericidin, the specific inhibitors of ubiquinone reduction. The relative rates of the NADH oxidase, quinone reductase, and HAR reductase reactions (1 : ~1 : >10, Table 1) are in accordance with the data on the steady-state iron–sulfur center reduction level: all the centers are almost completely reduced upon coupled or uncoupled respiration [7].

When catalytic activities of complex I are to be measured, it is important to realize that the NADH binding site is located in the matrix of mitochondria and their inner membrane is impermeable for NADH. Higher or lower activity measured for any particular preparation may thus reflect the accessibility of the enzyme active site,

Table 1. Catalytic activities of brain and heart submitochondrial fragments (30°C, pH 8.0)*

Reaction	Rate (2-electron-eq./min per mg) × 10^6			
	B_T-SMP	B_H-SMP	H-SMP [34, 45]**	Keilin–Hartree heart muscle preparation [42]***
NADH oxidase				
– oligomycin, + gramicidin	0.63	0.82	1.0	0.62
+ oligomycin	0.09	0.16		
+ alamethicin****	0.66	0.94		
Succinate oxidase				
– oligomycin, + gramicidin	0.15	0.17	0.94	0.45
+ oligomycin	0.05	0.05		
NADH:acceptor reductases				
NADH → Q_1		0.65	1.0	
NADH → HAR		8.0	10.0	
Aerobic reverse electron transfer				
succinate → NAD^+	not detectable	0.03	0.25	not detectable
succinate → ferricyanide		0.03		
$O_2^{\cdot-}$ generation	not detectable			not detectable
NADH → O_2		0.43×10^{-3}	0.46×10^{-3}	
+ rotenone		0.65×10^{-3}	0.68×10^{-3}	
succinate → O_2		0.53×10^{-3}	0.48×10^{-3}	
+ rotenone		0.25×10^{-3}	0.16×10^{-3}	

* Absolute activity values for different preparations of SMP were variable within ±20%.
** Activity measured at 25°C.
*** Measured in 50 mM K^+-phosphate buffer (pH 7.8, 25°C).
**** Final concentration of alamethicin and $MgCl_2$ is 20 μg/ml and 1 mM, respectively.

not its catalytic capacity. A useful tool for measurement of intramitochondrial enzymes activities is the antibiotic alamethicin, which was shown to induce free permeability of the mitochondrial membranes for low molecular mass compounds [43]. Evaluation of the membrane preparation orientation heterogeneity can thus be done using alamethicin. Alamethicin only slightly stimulated the NADH oxidase activity of B-SMP (Table 1), thus showing that about 90% of the potentially active complex I was accessible for NADH. It should be noted that the alamethicin test tells nothing about "unsealed", open membrane fragments, and the potential activity of complex I in right-side-out vesicles can be considered simply as contaminated protein, whereas open fragments contribute to uncoupled NADH oxidation.

The succinate oxidase activity of B-SMP was substantially lower (about 4-fold) than that of H-SMP or Keilin–Hartree preparation. It is not clear whether this is due to lower content of complex II in brain mitochondria or to qualitative difference of the enzyme in heart and brain tissues. If the first alternative is correct, the data it would contradict the ideas about structure of supercomplexes of the respiratory chain; at least tissue specific stoichiometry of supercomplexes arrangement should be assumed.

We were unable to register energy-dependent NAD^+ (or ferricyanide) reduction by succinate (reverse electron transfer) in B-SMP prepared from the "total" fraction of brain mitochondria. SMP derived from "heavy" mitochondria catalyzed this reaction at rates significantly lower than those seen in H-SMP (0.03 and 0.25 μmol per min per mg protein, respectively). The difference between SMP prepared from "total" and "heavy" mitochondria is most likely due to significant contaminations

of "open" particles in the former preparation. Although coupled (in the presence of oligomycin) NADH oxidase activity of B-SMP is not high (0.09 μmol per min per mg protein), this activity provides rapid oxidation of NADH, which is formed by reverse electron transfer. B_T-SMP catalyzed rapid NADH:cytochrome *c* reductase reaction (criteria for the presence of "open" membranes accessible for both NADH and cytochrome *c*). This activity was decreased by polylysine, an inhibitor of cytochrome *c*–complex III interaction, and apparent coupling efficiency as determined by the respiratory control ratio was increased in the presence of polylysine (results not shown).

Cytochrome *c* oxidase (complex IV) and complex I content values in brain and heart mitochondria and SMP derived from them are depicted in Table 2. Our data on heme *a* content in H-SMP are in close agreement with those previously published for heme *a* (0.65 nmol/mg protein) in Keilin–Hartree preparation (0.64 nmol/mg protein) [42]. Heme *a* content in B-SMP is considerably less (about 3-fold) than that in preparations derived from heart mitochondria. This may be due to either real difference in the cytochrome oxidase content or to contaminations of non-mitochondrial membrane in B-SMP. The content of complex I was determined as a titer of inhibitory effect of the specific inhibitors [46, 47]. The value determined for B-SMP was 2-fold less than that found in H-SMP.

Significant difference between brain and heart SMP is evident if relative contents, e.g. heme *a*/complex I, are compared (5.4 and 3.7 for heart and brain, respectively; Table 2). Detailed studies on the stoichiometry of respiratory complexes in mitochondria may elucidate an old, not solved yet problem that again became popular: does the respiratory chain operate as a supercomplexed unit [48], or do the components diffuse in the membrane and intermolecular electron transfer proceed via their interactions with mobile cytochrome *c* and ubiquinone [49]?

The data on complex I content and its specific activity can be used for calculation of the enzyme turnover numbers in fully uncoupled NADH oxidase reactions. These values were 250 and 140 sec^{-1} for the enzyme in the brain and heart preparations, respectively. The difference may be indicative of the tissue-specific enzyme isoform, or more likely, it is due to different phospholipid composition of the inner mitochondrial membrane in heart and brain: it is well established that catalytic activity of complex I depends on phospholipids [50, 51].

A peculiar property of complex I, which was originally discovered for heart SMP [52, 53] and later studied by our group [54], is that the enzyme exists in two functionally and structurally distinct forms (so-called A/D-transition). Active (A) form is insensitive to SH reagents, whereas catalytically inactive (de-active, D) form is capable of slow redox-dependent transformation into A-form, and this transition is prevented by SH reagents. The A/D-

Table 2. Contents of respiratory chain components in brain and heart mitochondrial preparations

Species, organ, preparation	Heme *a*, nmol/mg protein ($\Delta A_{605-630}$, $\varepsilon = 24 \times 10^3\ M^{-1} \cdot cm^{-1}$)	Complex I* (inhibitor titer, nmol/mg protein)	Heme *a*/ complex I
Sus scrofa domesticus (pig)			
brain			
mitochondria:			
"total fraction"	0.15		
"light"	0.10		
"heavy"	0.19		
B-SMP:			
B_T-SMP	0.17		
B_H-SMP	0.22	0.06	~3.7
Bos taurus (cattle)			
heart			
mitochondria:			
"heavy"	0.64		
H-SMP	0.65	0.12	~5.4

* Piericidin [44] and NADH-OH titers [46, 47] for different preparations of SMP were variable within ±20%.

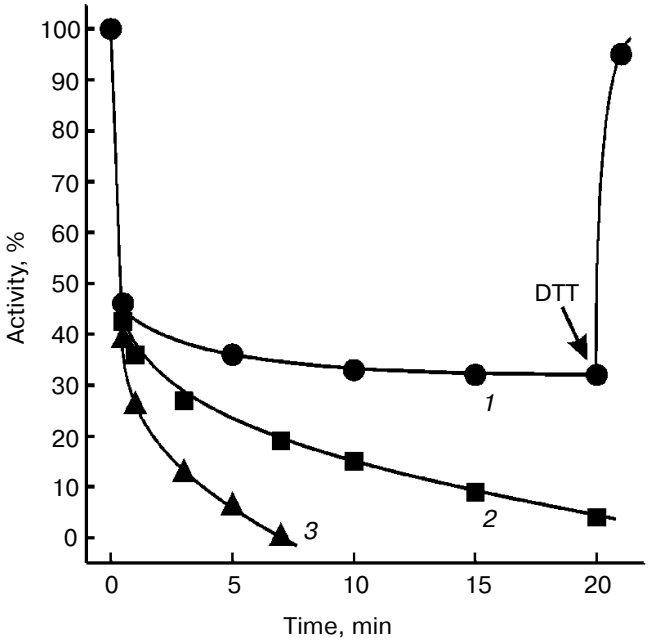

Fig. 1. Kinetics of inhibition of NADH oxidase activity by DTNB. B_T-SMP (1 mg protein per ml) were incubated in the standard mixture supplemented with 1 mM DTNB at 25°C (curve *1*), 30°C (curve *2*), and 37°C (curve *3*). The residual activity was measured as described in the "Materials and Methods" section. One hundred percent activity corresponds to the values measured without preincubation with DTNB at different temperature; those were 0.5, 0.6, and 0.74 μmoles/min per mg protein at 25, 30, and 37°C, respectively. The addition of 5 mM DTT is indicated by the arrow.

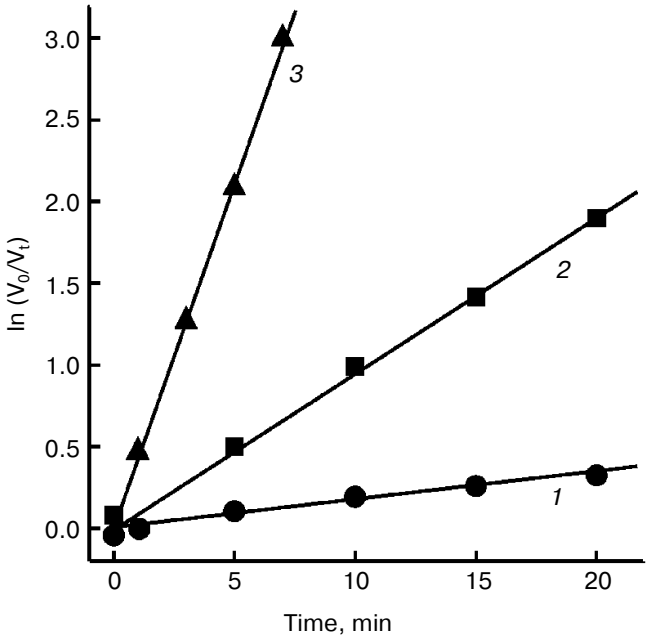

Fig. 2. Linear anamorphoses of DTNB-induced inhibition of activated (A-form) complex I at different temperatures. B_T-SMP were activated by preincubation with NADPH as described in "Materials and Methods". Kinetics of inhibition was followed as described in Fig. 1. Curves *1-3* correspond to incubation temperature 25, 30, and 37°C, respectively. The apparent first-order rate constants were 0.02, 0.1, and 0.4 min^{-1} at 25, 30, and 37°C, respectively.

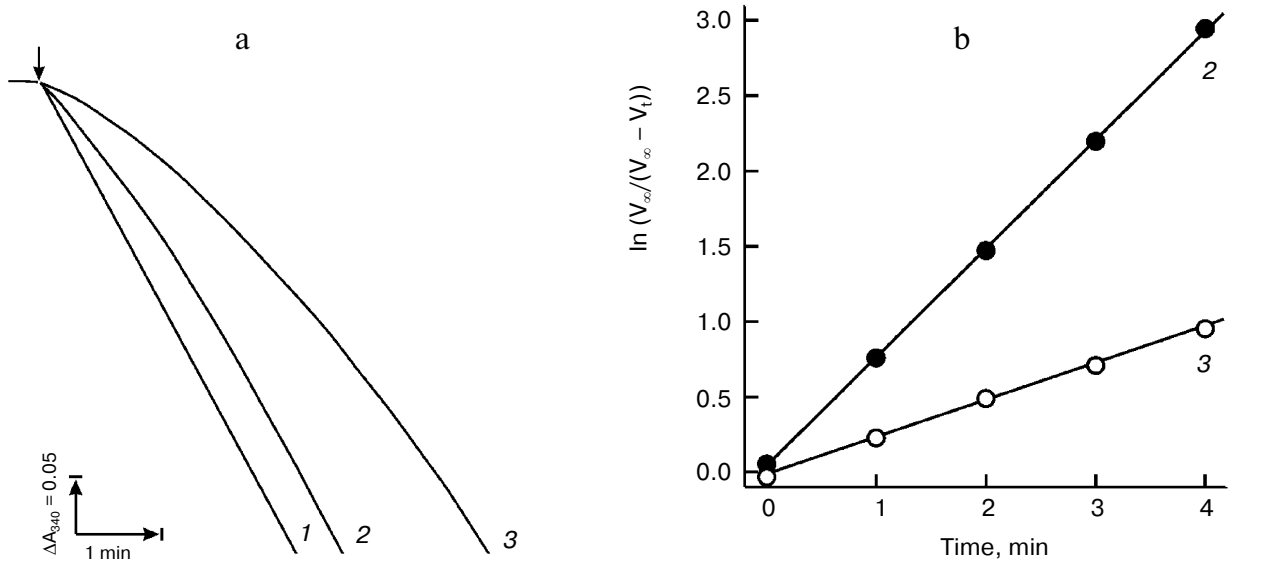

Fig. 3. Activation of de-activated complex I at different pH values. a) B_T-SMP were activated by NADPH (see "Materials and Methods") and their NADH:Q_1 reductase activity was followed at pH 7.0 (curve *1*). SMP were then de-activated by preincubation at 30°C for 15 min and their NADH:Q_1 reductase activity was followed at pH 7.0 (curve *2*) and 9.0 (curve *3*). b) Linear anamorphoses of curves *2* and *3* (first order rate constants were 0.7 and 0.2 min^{-1} for lines *2* and *3*, respectively).

transition has been observed in various preparations [55, 56] including cell culture [57] and intact perfused heart [58]. We evaluated some parameters of complex I A/D-transition in brain SMP.

The content of A- and D-forms in any complex I containing preparations can be quantified by the sensitivity of its NADH oxidase activity to SH reagents, particularly to DTNB [59]. If DTNB reacts with the D-form rapidly ("instantly" within the time resolution limit), the kinetics of irreversible inactivation is bi-phasic: the fraction of instant inhibition is proportional to the D-form content, whereas time-dependent inactivation corresponds to the slow A-to-D-form transition.

Figure 1 shows the time-dependencies of the residual NADH oxidase activity of B-SMP during their incubation with DTNB at different temperatures. The inactivation pattern shows that: 1) about half of total complex I in B-SMP as prepared was present as the D-form; 2) the de-activation was strongly temperature-dependent (it was practically absent at 25°C), and 3) neither A- nor D-forms were subjected to thermal denaturation: the inhibitory effect of DTNB was completely reversed by DTT. It should be noted that the activities observed after 2-3-min incubation in the assay mixture were taken as the residual activities because the lag-phase in NADH oxidation was dependent on fractional content of the D-form originally present in a sample. The de-activation process (slow phase of DTNB-induced inhibition) followed first-order kinetics (Fig. 2), and the activation energy as determined from the temperature dependence of the de-activation first-order rate constant was 230 kJ/mol, a value almost identical to that previously reported for bovine heart complex I [34].

The rate of activation (the length of lag-phase when NADH oxidation was initiated by the de-activated SMP) was strongly pH dependent (Fig. 3), whereas the NADH:Q_1 reductase and NADH oxidase activities of the A-form were almost the same within the pH range of 7-9 (the data are not shown).

The specific aim of this study was to develop a procedure suitable for studies on brain mitochondrial complex I. The major unsolved bioenergetic problem concerning complex I is the mechanism of coupling between oxidoreduction and vectorial proton translocation. There is no indication whatsoever for any possible difference in the coupling mechanism of the enzyme operating in different organs and tissues of any eukaryotes. Studies on the mechanism of coupling are possible only using tightly coupled enzyme preparations. In terms of coupling efficiency, brain SMP are certainly inferior to those derived from heart mitochondria. Although analysis of general characteristics of B-SMP suggests several ways to improve their coupling efficiency, these efforts do not seem worth pursuing. On the other hand, some regulatory properties of complex I relevant to its major functions, that is to maintain "correct" NADH/NAD^+ ratio in the mitochondrial matrix, may be various in different tissues. We found no differences in the catalytic properties of either complex I or the complete respiratory chain of brain and heart mitochondria. This does not mean that under physiologically relevant conditions complex I operates identically in heart or brain. Any differences in the operation of this key enzyme, if they exist, are likely due to external factors which control its activity, such as posttranslational modifications, intramitochondrial Ca^{2+} level, free fatty acid content, and the phospholipid composition of the membrane. B-SMP obtained as described in this paper seem to be suitable for further studies on the control mechanisms of complex I activity in nervous tissues.

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