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Review

NADH/NAD$^+$ interaction with NADH: Ubiquinone oxidoreductase (complex I)

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ABSTRACT

The quantitative data on the binding affinity of NADH, NAD$^+$, and their analogues for complex I as emerged from the steady-state kinetics data and from more direct studies under equilibrium conditions are summarized and discussed. The redox-dependency of the nucleotide binding and the reductant-induced change of FMN affinity to its tight non-covalent binding site indicate that binding (dissociation) of the substrate (product) may energetically contribute to the proton-translocating activity of complex I.

1. Introduction

Mitochondrial NADH:ubiquinone oxidoreductase (complex I) and its prokaryotic homologues (NDH-1) are intrinsic components of the respiratory chains that catalyze the first step of intramitochondrial or intracellular NADH oxidation (or NAD$^+$ reduction) and vectorial translocation of protons across the coupling membrane. Under "optimal" conditions (uncoupled NADH oxidase activity of inside-out bovine heart submitochondrial particles (SMP) at saturating NADH concentration, pH 8.0, 25 °C) the enzyme turnover is about 150 s^{-1} [1]. Approximately the same rotenone-sensitive steady-state turnover is seen with exogenous ubiquinone homologue, Q_1, as electron acceptor. The multiple iron–sulfur clusters [2] participating in intramolecular electron transfer from FMN, presumably the primary oxidant (reductant) of NADH (NAD$^+$), are almost completely reduced during the steady-state NADH-supported coupled or uncoupled respiration [3,4]. Reoxidation of the terminal iron–sulfur cluster N-2 thus seems to be the rate-limiting step of the overall NADH oxidase or NADH: ubiquinone reductase \reactions. The enzyme turnover in the rotenone-insensitive "diaphorase" activities of complex I as revealed with a number of artificial electron acceptors such as ferricyanide, hexaammineruthenium (III) (HAR), DCIP, menadione, cytochrome *c*, acetyl-NAD$^+$ (transhydrogenase reaction), oxygen (generation of superoxide radical) varies from less than 0.1 (superoxide generation) to 1000% of that measured in the natural NADH oxidase reaction [1].

NADH oxidation by ubiquinone is energetically coupled with translocation of 4 protons per pair of electrons transferred [5–7]. This stoichiometry eliminates the single Mitchellian redox loop [8] as the only mechanism of *pmf* generation in the NADH:ubiquinone region of the respiratory chain. Other possibilities such as energy-coupled redox-dependent substrate/product binding/release conformational change mechanism are worthy of consideration besides a number of hypothetical models that have been proposed for a direct redox-coupled proton-translocating loop mechanism at the N-2–ubiquinone junction site [9–16]. In fact, the NADH-induced conformational change of complex I has been qualitatively demonstrated [17–19]. To create any testable model for the conformationally-driven energy transduction model quantitative information on the parameters of the substrate (product) binding (release) is needed. Here the current state of knowledge of NADH, NAD$^+$, and their analogues interaction with complex I as related to the flavin redox state is briefly summarized and discussed.

2. NADH/NAD$^+$ binding affinities as emerged from the steady-state kinetics

Numerous reports have been published on NADH/NAD$^+$ affinities to complex I or to other enzymatically active preparations derived from the intact complex. These reports have used different approaches including the steady-state kinetics. Here only the data on the membrane-bound and purified dispersed complex I or so-called "high molecular weight" NADH dehydrogenase are discussed. The redox potentials of the iron–sulfur centers [20] and likely FMN are

Abbreviations: Acetyl-NAD$^+$, 3-Acetylnicotinamide adenine dinucleotide; DCIP, 2,6-dichlorophenol indophenol; FMN, flavin mononucleotide; FP, 2–3 subunit soluble fragment of complex I; HAR, hexaammineruthenium(III); NDH-1, prokaryotic homologue of the mitochondrial NADH:ubiquinone oxidoreductase (complex I); *pmf*, proton-motive force, SMP, submitochondrial particles; Q_1, 2,3-Dimethoxy-5-methyl-6-[3-methyl-2-butenyl]-1,4-benzoquinone. The iron–sulfur centers are designated according to Ohnishi's nomenclature

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Table 1
Apparent affinities of the substrate/product nucleotides to the active site(s) of the NADH dehydrogenase (ubiquinone reductase) as derived from enzyme steady-state kinetics

Enzyme[a] and assay conditions		NADH oxidation				NAD^+-reduction (succinate-supported reverse electron transfer		Ref
	Activity	Kinetic mechanism suggested	K_m^{NADH}	$K_i^{NAD^+}$	K_i^{NADH} (μM)	$K_m^{NAD^+}$	K_i^{NADH}	
SNDH, 30 °C, pH 7.8	Ferricyanide reductase		100					21
BHC-I, 25 °C, pH 7.5	Ferricyanide reductase	Ping-pong	100		50			22
B-SMP, 27 °C, pH 8.0	HAR reductase	Ordered	>40[b] (100)		No inhibition			23
B-SMP, 30 °C, pH 8.0	Oxidase		2	1 000; 1 600	No inhibition	7; 25	40; 80	24, 25
P-SBP, 25 °C, pH 7.0	Oxidase		5		No inhibition	20		26
P-SBP, 25 °C, pH 8.0	Oxidase		7			270		27
BHC-I, 20 °C, pH 8.0	Q_1 reductase	Ordered, ternary complex	2		No inhibition			28
BHC-I, 32 °C, pH 7.5	Acetyl-NAD^+ transhydrogenase	Ping-pong	100		160–260			29
	HAR reductase		90					29
	Superoxide generation		0.05[c]	Strong inhibition				30

[a] Abbreviations: SNDH, soluble high molecular weight NADH dehydrogenase derived from bovine heart submitochondrial particles; BHC-I, purified bovine heart complex I; B-SMP, bovine heart inside-out submitochondrial particles; P-SBP, inside-out plasma membrane vesicles from anaerobically grown *P. denitrificans*.
[b] At 0.5 mM HAR. Significantly higher K_m^{NADH} (100 μM) is seen at high (2 mM) concentration of HAR.
[c] Only 5-times molar excess of NADH over the enzyme concentration in the presence of NADH-regenerating system.

significantly modified in simpler "low molecular weight" fragments of complex I (such as FP, flavoprotein fragment of complex I. Although FP certainly bears the NADH binding site, these redox modifications are expected to modify also the apparent affinities to NADH/NAD^+ (see below). Before discussing quantitative data summarized in Table 1, some comments on the steady-state kinetics of NADH oxidation seem to be helpful.

The simplest (single NADH binding site and single site for the enzyme reoxidation) scheme describing NADH oxidation catalyzed by complex I is depicted in Fig. 1. Because two substrates (NADH and electron acceptor) are involved at least two alternative reaction pathways are conceivable: after NADH binding and intramolecular oxidoreduction (intermediates **1**, **2**, and **3**) an electron acceptor may oxidize the enzyme before dissociation of NAD^+ (the overall reaction proceeds via intermediates **1**, **2**, **3**, and **4**); alternatively, an electron acceptor may reoxidize product-free reduced enzyme only (intermediates **1**, **2**, **3**, and **5**). According to text-book steady-state kinetics the first alternative is characterized by a series of simple hyperbolic initial rate versus NADH concentration dependencies with different apparent K_m^{NADH} values, which are extrapolated to K_S^{NADH} when electron acceptor concentration is extrapolated to zero. For redox enzymes the K_S^{NADH} value thus obtained is not a measure of primary NADH binding to the active site ($\boldsymbol{K_1}$, Fig. 1) but it is always a product, $\boldsymbol{K_1 \cdot K_2}$, where $\boldsymbol{K_2}$ is the redox equilibrium constant for the intramolecular electron transfer between bound NADH and primary enzyme-bound electron acceptor, presumably FMN. The alternative mechanism (reoxidation of product-free reduced enzyme, intermediates **1**, **2**, **3**, and **5**) gives a series of parallel lines in 1/v versus 1/[NADH] graphs at different acceptor concentrations with apparent K_m^{NADH} extrapolating to zero when an electron acceptor concentration extrapolates to zero (ping-pong mechanism). It should be noted that if $\boldsymbol{K_2} \ll 1$, i.e. the intramolecular oxidoreduction is greatly shifted to the intermediate **3** (e.g. midpoint redox potential of FMN/$FMNH_2$ is significantly more positive than that of bound NADH/NAD^+), two alternative mechanisms become kinetically undistinguishable. Indeed, if so, the interconversion **2**→**3** becomes "irreversible" just like interconversion **3**→**5** is in the ping-pong mechanism where "irreversibility" is due to the initial rate limitation ([NAD^+]=0). It is also possible that the reaction proceeds both via **1**, **2**, **3**, **4**, and **1**, **2**, **3**, **5** intermediates with relative contributions of two pathways that depend on particular electron acceptor concentration. In this case deviations from simple hyperbolic rate-reactant concentrations dependencies are expected.

2.1. Binding of NADH

The apparent K_m^{NADH} values published in the literature for the reactions with different electron acceptors (Table 1) can, at first approximation, be divided into "high K_m" (rotenone-insensitive artificial

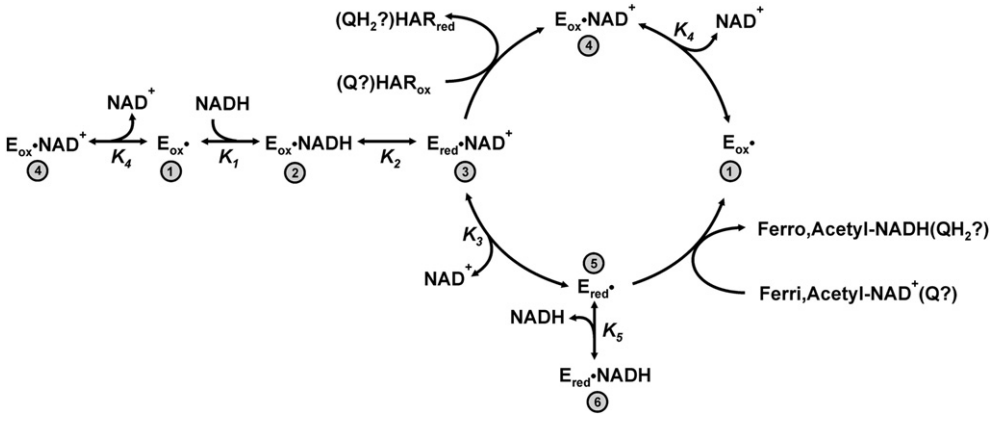

Fig. 1. Simplified scheme describing the steady-state NADH oxidation and NAD^+ reduction by complex I. Shadowed numbered circles are designation for distinct intermediates (see text for further explanation).