

# Kinetics and Regulation of Mammalian NADH-Ubiquinone Oxidoreductase (Complex I)

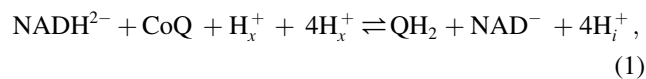
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**ABSTRACT** NADH-ubiquinone oxidoreductase (Complex I, European Commission No. 1.6.5.3) is one of the respiratory complexes that generate the proton-motive force required for the synthesis of ATP in mitochondria. The catalytic mechanism of Complex I has not been well understood, due to the complicated structure of this enzyme. Here, we develop a kinetic model for Complex I that accounts for electron transfer from NADH to ubiquinone through protein-bound prosthetic groups, which is coupled to the translocation of protons across the inner mitochondrial membrane. The model is derived based on the tri-bi enzyme mechanism combined with a simple model of the conformational changes associated with proton transport. To study the catalytic mechanism, parameter values are estimated by analyzing kinetic data. The model is further validated by independent data sets from additional experiments, effectively explaining the effect of pH on enzyme activity. Results imply that matrix pH significantly affects the enzyme turnover processes. The overall kinetic analysis demonstrates a hybrid ping-pong rapid-equilibrium random bi-bi mechanism, consolidating the characteristics from previously reported kinetic mechanisms and data.

## INTRODUCTION

NADH-ubiquinone oxidoreductase (Complex I, European Commission No. 1.6.5.3) catalyzes the reduction of lipid-soluble coenzyme Q (ubiquinone, CoQ) by water-soluble  $\text{NADH}^{2-}$ , which is the first step in the mitochondrial respiratory system,



where subscripts  $x$  and  $i$  indicate protons inside and outside of the matrix, respectively. Therefore, this biochemical reaction represents two coupled processes: reduction of ubiquinone (CoQ) to ubiquinol ( $\text{QH}_2$ ) by  $\text{NADH}^{2-}$ , transferring one free hydrogen ion to ubiquinone, and transport of four hydrogen ions out of the matrix across the mitochondrial inner membrane. Complex I, illustrated schematically in Fig. 1 A, is an L-shaped protein complex located in the mitochondrial inner membrane, with one arm protruding into the matrix space (1,2). It contains at least 41 subunits and 9 redox cofactors (a flavin mononucleotide and eight iron-sulfur (Fe-S) clusters) in mammalian cardiac mitochondria, more than any other complex in the electron transport system (3,4). These subunits are grouped into three modules, the NADH dehydrogenase module, the hydrogenase module, and the proton transporter module (1). The latter two modules couple by a yet unknown mechanism and are linked with a redox-driven proton translocation. The mechanism by which electron transfer and proton pumping are coupled

via Complex I has not been established, but it has been suggested that it involves conformational changes (5,6).

Recently, a number of *in vitro* experiments have been performed in an attempt to understand the catalytic mechanism of Complex I. Fato et al. (7) investigated the kinetics of Complex I from bovine heart mitochondrial membrane using  $\text{CoQ}_1$  and 6-pentylubiquinone as artificial electron acceptors and proposed a ping-pong catalytic mechanism for the enzyme. Nakashima et al. (8) used  $\text{CoQ}_1$  as the electron acceptor to analyze the activity of Complex I purified from bovine heart muscle. They proposed an ordered sequential mechanism with  $\text{CoQ}_1$  binding in the first step and  $\text{Q}_1\text{H}_2$  releasing in the last step. Hano et al. (9) also assumed that the kinetics of Complex I obeys an ordered sequential mechanism when they used DQ as the electron acceptor, and suggested that the maximal enzyme activity at the saturating concentrations of DQ and  $\text{NADH}^{2-}$  is not sensitive to pH. Sadek et al. (10) reported that pH and  $\text{Ca}^{2+}$  regulate the activity of Complex I from solubilized rat heart mitochondria.

The *in vivo* behavior of Complex I necessarily differs from that of purified Complex I in solution. One fundamental difference is that *in vivo* Complex I function is dependent on the electrostatic potential across the mitochondrial inner membrane (11–13). Thus, the membrane potential will have a direct effect on reduction of Q to  $\text{QH}_2$  and transfer of protons from the matrix space to the intermembrane space. In addition, pH in the matrix space also affects the electron transport rate (9,11,14). The primary motivation of this work is to understand kinetic data reported by different groups in terms of a mathematical model using a unified catalytic mechanism that can characterize and predict Complex I behavior under different conditions, both *in vitro* and *in vivo*.

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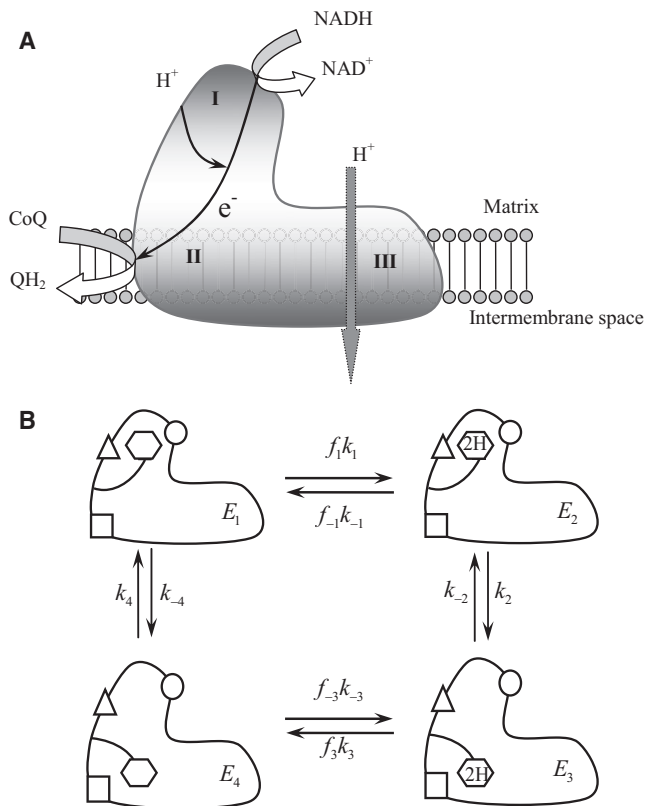


FIGURE 1 (A) Schematic representation of the mechanism of Complex I. The  $\text{NADH}^{2-}$  dehydrogenase module (I) accepts electrons from  $\text{NADH}^{2-}$  and transfers them to the hydrogenase module (II) via the flavin (FMN) and the iron-sulfur clusters (N1–N5). Further transfer to N2 and finally to ubiquinone (Q) is linked with a redox-driven proton translocation (black arrows). The conformational changes of the proton transporter module (III), which transfer protons from the matrix to the cytosol, are mediated by the overall electron transfer by a yet unknown coupling mechanism. (B) Kinetic scheme of the tri-bi enzyme mechanism in combination with the mechanism of conformational changes. The forward reaction is read in the clockwise direction. The enzyme has three binding sites: site 1 binds to the proton (triangles), site 2 binds to substrate A or the corresponding product P (circles), and site 3 binds to substrate B or the corresponding product Q (squares). It is assumed that the mechanism involves conformational changes that are accompanied by phenomenological transfer of the  $\text{H}_2$  moiety from sites 1 and 2 to site 3.

Long-chain lipid-soluble electron acceptors (such as  $\text{CoQ}_{10}$ ) are largely lipid-bound *in vivo*, as compared to those in aqueous solution. Due to the insolubility of natural ubiquinone in aqueous solutions, experimental assays are usually performed using the short-chain coenzyme Q homologs and analogs as substitutes. Fato et al. (7) evaluated the effects of using different soluble  $\text{CoQ}$  analogs, and concluded that the behavior of  $\text{CoQ}_1$  is the most similar to that of  $\text{CoQ}_{10}$ , because electron transfer from  $\text{NADH}^{2-}$  reduces  $\text{CoQ}_1$  at the same rate it reduces  $\text{CoQ}_{10}$  occurring at or near the physiological  $\text{CoQ}_{10}$  site, so that  $\text{CoQ}_1$  has kinetic rates comparable to those calculated from reduction of endogenous  $\text{CoQ}_{10}$ . In a similar way, based on a systematic examination of the assay conditions of Complex I

in the mitochondrial inner membrane using a series of coenzyme Q homologs, such as  $\text{CoQ}_0$ ,  $\text{CoQ}_1$ , and  $\text{CoQ}_2$ , and the analogs duroquinone and decylubiquinone, Estornell et al. (15) concluded that the  $\text{CoQ}_1$  is the most suitable homolog.

One major motivation for developing a mechanistic model for Complex I function is that a model may prove useful to better understand the role of Complex I in superoxide ( $\text{O}_2^-$ ) formation (16). The production of  $\text{O}_2^-$  is reported to be much higher in Complex I when the net flux in Eq. 1 is in the reverse direction than when it is in the forward direction (17). The mechanism of this phenomenon is unknown. Lambert and Brand (11) found that  $\text{O}_2^-$  production in isolated mitochondria from rat skeletal muscle is suppressed by rotenone and uncoupling agents, but not by nigericin. They suggested that the pH gradient across the mitochondrial inner membrane may play a role in  $\text{O}_2^-$  production. Kussmaul and Hirst (14) measured  $\text{O}_2^-$  production from pure Complex I isolated from bovine heart mitochondria. Their results show a positive correlation between the  $\text{NADH}^{2-}/\text{NAD}^-$  ratio and  $\text{O}_2^-$  production.

Another issue that may affect the analysis of *in vivo* Complex I activity is the  $\text{NADH}^{2-}$  binding state. It has been reported that mitochondrial  $\text{NADH}^{2-}$  is predominantly protein-bound, where  $\text{NAD}^-$  is mostly in the free state (18,19). Tischler et al. (19) estimated that the ratio of mitochondrial  $\text{NADH}^{2-}_{\text{free}}$  to  $\text{NADH}^{2-}_{\text{total}}$  in hepatocytes is in the range 8.5–22.5%, based on the lactate dehydrogenase redox couple at pH 7.0 and  $\beta$ -hydroxybutyrate dehydrogenase redox couple at pH 7.4. Therefore, it is important to account for  $\text{NADH}^{2-}$  binding in the elucidation of Complex I catalytic mechanisms *in vivo*. Because of binding, the reported apparent  $K_m$  values of  $\text{NADH}^{2-}$  are greater for submitochondrial particles (SMPs) and beef heart mitochondria (BHM) than for the purified enzyme: the latter is around 2  $\mu\text{M}$  (8,9), only ~20% of the apparent  $K_m$  for SMPs (9.2  $\mu\text{M}$ ) and BHM (8.7  $\mu\text{M}$ ) (7).

In this work, we develop a mechanistic model to describe Complex I activity in purified enzyme experiments as well as in the intact mitochondria *in vivo*. The model is derived from a tri-bi enzyme mechanism combined with a model of conformational changes associated with proton transport across the inner mitochondrial membrane. Parameter values of the proposed kinetic model are estimated by analyzing available kinetic data; the model is validated based on independent data sets from additional experiments.

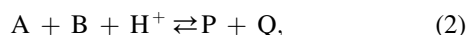
## METHODS

In this section, we first introduce a general kinetic model for conformational change in a tri-bi enzyme mechanism. The model is then further developed to account for proton transport and  $\Delta\Psi$  dependency to characterize the kinetic model of Complex I. Finally, we describe the method used to estimate the kinetic parameter values in the model.

## Kinetic model for conformational change in a tri-bi enzyme mechanism

Complex I is a multisubunit and multiredox component enzyme. Structure studies imply that Complex I has an L-shaped structure consisting of a membrane domain and a peripheral arm (20), with the majority of the peripheral arm located in the matrix (21). Recent structure and evolution studies have elucidated a modular structure of Complex I. It contains an NADH<sup>2-</sup> dehydrogenase module, a hydrogenase module, and a transporter module (Fig. 1 A). The hydrogenase and transporter modules are known to be involved in proton translocation and act as redox-driven and conformation-driven proton pumps, respectively (22).

The kinetic equation of our model for Complex I catalytic reaction is derived from a tri-bi enzyme mechanism combined with a model of conformational change. The derivation is inspired by a previously developed model for transcarboxylase (23,24). The basic chemical reaction of the tri-bi enzyme mechanism has the form



in which we have temporarily excluded the proton pumping component from the analysis. The model assumes that the enzyme has three binding sites: site 1 binds to the proton, site 2 binds to substrate A or the corresponding product P, and site 3 binds to substrate B or the corresponding product Q. In addition, it is assumed that the basic mechanism involves four conformational changes that accompany the transfer of a 2H moiety from sites 1 and 2 to site 3, as illustrated in Fig. 1 B.

Each binding state can exist in any one of the four conformational states illustrated in Fig. 1 B. The first site is either empty or proton-bound; the second site is either empty or bound to A or P; and the third site is either empty or bound to B or Q. Therefore, there are a total of 18 binding states associated with each of these four conformational states, yielding a total of  $4 \times 18 = 72$  distinct states in the model. The 72 states are denoted as  $E_{xyz}^i$ , where  $i \in \{1, 2, 3, 4\}$  indicates the conformational state,  $x \in \{\emptyset, H\}$  indicates the site-1 binding state,  $y \in \{\emptyset, A, P\}$  indicates the site-2 binding state, and  $z \in \{\emptyset, B, Q\}$  indicates the site-3 binding state. Using the lower-case  $e_{xyz}^i$  to indicate a fraction in each state, we have

$$e_{\text{total}}^i = \sum_{x \in \{\emptyset, H\}, y \in \{\emptyset, A, P\}, z \in \{\emptyset, B, Q\}} e_{xyz}^i. \quad (3)$$

For each conformation, we assume the rapid equilibration between 18 bound states, which means that the binding processes are much faster than conformational changing processes:

$$e_{\text{total}}^i = \left(1 + \frac{[H^+]}{K_H}\right) \left(1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}\right) \left(1 + \frac{[B]}{K_B} + \frac{[Q]}{K_Q}\right) e_{\text{free}}^i, \quad (4)$$

where  $e_{\text{free}}^i \equiv e_{\emptyset\emptyset\emptyset}^i$  indicates fraction of the free enzyme;  $K_H$ ,  $K_A$ ,  $K_P$ ,  $K_B$ , and  $K_Q$  are the dissociation constants for H<sup>+</sup>, A, P, B, and Q binding, respectively. It is assumed that these constants do not depend on the conformational state of the enzyme. (It has been shown that the binding events are not influenced by the electron transfer reaction inside the enzyme (9).) Furthermore, the presence of a reactant at one site does not influence the binding reaction at another, meaning that these constants are independent of each other. These simplifying assumptions are necessary to make the

model tractable, and are validated by comparison of model prediction to experimental data.

We define  $f_1$  and  $f_3$  as the fractions in conformations 1 and 3 that may undergo forward conformational transformations to states 2 and 4. In a similar way,  $f_{-1}$  and  $f_{-3}$  are the fractions in conformations 2 and 4 that are able to undergo conformational transformation in the reverse direction (see Fig. 1 B). Specifically, the model mechanism requires that proton binding at site 1 and A binding at site 2 are necessary for transition from conformational state 1 to state 2. Therefore,

$$f_1 = \frac{e_{HA\emptyset}^1 + e_{HAB}^1 + e_{HAQ}^1}{e_{\text{total}}^1} = \frac{\frac{[H^+]}{K_H} \frac{[A]}{K_A}}{\left(1 + \frac{[H^+]}{K_H}\right) \left(1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}\right)}. \quad (5)$$

In a similar way, we will have

$$f_{-1} = \frac{e_{\emptyset PB}^2 + e_{\emptyset PQ}^2 + e_{\emptyset P\emptyset}^2}{e_{\text{total}}^2} = \frac{\frac{[P]}{K_P}}{\left(1 + \frac{[H^+]}{K_H}\right) \left(1 + \frac{[A]}{K_A} + \frac{[P]}{K_P}\right)}, \quad (6)$$

$$f_3 = \frac{e_{\emptyset\emptyset B}^3 + e_{\emptyset AB}^3 + e_{\emptyset PB}^3 + e_{H\emptyset B}^3 + e_{HAB}^3 + e_{HPB}^3}{e_{\text{total}}^3} = \frac{\frac{[B]}{K_B}}{\left(1 + \frac{[B]}{K_B} + \frac{[Q]}{K_Q}\right)}, \quad (7)$$

and

$$f_{-3} = \frac{e_{\emptyset\emptyset Q}^4 + e_{\emptyset AQ}^4 + e_{\emptyset PQ}^4 + e_{H\emptyset Q}^4 + e_{HAQ}^4 + e_{HPQ}^4}{e_{\text{total}}^4} = \frac{\frac{[Q]}{K_Q}}{\left(1 + \frac{[B]}{K_B} + \frac{[Q]}{K_Q}\right)}. \quad (8)$$

The net turnover rate (reaction velocity) can be expressed as

$$v = \frac{V}{[E_{\text{total}}]} = k_2 [e_{\text{total}}^2] - k_{-2} [e_{\text{total}}^3]. \quad (9)$$

Application of the King and Altman method (see Segel (24)) to the scheme shown in Fig. 1 B yields the following expression for the net reaction velocity:

$$v = \frac{k_1 k_2 k_3 k_4 f_1 f_3 - k_{-1} k_{-2} k_{-3} k_{-4} f_{-1} f_{-3}}{\left( k_1 k_4 (k_2 + k_{-2}) f_1 + k_2 k_3 (k_4 + k_{-4}) f_3 + k_1 k_3 (k_2 + k_4) f_1 f_3 + k_{-1} k_{-2} (k_4 + k_{-4}) f_{-1} + k_{-3} k_{-4} (k_2 + k_{-2}) f_{-3} + k_{-1} k_3 (k_4 + k_{-4}) f_{-1} f_3 + k_1 k_{-3} (k_2 + k_{-2}) f_1 f_{-3} + k_{-1} k_{-3} (k_{-2} + k_{-4}) f_{-1} f_{-3} \right)}. \quad (10)$$

This complex expression can be obtained using the freely available KAPattern package tool for computer-aided derivation of the enzyme rate expressions (25). Substitution of the fractional occupancy distributions, defined by Eqs. 5–8, yields the following expression for the net reaction velocity in terms of the individual rate constants and dissociation constants:

$$v = \frac{k_1 k_2 k_3 k_4 K_P K_Q [A][B][H^+] - k_{-1} k_{-2} k_{-3} k_{-4} K_H K_A K_B [P][Q]}{K_P K_Q K_H k_2 k_3 k_4 [A][B] + K_P K_Q k_2 k_3 k_4 [A][B][H^+] + K_P K_Q K_H k_2 k_3 k_{-4} [A][B] + K_P K_Q k_2 k_3 k_{-4} [A][B][H^+] + K_P K_Q k_1 k_3 k_2 [A][B][H^+] + K_P K_Q k_1 k_3 k_4 [A][B][H^+] + K_P K_Q k_1 k_4 k_2 [A][B][H^+] + K_P K_Q k_1 k_4 k_{-2} [A][B][H^+] + K_P K_B k_2 k_1 k_{-3} [A][H^+][Q] + K_P K_B K_H k_2 k_{-3} k_{-4} [A][Q] + K_P K_B k_2 k_{-3} k_{-4} [A][H^+][Q] + K_P K_B k_1 k_4 k_2 [A][H^+][Q] + K_P K_B k_{-2} k_1 k_{-3} [A][H^+][Q] + K_P K_B K_H k_{-2} k_{-3} k_{-4} [A][Q] + K_P K_B k_{-2} k_{-3} k_{-4} [A][H^+][Q] + K_P K_B k_1 k_4 k_{-2} [A][H^+][Q] + K_P K_B K_Q k_1 k_4 k_2 [A][H^+] + K_P K_B K_Q k_1 k_4 k_{-2} [A][H^+] + K_A K_H K_Q k_4 k_{-1} k_3 [B][P] + K_A K_Q K_H k_4 k_{-1} k_{-2} [B][P] + K_A K_H K_Q k_3 k_2 k_4 [B][P] + K_A K_Q k_4 k_2 k_3 [B][H^+][P] + K_A K_Q K_P K_H k_4 k_2 k_3 [B] + K_A K_Q K_P k_4 k_2 k_3 [H^+][B] + K_A K_H K_Q k_{-4} k_{-1} k_3 [B][P] + K_A K_Q K_H k_{-4} k_{-1} k_{-2} [B][P] + K_A K_Q K_H k_{-4} k_2 k_3 [B][P] + K_A K_Q k_{-4} k_2 k_3 [B][H^+][P] + K_A K_Q K_P K_H k_{-4} k_2 k_3 [B] + K_A K_Q K_P k_{-4} k_2 k_3 [H^+][B] + K_A K_B K_H k_2 k_{-3} k_{-4} [P][Q] + K_A K_B k_2 k_{-3} k_{-4} [H^+][P][Q] + K_A K_B K_H k_{-2} k_{-3} k_{-4} [P][Q] + K_A K_B k_{-2} k_{-3} k_{-4} [H^+][P][Q] + K_A K_B K_H k_{-1} k_{-2} k_4 [P][Q] + K_A K_B K_H k_{-1} k_{-2} k_{-4} [P][Q] + K_A K_B K_H K_Q k_{-1} k_{-2} k_4 [P] + K_A K_B K_H K_Q k_{-1} k_{-2} k_{-4} [P] + K_A K_B K_P K_H k_{-3} k_{-4} k_2 [Q] + K_A K_B K_P k_2 k_{-3} k_{-4} [H^+][Q] + K_A K_B K_P K_H k_{-3} k_{-4} k_{-2} [Q] + K_A K_B K_P k_{-3} k_{-4} k_{-2} [Q][H^+]}$$

(11)

Defining kinetic constants in terms of groups of rate constants and using the Haldane relationship (24), Eq. 11 yields the following velocity equation for the mechanism of Fig. 1.

parameters have the units of concentration (mass/unit volume);  $V_1$  and  $V_2$  have the units of mass/unit time/mass protein. Full definitions are presented in the Supporting Material.

For a given value of the equilibrium constant for the tri-bi biochemical reaction, the twelve kinetic parameters cannot vary independently. They

are related to the equilibrium constant  $K_{eq}$  via the equilibrium relationship

$$v = \frac{\left(\frac{V_1}{K_{mB} K_{ia} K_{ih}}\right) ([A][B][H^+] - \left(\frac{1}{K_{eq}}\right) [P][Q])}{\left(\frac{K_{mH}}{K_{mB} K_{ia} K_{ih}}\right) [A][B] + \left(\frac{1}{K_{ia} K_{ih}}\right) [A][H^+] + \left(\frac{K_{mH}}{K_{mA} K_{ih} K_{iq}}\right) [A][Q] + \left(\frac{1}{K_{mB} K_{ia} K_{ih}}\right) [A][B][H^+] + \left(\frac{1}{K_{ia} K_{ih} K_{iq}}\right) [A][H^+][Q] + \left(\frac{K_{mA}}{K_{mB} K_{ia}}\right) [B] + \left(\frac{K_{mA}}{K_{mB} K_{ia} K_{ih}}\right) [B][H^+] + \left(\frac{K_{mQ}}{K_{mP} K_{ib} K_{iq}}\right) [B][P] + \left(\frac{K_{mA}}{K_{mB} K_{ia} K_{ih} K_{ip}}\right) [B][H^+][P] + \left(\frac{K_{mQ}}{K_{mP} K_{iq}}\right) [P] + \left(\frac{1}{K_{iq}}\right) [Q] + \left(\frac{1}{K_{mP} K_{iq}}\right) [P][Q] + \left(\frac{1}{K_{ih} K_{iq}}\right) [Q][H^+] + \left(\frac{1}{K_{ih} K_{ip} K_{iq}}\right) [H^+][P][Q]}$$

(12)

The kinetic constants in Eq. 12 are defined as

$$V_1 = \frac{\text{num}_1}{\text{Coef}_{ABH}}, V_2 = \frac{\text{num}_2}{\text{Coef}_{PQ}}, K_{mA} = \frac{\text{Coef}_{BH}}{\text{Coef}_{ABH}},$$

$$K_{mB} = \frac{\text{Coef}_{AH}}{\text{Coef}_{ABH}}, K_{mH} = \frac{\text{Coef}_{AB}}{\text{Coef}_{ABH}},$$

$$K_{mP} = \frac{\text{Coef}_Q}{\text{Coef}_{PQ}}, K_{mQ} = \frac{\text{Coef}_P}{\text{Coef}_{PQ}}, K_{ia} = \frac{\text{Coef}_{HQ}}{\text{Coef}_{AHQ}},$$

$$K_{ib} = \frac{\text{Coef}_P}{\text{Coef}_{BP}}, K_{ih} = \frac{\text{Coef}_Q}{\text{Coef}_{HQ}},$$

$$K_{ip} = \frac{\text{Coef}_{BH}}{\text{Coef}_{BHP}}, K_{iq} = \frac{\text{Coef}_{AH}}{\text{Coef}_{AHQ}}, \text{ and } K_{eq} = \frac{\text{num}_1}{\text{num}_2},$$

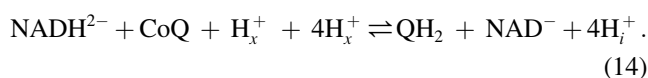
where we have used the shorthand notation from Segel (24); for example,  $\text{num}_1 = k_1 k_2 k_3 k_4 K_P K_Q$ ,  $\text{num}_2 = k_{-1} k_{-2} k_{-3} k_{-4} K_A K_B K_H$ ,  $\text{Coef}_{AB} = K_P K_Q K_H k_2 k_3 (k_4 + k_{-4})$ , and so on. Other than  $V_1$  and  $V_2$ , all of these

$$K_{eq} = \left(\frac{[P][Q]}{[A][B][H^+]}\right)_{eq} = \frac{\text{num}_1}{\text{num}_2} = \frac{K_{mP} K_{iq}}{K_{mB} K_{ia} K_{ih}} \frac{V_1}{V_2}. \quad (13)$$

In the next section, we apply the general form of the tri-bi enzyme mechanism to analyze experimental data on the kinetics of Complex I to determine whether the proposed mechanism can match the experimental data and to estimate the associated parameter values.

## Kinetic model of Complex I

Complex I is the first of the respiratory complexes providing the proton-motive force required for energy-consuming processes like the synthesis of ATP in the oxidative phosphorylation process in the cell. It is an energy-converting oxidoreductase that accepts electrons from a hydride donor ( $\text{NADH}^{2-}$ ) and passes them to a membrane-bound two-electron acceptor (ubiquinone) with translocation of four protons across the mitochondria intermembrane. The reference chemical reaction can be written as



The equilibrium constant for the reference reaction ( $K_{\text{eq,C1}}$ ) can be calculated as

$$K_{\text{eq,C1}} = \left( \frac{[\text{NAD}^-][\text{QH}_2][\text{H}_i^+]^4}{[\text{NADH}^{2-}]_{\text{free}}[\text{CoQ}][\text{H}_x^+]^5} \right)_{\text{eq}} = K_{r,\text{eq}} e^{-4F\Delta\Psi/RT} = e^{-(\Delta_r G_{\text{C1}}^0 + 4F\Delta\Psi)/RT}, \quad (15)$$

where  $K_{r,\text{eq}}$  is the equilibrium constant and  $\Delta_r G_{\text{C1}}^0$  is the standard free energy for the chemical reaction  $\text{NADH}^{2-} + \text{CoQ} + \text{H}^+ \rightleftharpoons \text{QH}_2 + \text{NAD}^-$ . The term  $e^{-4F\Delta\Psi/RT}$  accounts for the free energy cost of pumping four protons across the inner mitochondrial membrane, where  $\Delta\Psi$  is the potential difference, measured relative to the matrix, and  $F$  is Faraday's constant. The reference Gibbs free energy  $\Delta_r G_{\text{C1}}^0$  is computed using the basic thermodynamic data (298.15 K,  $I = 0.15$  M) listed in Xin et al. (26):

$$\begin{aligned} \Delta_r G_{\text{C1}}^0 &= \Delta_r G_{\text{NAD}^-}^0 + \Delta_r G_{\text{QH}_2}^0 - \Delta_r G_{\text{NADH}^{2-}}^0 - \Delta_r G_{\text{CoQ}}^0 \\ &= -0.70 + (-89.92) - 21.12 - 0.0 \\ &= -111.74 \text{ kJ/mol} \end{aligned} \quad (16)$$

$$V_{\text{C1}} = \frac{\left( \frac{1}{1 + \frac{[\text{H}^+]}{K_{\text{IH}}}} \right) \left( \frac{V_1}{K_{\text{mB}}K_{\text{ia}}K_{\text{iH}}} \right) \left( [\text{A}][\text{B}][\text{H}^+] - \left( \frac{1}{K_{\text{eq}}^{\text{app}}} \right) [\text{P}][\text{Q}] \right)}{\left( \frac{K_{\text{mH}}}{K_{\text{mB}}K_{\text{ia}}K_{\text{iH}}} \right) [\text{A}][\text{B}] + \left( \frac{1}{K_{\text{ia}}K_{\text{iH}}} \right) [\text{A}][\text{H}^+] + \left( \frac{K_{\text{mH}}}{K_{\text{mA}}K_{\text{iH}}K_{\text{iQ}}} \right) [\text{A}][\text{Q}] + \left( \frac{1}{K_{\text{mB}}K_{\text{ia}}K_{\text{iH}}} \right) [\text{A}][\text{B}][\text{H}^+] + \left( \frac{1}{K_{\text{ia}}K_{\text{iH}}K_{\text{iQ}}} \right) [\text{A}][\text{H}^+][\text{Q}] + \left( \frac{K_{\text{mA}}}{K_{\text{mB}}K_{\text{ia}}} \right) [\text{B}] + \left( \frac{K_{\text{mA}}}{K_{\text{mB}}K_{\text{ia}}K_{\text{iH}}} \right) [\text{B}][\text{H}^+] + \left( \frac{K_{\text{mQ}}}{K_{\text{mP}}K_{\text{ib}}K_{\text{iQ}}} \right) [\text{B}][\text{P}] + \left( \frac{K_{\text{mA}}}{K_{\text{mB}}K_{\text{ia}}K_{\text{iH}}K_{\text{ip}}} \right) [\text{B}][\text{H}^+][\text{P}] + \left( \frac{K_{\text{mQ}}}{K_{\text{mP}}K_{\text{iQ}}} \right) [\text{P}] + \left( \frac{1}{K_{\text{iQ}}} \right) [\text{Q}] + \left( \frac{1}{K_{\text{mP}}K_{\text{iQ}}} \right) [\text{P}][\text{Q}] + \left( \frac{1}{K_{\text{iH}}K_{\text{iQ}}} \right) [\text{Q}][\text{H}^+] + \left( \frac{1}{K_{\text{iH}}K_{\text{ip}}K_{\text{iQ}}} \right) [\text{H}^+][\text{P}][\text{Q}]}, \quad (19)$$

The apparent equilibrium constant is computed to include proton transfer across the membrane and  $\text{NADH}^{2-}$  binding dynamics in the model, which is an explicit function of the proton gradient and electrostatic gradient across the membrane:

$$K_{\text{eq,C1}}^{\text{app}} = \frac{[\text{NAD}^-][\text{QH}_2]}{[\text{NADH}^{2-}]_{\text{total}}[\text{CoQ}][\text{H}_x^+]} = \frac{[\text{NADH}^{2-}]_{\text{free}}}{[\text{NADH}^{2-}]_{\text{total}}} \frac{[\text{NAD}^-][\text{QH}_2]}{[\text{NADH}^{2-}]_{\text{free}}[\text{CoQ}][\text{H}_x^+]} = \frac{[\text{NADH}^{2-}]_{\text{free}}}{[\text{NADH}^{2-}]_{\text{total}}} \left( \frac{[\text{H}_x^+]}{[\text{H}_i^+]} \right)^4 e^{-(\Delta_r G_{\text{C1}}^0 + 4F\Delta\Psi)/RT}, \quad (17)$$

where  $[\text{NADH}^{2-}]_{\text{total}}$  represents the sums of free NADH ( $[\text{NADH}^{2-}]_{\text{free}}$ ) and protein-bound NADH ( $[\text{NADH}^{2-}]_{\text{bound}}$ ).

Here, we assume simple first-order binding of NADH to protein, with the bound fraction of the binding protein  $f_b = X_{\text{bound}}/X_0$  computed as  $f_b = [\text{NADH}^{2-}]_{\text{free}}/(K_n + [\text{NADH}^{2-}]_{\text{free}})$ . Then the amount of bound NADH is computed as  $[\text{NADH}^{2-}]_{\text{bound}} = X_0 f_b$ , where  $X_0$  is protein-NADH binding capacity in the matrix, and  $K_n$  is the protein-NADH binding dissociation constant. Solving for free  $\text{NADH}^{2-}$  as a function of total  $\text{NADH}^{2-}$ , we have

$$[\text{NADH}^{2-}]_{\text{free}} = \frac{1}{2} \left( [\text{NADH}^{2-}]_{\text{total}} - K_n - X_0 + \sqrt{([\text{NADH}^{2-}]_{\text{total}} - K_n - X_0)^2 + 4K_n[\text{NADH}^{2-}]_{\text{total}}} \right) \quad (18)$$

The mechanism involves both proton and electron transfers: the electron transfer from substrate  $\text{NADH}^{2-}$  to ubiquinone through a number of protein-bound prosthetic groups, flavin mononucleotides (FMNs), 6-8 iron-sulfur clusters, and quinones. It has been shown that the rate of electron transfer increases with pH because the flavin potential decreases, increasing the thermodynamic driving force for electron transfer (14). To account for this effect phenomenologically, we assume that a hydrogen ion can act as a noncompetitive inhibitor of electron transfer.

Hypothesizing that the conformational change tri-bi enzyme mechanism proposed in the previous section (Fig. 1 B) can explain the observed kinetics (8), and the flux of Complex I can be expressed as

where  $[\text{A}]$ ,  $[\text{B}]$ ,  $[\text{P}]$ ,  $[\text{Q}]$ , and  $[\text{H}^+]$  represent the concentrations of  $\text{NADH}^{2-}$ ,  $\text{CoQ}$ ,  $\text{NAD}^-$ ,  $\text{QH}_2$ , and hydrogen ion, respectively, in the matrix. Here,  $\text{CoQ}$  and  $\text{QH}_2$  (reactants B and Q) represent oxidized and reduced ubiquinol in a generic sense. The kinetic parameters, particularly the binding

constants for these reactants, are in general expected to depend on the particular form of ubiquinol present.

## Parameter estimation

The developed kinetic model has 12 adjustable parameters. Parameter values were estimated in a systematic manner in multiple steps by least-square fitting of the model-simulated outputs to the available experimental data in Figs. 2–5, as detailed in the Results section below. The FMINCON

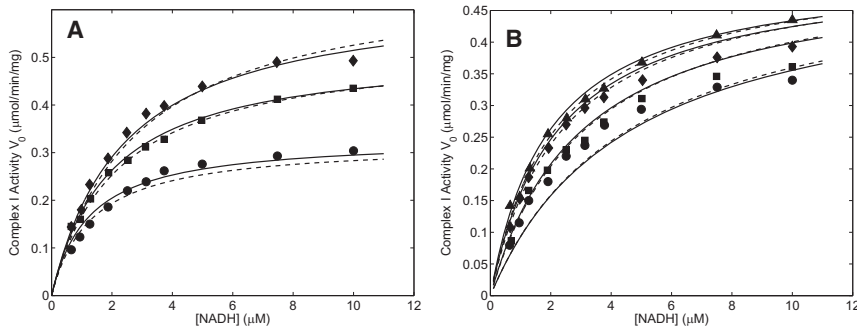


FIGURE 2 (A) Complex I activity as a function of  $\text{NADH}^{2-}$  concentration at different  $\text{Q}_1$  concentrations in the absence of products. Kinetic data were obtained from Fig. 4 of Nakashima et al. (8). The  $\text{Q}_1$  concentrations in the assay were  $50 \mu\text{M}$  ( $\blacklozenge$ ),  $25 \mu\text{M}$  ( $\blacksquare$ ), and  $10 \mu\text{M}$  ( $\bullet$ ). (B) Complex I activity as a function of  $\text{NADH}^{2-}$  concentration at different  $\text{NAD}^-$  concentrations. Kinetic data were obtained from Fig. 5 A of Nakashima et al. (8). The  $\text{NAD}^-$  concentrations were  $0 \mu\text{M}$  ( $\blacktriangle$ ),  $20 \mu\text{M}$  ( $\blacklozenge$ ),  $80 \mu\text{M}$  ( $\blacksquare$ ), and  $200 \mu\text{M}$  ( $\bullet$ ) in the assay.  $\text{Q}_1$  concentration was fixed to at  $25 \mu\text{M}$  in these assays. Solid lines are results of model fitting to the data points represented by symbols based on the optimization of kinetic parameters (Table 1). Dashed lines are results of model fitting based on the optimization of rate constants and dissociation constants (Table 1).

algorithm in MATLAB (The MathWorks, Natick, MA) was used to solve this nonlinear optimization problem. In addition, sensitivity analysis was performed to estimate the sensitivity of the least-square error to small changes in parameter values. The sensitivity was computed using (27)

$$S_i = \frac{\max(|E_i^*(x_i \pm 0.1x_i) - E_i^*(x_i)|)}{0.1E_i^*(x_i)}, \quad (20)$$

where  $E^*$  is the least-squared difference between model simulations and experimental data, and  $x_i$  is the optimized value of the  $i$ th parameter. The estimated sensitivity coefficients are listed in Table 1.

## RESULTS

### Kinetic parameters determination from bovine and rat mitochondrial Complex I

Nakashima et al. (8) conducted a number of experiments to study the steady-state kinetics of purified Complex I from bovine heart mitochondria, and proposed an ordered bi-bi sequential mechanism. Their data are used here to identify kinetic parameters for Complex I in bovine heart mitochondria using coenzyme  $\text{Q}_1$  as the substrate. Figs. 2 and 3 illustrate model fits to data obtained from Figs. 4 and 5, *a-c*, of Nakashima et al. (8): Fig. 2, A and B, plots the Complex I

activity versus  $\text{NADH}^{2-}$  concentration at different  $\text{CoQ}_1$  and  $\text{NAD}^-$  concentrations; Fig. 3, A and B, plots the Complex I activity as a function of  $\text{CoQ}_1$  concentration at different  $\text{NAD}^-$  and  $\text{QH}_2$  concentrations. All experimental data in Figs. 2 and 3 were obtained at pH 8.0 and  $20^\circ\text{C}$ . The reaction medium contains 0.1 M potassium phosphate and 0.1% dodecylmaltoside (8). Measured enzyme activity is expressed in  $\mu\text{mol}/\text{min}/\text{mg}$  protein. The ionic strength of the medium was estimated to be  $I = 0.25 \text{ M}$ . In fitting the data in Figs. 2 and 3, the thermodynamic variables used in the model were adjusted to the experimental conditions ( $T = 293.15 \text{ K}$ ,  $I = 0.25 \text{ M}$ ) using the procedure outlined in Beard and Qian (28).

The data from Figs. 2 and 3 were used to estimate 10 of the 12 adjustable parameter values in our model for Complex I by determining values at which the model best fits the data. To do the optimization in a systematic manner in multiple steps, Fig. 4 in Nakashima et al. was used first to determine the parameters  $K_{mA}$ ,  $K_{mB}$ ,  $K_{th}$ , and  $K_{mH}$ . For simplicity, we further assume that  $K_{th}$  equals  $K_{mH}$ . (Optimization was also performed without this assumption, which shows that these two parameters are not significantly different.) Using the data from Fig. 5 *c* in Nakashima

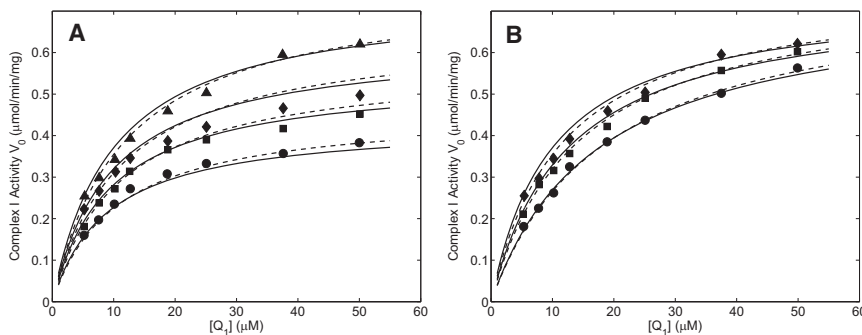
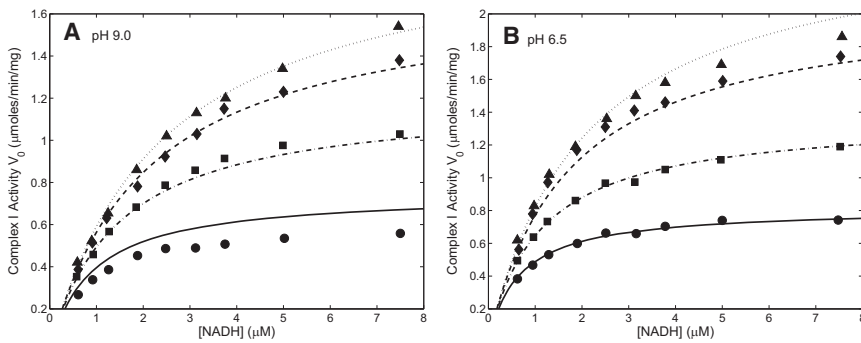


FIGURE 3 (A) Complex I activity as a function of  $\text{Q}_1$  concentration at different  $\text{NAD}^-$  concentrations. Kinetic data were obtained from Fig. 5 B of Nakashima et al. (8). The  $\text{NAD}^-$  concentrations in the assay were  $0 \mu\text{M}$  ( $\blacktriangle$ ),  $100 \mu\text{M}$  ( $\blacklozenge$ ),  $200 \mu\text{M}$  ( $\blacksquare$ ), and  $400 \mu\text{M}$  ( $\bullet$ ).  $\text{NADH}^{2-}$  concentration was fixed at  $6 \mu\text{M}$  in these assays. (B) Complex I activity as a function of  $\text{Q}_1$  at different  $\text{Q}_1\text{H}_2$  concentrations. Kinetic data were obtained from Fig. 5 C of Nakashima et al. (8).  $\text{Q}_1\text{H}_2$  concentrations in the assay were  $0 \mu\text{M}$  ( $\blacklozenge$ ),  $25 \mu\text{M}$  ( $\blacksquare$ ), and  $75 \mu\text{M}$  ( $\bullet$ ).  $\text{NADH}^{2-}$  concentration was fixed at  $6 \mu\text{M}$  in these assays. Solid lines are results of model fitting to the data points represented by symbols based on the optimization of kinetic parameters (Table 1). Dashed lines are results of model fitting based on the optimization of rate constants and dissociation constants (Table 1).



25  $\mu\text{M}$ , 50  $\mu\text{M}$ , 100  $\mu\text{M}$ , and 150  $\mu\text{M}$  DQ in the assay. No products were present in the assay. Solid, dash-dotted, dashed, and dotted lines are results of model fitting for the data points represented by symbols  $\bullet$ ,  $\blacksquare$ ,  $\blacklozenge$ , and  $\blacktriangle$ , respectively.

et al., two more parameters,  $K_{iq}$  and  $K_{ia}$ , may be determined. Finally, data from Fig. 5, *a* and *b*, in Nakashima et al. were used to determine the remaining four parameters,  $K_{ib}$ ,  $K_{ip}$ ,  $K_{mP}$ , and  $K_{mQ}$ . Because  $\text{NAD}^-$  and  $\text{QH}_2$  never occur together in these experiments, the  $[\text{P}][\text{Q}]$  term in the denominator of the flux expression is never nonzero. This means that our analysis is sensitive to the ratio of  $K_{mQ}$  to  $K_{mP}$ , not to the absolute values of these parameters.

The parameter values associated with the best fits of the model to the data are listed in Table 1 (*left four columns*) and the corresponding model predictions are plotted as solid lines in Figs. 2 and 3. For comparison, we also list in Table 1 the parameter values calculated by directly optimizing the rate constants  $k_{\pm i}$  and dissociation constants ( $K_H$ ,  $K_A$ ,  $K_P$ ,  $K_B$ , and  $K_Q$ ). The resulting values of rate constants  $k_{\pm i}$  and dissociation constants are listed in Table 1 (*right three columns*). The corresponding model fits based on direct optimization are also plotted as dashed lines in Figs. 2 and 3. Our model, based on both sets of parameter values, effectively reproduces the ordered bi-bi sequential kinetic pattern, consistent with the observation by Nakashima et al. (8).

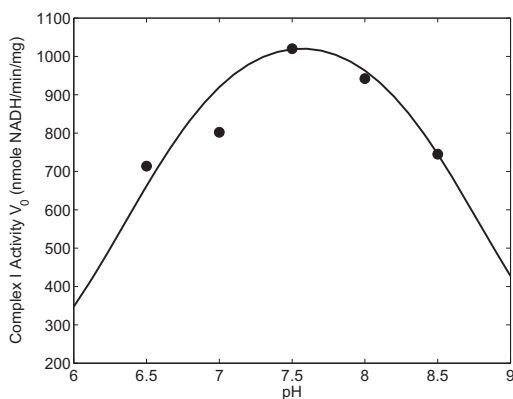


FIGURE 5 Effect of pH on the activity of Complex I from rat hearts. The measured flux as a function of pH was obtained from Fig. 2 *A* of Sadek et al. (10). The reaction medium contains 40  $\mu\text{M}$   $\text{Q}_1$  and 40  $\mu\text{M}$   $\text{NADH}^{2-}$ . The solid line is the result of model fitting to the data point represented by the solid circle.

FIGURE 4 (A) Complex I activity as a function of  $\text{NAD}^-$  concentration at pH 9.0. Kinetic data were obtained from Fig. 1 (*upper left*) of Hano et al. (9). Symbols  $\bullet$ ,  $\blacksquare$ ,  $\blacklozenge$ , and  $\blacktriangle$  represent data obtained in the presence of 25  $\mu\text{M}$ , 50  $\mu\text{M}$ , 100  $\mu\text{M}$ , and 150  $\mu\text{M}$  DQ in the assay. No products were present in the assay. The solid, dash-dotted, dashed, and dotted lines are results of model fitting for the data points represented by symbols  $\bullet$ ,  $\blacksquare$ ,  $\blacklozenge$ , and  $\blacktriangle$ , respectively. (B) Complex I activity as a function of  $\text{NAD}^-$  concentration at pH 6.5. Kinetic data were obtained from Fig. 1 (*upper right*) of Hano et al. (9). Symbols  $\bullet$ ,  $\blacksquare$ ,  $\blacklozenge$ , and  $\blacktriangle$  represent data obtained in the presence of

Hano et al. (9) measured the activities of Complex I from bovine heart, and studied the effect of pH on the enzyme activity using analogs of decylubiquinone (DQ) as electron acceptors. Fig. 4, *A* and *B*, shows model fits to the data obtained from Fig. 1 *A* in Hano et al. (9). The enzyme activity as a function of  $\text{NADH}^{2-}$  and DQ concentrations was measured at pH 9.0 and pH 6.5 (Fig. 4, *A* and *B*, respectively). The reaction medium contained 0.2% dodecylmaltoside, and either 0.1 M pyrophosphate buffer (Fig. 4 *A*) or 0.1 M potassium phosphate buffers (Fig. 4 *B*). The ionic strength of the medium was estimated to be  $I = 0.16$  M. The enzyme activity is expressed in  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ . The parameter values associated with the best fits to the data are also listed in Table 1 (*left*) in parentheses. The estimated parameter values show that the  $K_m$  of DQ is much greater than the  $K_m$  of  $\text{CoQ}_1$ , which is consistent with the observation that DQ has a low affinity for the  $\text{CoQ}$  sites in the dehydrogenases (7). Nakashima et al. reported average kinetic parameter values for  $\text{NADH}^{2-}$  and  $\text{CoQ}_1$  of  $K_m = 2.07$   $\mu\text{M}$  and 12.72  $\mu\text{M}$ , respectively. Hano et al. reported kinetic parameter values for  $\text{NADH}^{2-}$  and DQ of  $K_m = 2.42$  and 50.71  $\mu\text{M}$  and 1.68 and 67.33  $\mu\text{M}$  for pH 9 and pH 6.5, respectively. Those values can be directly compared to our  $K_m$  estimates of  $K_{m(\text{NADH}^{2-})} = 3.10$   $\mu\text{M}$ ,  $K_{m(\text{CoQ}_1)} = 17.66$   $\mu\text{M}$ , and  $K_{m(\text{DQ})} = 96.74$   $\mu\text{M}$ .

Sadek et al. (10) conducted experiments to study the regulatory role of pH and  $\text{Ca}^{2+}$  on the activity of Complex I from rat heart mitochondria. Fig. 5 illustrates the model fits to the data obtained from Fig. 2 *A* in that study (10), which was used to estimate the value of parameter  $K_{ih}$ . Experiments were conducted at 25°C. The reaction medium contains 10 mM MOPs, 10 nM antimycin A, 20  $\mu\text{M}$  KCN, and 40  $\mu\text{M}$   $\text{CoQ}_1$ . The ionic strength of the medium was estimated to be  $I = 0.18$  M. The Complex I reaction was initiated by the addition of  $\text{NADH}^{2-}$  (40  $\mu\text{M}$ ), and was measured as the rate of  $\text{NADH}^{2-}$  oxidation. The measured enzyme activity is expressed in  $\text{nmole NADH}^{2-} \text{min}^{-1} \text{mg}^{-1}$ . Model fits to the data are plotted as solid lines in Fig. 4. The results presented here show that the developed model is able to explain the effect of pH on enzyme activity,

**TABLE 1** Estimated kinetic parameter and rate constant values for Complex I model

Name (unit)	Kinetic parameters			Rate constants		
	Estimated values*	Calculated sensitivity	Calculated values <sup>†</sup>	Name (unit) <sup>‡</sup>	Estimated values	Calculated sensitivity
$V_{\max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ ) <sup>§</sup>	1.0 (3.96)	—	—	$k_1$ ( $\text{s}^{-1}$ )	184	0.28
$K_{m\text{A}}$ ( $\mu\text{M}$ )	3.10 (3.5)	3.06	—	$k_2$ ( $\text{s}^{-1}$ )**	149	0.37
$K_{\text{ia}}$ ( $\mu\text{M}$ )	0.11	0.08	0.12	$k_3$ ( $\text{s}^{-1}$ )	148	0.92
$K_{m\text{B}}$ ( $\mu\text{M}$ )	17.66 (96.74)	4.44	18.06	$k_4$ ( $\text{s}^{-1}$ )	527	1.04
$K_{\text{ib}}$ ( $\mu\text{M}$ )	13.52	0.05	20.73	$k_{-1}$ ( $\text{s}^{-1}$ )	1235	0.32
$K_{m\text{H}}$ (pH)	8.75 (9.36)	0.94	—	$k_{-2}$ ( $\text{s}^{-1}$ )	527	1.04
$K_{\text{ih}}$ (pH)	8.75 (9.36)	0.94	—	$k_{-3}$ ( $\text{s}^{-1}$ )	27	0.01
$K_{m\text{P}}$ ( $\mu\text{M}$ )	—	—	2.29	$k_{-4}$ ( $\text{s}^{-1}$ )	149	0.37
$K_{\text{ip}}$ ( $\mu\text{M}$ )	399.25	0.02	—	$K_{\text{CoQ}}$ ( $\mu\text{M}$ )	27.25	4.23
$K_{m\text{Q}}$ ( $\mu\text{M}$ )	$43.82K_{m\text{P}}$	0.11	105.08	$K_{\text{QH2}}$ ( $\mu\text{M}$ )	111	0.21
$K_{\text{iq}}$ ( $\mu\text{M}$ )	101.60	0.24	101.75			
$K_{\text{ih}}$ ( $\mu\text{M}$ )	0.43	0.39	—			
$X_0$ (mM) <sup>¶</sup>	3.5	—	—			
$K_n$ (mM) <sup>  </sup>	0.3	—	—			

\*Estimated values in parentheses are kinetic parameters for experiments using DQ as substrate.

<sup>†</sup>Parameter values were calculated from the direct optimization of rate constants  $k_{\pm i}$  (right three columns) and dissociation constants.

<sup>‡</sup>Based on definitions of the kinetic parameters  $K_{\text{H}} = K_{\text{ih}}$  and  $K_{\text{P}} = K_{\text{ip}}$ , because we assume that  $K_{m\text{H}} = K_{\text{ih}}$ , we can also derive  $K_{\text{A}} = K_{m\text{A}}$ .

<sup>§</sup>We cannot estimate the value of parameter  $V_1$  in the model without knowing the enzyme activity in the experimental setup. The value of  $V_{\max}$  reported in the table is equal to  $X \times V_1$ , where  $X$  is the enzyme activity. The optimization estimate (in  $\mu\text{mol min}^{-1} \cdot \text{mg}^{-1}$ ) is  $V_{\max} \approx 1.0$  for Figs. 2–5 and  $V_{\max} \approx 4.0$  for Fig. 6.

<sup>¶</sup>The protein binding capacity for  $\text{NADH}^{2-}$ .

<sup>||</sup>The protein-NADH binding dissociation constant.

\*\*For simplicity, we assume that the rate constants for conformational change are the same, which means that  $k_2 = k_{-4}$  and  $k_{-2} = k_4$ .

implying that matrix pH significantly affects the enzyme activity.

### Model validation with data from bovine heart submitochondrial particles

Fato et al. (7) studied the steady-state kinetics of Complex I in bovine heart mitochondria and submitochondrial particles, and proposed a ping-pong mechanism for the enzyme. Data from Fig. 2 of their study were used here to validate the catalytic mechanisms of our model. Fig. 6 plots Complex I activity versus  $\text{CoQ}_1$  concentration at different  $\text{NADH}^{2-}$  concentrations. All experimental data in Fig. 6 were obtained at pH 7.5 and  $30^\circ\text{C}$  (29). The reaction medium contains 50 mM potassium phosphate, 1 mM EDTA (29), 2 mM KCN, and  $4.6 \mu\text{M}$  antimycin A (7). The ionic strength of the medium was estimated to be  $I = 0.13$  M. Complex I activity was obtained spectrophotometrically by determining the change in absorbance at 240 nm (29). Measured Complex I activity is expressed in  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein.

The lines plotted in Fig. 6 are model predictions using kinetic parameters from Table 1, except for  $V_{\max}$ , which was allowed to vary to match the experimental data, and  $K_m$  for  $\text{NADH}^{2-}$ , which was set to  $8.7 \mu\text{M}$ . This value was used (rather than  $3.1 \mu\text{M}$ , estimated above) because Fato et al. conducted experiments in BHM and SMPs, not in purified enzyme conditions. Thus the apparent  $K_m$  is greater because of the  $\text{NADH}^{2-}$  binding. Our model effectively reproduced the apparent ping-pong kinetic pattern, consistent with the observations of Fato et al. (7). (Our anal-

ysis yields an estimated  $K_m$  for  $\text{CoQ}_1$  of  $17.66 \mu\text{M}$ , whereas Fato et al. reported a value of  $20 \mu\text{M}$  (7).) The ability of our model to match the data without adjusting any other parameters validates the model and demonstrates its robustness.

### Application to Complex I function in the intact mitochondria

To simulate Complex I operation in situ, the developed model (Fig. 1) is integrated into a biophysical model of

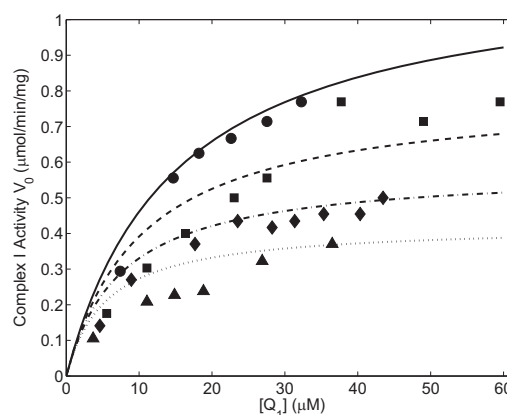


FIGURE 6 Model prediction of Complex I activity as a function of  $\text{Q}_1$  concentration at different  $\text{NADH}^{2-}$  concentrations. Symbols  $\blacksquare$ ,  $\blacklozenge$ ,  $\blacktriangle$ , and  $\bullet$  represent Complex I activity measured in the presence of  $4.27 \mu\text{M}$ ,  $7.13 \mu\text{M}$ ,  $14.19 \mu\text{M}$ , and  $75 \mu\text{M}$   $\text{NADH}^{2-}$  in the assay obtained from Fig. 2 of Fato et al. (7). The solid, dashed, dash-dotted, and dotted lines are model predictions to the data points represented by the symbols  $\bullet$ ,  $\blacksquare$ ,  $\blacklozenge$ , and  $\blacktriangle$ , respectively.



the mitochondrial respiratory system and oxidative phosphorylation (30). Substituting the model for Complex I into the integrated model, the new integrated model must be reparameterized to match data from the intact system. Specifically, this requires adjusting all the associated kinetic parameter values to match the same data set used for parameterization of the original model. The detailed procedure is described by Beard (30).

To incorporate charge transfer effects, it is necessary to explicitly consider the dependence of rate constants on membrane potential to account for the free energy cost of pumping four protons across the mitochondrial inner membrane. Recent electron tunneling simulations (31) and kinetics experiments (32) indicate that electrons flow rapidly from FMNs all the way down to iron-sulfur cluster N2, suggesting that the energy-converting reaction occurs at this cluster or further downstream during ubiquinone reduction. It is unlikely that the electron transfer within the NADH<sup>2-</sup> dehydrogenase module (I) is coupled with proton translocation, although it contains most of the known cofactors of Complex I. The role of FMNs and other cofactors in the NADH<sup>2-</sup> dehydrogenase module (I) may be to collect electrons and pass them to the hydrogenase module (II), so the conformational change that drives the proton translocation in the proton transporter module (III) is linked exclusively to the redox chemistry of ubiquinone reduction. That means that the proton pumping is tightly coupled with ubiquinone reduction and that binding of the substrate (product) may energetically contribute to the proton-translocation activity.

In our kinetic model (Fig. 1 A), the first step (I) represents NADH<sup>2-</sup> oxidation, the second step (II) represents electrons carried by tunneling through a chain of redox cofactors, the third step represents electrons from reduced cofactors transferring to the final acceptor, ubiquinone, to form ubiquinol, and the fourth step represents the conformational resetting of the enzyme. To account for the above assumptions, we modify the kinetic constant  $k_3$  to  $k_3 = k_3^0 \exp(-4F\Delta\Psi/RT)$ , where  $k_3^0$  is the rate constant when the membrane potential is zero. From a mechanistic perspective, this means that the last step of electron transfer to ubiquinone is accompanied by pumping protons across the membrane potential.

Fig. S1 in the Supporting Material illustrates a comparison between the new integrated model and experimentally measured values of NADH<sup>2-</sup>, membrane potential, oxygen consumption, matrix pH, and the cytochrome c reduced fraction from Bose et al. (33). The model equations and parameter values associated with these best fits between the model simulations and experimental data are presented in the Supporting Material. The assumption  $k_3 = k_3^0 \exp(-4F\Delta\Psi/RT)$  is validated by the fact that we are unable to produce reasonable matches between these data and model predictions when membrane potential dependence is incorporated into other rate constants.

## DISCUSSION

A mechanistic mathematical model to explain the catalytic mechanisms of Complex I has been developed. The model assumes that the enzyme catalyzes the reaction through a four-conformation-change process. The flux expression was derived based on the general form of the tri-bi enzyme kinetic mechanism. A general form of the model provides insights into the mechanisms of Complex I activity at various pH values. The developed kinetic model was parameterized by analyzing kinetic data. In total, 10 parameters (one is the  $K_{mQ}/K_{mP}$  ratio) were estimated in a systematic manner in multiple steps by fitting the model simulation to independent data curves (see Figs. 2–5), providing quantitative estimates of the model parameters. To quantitatively measure the degree to which the curves plotted are sensitive to the parameter values, we calculated the relative sensitivities to the adjustable parameters, listed in Table 1. A high sensitivity value indicates that changing a given parameter results in significant changes to the simulated curves used to identify the parameter values. Note that three of the adjustable parameters,  $K_{ia}$ ,  $K_{ib}$ , and  $K_{ip}$ , show relative sensitivity of <10%, indicating that model predictions are not sensitive to these parameters around the estimated values and that these parameters may not be identified well by the analysis presented here, given the data sets analyzed. (For example, only the curves in Fig. 2 B and Fig. 3 A are sensitive to changes in  $K_{ib}$  and  $K_{ip}$ .)

Correlations in parameter estimates were investigated by computing the Hessian matrix  $\partial^2 E/\partial x_i \partial x_j$ . Although we cannot assign covariance estimates to our parameter estimates because we do not have information on the statistical variation in the raw data analyzed, this Hessian matrix does reveal potential correlations between our parameter estimates. Specifically, with regard to the rate constant estimates, this analysis reveals significant correlations between estimates for  $k_1$  and  $k_3$  and estimates for  $k_{-1}$  and  $k_3$ .

### Catalytic mechanism (ordered bi-bi, random, or ping-pong?)

A number of Complex I catalytic schemes have been proposed in the literature based on steady-state kinetic experiments. Fato et al. studied the kinetics of Complex I from bovine heart membrane using CoQ<sub>1</sub> as the electron acceptor (7). The reciprocal plots of  $1/v$  versus  $1/\text{CoQ}_1$  at different NADH<sup>2-</sup> concentrations in that study are parallel straight lines, suggesting a random bi-bi mechanism with NADH<sup>2-</sup> first binding to the enzyme. Nakashima et al. also used CoQ<sub>1</sub> as the electron acceptor to analyze the activity of Complex I purified from bovine heart muscle (8). They proposed an ordered sequential mechanism with CoQ<sub>1</sub> binding as the first step and Q<sub>1</sub>H<sub>2</sub> releasing as the last step.

Our analysis demonstrates a hybrid ping-pong rapid-equilibrium random bi-bi mechanism, consolidating the

characteristics from the various mechanisms and data reported in the literature. In the absence of products, our flux expression can be expressed as

$$\begin{aligned} \text{flux}_{c1} &= \frac{1}{K_{mB}K_{ia}K_{iH}} \left( \frac{V_1[H^+]}{1 + \frac{[H^+]}{K_{iH}}} \right) \\ &= \left[ \left( \frac{K_{mH}}{K_{mB}K_{ia}K_{iH}} \right) + \left( \frac{[H^+]}{K_{mB}K_{ia}K_{iH}} \right) \right] + \frac{1}{[B]} \left( \frac{[H^+]}{K_{ia}K_{iH}} \right) \\ &\quad + \frac{1}{[A]} \left[ \left( \frac{K_{mA}}{K_{mB}K_{iA}} \right) + \left( \frac{K_{mA}[H^+]}{K_{mB}K_{ia}K_{iH}} \right) \right]. \end{aligned} \quad (21)$$

Based on this expression, at fixed pH, the model predicts the kinetic dependence of flux on substrate concentrations equivalent to that of the random bi-bi mechanism, matching the experimental observation from Fato et al. (7). The product inhibition patterns of our model are also consistent with most of the conclusions from Nakashima et al., including the competitive inhibition of QH<sub>2</sub> against CoQ, and the noncompetitive inhibition of NAD<sup>-</sup> against CoQ. However, Nakashima et al. also suggested a noncompetitive inhibition of NAD<sup>-</sup> against NADH<sup>2-</sup> in their model, whereas our model demonstrates that the inhibition of NAD<sup>-</sup> against NADH<sup>2-</sup> is competitive. Replotting the double reciprocal lines in Fig. 5 *b* from Nakashima et al. (8), we found that the data can also be fit by lines intersecting at the 1/*v* axis. Therefore, although our model does not agree with the ordered bi-bi mechanism proposed by Nakashima et al., it is able to fit their original data fairly well.

Our model suggests that the binding of NADH<sup>2-</sup> and CoQ to Complex I occur in random order, and that NADH<sup>2-</sup> oxidation and quinone reduction are not sequentially ordered. This is supported by the experimental reports in the literature, as summarized by Hirst (34). Sled et al. reported that the redox potential of the NADH<sup>2-}/NAD<sup>-</sup> couple is close to the two-electron potential of the FMN (35). Zu et al. showed in protein-film voltammetry experiments that NADH<sup>2-</sup> and NAD<sup>-</sup> are reversibly interconvertible at the subcomplex I<sub>λ</sub> of mitochondrial Complex I (36). Vinogradov argued that there are two kinetically undistinguishable mechanisms for Complex I depending on the order of substrate binding and the intramolecular oxidoreduction of the enzyme itself. The relative contributions of the two pathways may depend on particular electron acceptor concentrations (37). (These two pathways correspond to the ordered bi-bi and ping-pong mechanisms.)</sup>

The model does not account for the possible presence of a diffusion-limited step in the reaction of endogenous ubiquinone with the complex. Hackenbrock et al. (38) proposed a “random collision model” for the mitochondrial respiratory chain in that the presence of diffusion control in the activity of Complex I would have important physiological implications. There are no available experimental

data sets of diffusion control of Complex I interacting with exogenous ubiquinone.

### pH effect

Our model predicts that the matrix pH plays an important role in the regulation of Complex I activity. Specifically, in the absence of membrane potential, Complex I activity increases and then decreases, as matrix proton concentration increases from pH 6.5 to pH 9.0 (10). The optimal pH value corresponding to the maximal activity is ~7.5. The increase in activity is due to our assumption that protons participate in NADH<sup>2-</sup> oxidation as substrates to transfer a hydride to Complex I. This assumption is reasonable, since the hydride transfer reaction can avoid the formation of high-energy NADH<sup>2-</sup> radical intermediates (34). The decrease is due to our assumption that matrix protons also act as inhibitors of the enzyme. An increase in matrix proton concentration decreases the flavin potential in Complex I, decreasing the thermodynamic driving force for electron transfer from the NADH<sup>2-</sup> binding end to the CoQ binding end in Complex I (14). The competition of these two effects results in the bell shape of the pH dependence relationship (Fig. 5).

Furthermore, our model indicates that matrix pH has a pronounced effect on the binding of NADH<sup>2-</sup> to Complex I and the maximal apparent enzyme activity. This finding differs from the conclusion from Hano et al. (9), who conducted a number of experiments to study the effect of pH on the steady-state kinetics of bovine Complex I. They assumed that the enzyme obeys the ordered sequential model proposed by Nakashima et al. (8), and obtained different values of parameters at different pH values. Based on an analysis of the changes in parameter values, Hano et al. suggested that the maximal enzyme activity at the saturating concentrations of DQ and NADH<sup>2-</sup> is not sensitive to pH. Yet our model includes a dependence of enzyme activity on pH, and is able to reproduce fairly well the observations from both Hano et al. (9) and Nakashima et al. (8), showing that the substrate bindings are coupled to proton uptake either for proton pumping or for the formation of the product by the mechanical conformational changes. Such conformational changes couple the pumping to the reductive or oxidative phase during the catalytic turnover. In other words, the proton pump is driven by a redox-coupled conformational change in the pumping process.

In summary, we developed a detailed kinetic model for the NADH-ubiquinone oxidoreductases (Complex I) that accounts for the electron transfer from NADH<sup>2-</sup> to ubiquinone through protein-bound prosthetic groups, which is coupled to the translocation of protons across the inner mitochondrial membrane as well. The kinetic mechanism in our model is modified from the ping-pong bi-bi mechanism to a tri-bi enzyme mechanism that explicitly considers the proton binding and the conformational changes

associated with charge transport inside the complex. The developed model mechanism indicates a long-range interaction between the two binding sites of the substrates, induced by tight coupling between conformational change and electron transfer involving many subunits.

## SUPPORTING MATERIAL

One table, one figure, and additional references are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(10\)00807-6](http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00807-6).

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