Volume 244, number 2, 268–270 FEB 06814 February 1989

Hysteretic interaction of NADH and Mg^{2+} with mammalian NADH:CoQ reductase from beef heart

P.R. Tushurashvili, N.Z. Dekanosidze, N.P. Inasaridze, T.N. Kekelidze, M.A. Tsartsidze and B.A. Lomsadze

Department of Biophysics, Tbilisi State University, Tbilisi 380038, USSR

Received 28 November 1988

Preincubation of submitochondrial particles (SMP) from beef heart in a reaction mixture containing low concentrations of Mg^{2+} induces a time lag in the NADH:oxidase activity. Preconditioning of the SMP by NADH, but not by NAD^+, prevents the Mg^{2+}-related time lag. The data obtained show that there exists a tight binding site for Mg^{2+} regulating the rate of electron transfer from NADH to the natural acceptor. The ability of Mg^{2+} to form a catalytically inactive complex with the enzyme is regulated by NADH.

NADH dehydrogenase; Respiratory chain, Enzyme regulation

1. INTRODUCTION

The NADH:CoQ reductase region of the mammalian mitochondrial respiratory chain (NADH dehydrogenase, EC 1.6.99.3) is known as a complex membrane-bound enzyme, which catalyzes rotenone-sensitive electron transfer from NADH toward CoQ and vectorial transmembrane translocation of H^+ [1,2].

Various models of the reaction mechanisms have been discussed in the related literature [3,4]. However, the well-known experimental data on the hysteretic traits of the NADH:CoQ reductase are not included in these models.

These, among others, are the NADH- and time-dependent inactivation of the NADH oxidase with specific inhibitors [5,6], anomalous interaction with mercurials [7], temperature-dependent hysteretic interaction with N-EM [8] and NADH-dependent lag of the NADH oxidase reaction [9]. It should be noted that these experimental data have not yet been adequately explained.

Correspondence address: P.R. Tushurashvili, Department of Biophysics, Tbilisi State University, Tbilisi 380038, USSR

Abbreviations: SMP, submitochondrial particles; N-EM, *N*-ethylmaleimide

2. MATERIALS AND METHODS

Bovine SMP were prepared as described [10]. NADH oxidase activity was measured polarographically or spectrophotometrically in 0.1 mM Tris-HCl (pH 8.5). Polarographically the measurement was followed with a Clark-type platinum electrode in a final volume of 1 ml. In the spectrophotometric assay system NADH oxidation was registered in a final volume of 3 ml by the change in absorbance at 340 nm monitored by a Beckman spectrophotometer equipped with a recording amplifier. Succinate oxidation was monitored polarographically in the same reaction mixture containing 1 mM succinate instead of NADH. The NADH:ferricyanide reductase assay system contained, unless otherwise indicated, 1 mM ferricyanide and 2 mM KCN. Ferricyanide reduction was registered by the absorbance change at 420 nm. All activities were registered after temperature equilibration for 3 min at 25°C. All other details are indicated in the figure legends. Protein content was determined by the biuret method [11].

3. RESULTS AND DISCUSSION

A typical experiment demonstrating the specific effect of Mg^{2+} on the NADH oxidase reaction is shown in fig.1A. SMP catalyze the NADH oxidase reaction with a prolonged lag period after prein-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

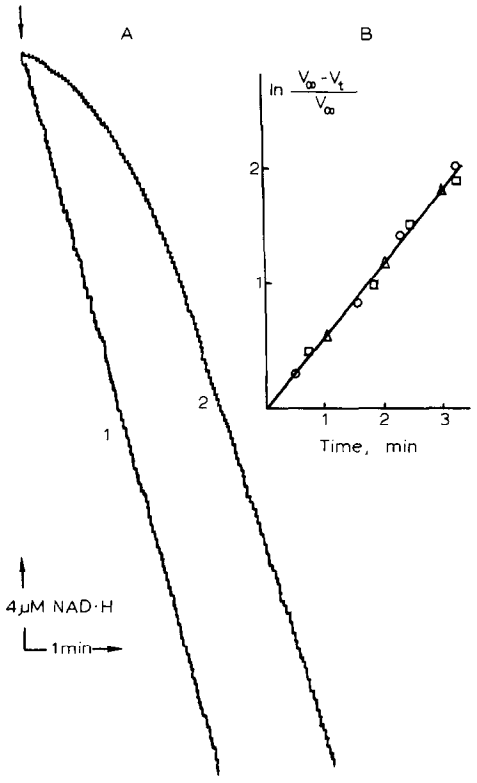

Fig.1. Effect of Mg^{2+} on the NADH oxidase reaction. (A) SMP (0.033 mg/ml) were preincubated with 0.13 mM K_2SO_4 (curve 1) or 0.1 mM $MgSO_4$ (curve 2) in the reaction mixture described in section 2 for spectrophotometric assay. The reaction was started by the addition of 50 μM NADH as indicated by the arrow. (B) Linearization of curve 2 is shown in the coordinates $\ln(V_\infty - V_t)/V_\infty$ vs t. SMP concentration (mg/ml): (○) 0.033, (□) 0.016, (△) 0.066.

cubation with 0.1 mM $MgSO_4$ or $MgCl_2$. Conversely, the NADH oxidase reaction of SMP, which were preincubated with K_2SO_4, KCl, NaCl, Na_2SO_4 or $CaCl_2$ (at concentrations giving equal ionic strength), is linear in time. Examination of the Mg^{2+} effect on various oxidation activities of the SMP shows that there is no lag which depends on Mg^{2+} in the case of NADH: ferricyanide reductase and succinate: oxidase reactions. These data indicate that Mg^{2+} reacts with the NADH: CoQ reductase region of the respiratory chain and controls the rate of the CoQ reduction [1]. Activation of the NADH oxidase reaction is a first-order process (fig.1B) and does not depend on SMP concentration over the range examined.

The time lag does not depend on the time of incubation of SMP with Mg^{2+}. EDTA completely abolished the action of Mg^{2+}. The time lag is hyperbolically related to the Mg^{2+} concentration. $K_i^{Mg^{2+}}$ calculated from these data is 20 μM. Preconditioning of the SMP with NADH, but not with NAD^+, abolishes the Mg^{2+}-related time lag (fig.2A). The ability of the NADH to abolish the Mg^{2+}-related lag of the NADH: oxidase is a hyperbolic function of the NADH concentration and K_m^{NADH} calculated from these data is 1 μM.

The unusual aspect of these experiments is that SMP are able to 'remember' the preconditioning by the NADH. Two alternatives can explain this

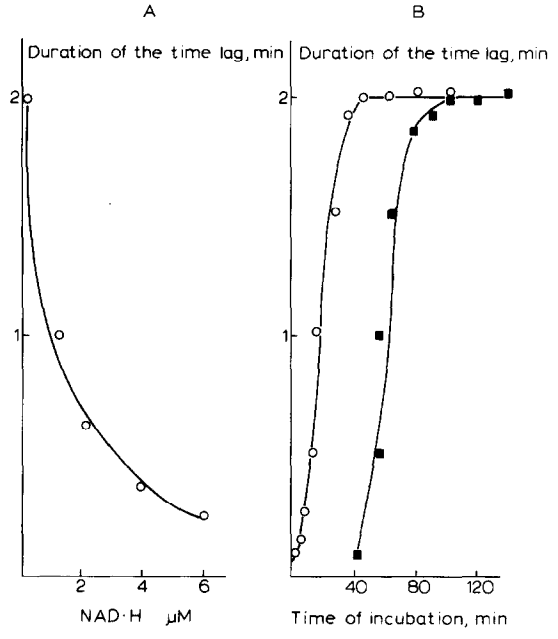

Fig.2. Effect of NADH on the NADH oxidase time lag. (A) SMP were preincubated in the reaction mixture as described in fig.1A (curve 1), comprising the indicated concentrations of NADH. After complete oxidation of NADH, $MgSO_4$ (0.1 mM) was added and the mixture incubated for an additional 5 min. The reaction was started by the addition of 50 μM NADH. (B) (○) Aliquots of SMP were preconditioned with 10 μM NADH as described in (A). After complete oxidation of NADH, 0.1 mM $MgSO_4$ was added and additionally incubated for the indicated time. Reaction was started by the addition of 50 μM NADH. The time lag was determined according to Frieden [13]. (■) Aliquots of SMP were preconditioned as described, and after 45 min incubation additional 10 μM NADH was added and incubation continued for the indicated time; the reaction was then started by the addition of 50 μM NADH.

paradox: (i) there must exist a site with high affinity for NADH binding on the NADH:CoQ reductase region of the respiratory chain; (ii) reduction by NADH of any redox centre of the NADH:CoQ reductase markedly changes the conformational state of the enzyme [12].

In special experiments we have shown that preincubation of SMP with stoichiometric concentrations of NADH and an NADH-regenerating system, comprising alcohol or lactic dehydrogenases and alcohol or lactate, has no effect on the Mg^{2+}-related time lag. These data point to the validity of the first alternative. However, the possibility cannot be excluded that the high-affinity binding site for NADH is formed after the reduction of the NADH:CoQ reductase. In any case, restoration of the Mg^{2+}-related lag must be time-dependent. As shown in fig.2B, the Mg^{2+}-related time lag abolished by preconditioning of SMP with NADH, is completely restored after 45 min aerobic incubation of the preconditioned SMP. The addition of a new portion of NADH abolished the Mg^{2+}-related time lag again.

The results discussed above are summarized in fig.3. According to the present data there are three states of the NADH:CoQ reductase: (i) E^A – ligand-free, active state; (ii) $E^i \cdot Mg^{2+}$ – catalytically inactive complex, that is formed only from the E^A state; and (iii) E^* – an active state that is formed by preconditioning with NADH. This state is unable to form an inactive complex with Mg^{2+}. There are two ways of formation of the E^* state: direct (i.e. from the E^A state) and Mg^{2+}-dependent (i.e. from the $E^i \cdot Mg^{2+}$ state). The Mg^{2+}-related direction is a slow, first-order process (k_{+2} = 0.55 min^{-1}). There is a certain competition between the two paths, which explains why the time lag depends on Mg^{2+}.

The principal result of the present study is the discovery of the existence of slowly interconvertible states of the NADH:CoQ reductase region of the respiratory chain, which can easily explain the numerous experimental data indicating hysteretic properties of the NADH:CoQ reductase.

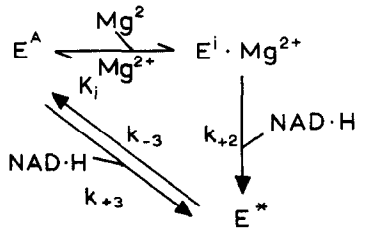

Fig.3. Interaction of Mg^{2+} and NADH with NADH:CoQ reductase. $K_i^{Mg^{2+}}$ dissociation constant of the $E^i \cdot Mg^{2+}$ state. k_{+2}, k_{+3}, k_{-3}, first-order rate constants, where $k_{+3} \gg k_{+2} > k_{-3}$. For other details, see text.

REFERENCES

[1] Hatefi, Y. (1985) in: The Enzymes of Biological Membranes (Martinosi, A. ed.) vol.4, pp.3–41, Plenum, New York.
[2] Ragan, C.J. and Hinkle, P.C. (1975) J. Biol. Chem. 250, 8472–8476.
[3] Ragan, C.J. (1986) in: Fourth European Bioenergetics Conference (EBEC Reports) vol.4, pp.6–7.
[4] Skulachev, V.P. (1986) Biokhimiya 51, 1925–1927.
[5] Burgos, J. and Redfearn, E.R. (1965) Biochim. Biophys. Acta 110, 475–483.
[6] Singer, T.P., Horgan, D.J. and Casida, J.Z. (1968) in: Flavins and Flavoproteins (Yagi, K. ed.) pp.192–213.
[7] Tyler, D.D., Butow, R.A., Gonze, J. and Estabrook, R.W. (1965) Biochem. Biophys. Res. Commun. 19, 551–555.
[8] Crenoma, T. and Kearney, E.B. (1965) J. Biol. Chem. 240, 3645–3652.
[9] Minakami, Sh., Schindler, F.J. and Estabrook, R.W. (1964) J. Biol. Chem. 239, 2049–2054.
[10] Lee, C.P. and Ernster, L. (1967) Methods Enzymol. 59, 158–164.
[11] Gornal, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 158–164.
[12] Kotlyar, A.B. and Vinogradov, A.D. (1988) Biokhimiya, in press.
[13] Frieden, C. (1970) J. Biol. Chem. 245, 5788–5799.