Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 681-689

EFFECT OF MONOVALENT CATIONS ON THE INHIBITION BY NAD^+ OF NADH OXIDATION IN SUBMITOCHONDRIAL PARTICLES

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Received January 9,1978

SUMMARY: Oxidation of NADH in submitochondrial particles, with O_2 or ferricyanide as electron acceptor, was inhibited by micromolar concentrations of NAD^+ when measured in 240 mM sucrose or, in a lesser extent, in 120 mM NaCl or LiCl. In 120 mM solutions of either KCl, RbCl, CsCl or NH_4Cl the inhibition by up to 100 μM concentrations of NAD^+ did not occur. The inhibition observed in the sucrose medium disappeared after solubilization of the particles with detergents and re-appeared when the membranes were reconstituted. The inhibitory effect was potentiated by palmitoyl-CoA. The possibility is discussed that the inhibition of NADH oxidation by low concentrations of NAD^+ and its release by K^+, Rb^+, Cs^+ and NH_4^+ depend on the interaction between NAD^+ and the negatively charged mitochondrial membrane.

Stimulatory effect of potassium ions on mitochondrial respiration is known since more than 20 years (1) and has been since repeatedly observed in studies on both intact mitochondria (2 - 7) and submitochondrial particles (8, 9). In most of these studies, the oxidation of NADH, but not that of succinate, was stimulated by K^+ (9, 10). It has been therefore concluded that potassium ions are required for maximum rate of the electron flow in the NADH-ubiquinone span and for the maximum efficiency of the first coupling site (6, 7, 10). On the other hand, however, stimulatory effect of potassium ions has been observed under uncoupled conditions (1, 3, 6, 9, 10). Moreover, monovalent cations may have an uncoupling effect under specific conditions (11). Therefore, it is not clear whether the effect of potassium ions is exerted on the coup-

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ling mechanism or rather on the initial span of the respiratory chain. Some controversies also concern the effect of other monovalent cations.

The present study was undertaken to get more information about the mechanism by which potassium ions affect mitochondrial respiration.

MATERIALS AND METHODS

Mitochondria were isolated from livers of albino rats (12), and non-phosphorylating submitochondrial particles were obtained as described by Lee and Ernster (13) in the presence of EDTA. Oxidation of NADH with O_2 or ferricyanide as electron acceptor was recorded spectrophotometrically at 340 nm at 25°C. Solubilization of the particles was performed using 1% Lubrol WX (Sigma Chemical Co., St. Louis, Mo., USA) or sodium cholate and the reconstitution of the membranes as described by Ragan and Racker (14) with the use of egg yolk lecithin.

Palmitoyl-CoA was synthesized by the procedure of Seubert (15). NADH was obtained from Boehringer (Mannheim, Federal Republic of Germany) and NAD^+ from Reanal (Budapest, Hungary). Other chemicals were of the highest purity commercially available.

RESULTS

Fig. 1 shows that the oxidation of NADH in submitochondrial particles was inhibited by micromolar concentrations of NAD^+ if the reaction was measured in the sucrose medium. No inhibition by concentrations of NAD^+ up to 100 uM was observed in 120 mM KCl, whereas in NaCl or LiCl solutions the inhibition was somewhat lower than in sucrose. A similar protection against inhibition by low concentrations of NAD^+, as in KCl solution, was found in 120 mM solutions of either RbCl, CsCl and NH_4Cl. Only concentrations of NAD^+ higher than 100 uM produced a substantial inhibition also in the KCl medium. The inhibition by micromolar NAD^+ concentrations and its abolishion by KCl were observed when ferricyanide was used as electron acceptor for NADH oxidation (Fig. 2). No effect of NAD^+ in either sucrose or salt media was observed on succinate oxidation.

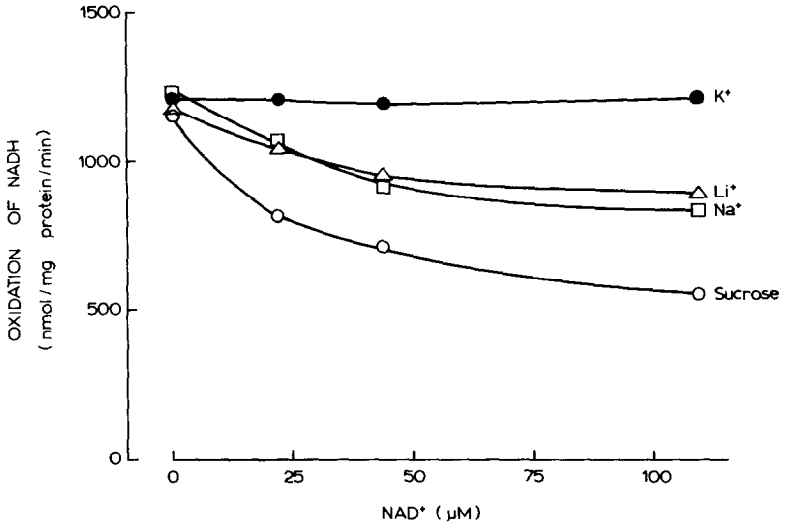

Fig. 1. Inhibition of NADH oxidation by low concentrations of NAD^+. Effect of the incubation medium. Oxidation of NADH was measured with O_2 as electron acceptor. The media contained either 240 mM sucrose or 120 mM solutions of KCl, LiCl or NaCl, buffered with 10 mM Tris-HCl (pH 7.4). The concentration of NADH was 46 μM and that of submitochondrial particles 62 μg protein/ml.

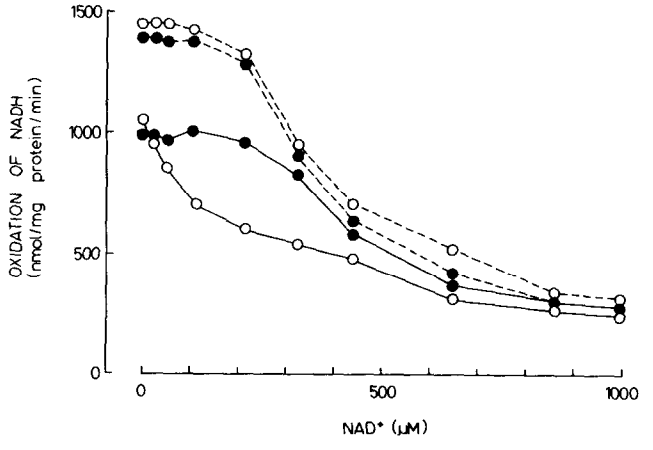

Fig. 2. Inhibition of NADH oxidation by higher concentrations of NAD^+. Effect of K^+ and the solubilization of the particles. Oxidation of NADH was measured with 0.84 mM ferricyanide as electron acceptor and in the presence of 1 mM KCN. NADH was 52 μM. Open symbols, 240 mM sucrose - 10 mM Tris-HCl (pH 7.4); closed symbols, 120 mM KCl - 10 mM Tris-HCl (pH 7.4). Solid lines, submitochondrial particles (2.6 μg protein/ml); dashed lines, particles solubilized in 1% Lubrol WX (1.3 μg protein/ml).

The inhibition by low concentrations of NAD^+ in sucrose medium could be completely released by 40 mM KCl, with half release at 10-20 mM KCl (Fig. 3). NaCl was less effective and never abolished the inhibition completely.

Different effects of various alkali metal cations on the inhibition by NAD^+ was not related to a possible different penetration of these ions across the membrane of submitochondrial particles, since a similar picture as that shown in Fig. 1 was observed when gramicidin was also present, i.e. under conditions when the membrane was fully permeable to all monovalent cations tested.

However, the inhibitory effect of low concentrations of NAD^+ disappeared when the particles were solubilized with detergents (Fig. 2). After reconstitution of the membranes by adding phospholipids and dialysing off the detergent (14) the inhibition in the sucrose medium re-appeared (Fig. 4). The experiment was performed with ferricyanide as electron acceptor because the solubilized material as well as the reconstituted particles were no longer able to react with molecular oxygen as electron acceptor.

This experiment suggested that the inhibitory effect of micromolar concentrations of NAD^+ was related to the existence of the membrane. This was further supported by the finding that the inhibition was affected by surface-active agents. This is exemplified in Fig. 5 for palmitoyl-CoA. This compound substantially inhibited NADH oxidation in sucrose and NaCl media in the presence of a fixed concentration of NAD^+. However, it is evident that palmitoyl-CoA was without effect in the absence of NAD^+. It can be therefore concluded that palmitoyl-CoA is not inhibitory per se but potentiates the inhibition produced by

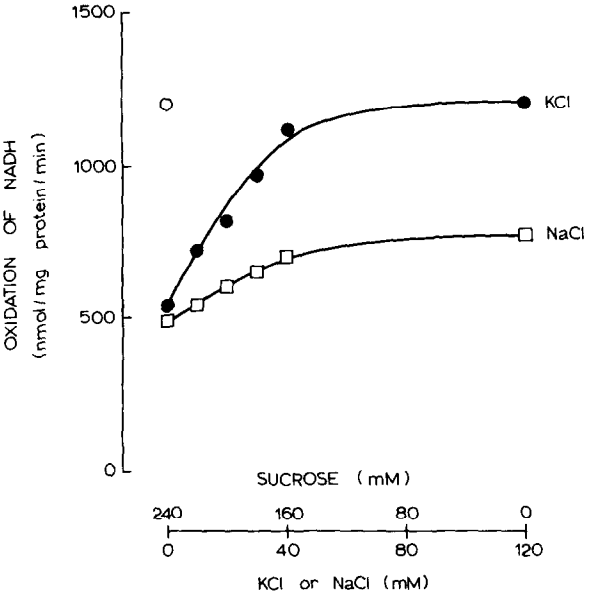

Fig. 3. Releasing of the inhibition by NAD^+ of NADH oxidation by KCl or NaCl. Experimental conditions were as in Fig. 1, except that sucrose was partly replaced by KCl or NaCl, as indicated in the abscissa, so that the tonicity of the medium remained constant. The medium contained 54 μM NAD^+, except for the point indicated by the open circle (○), where no NAD^+ was added.

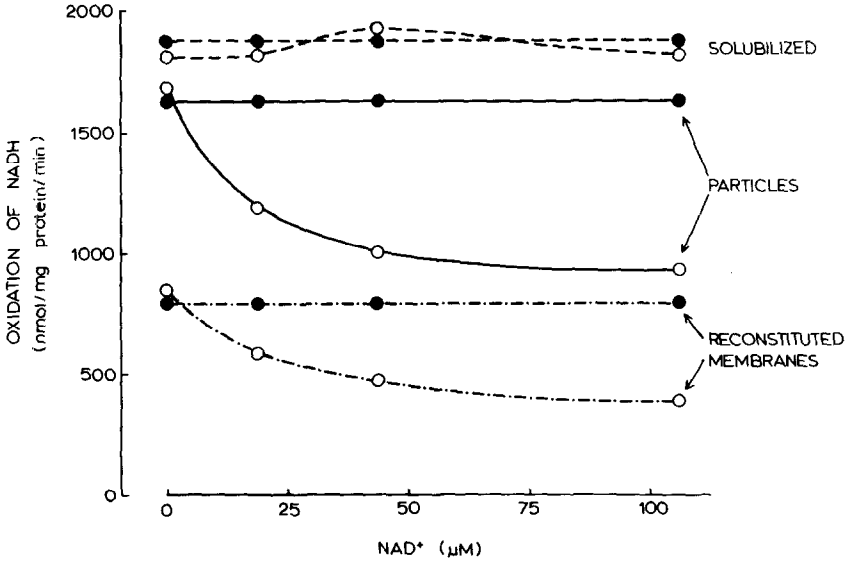

Fig. 4. Effect of solubilization and reconstitution of the membrane on the inhibition of NADH oxidation by NAD^+. Experimental conditions were as in Fig. 2, except that particles were solubilized in 1% sodium cholate. Open symbols, sucrose medium; closed symbols, KCl medium.

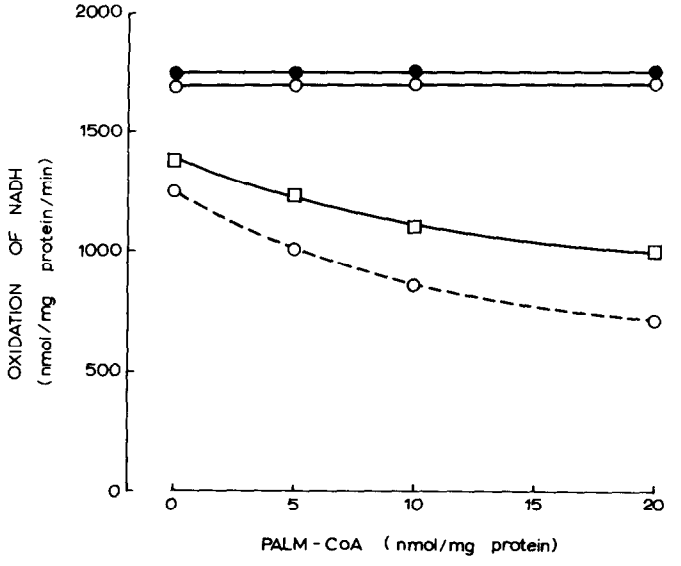

Fig. 5. Effect of palmitoyl-CoA on the oxidation of NADH in the presence and absence of NAD^+. Experimental conditions as in Fig. 2, except that the media contained in addition 1 mM $MgCl_2$ (in order to increase the binding of palmitoyl-CoA by the mitochondrial membrane, ref. 16).
O——O, Sucrose medium, no NAD^+; O- - -O, sucrose medium plus 54 μM NAD^+; ●——●, KCl medium plus 54 μM NAD^+; □——□, NaCl medium plus 54 μM NAD^+.

NAD^+. Similar results were obtained with other anionic surfactants, sodium dodecylsulphate and oleate, whereas the cationic detergent, cetyltrimethylammonium bromide, partly released the inhibitory effect of NAD^+. As expected, anionic surfactants increased, whereas the cationic surfactant decreased, the negative surface charge of the particles as determined by microelectrophoresis (these studies will be published elsewhere).

DISCUSSION

The inhibition of NADH oxidation by the reaction product, NAD^+, has already been observed on purified preparations of NADH dehydrogenase from both mammalian mitochondria (17) and

bacteria (18) and the competitive character of this inhibition postulated (18). The present study shows, however, that with submitochondrial inside-out particles the hyperbolic plot of the activity versus inhibitor concentration can be observed only in the absence of monovalent alkali metal cations. The protection against inhibition by micromolar concentrations of NAD^+ is exerted by K^+, Rb^+, Cs^+, NH_4^+ and, to a much lesser extent, Na^+ and Li^+. It therefore comes out that potassium and some other monovalent cations can maintain high rate of NADH oxidation by preventing product inhibition. This perhaps explains, at least partly, the activatory effect of K^+ on the respiration of mitochondria and submitochondrial particles observed so far (1 - 10).

The inhibition of NADH oxidation by low concentrations of NAD^+ depends on the existence of the membrane, as it largely disappears after solubilization of the particles (Fig. 2) and re-appears after reconstitution of the membrane (Fig. 4). Moreover, it is also affected by factors altering the surface charge of the membrane. It can be therefore speculated that the interaction between NAD^+ and the active center of NADH dehydrogenase, occurring at the outer side of submitochondrial particles (equivalent to the inner side of the inner mitochondrial membrane), depends on the surface charge. Since the pyridine ring of NAD^+ possesses a positive charge (in contrast with the pyridine ring of NADH which is neutral), its affinity to the active center of the flavoprotein NADH dehydrogenase could be increased by increasing the negative charge of the membrane in which the enzyme is situated. This explains the potentiating role of palmitoyl-CoA and other anionic surfactants on the inhibition by NAD^+.

In this context, the protective effect of K^+ and other alkali metal cations of higher atomic weight can be interpreted as promoting the release of the bound NAD^+ molecule from the active center of NADH dehydrogenase. Sodium and lithium, which have a smaller radius of the non-hydrated ion, are much less effective. This points to a certain steric requirement for the displacement of bound NAD^+ by monovalent cations. Different effects of various alkali metal cations on other enzymes have already been reported (19 - 21), but the molecular basis for these differences is still obscure.

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