AN ENERGY INDEPENDENT INTERACTION OF ATP WITH THE MITOCHONDRIAL NADH-DEHYDROGENASE: COMPETITIVE INHIBITION*

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1. Introduction

The first coupling site of oxidation phosphorylation has long been known to be associated with the mitochondrial NADH dehydrogenase. Gutman et al. [1] suggested that the coupling site is located between two components of the NADH-dehydrogenase: The Fe–sulfur complex giving the EPR signal at g=1.94 at 77°K and the piericidin inhibition site.

Further studies [2,3] employing low temperature EPR spectroscopy pointed out that the coupling site is between Fe–sulfur centers 1 and 2 of the NADH dehydrogenase. These conclusions were based on the observation that in presence of piericidin, addition of NADH to ETP_H led to a transient reduction of the NADH-dehydrogenase, but at the end of this cycle, Fe–sulfur center 2, which remained reduced, could be oxidized by addition of ATP. This ATP induced oxidation was sensitive to DNP or oligomycin.

This effect was regarded as a manifestation of intra enzymic reverse electron transport from the high potential Fe–sulfur centers [4] to the low potential ones.

Recently, Ohnishi proposed a different location for coupling site I [5,6]. In these studies pigeon heart mitochondria were kept under potential clamp at −438 mV, a potential where all of the Fe–sulfur centers are reduced. On addition of ATP a partial oxidation of center 1 was observed, but when both uncoupler and oligomycin were present, ATP could not oxidize center 1. This oxidized fraction, defined as center 1a, was associated with the coupling site. It was suggested that ATP can lower the midpotential of center 1a, an effect which is associated with energy tranducing carriers.

Support for this conclusion are the experiments of Albrecht and Slater [7] and Slater et al. [8]. Where addition of ATP to NADH reduced Mg-EDTA-particles led to partial oxidation of centers 1 and 2.

This location of coupling site 1 is incompatible with previous studies. Thus other mechanisms which may lead to the same effect were looked for. In the experiment of Ohnishi [5] redox mediators were employed to poise the redox potential. Recently this approach was critisized: an equilibrium between the redox mediators and the carriers is always assumed but it can not be taken for granted [9]. We can not exclude the possibility that ATP can have some indirect effect which perturbes the equilibration of center 1 with the redox mediators. On the other hand the experiments of Slater et al. [7,8] are more difficult to explain; in this case NADH was the reductant and it was never reported that ATP can perturb the interaction of NADH-dehydrogenase with its substrate. In order to investigate this possibility we looked at the effect of ATP on the catalytic oxidation of NADH by the NADH-dehydrogenase. As will be shown in this publication ATP as well as other nucleotides can interact with the NADH-dehydrogenase and affects its catalytic activity in a mode which is not associated with the phosphate potential or any energy linked effect.

2. Materials and methods

ETP_H were made of beef heart mitochondria accord-

* This study is dedicated to my late friend and colleague Mrs Nitza Silman Movshovitz.

ing to Hansen and Smith [10] NADH-$K_3Fe(CN)_6$ reductase activity was measured at V_{max} $K_3Fe(CN)_6$ as described by Minakami et al. [11] in the triethanolamine–HCl buffer (TEA). ATP, ADP, CTP and ITP were comercial preparation of the highest available purity.

3. Results

In order to measure the maximal catalytic capacity of the NADH-dehydrogenase we measured its NADH-$K_3Fe(CN)_6$ reductase activity [11].

The V_{max} was measured with constant NADH concentrations and variable concentrations of $K_3Fe(CN)_6$. The intercepts of the primary plots were drawn vs 1/NADH to give a secondary plot of the catalytic activity. Fig. 1 demonstrates that ATP inhibit the enzyme competitively with respect to the substrate. The K_i calculated from the slopes is 1.7–1.8 mM.

Table 1 demonstrates the effect of other nucleotides. In these experiments the inhibition was measured

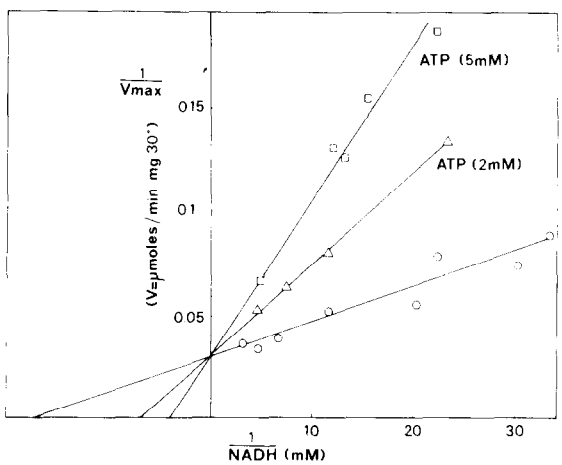

Fig. 1. Inhibition of the NADH-$K_3Fe(CN)_6$ reductase activity by ATP. ETP_H were suspended in 0.18 M sucrose. 50 mM Tris acetate, 5 mM $MgSO_4$ pH 7.4 at 1 mg/ml. The catalytic activity was measured at 30°C in 0.04 M TEA buffer pH 7.8, constant NADH concentration and variable $K_3Fe(CN)_6$. The observed rates were corrected for the blank rates which were determined for each concentration of NADH and $K_3Fe(CN)_6$. The intercepts of the primery plots are drown vs. the 1/NADH used in the assay. Protein concentration in the cuvette 8.3 μg/ml. (○–○–○), no ATP; (△–△–△)2 mM ATP; (□–□–□)5 mM ATP.

Table 1
Inhibition of NADH-$K_3Fe(CN)_6$ reductase activity by nucleotides

NADH (μM)	Inhibitor (mM)	NADH-DH activity at $V_{max}(K_3Fe(CN)_6)$ μmoles/min/mg 30°C
83	–	27
33	–	15
83	ATP(2)	14.2
33	ATP(2)	5.2
83	ITP(2)	18.7
33	ITP(2)	7.4
33	ADP(2)	5
33	ADP(2) + ATP(2)	2.8
83	–	2.4
33	–	13.7
33	ATP(2)	6.2
33	FCCP 1 μM + ATP(2)	6.9
10	–	6.3
10	+ 2 mM CTD	6.3

The enzymic activity was measured at V_{max} ($K_3Fe(CN)_6$) at the indicated NADH concentrations in 40 mM TEA buffer pH 7.8 30°C, using 25 μg protein of ETP_H in 3 ml vol. The number in parenthesis indicate the inhibitor concentration (mM).

with a constant NADH concentration at V_{max} with respect to $K_3Fe(CN)_6$. In order to augment the inhibition, low NADH concentrations were employed. It is evident that another purinenucleotide such as ITP is also inhibitory. ADP is also an inhibitor, and a combination of ADP plus ATP leads to additive inhibition. On the other hand, the pyrimidine nucleotide, CTP, is not inhibitory.

In order to evaluate the role of energy coupling on this inhibition, we first verified that the TEA buffer used for the NADH-dehydrogenase assay did not uncouple the ETP_H. This was monitored by measuring the ability of the ETP_H to carry reverse electron transport in the TEA buffer. Similar rates of ATP dependent NAD^+ reduction by succinate were measured in TEA buffer (0.065 μmoles/min mg 30°C) or in the sucrose–Tris–Mg system recommended by Ernster and Lee [12] (0.0695 μmoles/min mg 30°C). One μM of FCCP abolished completely reverse electron transport, but had no effect at all on the inhibitory effect of ATP (table 1).

4. Discussion

The competitive inhibition of the NADH-dehydrogenase by adenine nucleotides is not surprising. Both NAD^+ ($K_{i(NAD^+)}$ = 1.1 mM) [13] and adenosine–diphosphoribose [14] are competitive inhibition of the enzyme, with K_i competiable to that measured here ($K_{i(ATP)}$ = 1.7–1.8 mM). Thus it is likely that the structure of purine–ribose–phosphate is sufficient to impart the ability to compete with the NADH for its binding site. Substitution of the purine by pyrimidine abolishes this property.

It is of interest to point out that although ATP is a poor competitive inhibitor, at sufficiently high concentrations it might lead to effects on the NADH dehydrogenase, which are unrelated to the operation of coupling site I. The experiments of Slater et al. [7,8] are a case in point. In conjunction with high (5–25 mM) ATP concentration, they employed very concentrated particles suspension (40–60 mg/ml) which could greatly increase the apparent K_m for NADH [15]. When the ATP was added to the particles, which were maintained anaerobically at the expense of NADH, center 1 was oxidized. This effect was not shown to be sensitive to uncouplers or to energy transfer inhibitors [7].

An exploratory experiment was carried out [16] duplicating the conditions described by Albrecht and Slater [7]. It was observed that the addition of ATP (5 mM) oxidized about 50% of center 2 and almost all of center 1. The Hipip component (17) was also oxidized. These oxidations were largely prevented by CCCP (1 μM), but not the oxidation of center 1, which was still oxidized by more then 80%. This oxidation in the presence of uncoupler, is not likely to be an energy linked reaction, but a subsequent of a competitive inhibition.

One must consider the possibility that some of the effects attributed to the interaction of ATP with the phosphorylation system, should be reevaluated. Finally, the competitive inhibition of NADH dehydrogenase by adenine nucleotides should be considered while studying the mitochondrial intermediate metabolism. The intramitochondrial concentration of ATP and ADP can be 2–4 times higher then the K_i value with respect to NADH dehydrogenase, and the NADH concentration, under certain condition, can be comparable to its K_m [18]. Under such conditions the competitive inhibition of the oxidation of NADH may contribute to the fine balance of the metabolite concentration in the mitochondria.

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