MUTUAL INHIBITION BETWEEN NADH OXIDASE AND SUCCINOXIDASE ACTIVITIES IN RESPIRING SUBMITOCHONDRIAL PARTICLES

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1. Introduction

The dehydrogenases of the respiratory system can oxidize substrates at rates much higher than the respiration rate. When more than one substrate is oxidized, the rate of respiration is less than the sum of the rates of respiration on each substrate separately [1–4]. In such a case, not only are the dehydrogenases partially utilized, but they also inhibit each other. This type of mutual inhibition was postulated to operate at the junction of the flavoprotein and the CoQ_{10} [5], but a detailed mechanism was not proposed.

In this study we looked at the mutual inhibition between the two main electron donors of the respiratory system: NADH-dehydrogenase (NADH-DH) and succinic dehydrogenase (SDH). The interaction between them is reflected in the oxidase activity. Thus, we shall refer to the mutual inhibition between the NADH oxidase and the succinoxidase activities.

A recent hypothesis [6] suggested that the multiple control mechanism of SDH [7–10] operates to cope with the mutual inhibition between the NADH oxidase and succinoxidase activities. Consequently, this mutual inhibition should reflect the activation level of the SDH.

It was observed that the inhibition of the succinoxidase by the NADH oxidase is in inverse relationship to the activation level of the SDH; the higher the activation level, the lower the inhibition of succinoxidase by the NADH oxidase. The inhibition between the two oxidase activities was found to be "asymmetric" in the sense that the NADH oxidase can modify the succinoxidase activity much more than the succinoxidase can inhibit the NADH oxidase.

The results reported can be explained by assuming that the CoQ_{10} pool is, at least a certain degree, compartmentized with respect to the dehydrogenases [11]. The mutual inhibition between the oxidase activities is suggested to be the result of a spill-over of reducing equivalents over the compartmenting barrier. As a result of that, NADH-DH reduces CoQ_{10} in the pool available to SDH to such a level that the concentration of oxidized CoQ_{10} falls below the saturating level for the SDH.

2. Materials and methods

ETP_H was prepared, and NADH oxidase, succinoxidase, SDH and NADH-DH activities were assayed as detailed before [9, 12]. The oxidation rate of NADH or succinate, when both were present, was calculated from monitoring the respiration by oxygen electrode and at 340 nm. The rate of NADH oxidation in the presence of succinate was subtracted from the rate of oxygen uptake on both substrates to give the value for the succinoxidase activity. Deactivation of SDH was carried out as detailed before [9], and activation was achieved by 1 mM malonate at 30°.

3. Results

To measure the effect of succinate on NADH oxidation and vice versa, ETP_H preparations were used. Addition of NADH to ETP_H respiring on succinate accelerated the rate of oxygen consumption, yet the respiration rate with both substrates was less than the sum of respiration rates on each substrate separately. Addition of succinate to ETP_H respiring on NADH, led to 10–15% inhibition of the NADH oxidation

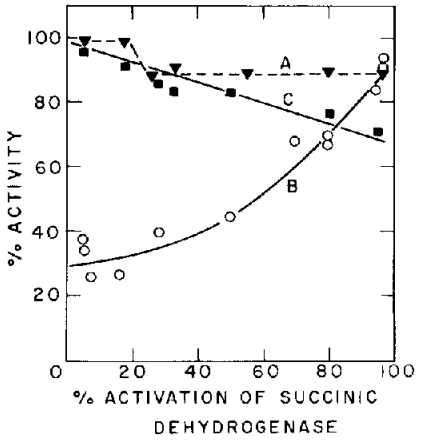

Fig. 1. The effect of activation level of succinate dehydrogenase on the mutual interactions between NADH oxidase and succinoxidase activities. ETP_H, (specific activity in μmoles/min mg at 30°: NADH-DH, 31; SDH, 1; NADH oxidase, 1.5; succinoxidase, 0.7), 10 mg/ml in 0.18 M sucrose, 50 mM Tris-acetate, 5 mM $MgSO_4$ were activated by 1 mM malonate at 30° and at desired times samples were withdrawn and cooled to 0°. Respiration was measured in 80 mM KPi, 50 μM EDTA, 1 mg BSA/ml, 30°, with 300 μM NADH, 16 mM succinate, or both. In the assay, the malonate was diluted to less than 50 μM. A) (▼---▼---▼) The inhibition of NADH oxidase by addition of succinate. B) (○—○—○) The percent of succinoxidase activity in presence of NADH *vs.* succinoxidase in the absence of NADH. C) (■—■—■) The contribution of NADH oxidation to the total respiration rate in presence of succinate.

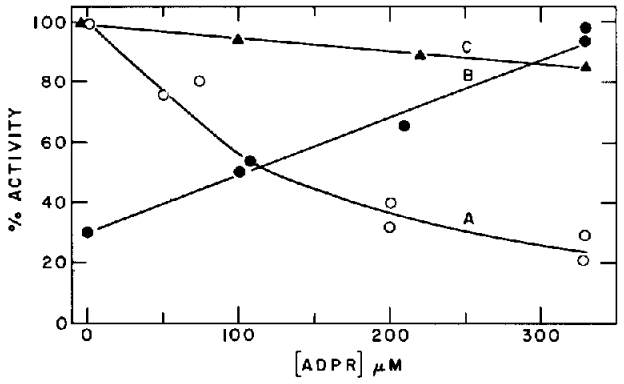

Fig. 2. The effect of ADPR on the inhibition of succinoxidase in the presence of NADH. The respiration on NADH, (0.5 mM), succinate (20 mM) or both of them was measured with non-activated ETP_H as in fig. 1. NADH-Fe $(CN)_6^{3-}$ reductase activity was measured at V_{max}. A) (○—○—○) Inhibition of NADH-Fe $(CN)_6^{3-}$ reductase activity. B) (●—●—●) The percent of succinoxidase activity in presence of NADH *vs.* succinoxidase in absence of NADH. C) (▲—▲—▲) Inhibition of NADH oxidase on addition of succinate.

rate measured at 340 nm. No inhibition was observed in non-activated preparations. The inhibition attained its maximal value in the range of 20–30% activation (fig. 1, line A).

When the contribution of succinoxidase to the mixed substrate respiration was computed, it was found to be less than the rate in the absence of NADH. In non-activated preparations, NADH lowered the succinoxidase activity to 30% of its full activity, while in fully activated particles, 80–90% of succinoxidase activity was measured in the presence of NADH (fig. 1, line B). The contribution of NADH oxidase to the respiration rate on both substrates is given in fig. 1, line C. It falls from 95% in non-activated ETP_H to 70% with fully activated particles.

The rate of electron flux from NADH to oxygen is practically invariable, while the respiration on succinate is a function of two variables: the activation level, and the inhibition by NADH oxidase. The higher the potential activity of SDH, the lesser is its inhibition on addition of NADH, as if the ratio between electron fluxes from the two flavoproteins determines the extent of the inhibition of succinoxidase. According to this model, lowering the activity of NADH-DH without changing the SDH activity, will have the same effect as an increase of the SDH activity while keeping the NADH-DH activity constant. This experiment is shown in fig. 2. Line A denotes the inhibition of the NADH-Fe $(CN)_6^{3-}$ reductase activity (measured at V_{max}) by increasing the concentration of ADPR, (adenosine diphosphoribose), a competitive inhibitor of NADH linked dehydrogenases. As the NADH-Fe $(CN)_6^{3-}$ reductase of this preparation is 20 times higher than its NADH oxidase activity, even the highest ADPR concentration did not inhibit the NADH oxidase.

In the absence of ADPR, NADH inhibits the succinoxidase activity by 70%, typical for a non-activated ETP_H. On increasing the ADPR concentration, the inhibition decreased, parallel to the decrease of the NADH-Fe $(CN)_6^{3-}$ reductase activity (fig. 2, line B). At 330 μM ADPR, no inhibition of succinoxidase was observed, a characteristic for the interaction of activated succinic dehydrogenase with NADH oxidase. It should be noted that ADPR does not activate the SDH.

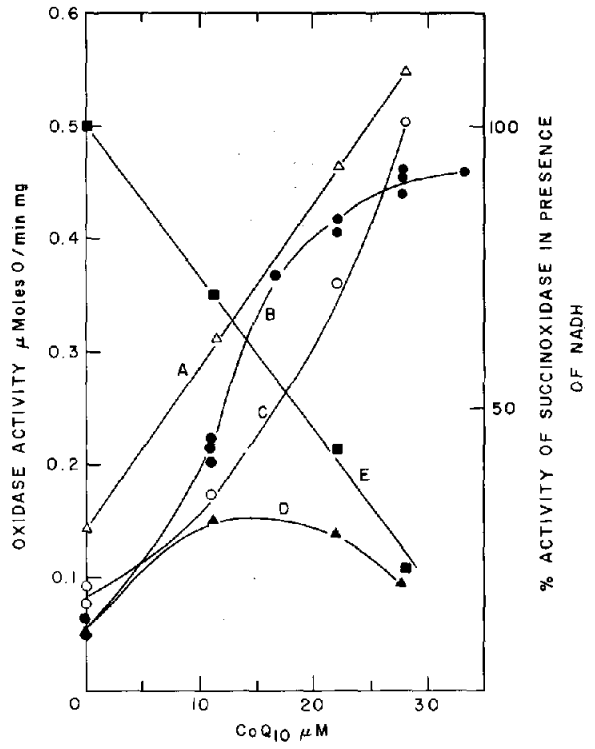

Fig. 3. The effect of reincorporation of CoQ_{10} on the oxidase activities of CoQ_{10} depleted ETP_H. Lyophilized ETP_H were extracted 5 times with pentane and dried in vacuum, resuspended in sucrose-Tris buffer as in fig. 1 and activated. Oxidase activities were assayed as in fig. 1 with 0.16 mg protein/ml. CoQ_{10} was added as ethanolic solution. A) (△—△—△) The rate of respiration on NADH plus succinate. B) (●—●—●) Succinoxidase activity. C) (○—○—○) NADH oxidase activity. D) (▲—▲—▲) Succinoxidase activity measured in the presence of NADH. E) (■—■—■) The percent of succinoxidase measured in the presence of NADH *vs.* succinoxidase in the absence of NADH. The maximal activity of NADH oxidase was attained at 60 μM of CoQ_{10} with a value of 0.8 μmoles 0/min/mg.

The addition of ADPR simulated the effect of activation also in allowing succinoxidase to inhibit NADH oxidase by 10% (fig. 2, line C).

To localize the site of the mutual inhibition at the level of CoQ_{10}, ETP_H was depleted of CoQ_{10} and the effect of its reincorporation on the oxidase activities was studied. As seen in fig. 3, both succinoxidase and NADH oxidase are restored, (lines B and C). The inhibition of succinoxidase by NADH increases with increasing concentration of CoQ_{10} (line E). The absolute rate of the succinoxidase in the presence of NADH approaches a maximal value and then drops, while in the absence of NADH the succinoxidase activity increases in a normal saturation curve.

4. Discussion

Measurements of the gross reduction of CoQ by either NADH or succinate, led Klingenberg and Kruger to conclude that the CoQ_{10} is a homogeneous pool [13].This model could explain the sigmoidal inhibition curve of Antimycin, but failed to account for the loss of sigmoidicity by treatment with detergents [16].

The compartmentation of the CoQ_{10} pool was suggested when it was observed that, in CoQ_{10} depleted preparations, the concentration of CoQ_{10} restoring the activity of succinoxidase was lower than that needed for NADH oxidase [11, 15]. This hypothesis was further supported by the effects of piericidin A [12]. Piericidin A bound at the non-specific sites was observed to inhibit electron transport from NADH-DH to cytochrome *b* or energy linked NAD reduction by succinate. It had no effect on succinoxidase nor on NADH-CoQ_2 reductase activities. Furthermore, at its non-specific sites piericidin inhibited the activation of SDH by endogeneous $CoQ_{10}H_2$, though it did not prevent the reduction of the CoQ_{10} [9]. This was explained by assuming that non-specifically bound piericidin enhanced the compartmenting barriers.

The mutual inhibition between oxidase activities was suggested to be a result of a competition of dehydrogenases for the CoQ_{10} [5]. When considering the ratio of CoQ_{10} to SDH or NADH-DH, 30:1 and 100:1, respectively, it is difficult to visualize a competition of this kind. On the other hand, if each dehydrogenase is associated with an isolated "sub-pool" of CoQ_{10}, no inhibition will ensue. To explain the mutual inhibition, we combine the "sub-pool" concept with a mechanism permitting a competition between the dehydrogenases. We propose that the "sub-pools" of CoQ_{10} interact with each other. Originally, the redox state of each "sub-pool" is determined by the rate of its reduction by the dehydrogenase and oxidation by cytochrome *b*. The "sub-pools", separated by an unidentified mechanism, would reduce each other whenever the difference in the redox

potential between them overcomes the effects of compartmentation. If we assume that cytochrome *b* will react with the same rate constant with all the "sub-pools", then we may conclude that the dehydrogenase activity is the factor which affects most the redox state of the "sub-pool".

As the activity of NADH-DH is higher than that of SDH, it's "sub-pool" will be more reduced than that of SDH. When the difference in potential becomes high enough to overcome the compartmenting barrier, NADH-DH will contribute to the reduction of the SDH "sub-pool". As a result of that, the concentration of oxidized CoQ_{10} in the SDH "sub-pool" will decrease and the rate of oxidation of reduced SDH by CoQ_{10} will be slowed. The inhibition of succinate oxidation is masked by an increase of overall electron flux to oxygen, enhanced by the higher content of $CoQ_{10}H_2$ available to react with cytochrome *b*. The same mechanism operates in the other direction. By increasing the activity of SDH, we could gradually lower the contribution of NADH oxidase to the overall respiration (fig. 1), an effect that is due to both inhibition of NADH oxidase and increased contribution of succinoxidase to the mixed substrate respiration.

Inhibition of NADH-DH by ADPR lowers the reduction state of the NADH-DH "sub-pool" and will minimize it's interaction with the SDH "sub-pool". The observed decrease of the inhibitory effect of NADH on succinoxidase with increasing ADPR concentration is compatible with this hypothesis (fig. 2).

Finally, the effect of the content of CoQ_{10} on the mutual inhibition was tested. By adjusting the concentration of CoQ_{10} in the reincorporation experiment, (fig. 3), we could demonstrate that, with low levels of CoQ_{10}, where succinoxidase is restored to higher levels than NADH oxidase, the inhibition by NADH was quite low (fig. 3, line E). At higher levels of CoQ_{10}, where NADH oxidase increased more steeply than succinoxidase, the inhibitory effect of NADH increased too. This is depicted in the downward deflection of succinoxidase measured in the presence of NADH (fig. 3, line D).

It seems that the mutual inhibition in multi-substrate respiration is a very sensitive probe which enables one to discriminate between fractions of the CoQ_{10} pool, a property that is naturally masked when the parameter measured is the total amount of reduction.

In conclusion, a hypothesis is suggested, which describes the CoQ_{10} to be divided into pools. Each pool is reduced by it's specific dehydrogenase and can interact, in a spill-over mechanism, with other pools. The final steady state reduction of each pool, determined by the influx and efflux of electrons, will be reflected in the rate of oxidation of the reduced dehydrogenase by the CoQ_{10}.

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