MODULATION OF MITOCHONDRIAL SUCCINATE DEHYDROGENASE ACTIVITY,
MECHANISM AND FUNCTION*

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Summary

The mitochondrial succinate dehydrogenase (E.C. 1.3.3.99) is subjected to apparently com-
plicated regulatory mechanism. Yet, systematic
analysis of the mechanism reveals the simplicity
of the control. There are two stable forms of
the enzyme; the non-active form stabilized as
1:1 complex with oxaloacetate and the active
form stabilized by binding of activating ligands.
This model quantitatively describes either the
equilibrium level of active enzyme or the kine-
tics of activation-deactivation, in the presence of
various concentrations of opposing effectors.
The site where the regulatory ligands interact
with the enzyme is not the substrate bonding
site. The marked differences of dissociation
constants of the same ligand from the two sites
clearly distinguish between them.

This model is fully developed for simple cases
where the activating ligands are dicarboxylic
acids or monovalent anions. On the other hand
with activators such as ATP or CoQH₂, quanti-
tation is still not at hand. This stems from the
difficulties in maintaining determined, measura-
ble, concentrations of the ligand in equilibrium
with the membranal enzyme.

While in active form the histidyl flavin moity
of the enzyme is reduced by physiological
substrate (succinate; CoQH₂). The non-active
form is not reduced by these compounds, only
strong reductants with low redox potential re-
duce the non-active enzyme. It is suggested that
deactivation is a simple modulation of the redox
potential of the flavin form E'<0 mV in the
active enzyme to E'<−190 mV. The switch
from one state to another might be achieved by
distortion of the planar form of oxidized flavin
to the bend configuration of the reduced flavin.
Thus, in the active enzyme such distortion will
destabilize the oxidized state of the flavin,
shifting the redox potential to the higher value.
The binding of oxaloacetate to the regulatory
sites releases the distorting forces by relaxing
the conformation of the enzyme. Consequently,
the flavin assumes its planar form with the low
redox potential. This assumption is supported
by the spectral shifts of the flavin associated
with the activation deactivation transition.

The suicidal oxidation of malate to oxaloacetate,
carried by the succinate dehydrogenase,
plays an important role in modulating the
enzyme activity in the mitochondria. This
mechanism might supply oxaloacetate for deac-
tivation in spite of the negligible concentration
of free oxaloacetate in the matrix. The oxida-
tion of malate by the enzyme is controlled by
the redox potential at the immediate vicinity of

* An invited article.

* Abbreviations: SDH–succinate dehydrogenase (succinate:
acceptor oxidoreductase (E.C. 1.3.99.1)); OAA–
oxaloacetate; Act–activator; Eₐ, Eₐₐ–active and non
active forms of the enzyme, respectively; K′eq–apparent
equilibrium constant; K'd–apparent dissociation constant;
K Act, K OAA–dissociation constant of the respective ligand
from the enzyme; k'a, k'd–the apparent rate constants of
activation and deactivation, respectively; kₐ, kₐ–the true
rate constant of activation and deactivation respectively;
ETP, ETP H–non phosphorylating and phosphorylating sub-
mitochondrial particles; PMS–phenazine methosulfate;
DCIP–dichlorophenol indophenol; CoQ–ubiquinone;
TTFA–Thenotrifluoacetone; NEM–N methyl Maleimide.
the enzyme, and is imposed by the redox level of the membranal quinone.

Finally, the modulation of succinate dehydrogenase activity is closely associated with regulation of NADH oxidation through the mutual inhibition between oxidases (Gutman, M. in Bioenergetics of Membranes, L. Packer et al., ed. Elsevier 1977, p. 165). The consequence of these interactions is the selection for the main electron donor for the respiratory chain, during mixed substrate respiration, according to the metabolic demands from the mitochondria.

1. Activation of succinate dehydrogenase, in retrospect

In 1955 Kearney et al. reported that the catalytic activity of SDH is much higher when assayed in phosphate buffer than in other buffers such as glycylglycine or imidazol. The level of activity was function of phosphate concentrations. In 1957 Kearney explained this observation as activation by substrate or its analogs (malonate and phosphate). The final level of activation was correlated with the activator concentration. The slowness of the reaction and the high energy of activation 33 Kcal/mol suggested that the phenomenon represents a conformational change of the enzyme and not a release of inhibitor from the enzyme. Even now, when the nonactive form is identified with the SDH-OAA, activation is regarded as conformational change taking place within the complex, followed by release of OAA and stabilization of the active enzyme by the activator. The activation was correlated with distinct spectral changes (maximum 508 nm, minimum at ~570 nm). The spectral change is not reduction of the enzyme, as a matter of fact the nonactive enzyme is not reducible by substrate.2

In her studies Kearney observed that activation could not be reversed by dialysis, but somewhat later, when gel filtration was employed to remove the activator3,4 the reversibility of activation was detected. This led Kimura et al.4 to suggest that activation might regulate the activity of the enzyme in the mitochondria. Furthermore, the physiological regulator could not be succinate or fumarate, these compounds were easily removed from the mitochondria yet no change in activation ensues. Accordingly the physiological activator was speculated to be a tightly bound competitive inhibitor. The free enzyme was assumed to be in the nonactive configuration (E NA) while activator stabilized the active enzyme in an effector-enzyme complex (EA Act). At that time, the reasoning was sound but it turned to be that the tightly bound inhibitor did not act as activator but functioned as a negative modulator. Wojtczak et al. in 1969 noted that addition of OAA to active SDH, in presence of substrate and oxidant cause a delayed inhibition. The inhibition could be reversed by increasing the succinate concentration. Wojtczak, et al. speculated that either OAA forms a very tight complex with the enzyme or alternatively, it induces a conformation change from active to nonactive form. Both alternatives turned out later to be true. Furthermore, Wojtczak identified the effect of OAA as reversal of the activation. “It may be speculated that both processes have a common background and that, in fact, the enzyme isolated by Kearney was already inhibited by previous contact with OAA”. The presence of OAA in inactive enzyme was later confirmed, and thoroughly quantitated by Kearney and her colleagues.6,7,8. The competitive relationship between OAA and succinate perpetuated a concept that both ligands interact at the substrate binding site9,10, though such a relationship does not necessitate the involvement of the substrate binding site11. Much later Gutman12 demonstrated that the regulatory site is distinct from the substrate binding one. The relative slow deactivation following addition of OAA to submitochondrial particles respiring on succinate was utilized by Zimakova et al.13 for kinetic analysis of the deactivation. Though there was no distinction between binding of OAA and deactivation, parameter fitting and computer simulation yield dissociation constant comparable to those obtained by equilibrium methods.4

Before the role of OAA was fully appreciated, two other modes of activation were discovered. Both can be regarded physiological, as the activators CoQH2,14,15,16 and ATP17 are native in the mitochondria. Regulation by these compounds could explain the accumulation of succinate in state 3 mitochondria. Regulation by these compounds could explain the accumulation of succinate in state 3 mitochondria10. As demonstrated by Gutman et al.17 the modulation of the enzyme activity in a sub-cellular organelle, takes place with amplitude and response time suitable for physiological functionality.