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O2k-Protocols

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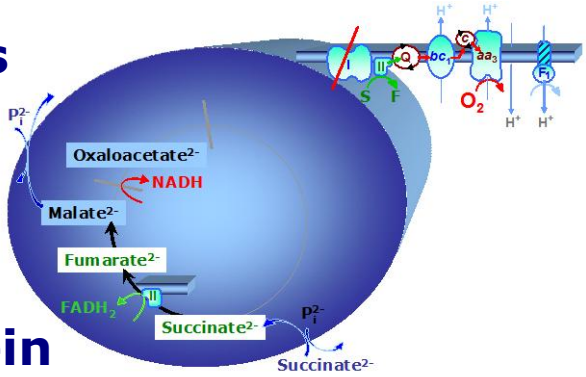
Mitochondrial Pathways to Complex II, Glycerophosphate Dehydrogenase and Electron-Transferring Flavoprotein



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Introduction

Complex II (CII) is the only membrane-bound enzyme in the tricarboxylic acid cycle and is part of the mitochondrial electron transfer system. The flavoprotein succinate dehydrogenase is the largest polypeptide of CII, located on the matrix face of the inner mitochondrial membrane. Following succinate oxidation, the enzyme transfers electrons directly to the quinone pool (Cecchini 2003; Sun et al 2005). Whereas CI is NADH-linked to the dehydrogenases of the tricarboxylic acid cycle *upstream* of coenzyme Q, CII is $FADH_2$–linked *downstream* with subsequent electron flow to Q.

1. Succinate+Rotenone: S(Rot)

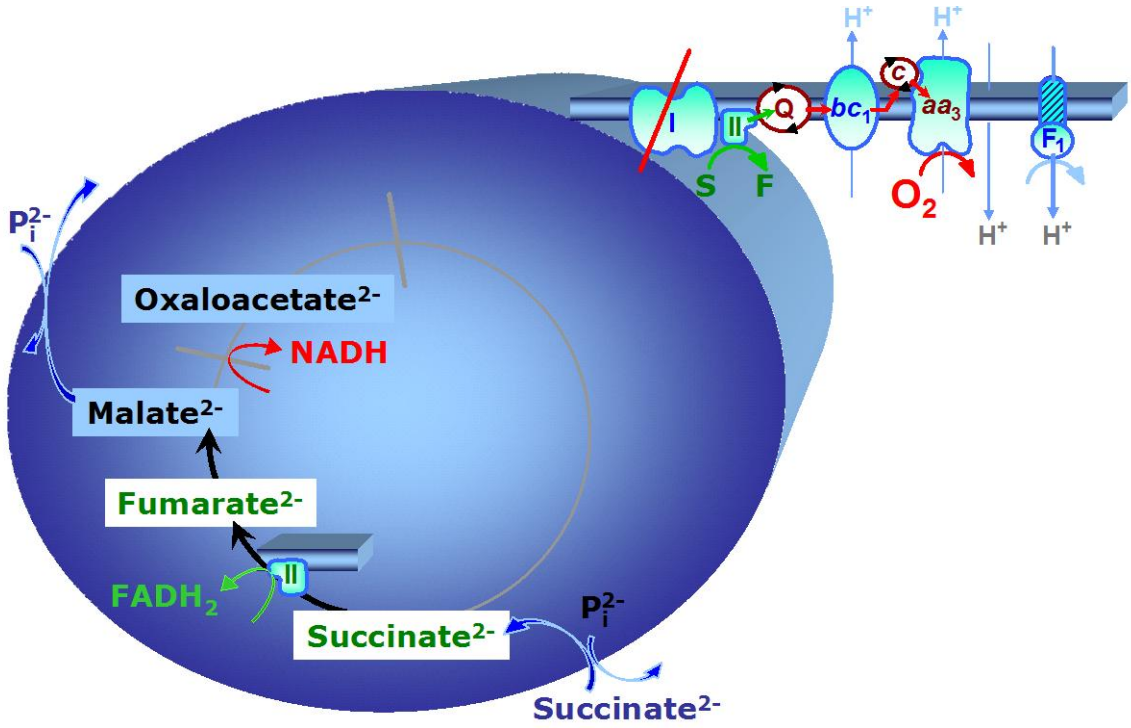

Figure 1. Succinate, S(Rot), supports electron flux exclusively through CII via flavin adenine dinucleotide ($FADH_2$). After inhibition of CI by rotenone (Rot; or amytal, piericidine), the NADH-linked dehydrogenases become inhibited by the redox shift from NAD^+ to NADH.

Carrier for succinate

The dicarboxylate carrier catalyses the electroneutral exchange of $succinate^{2-}$ for HPO_4^{2-}, and accumulation of malate is also pevented by exchange of malate and inorganic phosphate.

Succinate dehydrogenase is activated by succinate, which explains in part the time-dependent increase of respiration in isolated mitochondria after addition of succinate+rotenone and ADP (Fig. 1).

2. Succinate: S

When succinate is added without rotenone (Fig. 2), oxaloacetate is formed from malate by the action of malate dehydrogenase. Oxaloacetate cannot permeate the mitochondrial inner membrane, accumulates, and is a more potent competitive inhibitor of succinate dehydrogenase than malonate even at small concentration (Lehninger 1970, p. 352). Reverse electron flow from CII to CI is known to stimulate production of reactive oxygen species under these conditions. Surprisingly, addition of malate inhibits superoxide production with succinate, probably due to the oxaloacetate inhibition of CII (Muller et al 2008).

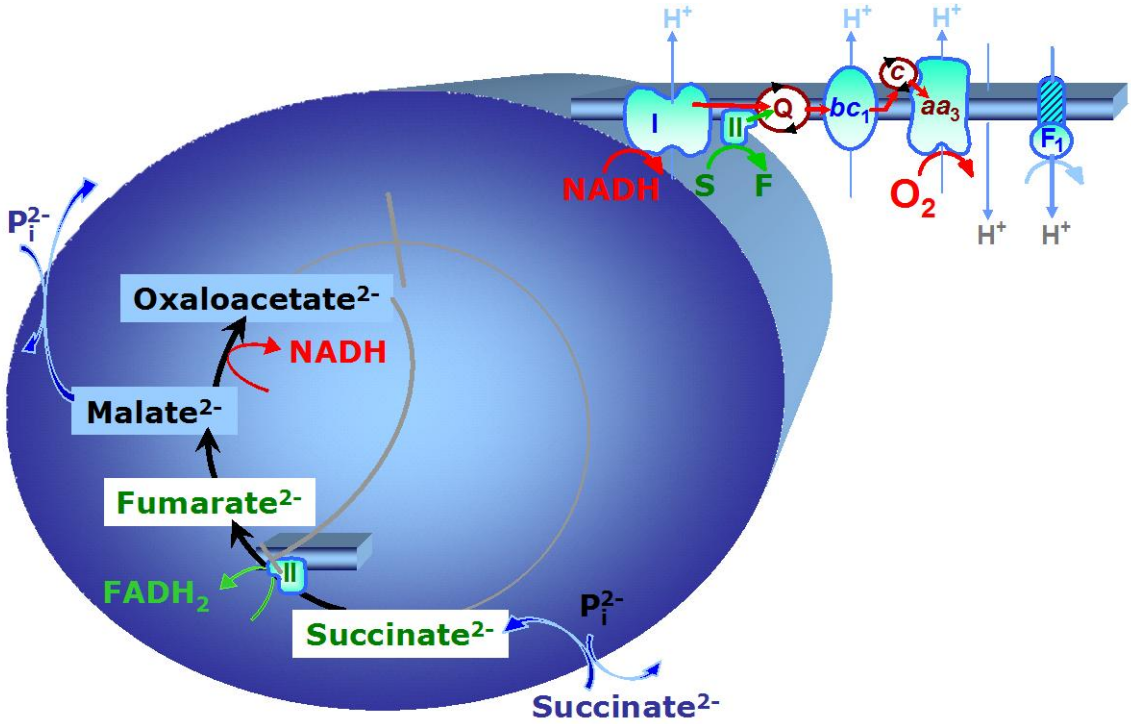

Figure 2. OXPHOS capacity with succinate alone, S_D, is underestimated at 30-40% of flux with succinate+rotenone, $S(Rot)_D$, in human and rat skeletal muscle mitochondria, due to inhibition of succinate dehydrogenase by accumulating oxaloacetate (Ernster and Nordenbrand 1967; Capel et al 2005).

3. Glycerophosphate: Gp

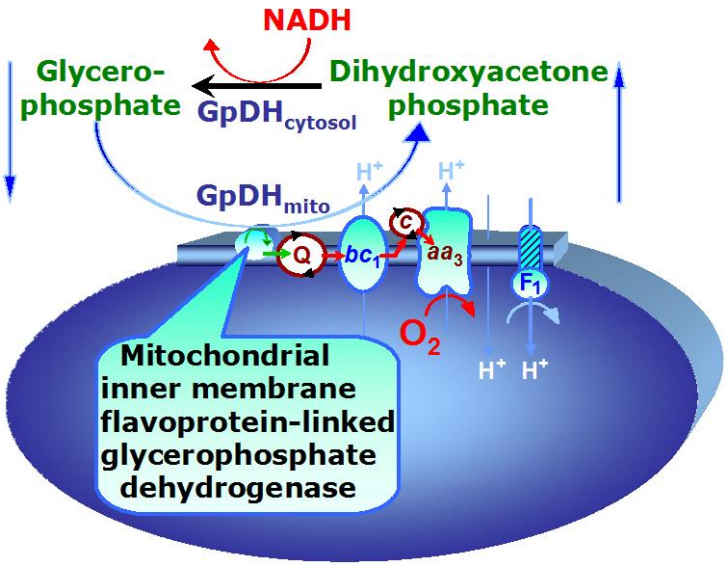

Figure 3. The glycerophosphate shuttle represents an important pathway, particularly in liver, of making cytoplasmic NADH available for mitochondrial oxidative phosphorylation. Cytoplasmic NADH reacts with dihydroxyacetone phosphate catalyzed by cytoplasmic glycerophosphate dehydrogenase. On the outer face of the inner mitochondrial membrane, mitochondrial glycerophosphate dehydrogenase oxidizes glycerophosphate back to dihydroxyacetone phosphate, a reaction not generating NADH but reducing a flavin prosthesic group. The reduced flavoprotein donates its reducing equivalents to the electron transfer system at the level of CoQ.

Glycerophosphate oxidation (Fig. 3) is 10-fold higher in rabbit gracilis mitochondria (fast-twitch white muscle; 99% type IIb) compared to soleus (slow-twitch red muscle; 98% type I; Jackman and Willis 1996). Activity is comparatively low in human vastus lateralis (Rasmussen and Rasmussen 2000). Glycerophosphate is an important substrate for respiration in brown adipose tissue mitochondria (Rauchova et al 2003).

4. Electron-Transferring Flavoprotein

Electron-transferring flavoprotein (ETF) is located on the matrix face of the inner mitochondrial membrane, and supplies electrons from fatty acid *β*-oxidation to CoQ.

5. Notes - Pitfalls

5.1 Ponsot et al (2005) *J Cell Physiol* 203: 479-486. '.. the mitochondrial form of GPDH, which produces $FADH_2$ within the mitochondrial matrix and provides electrons to Compoex II of the phosphorylation chain'. – The mitochondrial glycerophosphate dehydrogenase (GpDH), located on the outer side of the inner mitochondrial membrane, does not provide electrons to CII, but feeds electrons into the Q-cycle entirely independent of CII. $FADH_2$ is not produced within the mitochondrial matrix. Electron transfer takes place from the mitochondrial inner membrane flavoprotein-linked glycerophosphate dehydrogenase to CoQ.

5.2. In the original printed edition of 'Mitochondrial Pathways and Respiratory Control' (2007), the term 'electron transport' has been used synonymously for 'electron transfer'.

References: Gnaiger_2012_MitoPathways_References.pdf