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# Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution☆

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ABSTRACT

This paper explores the bioenergetics and potential co-evolution of denitrification and aerobic respiration. The advantages and disadvantages of combining these two pathways in a single, hybrid respiratory chain are discussed and the experimental evidence for the co-respiration of nitrate and oxygen is critically reviewed. A scenario for the co-evolution of the two pathways is presented. This article is part of a Special Issue entitled: The evolutionary aspects of bioenergetics systems.

## 1. Introduction

After the evolution of oxygenic phototrophy, molecular oxygen has become the major electron acceptor on Earth; it is responsible for the oxidation of most of the organic matter originating from primary production. Bacteria are also able to use several other electron acceptors for respiration. Among these electron acceptors, nitrate is common in the environment and has an oxidative potential which approximates that of oxygen. The most studied and evolutionary most widespread form of nitrate respiration is known as denitrification. In this process, nitrate is reduced stepwise to nitrite, nitric oxide, nitrous oxide and finally nitrogen ($N_2$).

In the past century, mankind has become dependent on the industrial production and agricultural use of chemical fertilizers. This has created an important new source of nitrate for the biosphere. Part of the applied fertilizer is oxidized to nitrate by nitrifying bacteria, detaches from the negatively charged clay particles and is washed into the surface waters via rainwater or irrigation. On top of this, the burning of fossil fuels also contributes fixed nitrogen in the form of ammonia deposition. It is estimated that currently every one out of two nitrogen atoms in the biosphere originates from fertilizers or fossil fuels [1]. This has increased the importance of denitrification relative to aerobic respiration in aquatic habitats. Because denitrification leads to the emission of nitrous oxide (a potent greenhouse gas and ozone scavenger) to the atmosphere, it is currently actively researched by environmental scientists.

Denitrification and aerobic respiration depend on the same core respiratory machinery. This machinery consists of the NADH dehydrogenase (complex I), the quinone pool, the $bc_1$ complex (complex III) and cytochrome *c*. Each of the two pathways adds its own specific modules to this backbone. Aerobic oxidation requires a terminal oxidase (complex IV) which accepts electrons either from cytochrome *c* or the quinone pool. Denitrification consists of four modules: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase. Organisms that contain at least two or three of these enzymes and produce nitrous oxide or dinitrogen gas will be referred to as "denitrifiers".

As far as we know, all denitrifiers are also capable of aerobic respiration, and the simultaneous "plugging in" of all modules into the backbone would lead to a highly branched respiratory chain in these organisms. Although the four steps of denitrification operate in series from the perspective of the electron acceptor ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), they operate in parallel from the perspective of the respiratory chain (all accept their electrons from cytochrome *c* or the quinol pool) (see Figs. 1–3).

The discovery of "intra-aerobic" denitrification has complicated matters even further. This process adds yet another module to the already rich inventory, namely the dismutation of nitric oxide into

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*Abbreviations:* Complex I, NADH dehydrogenase; Complex III, $bc_1$ complex; Complex IV, terminal oxidase; FMN, flavin-mono-nucleotide; ISP, iron sulfur protein; Mo-*bis*MGD, molybdenum *bis* molybdopterin guanine dinucleotide; Nap, periplasmic nitrate reductase; Nar, membrane bound nitrate reductase; Nir, nitrite reductase; Nod, nitric oxide dismutase; Nor, nitric oxide reductase; Nos, nitrous oxide reductase; Nrf, nitrite:ammonia oxidoreductase; Paz, pseudoazurin; Q, quinol; TAT, twin-arginine translocation

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oxygen and nitrogen ($N_2$) [2] (Fig. 4). The dismutation requires no electrons but would in theory enable the operation of a hybrid denitrification/aerobic respiratory chain. Such as chain starts out with the nitrate and nitrite reduction into nitric oxide. Subsequently, the nitric oxide is dismutated into oxygen and the final part of the chain proceeds aerobically via complex IV. Members of the heme-copper oxidase superfamily or a divergent multicopper oxidase have been implicated in the catalysis of the dismutation. It still needs to be investigated how widespread nitric oxide dismutation is and whether the intracellularly produced oxygen is restricted to serve the oxidation of recalcitrant electron donors such as methane. However, it opens up interesting theoretical possibilities that will partly be explored in this paper.

In the following section the enzymology of the different respiratory modules will be briefly summarized. For more detailed information on individual enzymes, the reader is referred to excellent specialized reviews previously published (see below). Next, the bioenergetics of (combinations of) the different denitrifying and aerobic respiratory chains are discussed in terms of their catabolic energy efficiency. The paper continues with an overview of the current experimental evidence that the two pathways may function simultaneously in one complex respiratory network. Finally, a scenario is presented for a possible shared evolutionary origin of aerobic respiration and denitrification.

## 2. A brief summary of the enzymes, the modules of the denitrifying/aerobic respiratory chain

What follows is a brief introduction of the respiratory backbone and the different modules necessary for aerobic respiration and denitrification. The canonical, best-studied forms of the enzyme complexes are presented (Fig. 2). Deviations are known (e.g. in mononderm bacteria and archaea, both lacking a periplasm [3], Fig. 3) but have only been explored partially. It is likely that even more exceptions will be discovered in the future. To acknowledge the different types of quinols used by different organisms (menaquinol, ubiquinol, etc.), we refer to these molecules as the “quinone/quinol pool.”

Both aerobic respiration and denitrification handle compounds that easily give rise to reactive radicals. These can cause oxidative damage to the cell [4]. Many of the enzymes involved also depend on iron for catalysis or electron transfer and even more radicals are formed in the presence of iron (due to the Fenton reaction [5]). Therefore, apart from the core enzymes described here, more enzymatic machinery will be in place to quench the formation of radicals. Because of the highly branched nature of the denitrifying respiratory chain, imbalances in electron donor supply can potentially lead to (temporal) incomplete denitrification and build up of nitrite or nitric oxide. Binding of nitric oxide to specific carrier molecules (such as cytochrome *c*′) and the use of sensors and regulators may help to prevent this scenario [6]. These aspects are not addressed here and we focus on the structural parts of the pathway.

Figs. 1–3 illustrate three examples of the integration of the different modules into the backbone respiratory chain.

### 2.1. NADH dehydrogenase (complex I)

Together with the quinone pool, complex III and cytochrome *c*, NADH dehydrogenase constitutes the backbone of the respiratory chain (reverse electron transport is also possible, but is not discussed further here). Bacterial complex I consists of up to 14 subunits (550 kDa total), encoded by the genes *NuoABCDEFGHIJKLMN* [7,8]. The electrons are transduced from NADH (−0.32 V) to Flavin-mono-

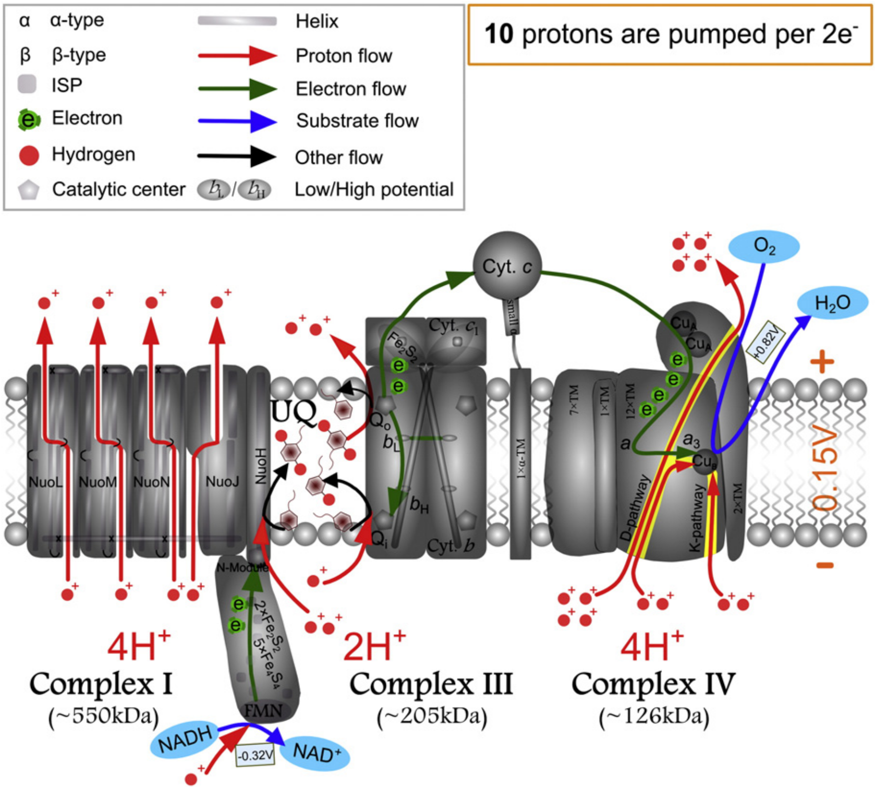

**Fig. 1.** The canonical respiratory chain of oxygen respiration. Electrons are transferred from NADH to complex IV via complex I, ubiquinone/ubiquinol, complex III and cytochrome *c*. Protons are pumped across the membrane by complex I, complex III (Q-cycle) and complex IV. Overall, the contribution to the proton motive force is 10 protons per electron pair.

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