Energy metabolism, oxygen flux, and iron in bacteria: The Mössbauer report

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Iron is the most common transition metal cofactor across biological systems. As the earth transitioned from an anaerobic to aerobic environment, cellular mechanisms evolved to protect against iron-mediated oxidative damage, but the molecular details of these protective strategies remain unclear. In this report, the Lindahl group has combined spectroscopic, biochemical, and genetic approaches to inventory iron in *Escherichia coli* as a function of bacterial oxygen metabolism. Their results suggest that ferrous iron functions as an oxygen sink that is modulated by a "respiratory shield" of electron flux in the bacterial plasma membrane.

The earth's ecosphere converted from reducing to oxidative ~2.5 billion years ago, the boundary between the Archean and Proterozoic eons (1). This change meant a shift in iron's redox equilibrium from soluble Fe^{II} to Fe^{III}, which is subsequently rendered insoluble by hydrolysis to $[FeO(OH)]_n$ (2). Organisms at this boundary—archaeal and bacterial anaerobes—were totally dependent on the ferrous iron pool in which they evolved. For example, the terminal electron transport pathway that fueled ATP synthesis at their plasma membranes was an enzyme cohort largely assembled using nutrient ferrous iron. In regard to energy metabolism, what distinguished nascent aerobes was a terminal dioxygen reductase coupled to this pathway, cytochrome *c* oxidase (3). Wofford *et al.* (4) provide a combination of ^{57}Fe Mössbauer and iron EPR spectroscopy along with genetic manipulation and biochemical fractionation to suggest that this terminal respiration, in addition to using the free energy derived from reduction of dioxygen, assisted also in keeping the cytoplasm microaerobic and thus "protected" the cell's essential ferrous iron. When this protection is lost through run-down of terminal respiration in the bacterial inner membrane, oxygen tension increases in the cytoplasm, oxidizing a fraction of what is generally referred to as the labile iron pool of ferrous iron (5). This autoxidation results in the accumulation of a cytoplasmic pool of ferric iron nanoparticles. The Wofford *et al.* (4) study thus tells a compelling story about evolutionary aerobiosis and provides a compelling example of the power of precise spectroscopy in cell biology research.

Previous investigations of iron speciation in cells have utilized a variety of methods, including Mössbauer spectroscopy. Mössbauer is likely not the most familiar of spectroscopies, and yet it is a remarkably straightforward method. Briefly, it is γ-photon absorption spectroscopy. In iron Mössbauer, the photon comes from the first excited nuclear state of ^{57}Fe in the source and is captured (absorbed) by the ^{57}Fe in the sample (natural abundance 2.1%) resulting in a nuclear quantum transition that can be visualized as a loss of signal (6). This transition is called an isomer shift, but can be thought of as functionally equivalent to a chemical shift in NMR, where the iron species in the sample will have a specific readout. This isomer shift, δ, is therefore informative about the redox and electronic spin state of the metal. Broadly speaking, δ increases with spin state and with the increase in *d*-electron spin density in Fe^{II} compared with Fe^{III}. Moreover, as in NMR, additional spectroscopic details can reveal the nuclear quantum state (via quadrupole splitting, Δ) or the electronic environment (via hyperfine splitting) (6). For example, quadrupole splitting occurs when the angular momentum quantum number, I, is >1/2 and is in a nonspherical electric field typical of an asymmetric ligand environment. Wofford *et al.* (4) use this splitting as an important diagnostic in their survey of cellular iron species. These elements of Mössbauer spectra are illustrated by some of their most significant data in Fig. 1*A*. Finally, Mössbauer has exceptional resolution but low sensitivity, meaning that spectral data accumulation takes hours not minutes; it is a credit to the authors that they have made the commitment to fully exploit this technique in the elucidation of cellular iron metabolism.

There are several unique take-home messages provided by the data presented by Wofford *et al.* (4). Previous work had provided evidence both for and against the storage of large amounts of iron as Fe^{III} in ferritin (*i.e.* bacterioferritin), which could be used to protect cellular iron and avoid oxygen toxicity (7). In their system, using *E. coli* without overexpression of any proteins, Wofford *et al.* observed only a small amount of iron that displayed ferritin-like features, helping to resolve this controversy. Another novel observation is that the nonheme, high-spin Fe^{II} spectral signature of iron coordinated by O and N ligands is contributed by two biochemically separable species. Importantly, when cellular respiration in the form of electron
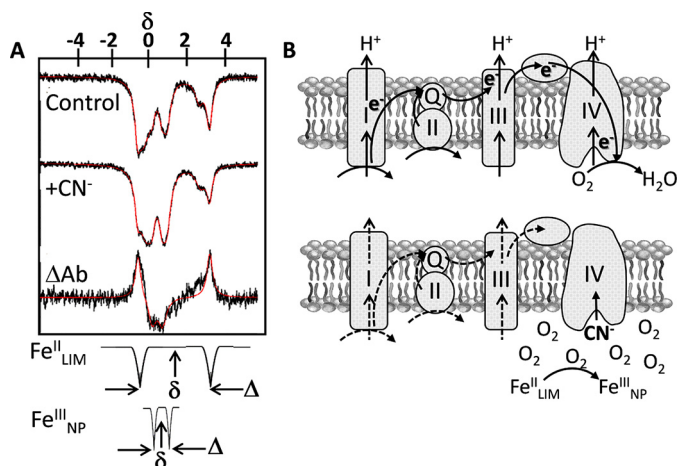
Figure 1. Using Mössbauer to track conversion of labile ferrous iron into ferric iron nanoparticles. *A*, illustrating how Wofford *et al.* use Mössbauer to catalogue cell iron species. Respiratory cyanide poisoning leads to conversion of Fe^{II} in a low-molecular-mass pool (Fe^{II}_{LIM}) into a pool of Fe^{III} nanoparticles Fe^{III}_{NP}. These two components of the experimental traces are idealized at the bottom of the panel. Note the typically larger δ for Fe^{II} compared with Fe^{III}; the relatively large quadrupolar splitting, Δ, indicates that this Fe^{II} is high-spin. The trace labeled "ΔAb" is functionally the difference spectrum between the $+CN^-$ and control spectra in which a fraction of the Fe^{II}_{LIM} in the control is replaced by an equivalent fraction of Fe^{III}_{NP}. These spectra are taken from Fig. 7 in Wofford *et al.* (4). *B*, the "respiratory shield" hypothesis illustrating how CN^- inhibition of terminal reduction of O_2 results in an increase in the cytoplasmic oxygen tension, the subsequent autoxidation of the LIM pool of ferrous iron, and the accumulation of ferric iron nanoparticles.

flux to O_2 via complex IV was inhibited by the addition of CN^-, one of these species, designated Fe^{II}_{LMM}, decreased (Fig. 1*A*). A significant fraction of this iron was converted to what the authors have characterized as magnetically ordered ferric oxyhydroxide nanoparticles, importantly differing from previous assignments of this species as ferritin iron. Based on biochemical fractionation, the authors further linked this Fe^{II}_{LMM} species to what is commonly referred to as the cellular labile iron pool (4). The demonstration that this iron pool is sensitive to autoxidation and deposited as inert iron nanoparticles is a novel finding. Their model of this respiratory shield is illustrated in Fig. 1*B*.

The Lindahl group has previously demonstrated a similar link between diminished respiratory electron flux and iron nanoparticle deposition in mitochondria (7). Their conclusion is that whether in the bacterial inner membrane or in the mitochondrial matrix, aside from providing the H^+-gradient that supports ATP synthesis, respiration maintains a microaerobic environment that stabilizes the ferrous iron required in the assembly of heme and FeS clusters. In this context, it is noteworthy that mitochondrial "anaerobiosis" also supports the assembly of an active nitrogenase, further evidence of this "respiratory shield" (8). After all, as Lynn Margulis (Sagan) pointed out over a half-century ago, mitochondria are simply repurposed bacteria (9). Wofford *et al.* (4) bring the story of evolutionary aerobiosis and the appearance of the eukaryotic cell full circle. It is a paper worth reading.

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