Chapter 1
Iron, an Element Essential to Life

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Abstract Iron plays a vital role in virtually all living organisms. This element is the second most common metal after aluminum in the earth's crust. Its abundance and the flexibility of its electronic structure made iron particularly suitable for life. Indeed, the Fe^{3+}/Fe^{2+} couple covers a wide range of redox potentials which can be finely tuned by coordinated ligands, conferring on it a key catalytic role in various fundamental metabolic pathways. However, as ferrous iron catalyzes the production of cell-damaging reactive oxygen speices $OH°$ via the Fenton reaction, excess iron or incorrect storage of this metal can be deleterious to organisms. Despite its abundance, iron is not easily bioavailable under aerobic conditions because the oxidized ferric form displays low solubility. Confronted with shortages of iron, organisms with aerobic lifestyles express specific mechanisms for its acquisition. Thus, iron is often a stake in competition between organisms of the same ecological niche and holds a peculiar position at the microbe–host interface. This chapter illustrates the importance of this metal in biological systems.

Keywords Iron biochemistry history · Iron biology history

Iron is a trace element essential to almost every living cell, in microbes, plants, and animals. Vital processes such as photosynthesis, respiration, DNA synthesis, nitrogen fixation, and detoxification of free radicals depend on the activity of iron-containing enzymes and proteins. Proteins using iron as a metal cofactor display great diversity. Enzymes containing iron play important roles in the basic biochemical mechanisms, proteins containing iron–sulfur clusters, or heme mediate redox and electron transfer reactions. Hemoglobin and leghemoglobin present in

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root nodules of nitrogen-fixing plants provide another representative class of iron proteins which bind oxygen. Why is iron involved in so many metabolic processes? The catalytic function of iron relies on its electronic structure. Its position in the middle of the elements in the first transition series implies that it can exist in various oxidation states, principally ferrous (Fe^{2+}) and ferric (Fe^{3+}). In addition, it can undergo reversible changes in its oxidation states which differ by one electron. Remarkably, its redox properties can be modified by its ligand environment and fine-tuning by well-adapted coordinated ligands makes iron-containing enzymes to display redox potentials able to cover a wide range of nearly 1 V. Owing to the flexibility of its electronic equipment, the iron atom is thought to have played a pivotal role in the history of the earliest Earth ecosystems. In this introduction, we wish to illustrate the importance of iron in biological systems by briefly depicting the evolutional path of this element through the geological times.

Iron is the fourth most abundant element in the Earth's crust after oxygen, silicon, and aluminum, making up 4.7 % of the total crust mass (Williams and Fraùsto da Silva 1999). Because of its abundance in the prebiotic world, iron is believed to have been selected as catalyst in the former energy producing chemical reactions. Interestingly, for the authors of the theory of a chemoautotrophic origin of life, who consider that the prime source of energy for carbon fixation is of chemical nature, the necessary reducing power arises from the oxidative formation of pyrite (FeS_2) from ferrous sulfide (FeS) and hydrogen sulfide (H_2S) (Wachtershaüser 1990; Huber and Wachtershaüser 1997). In this world, that is 4–2.4 billion years ago, the atmosphere was anoxic and essentially composed of nitrogen, carbon dioxide, and water (Williams and Fraùsto da Silva 1999). Iron was present in the reduced state, quite soluble, and thus available for life. But the idea that oxidation of ferrous iron was taking place naturally, in the absence of free oxygen, was a matter of controversy (Canfield et al. 2006). The discovery of ferric oxides contained in banded iron formations (BIF) dated from the Archean and Proterozoic ages (3.5–2.5 billions years ago) was in this respect of prime interest (Dietrich et al. 2006). It thus became plausible that anaerobic ferrous iron oxidation could have occurred before the evolution of oxygenic photosynthesis. Such a hypothesis was strengthened by the discovery of purple, nonsulfur, phototrophic bacteria able to oxidize Fe^{2+} to Fe^{3+} and reduce carbon dioxide to organic matter in the absence of oxygen (Widdel et al. 1993). Finding nitrate-reducing bacteria which gain energy for growth by oxidizing ferrous iron in an anaerobic manner was also considered a convincing argument (Straub et al. 2001).

With the rise of oxygen into the biosphere, around 2.3 billions years ago, there was a considerable change in the redox balance on Earth (Williams and Fraùsto da Silva 1999). It is believed that oxygenic photosynthesis evolved before the atmosphere became permanently oxygenated. One consequence was a progressive transformation in the availability of elements with certainly dramatic effects on anaerobic indigenous populations (Raymond and Segrè 2006). Very likely, sulfide and ferrous iron were the first reducing chemicals to have been removed from the ocean by dioxygen: they were oxidized to sulfate and ferric ion, the latter precipitating as ferric hydroxides. In this regard, the stalk-like morphologies

observed in contemporary lithotrophic iron-oxidizing bacteria, also identified in filamentous Fe microfossils might be important markers of the Earth's oxygen history (Chan et al. 2011). Indeed, these bacteria are present in freshwater and marine environments in which redox gradients of oxygen and ferrous iron exist. Analysis of the stalk of *Mariprofundus ferroxydans* revealed the formation of a biomineralized structure containing carboxyl-rich polysaccharides and granular iron oxyhydroxides (Singer et al. 2011). Excreted from the cell as fibrils, these structures are supposed to enhance the elimination of the ferric waste product of Fe-oxidation metabolism.

Ferric hydroxides are insoluble at pH > 4. The solubility constant Ksp of $Fe(OH)_3$ is 10^{-38}. At pH 7, Fe^{3+} is available at 10^{-17}M, which is far below the micromolar concentrations required for microbial growth (Neilands 1991). The solubility of iron decreases three orders of magnitude per pH unit. In soils, iron is present as insoluble hydrated ferric oxides commonly described as rust (Loeppert et al. 1994). Their dissolution takes place by reduction or complexation, organic components present in the rhizosphere playing a major role in these processes (Lindsay and Schwab 1982). However, about 30 % of croplands are too alkaline for optimal plant growth. Confronted with a lack of iron availability, living organisms have developed adapted mechanisms to acquire this metal from their environment. They elaborated high-affinity uptake systems, based on the expression of plasma membrane-bound ferric reductases or the production of ferric-specific chelating molecules. For instance, fungi and plants use ferric reduction (reviewed in Kornitzer 2009; Labbé et al. 2007; Morrissey and Guerinot 2009). In body fluids of vertebrates and some invertebrates, mainly worms and insects, ferric iron is bound to transferrin or transferrin-like proteins (reviewed in Gkouvatsos et al. 2011). Microorganisms and certain plants such as grasses excrete sidero-phores, which are high affinity Fe^{3+} scavenging/solubilizing small molecules that, once loaded with iron, are specifically imported into cells (Neilands 1995; Kobayashi et al. 2010; Krewulak and Vogel 2008, see Fig.1). Microbial sidero-phores vary widely in their overall structure, which accounts for the specific recognition and uptake by a given microorganism, but the iron-chelating functional groups, catechol, hydroxamate, and carboxylate, are well conserved (Budzikiewicz 2010).

In aerobic environments, iron catalyzes the single electron reduction of oxygen giving rise to oxidizing radicals, which may be very damaging to biomolecules. Iron toxicity is involved in lipid peroxidation, protein degradation, and DNA mutations. Living cells protect themselves by strictly controlling their intracellular concentration of iron, which requires the coordinated regulation of the synthesis and action of proteins involved in acquisition, utilization, and storage of the metal. When present in excess, iron is stored in a nontoxic form, in ferritins. Ferritins constitute a remarkable family of iron proteins, widespread in all domains of life. They are organized in a 24-subunit shell surrounding a central cavity, and owing to their ferroxidase activity, they oxidize excess of ferrous ions and store the ferric form in a bioavailable mineral core (Theil 2003; Briat et al. 2010; Andrews 1998). Ferritin is considered as an ancient protein which has evolved to solve the problem

of iron/oxygen chemistry and metabolism. When intracellular levels are low, the cell is able to increase its acquisition of iron by either mobilizing stored iron or utilizing external sources. The cell has also the ability to prioritize its iron utilization so that iron-containing proteins preferentially receive iron, at the same time avoiding toxic side reactions. For instance, biosynthesis of iron–sulfur proteins involves complex protein machineries that build iron–sulfur clusters from intracellular iron and sulfur and transfer them to their cognate protein acceptors (Xu and Møller 2011). Present in all organisms, these machineries protect the cellular surroundings from the potentially deleterious effects from free iron and sulfur. Numerous studies focused on diverse organisms, from bacteria to humans, point to the existence of proteins acting both as iron sensors and as regulators of gene expression for functions participating in the maintenance of iron homeostasis (Hentze et al. 2010; Kaplan and Kaplan 2009; Ivanov et al. 2012). The best studied iron-responsive transcriptional regulator in Gram-negative bacteria certainly is the ferric uptake regulator (Fur) protein which controls the expression of iron acquisition and storage systems (Hantke 2001). Fur protein acts as a dimer, each monomer containing a non-heme ferrous iron site. If the cellular iron level becomes too low, the active Fur repressor loses Fe^{2+}, its co-repressor, and is no longer able to bind to its operator sites. Interestingly, it was found that genes other than those involved in iron homeostasis can be Fur-regulated. Depending on the bacterial niche considered, Fur can regulate genes involved in pathogenicity, redox-stress resistance, or energy metabolism. However, the Fur family of bacterial iron regulatory proteins is not unique. In rhizobia for instance, new iron sensory proteins were identified that do not strictly conform to the Fur paradigm (Rudolph et al. 2006).

Mainly present as a chelated element, iron often constitutes a growth-limiting factor and the stake in an ardent competition between various members of a particular ecological niche or habitat. In the rhizosphere, siderophores released by bacteria and fungi can capture iron from natural chelates, thus depriving of iron microorganisms that produce siderophores in lower concentrations or with a lower affinity for this metal (Kloepper et al. 1980; Lemanceau and Alabouvette 1993; Haas and Defago 2005; Robin et al. 2007). Another example of iron competition resides in pathogenic microorganisms that invade vertebrate hosts. The topic of iron and infection arose when Schade and Caroline (1944), discovered the presence of transferrin in blood and ovotransferrin in egg whites, and noted that these proteins inhibited the growth of certain bacteria. Close attention has then been drawn to iron as a clue to bacterial virulence and animal host defense (Bullen 1981; Payne 1989; Litwin and Calderwood 1993; Schaible and Kaufmann 2004; Weinberg 2009). Host antimicrobial mechanisms include the well-known iron-withholding strategy and are considered as a part of the vertebrate innate immune system (Ong et al. 2006; Nairz et al. 2010). The problem of iron availability and toxicity for plant pathogens and rhizobial symbionts has been raised more recently (Expert and Gill 1991; Leong and Neilands 1982; Expert et al. 1996; Franza and Expert 2010; O'Brian and Fabiano 2010). Knowledge of strategies of iron acquisition and control of iron homeostasis in microorganisms living in close

associations with plants has progressed considerably in recent years (Barton and Abadia 2006). Much attention has also been turned to molecular mechanisms involved by plants to cope with iron deficiency. These studies have opened perspectives to further understanding the effects of iron in plant–microbe interactions. In the following chapters, we will consider the role of iron in microbial virulence, plant defense, and symbiotic legume associations with particular attention to *Erwinia*, *Rhizobium,* and related genera, the studies of which have most contributed to broaden the concept of iron modulators in plant–microbe interactions.

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