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Review

Enzymes with an heterodinuclear iron–manganese active site: Curiosity or necessity?

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ABSTRACT

This review analyzes the currently available data on true and purported FeMn enzymes with a particular emphasis on their specific physical properties. The characterization of the purple acid phosphatase from *sweet potato* and the current view of the hydrolysis mechanism are presented. The controversy associated with the discovery of the class Ic ribonucleotide reductase from *Chlamydia trachomatis* is discussed in the light of its extensive reactivity and physical studies. The amine oxygenase AurF is presented also albeit it is not exactly an FeMn enzyme but its case is particularly enlightening of the difficulties in assessing which is the right metal of an enzyme. Then, the very recent emergence of a new class of FeMn oxidases is highlighted. Lastly, examination of potential model compounds reveals the paucity of reported examples

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Amine oxygenase
Bimetallic sites
Fe–Mn center

and therefore the need to develop this area. General considerations on biologically active metals and their substitution in hydrolases and redox active proteins are provided and possible reasons for the choice of the peculiar FeMn active site over the more classical diiron center are considered.

1. Introduction

It is generally admitted that about half of all enzymes require a metal ion to function [1] and the emergence of bioinformatic techniques has allowed one to delineate the main features of the metallome [2]. In this context the first row transition metals, i.e. manganese, iron, cobalt, nickel and copper, occupy a critical position, being involved in many essential hydrolytic and/or redox enzymatic systems. In many cases this function is associated with a specific property of the metal ion, i.e. its redox potential, so its replacement by any other metal ion will lead to the loss of the function. As a consequence, delivering the right metal to the right protein is generally an issue of utmost importance [1,3]. It has been shown that the concentration of free Cu^{2+} ion is maintained at an extremely low value [4], and this is probably the case for other metals. It is now recognized that all metal ions are taken charge of by sophisticated sensor, transport and storage systems, that fulfill the requirements of the cells by regulating the availability of the necessary metals and expelling the toxic ones [3].

The duality of iron and manganese is especially interesting. Indeed both metals are of utmost biological importance and possess very similar charges and ionic radii (Table 1). As a consequence, in non-heme enzymes they have highly similar coordination environments, mainly composed of combinations of histidines and aspartates/glutamates. On the other hand, whereas very specific functions are associated with each metal, i.e. O_2 production for Mn and O_2 transport and reduction for Fe, they fulfill a few common ones. This complex duality is illustrated by comparisons of the recently described Fe- and Mn-dependent homoprotocatechuate 2,3-dioxygenases [5] and of superoxide dismutases (SOD) [6]. The former enzymes have been shown by X-ray crystallography to have identical active site structures whatever the metal involved. Moreover, it was recently shown that a reconstitution of the Fe enzyme with Mn or the reverse gave an enzyme with similar activity [5]. This unsensitivity of the catalyzed reaction to the involved metal has been assigned to the fact that whereas the overall reaction implies O_2 activation and electron transfers, the metals do not change oxidation state and operate through their acidic properties only [5].

The situation of the SOD enzymes is more complicated. Indeed, while both Fe- and Mn-SOD have been shown by X-ray crystallography to possess identical active sites, in most cases replacing Fe by Mn in a Fe-SOD (or the reverse) leads to an inactive enzyme. In fact, this substitution preserves the activity only in the so-called cambialistic SOD family, and understanding these differences has been attracting active research for many years [6]. The generally accepted mechanism states that during catalysis the metals shuttle between the oxidation states +II and +III in the superoxide reduction to peroxide and back in the oxidation of superoxide to dioxygen. Of course in this case, the overall reaction is more sensitive to the redox potentials of the metals and to the H-bond network involved in the proton transfers to the active site [6].

Table 1
Ionic radii of biologically important first row transition metals (high-spin state).

Ion	Electronic configuration	Coordinance	Ionic radii
Mn^{2+}	$3d^5$	6	0.83
Mn^{3+}	$3d^4$	6	0.645
Fe^{2+}	$3d^6$	6	0.78
Fe^{3+}	$3d^5$	6	0.645
Co^{2+}	$3d^7$	6	0.745
Co^{3+}	$3d^6$	6	0.61
Ni^{2+}	$3d^8$	6	0.69
Cu^{2+}	$3d^9$	5	0.65

This Fe/Mn duality leads to an even more complicated situation for dinuclear metal sites. Indeed, in this case apart from the two homonuclear dimetal sites, FeFe and MnMn, a third possibility exists to have an heterodimetal site, FeMn. Structural characterization of numerous diiron [7,8] and dimanganese [9,10] enzymes has shown that they use the same combinations of histidines and aspartates/glutamates to hold the two metals in close proximity (ca. 2.5–4.0 Å, depending on their oxidation states). Controversies have arisen at times concerning the true nature of certain enzyme active sites, especially some purple acid phosphatase, ribonucleotide reductase and arylamine oxygenase. For the former two enzymes, the existence of the heterodimetal site FeMn has been definitely established [11,12], while for the latter it was proposed [13] but experimental evidence gathered afterwards strongly support a diiron active site [14]. The aim of this review is to analyze the currently available data on FeMn enzymes with a particular emphasis on their specific physical properties. In addition the very few available model compounds reported so far will be presented. Finally, possible reasons for the choice of the peculiar heteronuclear active site over the more classical diiron center will be considered.

2. The FeMn purple acid phosphatase

2.1. Presentation

Purple acid phosphatases (PAP) belong to the large family of dinuclear metallohydrolases that encompasses diverse phosphatases, RNA and DNA polymerases and peptidases to mention a few [15,16]. Metallohydrolases assemble at their active sites various homometallic pairs Zn_2, Ni_2, Co_2, Fe_2, Mn_2 and Mg_2 and even a few heterometallic ones FeZn and FeMn. The PAPs' active site comprises a ferric ion associated with a divalent metal that is Fe^{II} for mammalian enzymes and Zn^{II}, Fe^{II} or Mn^{II} for those of plant origin. They owe their name to the fact that their phosphatase activity maximizes at acidic pH and that they exhibit a purple color attributed to a tyrosinate → Fe^{III} charge transfer transition. While the Fe^{III} ion is strongly bound, the M^{II} ion is more labile and can be exchanged easily in uteroferrin without a significant loss of activity [17].

2.2. Structural and physical characterization

X-ray structures of PAP enzymes of mammalian [18] and plant [19,20] origins have been reported and reveal a high degree of homology of the active site whatever the metal pair involved $Fe^{III}Fe^{II}$, $Fe^{III}Zn^{II}$ or $Fe^{III}Mn^{II}$. Most plant PAPs are homodimers with a monomer subunit of ~55 kDa. It is common that several PAP isoforms exist within an organism and sweet potato possesses three isoforms with the three types of dimetal centers $Fe^{III}Fe^{II}$, $Fe^{III}Zn^{II}$ or $Fe^{III}Mn^{II}$ [11,21,22]. The presence of $Fe^{III}Mn^{II}$ center in isoform 2 was demonstrated by Schenk et al. [11] and a similar center was reported very recently in diphosphonucleotide phosphatase/phosphodiesterase from yellow lupin [23]. Fig. 1 depicts the structure of the active site of the PAP from sweet potato isoform 2 with a FeMn pair complexed by a phosphate anion [20].