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MODELS FOR THE ACTIVE SITE STRUCTURE OF PURPLE ACID PHOSPHATASES

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ABSTRACT

This review article examines the current and most relevant models proposed for purple acid phosphatases (PAPs). It also presents a new possibility for the active site structure of mammalian PAPs based on the work that we and other researchers have done in bioinorganic chemistry.

RESUMO

Este artigo de revisão bibliográfica faz uma análise dos modelos mais relevantes, propostos, para as fosfatases ácidas púrpuras (PAPs). Apresenta também uma nova possibilidade para a estrutura do sítio ativo das PAPs de mamíferos baseada no trabalho que nós e outros pesquisadores tem realizado em química bioinorgânica.

KEYWORDS: purple acid phosphatases, model complexes, active site, bioinorganic chemistry.

INTRODUCTION

"One of the great intellectual challenges presented to Science by Nature is a proper understanding of how enzymes work". This was the introduction to a recent paper[1], in order to establish ideas about enzymes mechanisms. Quite apart from this fascinating challenge, a proper understanding of how enzymes work holds out the promise of obtaining synthetic analogues, capable of closely approach the properties of the natural biomolecules. From a complete characterization of the model compounds, and comparing their properties with the same properties of the enzymes, the scientists should be able to have more precise ideas about the active site structure of the proteins.

Purple acid phosphatases are non-heme metallobiomolecules, so-termed because their pH optimum for enzymatic activity normally lies in the rang 4.9 - 6.0, and their typical intense pink or violet coloration. These enzymes are involved in the hydrolysis of phosphoric acid esters and anhydrides, such as adenosine triphosphate, ATP and others[2].

$$RCH_2O\text{-}PO_3^{2-} + H_2O \rightarrow RCH_2OH + HPO_4^{2-}$$

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PAPs have been isolated from a variety of mammalian tissue , such as , porcine allantoic fluid (referred as uteroferrin), rat bone and spleen, human spleen, bovine spleen (referred to as bovine spleen in the text) and certain plant such as sweet potato, red kidney bean, soybean, and microbial sources. The physiological function(s) on these enzymes has yet to be established[2]. Mammalian PAPs characterized so far are monomeric diiron proteins with molecular weigh of approximately 35 kDa. The plant enzyme from the kidney bean, is in contrast a homodimeric , $[Fe^{III}Zn^{II}]_2$ proteins, of 111 kDa. The most extensively studied PAPs are uteroferrin and bovine spleen. The active site of these last two enzymes consists of a homonuclear diiron complex with two accessible oxidation states[3a] : a catalytically active pink form, $[Fe^{II}\ Fe^{III}]$, λ_{max} = 505-515nm ; $\varepsilon \approx 4000\ M^{-1}\ cm^{-1}/Fe_2$, and an enzymatically inactive purple form, $[Fe^{III}\ Fe^{III}]$, λ_{max} = 550-570 nm; $\varepsilon \approx 4000\ M^{-1}\ cm^{-1}/Fe_2$. The absorptivity of the visible band is roughly additive for successive phenolate coordinated to Fe(III) complexes[3b,c] and model complexes[3d], amounting to $\approx 2 \times 10^3\ M^{-1}cm^{-1}$ per phenolate ligand .This suggests that only one of the iron centers in the active site of uteroferrin and bovine spleen has a visible chromophore. The pink and purple colors of PAPs are due to tyrosinate-to-iron(III) charge transfer transitions [3a]. Therefore, based on the values for the extinction coefficients found in both PAPs, one can speculate about the possibility of two tyrosines residues being coordinated to only one iron center, the non-reducible site . Resonance Raman spectra of the purple and pink forms, using visible excitation, clearly demonstrated the presence of tyrosine ring modes [4]. The presence of histidine imidazole, as a probable ligand to both iron sites, has been demonstrated by NMR[5] and ENDOR[6] studies. Addition of phosphate to the pink form under anaerobic conditions, produces a phosphate complex which is gradually oxidized, in the presence of air, to the purple color[7]. The same purple complex is produced by addition of phosphate directly to the oxidized enzymes, but stronger oxidants such as hydrogen peroxide or ferricyanide, are required for conversion of the reduced pink form to the oxidized purple form in the absence of phosphate[8].

Despite a great deal of characterization of uteroferrin and bovine spleen (absorption spectra[2b,c], circular dichroism spectra[9] , HNMR[5], Mössbauer[10] and EPR[6a] spectra, resonance Raman spectra[4], EXAFS[11], magnetic susceptibility studies[2c,12] , and electrochemical studies[13]) and substantial efforts in several laboratories, crystals suitable for high-resolution X-ray diffraction studies have not been obtained for these PAPs. On the other hand, there exists information about bridging and terminal carboxylato[5b] and hydroxo[13,14] groups coordinated to the dinuclear iron center of uteroferrin and bovine spleen.

More recently, the crystal structure of the enzyme from red kidney bean was determined by the multiple isomorphous replacement method[2d], with resolution of 2.9Å. The iron ion is coordinated by Tyr-167, by the Nε of his-325, and by a monodentate carboxylate, Asp-135. The zinc ion is ligated by the Nε of His-286, the Nδ of His-323, and the amide oxygen of Asn-201. The two metal ions, in the active site structure, are bridged by the monodentate carboxylate group of Asp-164 and the metal-to-metal distance was refined to 3.1Å. Based on the observed coordination geometry, the reaction path and on spectroscopic studies, the researchers[2d] proposed three exogeneus ligands in the coordination sphere of the enzyme, so the active site structure was modeled as shown in Figure1 . The exogenous ligands have to be elucidated better by further studies and synthetic analogues should help to solve this challenge.

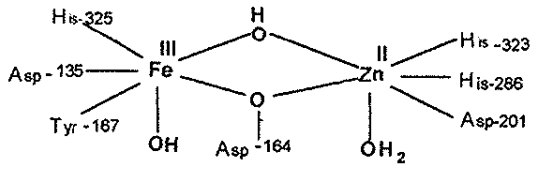

Figure 1. Proposed active site structure of purple acid phosphatases from red kidney bean[2d].

A growing number of dinuclear synthetic analogues of bovine spleen and uteroferrin , containing polypodal ligands having pyridine[15], 1-methylimidazole[16], pyridine-phenolate[17a-d], 1-methylimidazole-phenolate[17c] as pendant arms have been reported. However, most of the model complexes do not exhibit neither the characteristic spectral nor electrochemical properties, $\varepsilon^{o'} = 0.367$ V vs NHE at pH = 5.0 for the redox couple Fe_2^{III} - Fe^{II} Fe^{III}, found in uteroferrin[13]. The coordination environment of the dinuclear iron moieties of PAPs are not yet defined although, the presence of some ligands has already been unequivocally established.

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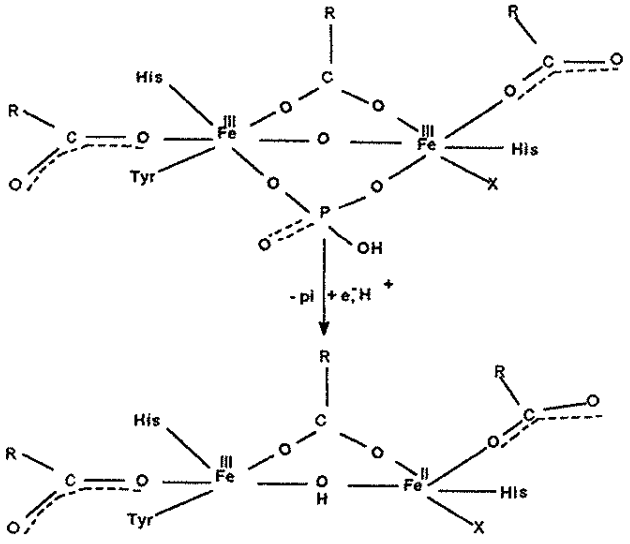
- Proposals based on enzymatic properties

Based on the properties of Bovine Spleen, B.A . Averill and coworkers[2c] proposed a model for the active site structure of mammalian purple acid phosphatases illustrated in Figure 2.



pi = Inorganic phosphate; His = histidine; Tyr = tyrosinate; X= ?

Figure 2. Active site structure of mammalian PAPs proposed by B.A .Averill and coworkers[2c]

The hypothesis of a μ-oxo bridging group was mainly based on the results from SQUID magnetization measurements[2c], J≈ - 150 cm^{-1}. However, neither EXAFS[11] nor resonance Raman[4] and electrochemical studies[13] have shown any evidence for such a bridge. In addition, a recent magnetic susceptibility study[12] of the oxidized form of bovine spleen reveals an antiferromagnetic coupling constant much smaller, J = -15 cm^{-1}, that indicates the lack of a μ-oxo bridge in the dinuclear iron center of the enzyme. More recently, on the basis of the pH dependence of the activity of the EPR spectra and the visible spectral shifts of the phosphate-saturated reduced and oxidized forms of bovine spleen, a dinuclear iron site bridged by carboxylato and hydroxo groups, was proposed by H. Witzel and collaborators[14].

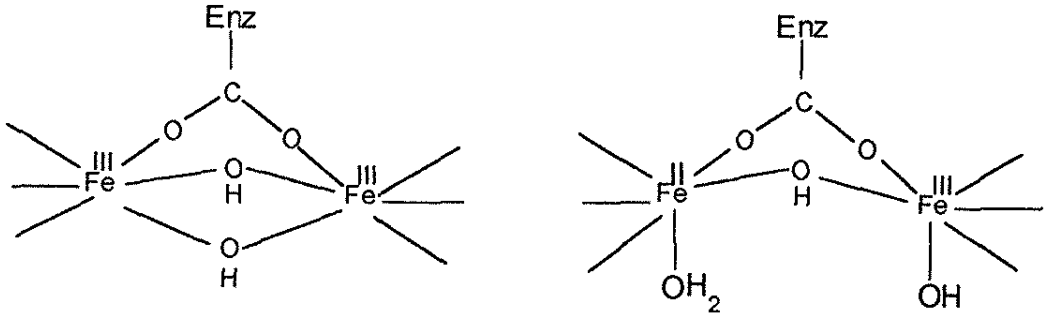


Figure 3. Active site structure of mammalian PAPs proposed by H. Witzel and collaborators[14].

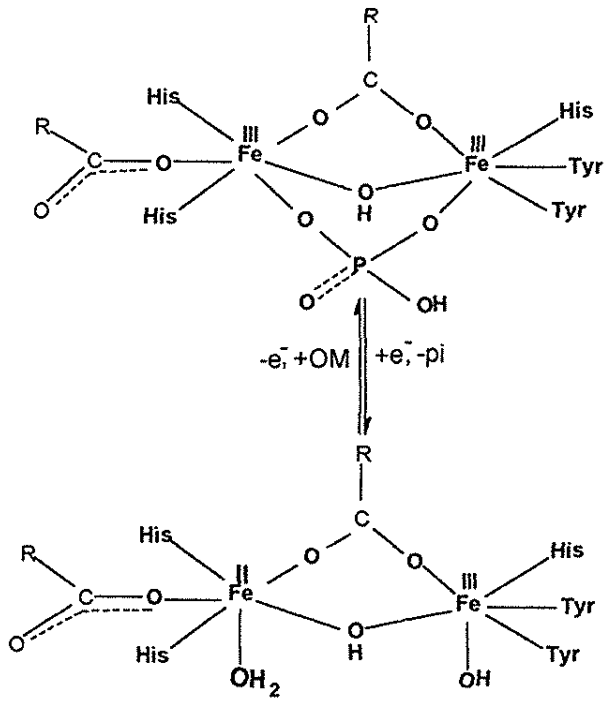
- Proposal based on bioinorganic chemistry approach

The model, illustrated in Figure 3, fails to show the other ligands coordinated to the active site of the enzyme. One can imagine the degree of difficulties that arise when one tries to propose an active site for the enzyme based only on properties. On the other hand, if we take in account the extinction coefficient of bovine spleen and uteroferrin[3a,15b], its is possible to predict[3b-d] the presence of at least one tyrosinate group, as a terminal ligand, in the dinuclear iron center of these PAPs. Taking this into consideration, we synthesized and fully characterized a new complex, $[Fe_2^{III}(BBPMP)(CH_3COO)_2]ClO_4 . H_2O$[17a], where BBPMP is the anion of 2,6-bis [(2-hydroxybenzyl)(2-pyridylmethyl)aminomethyl]-4- methylphenol as a new ligand to obtain synthetic analogues for mammalian PAPs. In the dinucleating ligand, phenolates and pyridines were employed to simulate tyrosinates and histidines respectively, in the enzymes. However, the complex is blue, λ_{max} = 601 nm; ε = 7700 M^{-1}/Fe_2, and the redox potential, $\varepsilon^{o'}$ = -0.71 V vs NHE for the redox couple Fe_2^{III} - Fe^{II} Fe^{III}, is shifted to a more cathodic potential when compared to the value reported for uteroferrin[13]. Fortunately, we realized that it is possible to adjust the chromophore of a precursor complex in order to obtain a suitable model complex for this property in the protein[18,19] .We recently reported a new unsymetrical N_5O_2-donor dinucleating ligand, 2-[((2-pyridylmethyl)amino)methyl]-6-[((2-hydroxybenzyl)(2-pyridylmethyl)amino)methyl]-4-methylphenol -(H_2BPBPMP) - and its first Fe^{II} Fe^{III} complex, $\varepsilon^{o'}$ = 0.38 V vs NHE for the redox couple Fe_2^{III}- Fe^{II} Fe^{III}, as a suitable synthetic analogue that mimics the redox potential of the enzyme. Based on the value found for

the redox potential of the synthetic analogue and of uteroferrin[13] we proposed a ratio of 2:3 for the number of tyrosines and histidines coordinated to the active site of the protein[18,20]. Interestingly, circular dichroism studies[9] also suggest that two tyrosines residues are coordinated to the dinuclear iron center of uteroferrin.

In contrast to the mammalian PAPs, the plant enzymes are insensitive to mild reductants such as ascorbate or dithioerythritol, and no change in the electronic absorption spectrum of oxidized and reduced species is observed[2a,c]. The kidney bean PAP exhibits a visible absorption spectrum, λ_{max}= 560 nm; $\varepsilon \approx 3400$ $M^{-1}cm^{-1}$/dimer. When compared to the chromophoric properties of uteroferrin and bovine spleen this indicates that the reducible site of mammalian PAPs should be similar to the Zn^{II} site of the plant enzyme . However, by the same comparison, this also suggests that the non-reducible site of uteroferrin and bovine spleen must be different from that found [2d] in the enzyme from the red kidney bean. Therefore, based on the active site structure of the kidney bean enzyme and its properties when compared to the corresponding properties of mammalian PAPs and synthetic analogues[18,20] for uteroferrin and bovine spleen it is very reasonable to think about two tyrosines and three histidines residues coordinated to the dinuclear iron site of mammalian purple acid phosphatases. Very recently, we described the synthesis and characterization of a new model complex suitable to mimic the chomophoric properties of the oxidized purple form of mammalian PAPs coordinated to phosphate[21]. The synthetic analogue, $[Fe_2^{III}(BBPMP)(\mu\text{-}OH)(\mu\text{-}O_2P(OPh)_2)].ClO_4$, λ_{max} = 560 nm; ε = 4480 $M^{-1}cm^{-1}$ /Fe_2, presents bridging hydroxo and diphenyl phosphate groups. Taking this into consideration, we can ascribe active site function of mammalian PAPs according to the following ideas: when the organism needs phosphate one of the Fe^{III} site of the oxidized purple form of the enzyme coordinated to this metabolite is reduced by a biological reducing agent, like NADH or NADPH, and the generated pink form free from phosphate is able again to catalyze the hydrolyze of orthophosphate monoesters[2a-c]. Thus, based on a bioinorganic chemistry approach, we present a new possibility for the active site structure of bovine spleen and uteroferrin and the way they could work (Figure 4).

Among the persisting problems which circumvent understanding of the binuclear iron center of uteroferrin and the purple acid phosphatases are the identity of the ligands of each iron atom and how these change in nature or arrangement during redox chemistry[2b]. In this review article we proposed a new possibility to model the dinuclear iron center of mammalian purple acid phosphatases based on their properties when compared to the same properties of some well characterized synthetic analogues and also based on the crystal structure of the enzyme from red kidney bean[2d]. Much more work has to be done in order to solve the challenges of understanding better the structure, the mechanism and the physiological role(s) of PAPs. Enzyme mimics remains an area of definite promise and bioinorganic chemists should be inspired and prepare new synthetic analogues to model the active site structure of purple acid phosphatases.



pi= inorganic phosphate; His= histidine; Tyr= tyrosinate; OM= orthophosphate monoesters.

Figure 4. New proposed active site structure of mammalian PAPs .

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