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Review

Purple acid phosphatase: A journey into the function and mechanism of a colorful enzyme

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ABSTRACT

Purple acid phosphatases (PAPs) catalyze the hydrolysis of a wide range of phosphomonoester and amide substrates. These enzymes have been identified and characterized from numerous plant and animal sources, and it is likely that a limited number of bacterial organisms also utilize this catalyst. The biological roles of this enzyme are diverse, including bone resorption, microbial killing and possibly iron transport in animals, and phosphate acquisition in plants. While animal and plant PAPs share less than 20% amino acid sequence identity and differ (with a couple of exceptions) greatly in size (35 kDa *vs.* 55 kDa per monomer) and oligomeric structure (monomer *vs.* homodimer), their catalytically relevant active sites are highly conserved, with seven invariant amino acid side chains coordinating an Fe^{3+} and an M^{2+} (M = Fe or Zn, Mn in animal or plant PAPs, respectively). Recent functional studies have indicated that PAPs are rather flexible in terms of the precise mechanistic strategy they may employ. Here, we review advances that have facilitated detailed insight into how these enzymes operate. The knowledge gained is not only of interest for coordination chemists and biochemists who focus on the physicochemical and mechanistic properties of the active site metal ion center in a metalloenzyme, but also for medicinal chemists who aim to exploit PAP as a target for the development of novel chemotherapeutics to treat osteoporosis.

1. Introduction

Purple acid phosphatases (PAPs) belong to the family of dinuclear metallohydrolases, members of which also include Ser/Thr protein phosphatases, the 3′-5′-exonuclease (*i.e.* proof reading activity) of DNA polymerases, arginase, aminopeptidases and urease [1–3]. Common to all these enzymes is that they require two closely spaced metal ions forming a dinuclear center to carry out a hydrolytic reaction, and, consequently, they employ variants of a common mechanistic scheme to carry out their respective functions. PAPs are among the most studied representatives of this large enzyme family and, due to their specific function in bone metabolism, they have become a lucrative target for the development of novel chemotherapeutics to treat osteoporosis and related bone ailments [2,4]. In this review we focus predominantly on the structure of the dinuclear active site and its contribution

Abbreviations: ATP, adenosine triphosphate; EPR, electron paramagnetic resonance; PAP, purple acid phosphatase; ROS, reactive oxygen species; TRAcP, TRAP, tartrate-resistant acid phosphatase.

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to catalysis. A discussion on the biological function and potential application of PAP is also given as an illustration of how the combined endeavors of coordination, biological and medicinal chemists can lead to the development of new strategies to combat a debilitating human disease.

2. Biological roles and medicinal significance

PAPs have been implicated in a number of biological functions. The physiological roles mammalian PAPs have been associated with are (i) iron transport [5], (ii) the generation of reactive oxygen species (ROS) as an immune response [6] and, foremost, (iii) increased bone resorption [4,7]. The role of PAP as an iron transporter does not require enzymatic activity, and appears to be relevant only in pigs, where a sharp peak of PAP expression levels in the uterine fluid of a pregnant sow was observed approximately two months after the onset of pregnancy [5]. Mammalian PAPs are bifunctional, catalyzing both hydrolytic reactions and peroxidations [6,8–10]. The latter are facilitated by the redox-active iron in the dinuclear active site, and the mechanism is believed to be based on Haber–Weiss–Fenton-type reactions:

$$Fe^{3+}\text{–}Fe^{2+} + H_2O_2 \rightarrow Fe^{3+}\text{–}Fe^{3+} + OH^{\bullet} + OH^{-} \quad (1)$$

$$Fe^{3+}\text{–}Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{3+}\text{–}Fe^{2+} + O_2 \quad (2)$$

Mammalian PAPs are abundantly expressed in osteoclasts, activated macrophages and dendritic cells [11–13]. The high expression levels of PAP in macrophages led to the suggestion that this enzyme may play an important role in the immune defense system, producing, *via* the continuous oxidation and reduction of the di-iron center, both hydroxyl and superoxide radicals [10]. In support of this proposal, it could be shown *in vivo* that the overexpression of PAP in a macrophage-like cell line leads to increased ROS production and an enhanced capacity of bacterial killing [10]. Both, increased PAP and ROS levels, are also observed at the resorptive interface formed between osteoclasts and bone, where they are required for active resorption [14,15]. In osteoclasts the phosphatase activity of PAP also plays a crucial role in bone metabolism by dephosphorylating bone matrix proteins such as osteopontin [7]. Osteopontin is believed to be involved in the adhesion of osteoclasts to the bone surface, and upon its dephosphorylation is no longer capable of supporting adhesion [7]. Thus, PAP may be important for efficient bone resorption by enabling the migration of osteoclasts to new resorptive sites. In support of this hypothesis transgenic mice, in which osteoclasts overexpress and secrete PAP into the bone-resorptive space, display increased levels of resorption with the concomitant symptoms of mild osteoporosis [16], while the PAP knock-out transgenic animals reveal osteopetrotic symptoms [17]. Consequently, PAP has become a target for the development of anti-osteoporotic chemotherapeutics [18–24]. Finally, since PAP is also an efficient ATPase it has been speculated that this enzyme also plays a crucial role in the ATP-dependent regulation of bone calcium homeostasis [25].

Due to its bifunctionality (hydrolysis and peroxidation), a variety of biological roles have also been proposed for plant PAPs. Assignments of specific functions have been rendered difficult due to the occurrence of multiple isoforms [26–35]. Also, the metal ion composition of plant PAPs is more diverse than that of their mammalian counterparts. While the latter have redox-active di-iron centers [36,37], the majority of plant PAPs have Fe^{3+}–Zn^{2+} or Fe^{3+}–Mn^{2+} centers [38–40]. A di-iron center has however been reported for an *in vitro* expressed isoform of sweet potato PAP [41]. Since evidence has accumulated that indicates that at least some of the various isoforms of plant PAP may be secreted it has been speculated that a major function of these enzymes is the mobilization/scavenging of inorganic phosphate from organophosphates in the soil [27–29,34,42].

Phosphorus is essential for both energy metabolism and the biosynthesis of a variety of biomolecules, and phosphorus deficiency in plants has been implicated in stunted growth [43,44]. The observed up-regulation of PAP expression during phosphate-starved growth conditions for tomato [27,28], *Arabidopsis thaliana* (AtPAP17 and AtPAP26) [31,33], *Medicago truncatula* [45], duckweek [46], white lupin [47], rice [48] and potato (StPAP2 and StPAP3) [30], and the broad range of substrates utilized by PAP support a role for this enzyme in phosphate metabolism [27,28,38,39,49]. One of the isoforms of soybean PAP (GmPhy) also hydrolyzes phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate), a major compound for storage of phosphorus in plant seeds [50]. In addition to histidine acid phosphatases and β-propeller phytases, some PAP isoforms form a third distinct group of phytases [51]. GmPhy is the only PAP with phytase activity that has been characterized to date, but the high degree of sequence homology between this enzyme and various PAPs including one from rice and *M. truncatula*, and several isoforms from *A. thaliana* (AtPAP13, AtPAP15, AtPAP23) indicates that PAPs with phytase activity are common amongst plants [52].

It could also be shown that phosphate-starved tomato expresses and secretes two unusual monomeric PAPs (84 kDa and 57 kDa, respectively) which hydrolyze a broad range of substrates (*e.g.* pyrophosphate, ATP and phosphoenolpyruvate) but also display peroxidase activity [27]. It is not yet clear what specific purpose the peroxidase activity of these extracellular tomato PAPs may serve. An intracellular, heterodimeric tomato PAP [28] and an *A. thaliana* PAP isoform (AtPAP17) [31] are also expressed in response to phosphate starvation, and like the secreted tomato enzymes have both hydrolytic and peroxidase activities. Similar to experiments with mammalian PAPs, an increase of the extracellular concentration of ROS is observed in plants in response to a pathogen attack [53]. It is thus possible that extracellular PAPs in plants have dual functions, including phosphate acquisition during phosphate-starved growth conditions and microbe killing during pathogen attack. However, a correlation between pathogen-induced ROS production and enhanced PAP expression levels still needs to be established. In contrast, intracellular PAPs with peroxidase activity may play an important role in respiratory electron transport and O_2 uptake during biotic and abiotic stresses [28]. It has also been speculated that PAP-generated ROS are up-regulated during senescence, salt stress as well as abscisic acid response [31,54,55]. Furthermore, since the *A. thaliana* PAP isoform AtPAP26 is induced by oxidative stress it has been speculated that this enzyme is associated with intracellular ROS production mainly during cellular degenerative processes and autolysis [33].

3. General enzymatic properties

Apart from the requirement for two closely spaced metal ions in their active sites [1,2,4,56,57], a feature common to all members of the family of metallohydrolases, PAPs are characterized by several distinct features, including (i) glycosylation (between 5% and 10% of their molecular mass is due to their carbohydrate content [38,39,58–60]), (ii) their characteristic purple color due to a charge-transfer transition from a conserved tyrosine ligand to a ferric ion in the active site (λ_{max} = 510–560 nm; ε = ~3000–4000 M^{-1} cm^{-1}; discussed in more detail below) [61–63], and (iii) their resistance towards inhibition by L-tartrate [64]. Thus, PAPs are frequently referred to as tartrate-resistant acid phosphatases (TRAcPs or TRAPs).

PAPs have been identified in, and extracted from various plant, animal and fungal sources and are likely to occur in only a limited