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Structural relationship between the mammalian Fe(III)–Fe(II) and the Fe(III)–Zn(II) plant purple acid phosphatases

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Abstract The primary structure of uteroferrin (Uf), a 35 kDa monomeric mammalian purple acid phosphatase (PAP) containing a Fe(III)–Fe(II) center, has been compared with the sequence of the homodimeric 111 kDa Fe(III)–Zn(II) kidney bean purple acid phosphatase (KBPAP). The alignment suggests that the amino acid residues ligating the dimetal center are identical in Uf and KBPAP, although the geometry of the coordination sphere might slightly differ. Secondary structure predictions indicate that Uf contains two $\beta\alpha\beta\alpha\beta$ motifs thus resembling the folding topology of the plant enzyme. Guided by the recently determined X-ray structure of KBPAP a tentative model for the mammalian PAP can be constructed.

Key words: Purple acid phosphatases; Sequence alignment; Structure prediction; Ancestor enzyme; Oxygen activation

1. Introduction

Purple acid phosphatases (PAPs) catalyse the hydrolysis of activated phosphoric acid esters and anhydrides in the pH range from 4 to 7. They have in common a dinuclear metal center with a tyrosine→Fe(III) charge transfer transition responsible for their characteristic purple colour and differ from other acid phosphatases in their insensitivity to tartrate inhibition (for review see [1–3]).

PAPs obtained from mammalian sources, including porcine uterine fluid (uteroferrin, Uf), bovine spleen, macrophages, and osteoclasts are monomeric Fe(III)–Fe(II) proteins of approximately 35 kDa. In addition to the hydrolytic function a role in the activation of dioxygen has been discussed for the mammalian PAPs [4,5]. An X-ray structure of one of the mammalian PAPs is not yet available. NMR studies reveal the ligation of one tyrosine and one $N\varepsilon$-coordinated histidine to iron(III) and the ligation of one N_δ-coordinated histidine to iron(II) in uteroferrin and the bovine spleen enzyme [6]. Definitive information about the remaining protein ligands could not be obtained, although further paramagnetically shifted proton signals indicate the presence of two carboxymethylene groups, besides some unassignable signals [7]. EPR [8–10], Mössbauer [11], and magnetic properties [9,12] of mammalian PAPs implicate the presence of a μ-hydroxo and/or a μ-carboxylato group bridging both metals. Other diiron proteins, like hemerythrin, ribonucleotide reductase, and methane monooxygenase, involved in transportation and activation of dioxygen, share similar core units, as revealed by their X-ray structures [13–15]. An antiparallel four-helix-bundle is a common tertiary structure motif for these diiron proteins.

The intensively studied plant purple acid phosphatase from kidney bean (KBPAP) is a homodimeric Fe(III)–Zn(II) enzyme of 111 kDa [4,16–18]. Compared to the mammalian PAPs the kidney bean enzyme shows a very similar substrate specificity and a nearly identical pH-optimum. Furthermore, in the kidney bean enzyme Zn(II) can be exchanged for Fe(II) [16] as well as in the mammalian enzymes Fe(II) can be exchanged for Zn(II) without affecting the activity [17]. The similarity between the Fe(III)–Fe(II) kidney bean enzyme and the native Fe(III)–Fe(II) mammalian PAPs regarding the VIS, EPR, and Mössbauer data [4,16,18] and regarding the interaction with substrates and oxoanions inhibiting the phosphatase reaction indicates a high degree of conformity in the active site.

The recently determined X-ray structure of KBPAP [19] revealed the geometry of the catalytic Fe(III)–Zn(II) center. A schematic view is shown in Fig. 1. Fe(III) is coordinated by Tyr^{167}, by the N_ε of His^{325}, and by a monodentate carboxylate, Asp^{135}. Zn(II) is ligated by the N_ε of His^{286}, the N_δ of His^{323} and the carboxamide oxygen of Asn^{201}. The two metal ions are bridged by the monodentate carboxylate group of Asp^{164}. In agreement with kinetic and spectroscopic data a μ-hydroxo bridge and two terminal solvent molecules, an aqua ligand at Zn(II) and a hydroxo ligand at Fe(III), were modeled to complete the coordination sphere of the Fe(III)–Zn(II) center. Thus, the ligands revealed by ^{1}H NMR for the mammalian PAPs [6] are also present in the Fe(III)–Zn(II) plant enzyme. Some differences in the redox behaviour and in the visible spectra, however, indicate that at least the geometry of the active sites cannot be exactly identical.

No similarity in the amino acid composition and only very low homology in the sequence (20% identity) could be observed between the kidney bean enyzme and the mammalian proteins. Thus the nature of the protein ligands completing the coordination sphere of the diiron core in the mammalian enzymes remains unclear. Also the question, whether the 35 kDa mammalian PAPs are structurally related to the dimeric 111 kDa plant enyzme or exibit a tertiary structure similar to those of the other diiron proteins remains open. According to the CD data and an average secondary structure prediction the mammalian enzymes belong to the α/β-type structures with alternating α helices and β strands [20] as also found for the kidney bean structure. In this report we present an alignment of the sequences of Uf and KBPAP that suggests that both enzymes

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Abbreviations: Uf, uteroferrin; KBPAP, kidney bean purple acid phosphatase; PAP, purple acid phosphatase.

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exhibit significant structural homology in the protein fold as well as a similar active site structure. Since Uf and the other mammalian PAPs show over 90% homology [21–25] our conclusions hold for all mammalian PAPs characterized so far.

2. Materials and methods

The sequence of Uf (ProteinBank accession number P09889) [21,22] was aligned to the sequence of KBPAP (accession number P80366) [26] using the program Bestfit of the Husar package and the alignment was subsequently refined by eye. Based on the primary structure the secondary structure of Uf was analysed by the joint prediction method [27]. Using the Husar program package the calculations were carried out via the program Predict combining the results of the Alb, Simpa, and Gor methods [28–30].

Guided by the three-dimensional structure of KBPAP a model for the catalytic domain (residues 1–226) of Uf was constructed. Model building was performed with the program O [31]. The side chains of KBPAP were replaced by the corresponding side chains as derived from the sequence alignment to Uf. The positions of the Cα-atoms of inserted residues and those of Cα-atoms around deleted residues, as well as the corresponding side chain rotamers were calculated using the O database [32]. The model could be built without any steric hindrance. No subsequent energy minimization was performed on the model. The additional loop (residues 147–161) and the elongation of helix α1 (residues 22–35) were both omitted in the model of Uf, because of missing corresponding features in the reference structure of KBPAP.

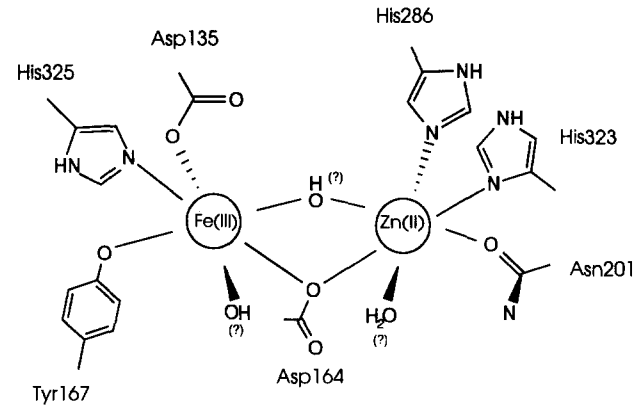

Fig. 1. Schematic view of the dimetal site of KBPAP according to the X-ray structure [19]. Fe(III) is coordinated by one tyrosine, one histidine, one aspartic acid, and one terminal hydroxo group, whereas Zn(II) is ligated from one asparagine, two histidines, and one terminal aqua ligand. Both metals are bridged by a μ-hydroxo group and a monodentate apartic acid. X-Ray studies could not unambiguously detect the three exogenous solvent molecules. In agreement with spectroscopic and kinetic data they have been modeled in order to complete the coordination sphere of the Fe(III)–Zn(II) center and are here marked with question marks.

3. Results

In spite of only very low homology between the kidney bean enzyme and uteroferrin as a member of the mammalian PAPs the sequences of both enzymes could be aligned as shown in Fig. 2. The alignment displays blocks of significant sequence homology, especially in the region of the metal-coordinating residues of KBPAP (encircled in Fig. 2). With this alignment, the residue Asp^{135} of KBPAP corresponds to Asp^{14} in Uf located in the sequence motif Gly-Asp-X-Gly. Asp^{164} and Tyr^{167} of KBPAP align with Asp^{52} and Tyr^{55} of Uf found in the motif Gly-Asp-X-X-Tyr. The residue Asn^{201} corresponds to Asn^{91} in a homologous block of four residues Ala-Gly-Asn-His. His^{286} is conserved in the motif Val-X-X-His-X-Pro, corresponding to His^{184} in Uf. His^{323} and His^{325} align with His^{211} and His^{213} of Uf located in the motif Gly-His-X-His.

Thus the coordination sphere of the diiron center in Uf might be described as follows: Fe(III) is coordinated by Tyr^{55}, by the N_ε of His^{221}, and by the carboxylate group of Asp^{14}. The Fe(II) ion is ligated by the N_ε of His^{184}, the N_δ of His^{219}, and by Asn^{91}. The two metal ions are bridged by the carboxylate group of Asp^{52}. In the kidney bean structure residues His^{202} and His^{295}, which are close to the dimetal center and proposed to interact with the substrate [19], can be aligned in Uf with His^{92} and His^{193}. His^{296}, a third histidine in the dimetal surrounding of KBPAP, is substituted by a glycine in the mammalian enzyme.

The structure of the dimeric KBPAP consists predominately of β sheets (35% β strand, 14% α helix) [19]. Each monomer consists of two domains (Fig. 3A). The N-terminal domain of approximately 120 amino acid residues is composed of two sandwiched β sheets, containing strands β1 to β7. The function of the N-terminal domain in the kidney bean enzyme remains unclear. According to the alignment in Fig. 2 this domain is absent in the mammalian enzymes. The catalytic C-terminal domain of KBPAP can be classified as an α/β-type structure with the active site located at the carboxy-end of two sandwiched βαβαβ-motifs. The first βαβαβ-motif (residues 126–198) comprises the parallel strands β8 to β10 connected by the helices α1 and α2, whereas the second βαβαβ-motif (residues 245–322) comprises the parallel strands β12 to β14 connected by the helices α4 and α5. The two sandwiched motifs, each comprising three parallel β strands and two α helices, are connected by the short helix α3 and β strand β11. All ligands to the dimetal core are contributed from the loops at the carboxy-ends of five of the six strands in these two motifs (β8 to β10, β13 to β14).

Comparing the predicted secondary structure of Uf to the secondary structure of KBPAP, as revealed by the X-ray structure [19], a similar folding topology is evident (Fig. 2). According to the secondary structure analysis the two βαβαβ-motifs of KBPAP are also present in the mammalian enzyme. In analogy to the kidney bean enzyme the first β sheet may be built up from strands β1 to β3, with the tentative parallel strands connected by the α helices α1 and α2. Obviously helix α1 in Uf is longer than the corresponding helix in the KBPAP structure thus causing a gap in the alignment, whereas helix α2 is shorter. Nevertheless the parallel β strands of the first sheet (β1 to β3) are of similar length as the cooresponding strands in the KBPAP structure and may be arranged like in the kidney bean enzyme. The secondary structure prediction for Uf suggests that the second βαβαβ-motif building the catalytic domain of the kidney bean enzyme is also present in the mammalian enzyme. It is formed by the strands β5 to β7 and by the helices α4 and α5. The loop between strand β4 and helix α4 is predicted to be longer than the corresponding loop in KBPAP, causing a gap of 15 residues lost in the kidney bean enzyme.

Based on these considerations a three-dimensional model of the active site containing domain of Uf could be constructed guided by the X-ray structure of the kidney bean enzyme (Fig. 3B). It is noteworthy that in the mammalian enzymes residue Arg^{155} is a well-accessible site for proteolytic cleavage [33]. Therefore it has been proposed to be located in an exposed loop

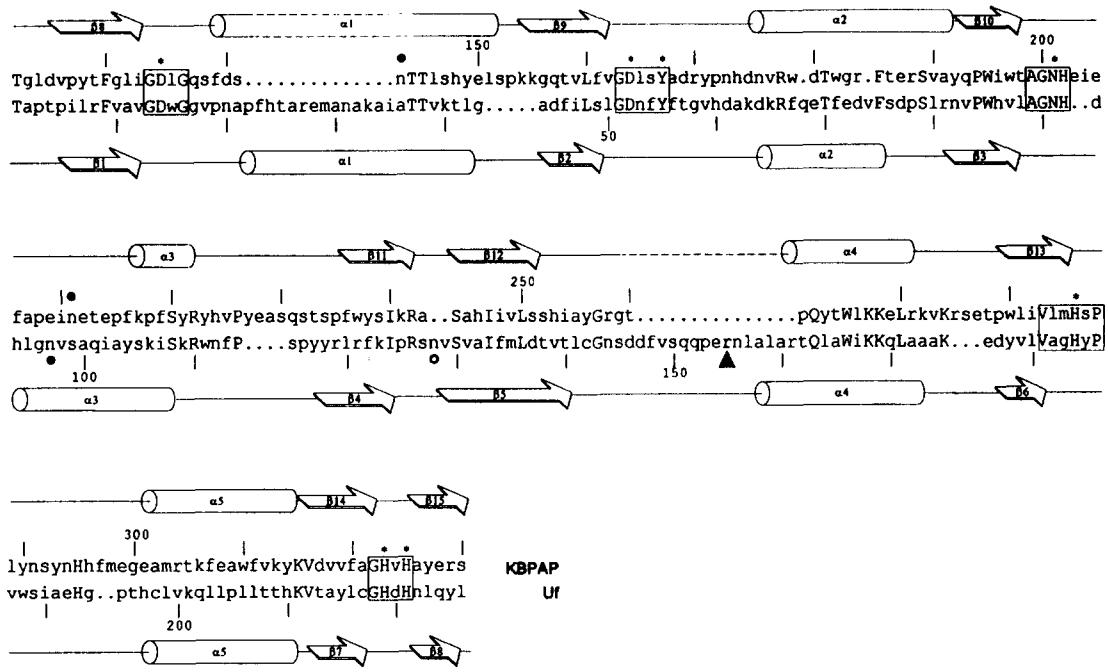

Fig. 2. Alignment of the sequence of Uf (residues 1–226) and that of KBPAP (residues 122–330) generated by the program BestFit of the Husar program package and subsequent refinement by eye. Conserved residues are printed in capital letters. The conserved metal ligands are indicated by asterisks. All of them are also present in the other mammalian PAPs. The secondary structure of KBPAP is derived from the crystallographic results [19] and that of Uf is based on the prediction calculated by the joint prediction method [27]. Horizontal columns and arrows indicate α helices and β strands, respectively. The triangle at Arg^{155} of Uf shows the site cleaved by limited proteolysis in the mammalian PAPs [33]. Circles indicate glycosylation sites for both enzymes [34,35]. The open circle at Asn^{128} reveals that this site is glycosylated in bovine spleen PAP [33] and also in in vitro secreted Uf, but not in Uf secreted by pig uterus [34]. Residues 227 to 311 of Uf and 331 to 432 of KBPAP have been omitted from the alignment. These regions show nearly no homology in the sequence and obviously exhibit a different folding topology as suggested by secondary structure predictions.

in agreement with its location in the structural model presented here. According to this prospective model for Uf the glycosylation site Asn^{97} of Uf occupies a position similar to that of glycosylated Asn^{211} of KBPAP, shifted only by two residues, and is located on the surface of the enzyme. A second glycosylation site Asn^{128} is present in bovine spleen PAP [33] and in Uf secreted in vitro [34]. In the model for Uf this residue is located in a small turn connecting the strands β4 and β5. Whereas in the kidney bean enzyme the corresponding site is shielded by the N-terminal domain, Asn^{128} is accessible in the modeled structure of Uf. Cys^{142} and Cys^{198} are neighboured in the three-dimensional Uf model with the sulphur atoms about 5 Å apart and might form an intramolecular disulfide bridge. This is in agreement with the observation that rat spleen PAP cleaved at the arginine residue in the exposed loop is separated into two fragments only after subsequent reduction with β-mercaptoethanol [25,38].

4. Discussion

At first sight the different molecular masses, the amino acid compositions and sequences, as well as the evolutionary distance between the mammalian and the plant enzyme suggest no relationship between these enzymes. However, the kinetics of the phosphatase reaction and a number of spectroscopic data, especially the Mössbauer results [4], implicate that the active center may be analogously constructed. Since the N-terminal domain of KBPAP does not contain amino acids ligated to the dimetal site and is probably not involved in the catalytic process it seems reasonable that this domain is absent in the smaller mammalian enzymes.

Indeed starting with amino acid 122 of KBPAP and aligning the following sequence to that of uteroferrin, we find blocks of four to six residues with a high degree of homology, containing all metal ligating residues of KBPAP. This result is striking as not only the succession of the blocks is identical, but also the distance of ligating residues within these blocks is conserved, for example Asp^{164}/Tyr^{167} or His^{323}/His^{325} in KBPAP and Asp^{52}/Tyr^{55} or His^{219}/His^{221} in Uf. Furthermore secondary structure predictions of Uf indicate that the mammalian enzymes contain two $\beta\alpha\beta\alpha\beta$-motifs thus resembling KBPAP. It seems unlikely that the domains involved in catalysis in both enzymes are constructed in such a similarity just by chance. We are convinced that they are derived from an ancestor enzyme, either containing Zn(II) or Fe(II), and that despite of the low homology in the sequence of KBPAP and Uf the structure of the catalytic domain has been conserved.

As long as we do not know more about the physiological function of mammalian and plant PAPs, it is difficult to speculate about the function of the ancestor enzyme. It is difficult to imagine that these enzymes with high ATPase and tyrosine-phosphatase activity are involved in metabolic processes in the cytosole. More probable is a physiological role in certain compartments and there maybe with a completely different function.

In macrophages PAP is expressed only after phagocytosis

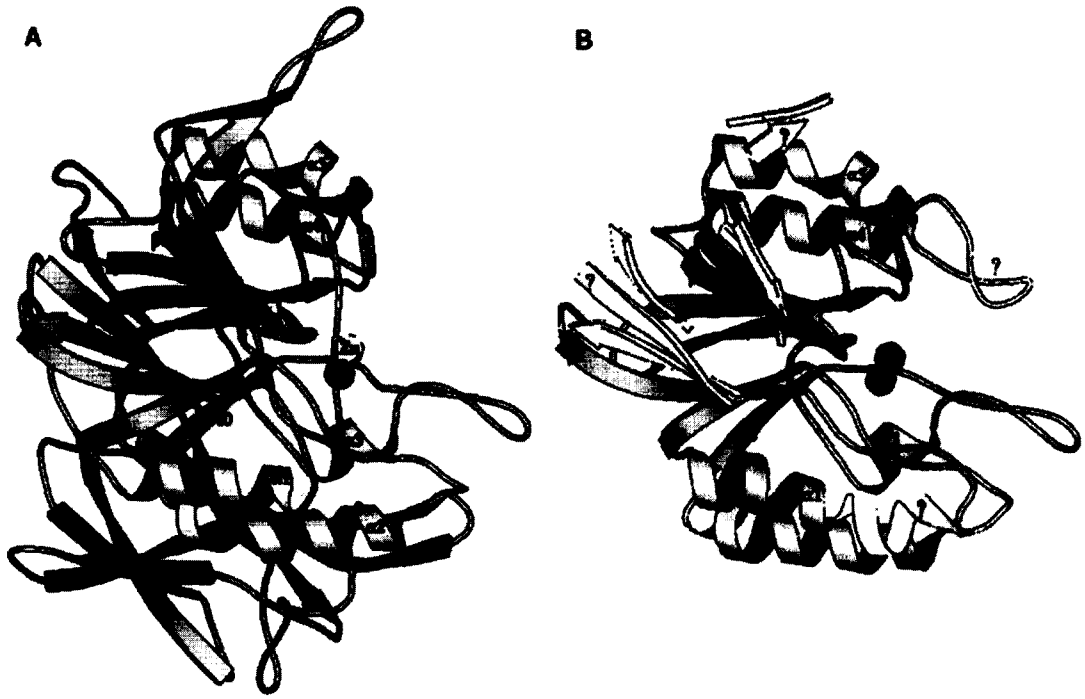

Fig. 3. (A) Ribbon diagram of a KBPAP monomer prepared by Molscript [35]. The loop at the top contains the cysteine residue forming the disulfide bridge to the other subunit. Additional interactions between both subunits occur between the helices α5 and between the loops from residues 253 to 260. The N-terminal domain is located at the bottom of the figure. Labeling is according to Fig. 2. (B) Ribbon diagram for the tentative dimetal binding domain of Uf prepared by Molscript [35]. The N-terminal domain present in the kidney bean enzyme is removed. The additional loop in Uf (residues 147–161) and the elongation of helix α1 (residues 22–35) were both omitted, because of missing corresponding features in the reference structure of KBPAP. These parts are printed in white with dashed lines and are marked with question marks. The tertiary structure of the remaining part of Uf (residues 227–311) is not predictable because of a obviously different folding topology compared to the remaining C-terminal part of KBPAP (residues 331–432). The positions of the corresponding β strands in KBPAP are indicated by dashed white arrows also marked with question marks. Labeling is according to Fig. 2.

[39]. In spleen cells the enzyme is stored in lysosome-like vesicles surrounding the phagocytized erythrocytes [40]. This indicates that the enzyme might be involved in the first step of the degradation of phagocytized particles, e.g. microbes or erythrocytes. In presence of inorganic phosphate the redox potential of the Fe(III)–Fe(II)-system is shifted ~200 mV to the negative site [41,42]. It has been discussed that the redox shift might allow that Fe(II) interacts with oxygen leading to the formation of superoxidanion-equivalents [4]. Justification for such a suggestion is the immediate disappearence of the EPR signal after addition of phosphate to the Fe(III)–Fe(II) species, whereas the oxidation of Fe(II) to Fe(III) is much slower. Oxidation might therefore occur only after dismutation of two superoxidanion-equivalents. Since after phagocytosis the formation of NO is also induced, peroxynitrite might be formed [43,44], capable to oxidize unsaturated fatty acids thus causing the breakdown of the membrane potential as primary step of degradation. Thus the phosphatase activity might be required for the self-activation of the oxygen activating function of these enzymes, which is demonstrated by chemiluminescence experiments [5]. Degrading membrane potentials might also be the function of uteroferrin inducing the apoptose of the mucosa cells in the uterus. It is known that the expression of Uf is progesteron induced.

In plants the formation of superoxidanion equivalents is not required. However, an interaction with oxygen also occurs in the presence of ascorbate (Löcke et al., unpublished results). Fe(III) can be reduced to Fe(II), which has such a low redox potential that it is immediately oxidized in the presence of oxygen leading to Fe(III) and finally water. Thus in the plant oxygen can be removed at the expense of ascorbate in order to diminish the formation of oxygen radicals in the seed. This would explain, why the plant enzyme uses the Fe(III)–Zn(II) system although both Fe(II) and Zn(II) are available in the cell. Because of the selective incorporation of the divalent metal ion into the semiapoenzymes of Uf and KBPAP small differences in the geometry of the dimetal sites are expectable.

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