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Sequence homology between purple acid phosphatases and phosphoprotein phosphatases

Are phosphoprotein phosphatases metalloproteins containing oxide-bridged dinuclear metal centers?

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The amino acid sequences of mammalian purple acid phosphatases and phosphoprotein phosphatases are shown to possess regions of significant homology. The conserved residues contain a high percentage of possible metal-binding residues. The phosphoprotein phosphatases 1, 2A and 2B are proposed to be iron-zinc metalloenzymes with active sites isostructural (or nearly so) with those of the purple phosphatases.

Protein phosphatase; Purple acid phosphatase; Sequence homology

1. INTRODUCTION

Phosphoprotein phosphatases (PPs) are a class of mammalian regulatory enzymes that catalyze the dephosphorylation of phosphoserine and phosphothreonine proteins [1,2]. Phosphoprotein phosphatase 1 (PP1), which is inhibited by inhibitor-1 and -2, generally occurs in a glycogen- or myosin-bound form. The type 2 enzymes (insensitive to the above inhibitors) are further subdivided into three classes. PP2A is a cytosolic enzyme that possesses broad reactivity, while PP2B, better known as calcineurin, is a Ca^{2+}- and calmodulin-binding protein that dephosphorylates several brain-specific phosphoproteins. PP1 and PP2A contain a catalytic, ~35 kDa subunit and a variety of regulatory subunits. However, PP2B is comprised of a catalytic 60 kDa subunit and a smaller 19 kDa subunit; PP2C is composed of a single 46 kDa subunit.

The nucleotide sequences of the cDNA coding (or partially coding) for the catalytic subunits of PP1, PP2A, and PP2B have been determined from a variety of sources [3–14]; the deduced amino acid sequences have very high degrees of homology (~50%), indicating that the catalytic subunits are closely related (and probably derived from a common ancestor). The amino acid sequence of PP2C appears to be unrelated to those of the other PPs [15], and the in vivo function of the enzyme is presently in question. Very recently, the nucleotide sequence encoding some proteins from certain fungi and *Drosophila* have been shown to be highly homologous (50–90%) to those of mammalian PP1 and PP2A [13,16–20], suggesting that the phosphoprotein phosphatases may be widely distributed.

Mammalian purple acid phosphatases (PAPs) are novel enzymes of molecular mass ~37 kDa that contain an oxide-bridged dinuclear iron active site. The amino acid sequences of the enzymes from bovine spleen, porcine uterine fluid, and human placenta are highly homologous (~90%), again indicating a close relationship [21,22]. A possibly related purple iron-zinc enzyme has been isolated from red kidney beans [23]; it differs significantly from the mammalian enzymes in both molecular weight and amino acid composition. Yet another purple phosphatase has been isolated from fungi [24,25], but the metal composition of this form is unknown.

Herein, we describe regions of the amino acid sequences of the catalytic subunits of PPs and mammalian PAPs that display surprising homology and may contain conserved metal-binding ligands.

2. RESULTS AND DISCUSSION

Given the similarities in size and function between PAPs and the catalytic subunits of PP1 and PP2A (both mammalian PAPs and PPs possess phosphoserine/phosphothreonine protein phosphatase activity [26,27], and all the enzymes are able to hydrolyze the artificial substrate *p*-nitrophenylphosphate [28–31]), the amino acid compositions of the human placental PAP and rabbit skeletal muscle PP2A proteins were

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compared (Table I). Using a statistical technique for testing the significance of amino acid composition indexes [32,33], the two proteins were found to have about a 90% probability of possessing significantly related sequences (DI = 10.4, D = 0.0635, SΔQ = 40.4). The major differences in the composition of the two enzymes are in the mole fractions of alanine, glutamate and aspartate, and cysteine. The higher mole fraction of carboxylates is reflected in the overall charge of the enzymes. PP2A, which possesses the greater number of carboxylates, binds to DEAE-cellulose, while PAPs are strongly cationic. The amino terminus of PAP is largely responsible for the relatively high content of alanine. This hydrophobic region of the protein has been found to be highly homologous to the noncatalytic F_o subunit of maize mitochondrial ATPase [34].

A search of Genbank revealed no significant similarities of the human PAP with the rabbit PP2Aα, confirming an earlier report [22]. However, careful inspection of the sequences of a representative PAP and PP did reveal a region of appreciable sequence homology between the two proteins. Between residues 186–237 of the human PAP [22] and residues 51–103 of the rabbit PP2Aα enzyme [7], 16 residues were conserved (~30% absolute homology). Extension of the comparison to other available PAP and PP sequences and partial sequences (Fig. 1) showed that the region of sequence homology was significantly larger, but that the sequences of the phosphoprotein phosphatases had two sizeable insertions compared to the sequences of the purple acid phosphatases (Fig. 2). Of note also is the fact that the amino terminus of the PAP sequences,

Table I

Comparison of amino acid content of rabbit PP2Aα and human PAP[c]

Amino acid	Mole fraction PP2A[a]	Mole fraction PAP[b]
Met	0.0194	0.0132
Asp	0.0777	0.0559
Asn	0.0388	0.0428
Trp	0.0162	0.0164
Thr	0.0583	0.0559
Ala	0.0388	0.0789
Leu	0.100	0.0921
Ile	0.0421	0.0362
Gln	0.0453	0.0427
Glu	0.0647	0.0395
Pro	0.0421	0.0559
Ser	0.0485	0.0625
Gly	0.0744	0.0789
Arg	0.0647	0.0625
Phe	0.0453	0.0789
Val	0.0680	0.0625
Tyr	0.0518	0.0559
Cys	0.0324	0.0724
His	0.0324	0.0362
Lys	0.0388	0.0461

[a] 309 residues; [b] 304 residues; [c] DI = 10.4, D = 0.00635, SΔQ = 40.4

Fig. 1. Sequence comparison of purple acid phosphatases and selected protein phosphatases. Numbers correspond to positions in the rabbit PP2A sequence. PPN, protein phosphatase N; X (in sequences), isoleucine or leucine; ·, not determined; *, possible metal ligands. [a] [22]; [b] [21,22]; [c] [21]; [d] [7]; [e] [3,4]; [f] [4]; [g] [8]; [h] [9]; [i] [44]; [j] [45]; [k] [6]; [l] [16]; [m] [13]. Conserved residues in all or all but one sequence are notated with a bar at the top and bottom of the column and capitalized; conservative substitutions are capitalized. For convenience, the list of PP sequences is not inclusive; however, the conserved metal binding ligands are unaffected by incorporation of the additional sequences [5,10,11,17–19].

which contains the region homologous to the F_o subunit of ATPase and the glycosylation site, has no counterpart in the PP sequences. As can be readily observed in Fig. 1, possible metal-binding ligands (indicated by asterisks) constitute a large portion of the conserved residues between the PAP and PP sequences, i.e. two tyrosines (80 and 264), two histidines (59 and 240), two aspartic acids (86 and 131), and one glutamic acid (95) (numbers correspond to the rabbit PP2Aα sequence). The homology between the sequences may,

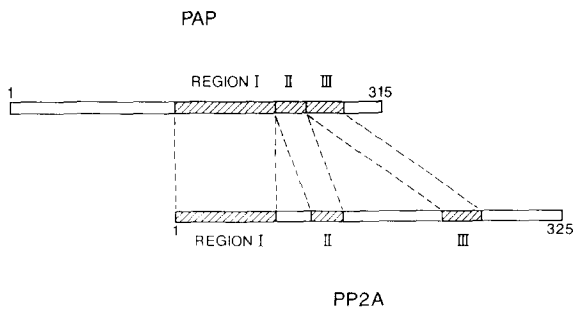

Fig. 2. Comparison of the position of the homologous regions (hatched) of the PP and PAP sequences. Numbering corresponds to the human PAP and rabbit PP2A sequences.

therefore, reflect the presence of a common or at least related metal-containing active site in both proteins. The question thus arises as to whether protein phosphatases contain a dinuclear active site resembling that of the PAPs.

While numerous metal analyses indicate that mammalian PAPs possess two iron atoms per protein molecule (e.g. [35]) and plant PAPs appear to possess one atom each of iron and zinc [23,36], analytical data have been reported for only two PPs. PP2B (calcineurin) has been shown to contain one zinc atom and one iron atom per catalytic subunit (Table II) [30]. Metal analyses on a preparation that probably consisted of a mixture of the catalytic subunits of PP1 and PP2A indicated that no metals were present in stoichiometric amounts [37]. However, it is of particular interest that an order of magnitude more zinc and iron were found than any other transition metal (Table II) [37]; this suggests that the enzymes may be Fe-Zn metalloenzymes whose active site is susceptible to metal loss during isolation. This may also help explain the very low recoveries of phosphatase activity in the isolation of PP2A and PP1; for example, 30% of this activity was lost for the analysis sample by dialysis against chelex-treated buffer to remove free or weakly associated metals [37]. Of note also is that one iron of the mammalian PAPs can be readily removed and replaced with zinc [38,39]; the resultant mixed-metal enzyme possesses spectroscopic and kinetic properties almost identical to those of the native plant Fe-Zn enzymes. Conversely, zinc can be removed from the plant enzymes and replaced by iron with similar results [40]. Consequently, protein phosphatases may be metalloenzymes that possess active sites similar or identical to those of plant purple acid phosphatases and similar to those of mammalian purple acid phosphatases where an iron has been replaced by an atom of zinc. Efforts to isolate PP2A without this loss of activity in order to obtain better metal analysis data are in progress.

Also worthy of brief note is the recent report of an exotic protein phosphatase encoded in the genome of bacteriophage λ [41,42]. While the amino-terminal half of the protein shows approximately 35% absolute homology to PP1 or PP2A, the carboxy-terminal half displays less than 2% homology. Based on the results presented above, this enzyme would be expected to possess the conserved ligands to the zinc site of the PPs but not the iron site. Therefore, this enzyme appears either to be a monozinc phosphatase or to possess a radically different second metal binding site.

Table II

Metal analyses of protein phosphatases

Metal	Mol metal ion/mol protein		
	Calcineurin (PP2B)		PP1[a,b]
Zn	0.6–0.9[c]	~1.0[d]	0.11
Fe	0.7–0.9	NR[e]	0.23
Co	0.04	NR	<0.0015
Cu	<0.02	NR	0.014
Mn	<0.02	<0.1	0.008
Ni	<0.02	<0.1	<0.0015
Sn	<0.02	NR	<0.062
Cr	<0.02	NR	NR
Mo	<0.02	NR	NR
V	<0.02	NR	NR
Cd	NR	NR	0.0004

[a] [37]; [b] probably mixture of PP1 and PP2A; [c] [30]; [d] [43]; [e] NR, not reported

3. CONCLUSION

As predicted from amino acid composition comparisons of human purple acid phosphatases and rabbit phosphoprotein phosphatase 2A, regions of homology between the sequences of PAPs and PPs have been identified. A high percentage of the conserved residues are possible metal ligands, i.e. tyrosine, histidine, and glutamic and aspartic acids. Combined with previous analytical studies, these results indicate that phosphoprotein phosphatases 1, 2A and 2B are probably iron-zinc metalloenzymes with active sites isostructural or nearly so to those of their purple relatives.

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