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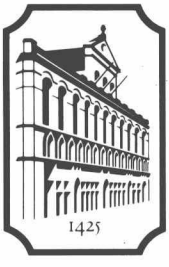

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THE LOW Mr CYTOSOLIC PHOSPHOTYROSINE PROTEIN PHOSPHATASE

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INTRODUCTION

During the past few years, very significant progress has been made in the understanding of cell growth control (1-3). Protein tyrosine phosphorylation is a key event in the regulation of cell division, differentiation, and development. Most growth factor receptors and several oncogene products possess tyrosine protein kinase (PTK) domains, and many dominant oncogenes are mutated versions of transmembrane or non-transmembrane PTKs. In addition, activation of PTKs appears to be a mandatory prerequisite for cell growth stimulation. A large family of transmembrane as well as intracellular PTKs has been characterized (4-6). In normal cells, the receptor PTK activity depends upon the presence of specific growth factors, whereas the oncogene products elicit unregulated PTK activity, leading to uncontrolled cell proliferation and tumorigenesis. A better understanding of the diversity and complexity of the signal transduction pathway through protein tyrosine phosphorylation has come from the recent identification and description of a heterogeneous family of protein tyrosine phosphatases (PTPases) (7-10) and the balance between PTKs and PTPases results in the level of protein tyrosine phosphorylation in cells. Thus the phosphorylated state of any given protein into a cell is the result of a balance of the kinase and phosphatase activities, and PTPases have been proposed as key regulatory elements of cell growth control (11).

A wealth of information is available on PTKs, characterized more than a decade earlier than PTPases, whereas little is known about PTPases, although they are believed to be involved in cell growth control in many ways (reviewed in 12). Nevertheless, the study of PTPases is greatly aided by the knowledge gained from prior studies about PTKs, and current work also suggests that PTKs and PTPases are equally important in many biological processes ranging from cell growth control to cell differentiation and development. In particular, the oncogenic potential of PTKs, and the

ability of PTPases to counteract this effect by their antiproliferative action, has led to the suggestion that the genes coding for PTPases may be considered to be genes involved in tumor suppression or even anti-oncogenes (13). At present, this hypothesis is supported by considerable experimental evidence.

The existence of PTPases was first predicted in 1979 to explain the rapid loss of phosphorylation of *in vitro* phosphorylated membrane proteins (14). However, two key contributions to PTPase knowledge were the purification to homogeneity of the main PTPase in human placenta (PTP 1B), allowing the determination of its amino acid sequence (15, 16), and the demonstration that Leukocyte Common Antigen (LCA, or CD45) shared homology with the catalytic domain of PTP 1B (17), indicating that PTPases can be considered to be a family of structurally related molecules.

At present, over twenty four mammalian PTPases are known (18), each containing at least one PTPase domain. Individual PTPase domains are approximately 300 amino acid residues long, of which 40 residues appear to be highly conserved. Molecular cloning of many PTPases has revealed that these enzymes, like PTKs, can be grouped into two families: transmembrane and non-transmembrane. Transmembrane PTPases, such as CD45 and LAR, are believed to be receptors for as yet unidentified ligands. They contain extracellular regions which, in many cases, are distinct in size and sequence. The extracellular regions, in some cases, contain varying numbers of immunoglobulin-like and/or fibronectin type-III domains, carbonic anhydrase domains, or domains which are unrelated to other proteins (18). The receptor-like extracellular regions are connected to the intracellular (catalytic) domains by a short transmembrane segment. Much progress has been made in the knowledge of the structure of many non-transmembrane PTPases. The non-transmembrane (cytoplasmic) PTPases are small soluble proteins with a single catalytic domain and can be grouped into subfamilies on the basis of structural features of their non-catalytic regions. Some of these enzymes are able to associate with other signaling proteins through specific SH^2 domains (19).

In addition to the PTPases grouped in the above-mentioned families, nonlysosomal acid phosphatases, with a pronounced PTPase activity but structurally and functionally different from the superfamily of the major PTPases, have also been described. There is no evidence that any nonlysosomal acid phosphatases contain the PTPase catalytic domain; therefore, they are not related to the major PTPases. It is well known that there are multiple superfamilies of Ser/Thr protein phosphatases and that they are structurally different from each other (20-23). It is therefore possible that there is also more than one superfamily of PTPases and that

nonlysosomal acid phosphatases represent a different superfamily of PTPases.

In screening for new members of the soluble PTPase family, we have identified and studied a one-domain, soluble PTPase, once known as low Mr acid phosphatase, which is characterized by a low molecular weight (about 18 kDa) and by a reaction mechanism similar to those proposed for other PTPases.

THE LOW Mr CYTOSOLIC PHOSPHOTYROSINE PROTEIN PHOSPHATASE

General features

The low Mr cytosolic PTPase (EC 3.1.3.48.), originally studied as low Mr soluble acid phosphatase (EC 3.1.3.2), has a relatively short history. The presence in mammalian tissues of low Mr acid phosphatase isoenzymes was demonstrated in 1969 by Heinrikson (24), who also purified such an enzyme from bovine liver and more recently by Waheed et al. (25) who purified a human placental acid phosphatase possessing phosphotyrosyl protein phosphatase activity, with a Mr of about 17,000. Some years after Heinrikson's work, De Araujo *et al.* localized the enzyme in the cytosol (26) and then Lawrence and Van Etten (27) reinvestigated the enzyme from bovine liver. The latter authors reported a purification of the enzyme to homogeneity and criticized Heinrikson's data, mainly with regard to amino acid composition and specific activity, which they found to be about twice as high as that reported in the preceding report. More recently, the enzyme has been purified to homogeneity and in good yield from bovine brain (28), and characterized with respect to amino acid composition, molecular weight, pH optimum, Km and Vmax on varying substrates, and Ki with varying inhibitors (28). The enzyme has also been purified from bovine heart (29, 30), human (31) and rat liver (32, 33), where it elicits apparent Mr values ranging between about 14,000 and 17,000. In rat liver, two isoenzymes, named AcP1 and AcP2, have been isolated, characterized and sequenced (32, 33). Several isoenzymes have also been purified from human erythrocytes (34), in which three alleles (*AcP1A*, *AcP1B*, and *AcP1C*) have been identified (35), each of which generates two isoenzymes, named *fast* (f) and *slow* (s) on the basis of their electrophoretic mobilities. The human erythrocyte isoenzymes Bfast and Bslow have also been sequenced (36, 37). Rat liver AcP1 and AcP2, as well as the erythrocyte f and s forms, differ from each other in substrate affinity and sensitivity to activators and inhibitors. By molecular cloning,

the sequences of two isoenzymes (HAAPa and HAAPb) from 3T3-L1 cells have also been determined (38). These isoenzymes correspond to rat liver AcP1 and AcP2 and show sequences which are 99% identical to those of the two isoenzymes from human erythrocytes; in addition to *p*-nitrophenylphosphate (PNPP), the partly purified isoenzymes are able to dephosphorylate the Tyr-phosphorylated adipocyte lipid binding protein. Finally, two low molecular weight isoenzymes (Mr about 12,000), differing from each other with regard to Mr, isoelectric point and some kinetic properties, have been purified from avian pectoral muscle (39). These findings also clearly demonstrate that the enzyme is widely distributed in mammalian and other vertebrate tissues.

The low Mr PTPase catalyzes very efficiently the hydrolysis of arylphosphates such as PNPP and l-phosphotyrosine and of acyl-phosphates, both natural and synthetic, such as carbamoylphosphate, acetylphosphate, and benzoylphosphate (24, 29, 31, 40, 41), whereas it has low activity on alkylphosphates (with the exception of flavin-mononucleotide); the enzyme also possesses a sharp PTPase activity on several protein and peptide substrates (29, 38, 40-43) whereas it is not active on Ser/Thr phosphorylated proteins (29, 42).

Structure

The low Mr cytosolic PTPase from bovine liver was first sequenced in 1989 by Camici *et al.* (44). The protein is a single domain enzyme of 157 amino acid residues (Mr 17,953) containing eight cysteines all in the reduced form and an acetylated *N*-terminal residue. No general sequence homologies are present between this enzyme and the two known acylphosphatase isoenzymes, the metalloproteins porcine uteroferrin and purple acid phosphatase from bovine spleen (both eliciting acid phosphatase activity), and other phosphatases such as the protein phosphatases specific for Ser/Thr or Tyr-phosphorylated residues. The sequences of the two isoenzymes from rat liver (AcP1 and AcP2) and of two isoenzymes from human erythrocytes (Bfast and Bslow) have been determined in our and other laboratories (32, 36, 37). The polypeptide chains of all these isoenzymes consist of 157 amino acid residues, and the isoenzyme pairs differ from each other only in the 40-73 region (Figure 1), suggesting that the two isoenzymes originate from a single gene through an alternative splicing mechanism. AcP2 and Bslow correspond to the bovine liver enzyme. No general homology exists between each isoenzyme and PTPases, both receptor-like (LAR, CD45) and non receptor-like (human placenta PTP 1B, cdc25 and rat brain PTP-1) (45). AcP1 and AcP2 are also distinct from rat liver PTP-1 and PTP-2, which show higher molecular

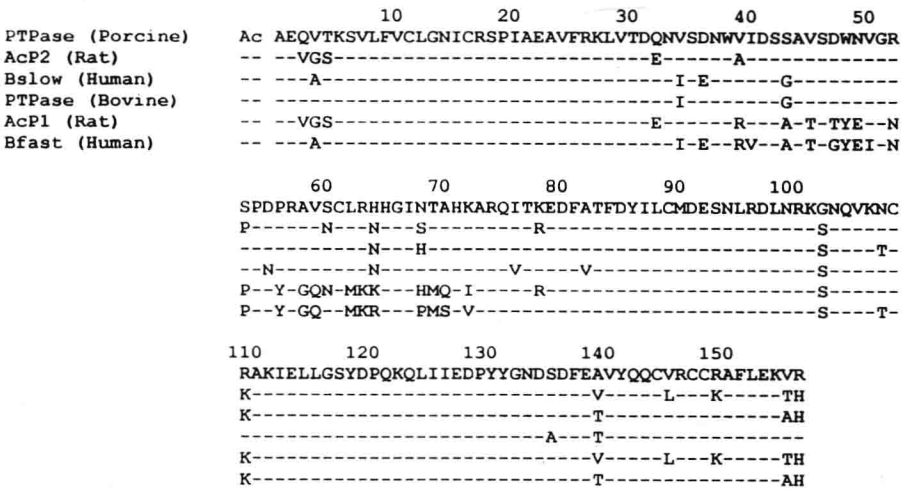

Figure 1. Alignment of the amino acid sequences of six type 1 (AcP1 and Bfast) and type 2 low Mr PTPases from various species.

weights (32, 45). Nevertheless, AcP1 and AcP2 (as well as Bslow and Bfast and the bovine liver enzyme) share some similarities with all PTPase family members, though these are limited to the active site region (46) (Figure 2).

In spite of a number of data reported on the primary structure of this and other PTPases, at present no information is available on the secondary and tertiary structure of these enzymes. Recently, crystals of the bovine liver enzyme of approximately 0.2-0.3 mm in all dimensions, suitable for X-ray diffraction studies, have been grown (Figure 3). In preliminary experiments, these crystals diffract to better than 2.0 Å, and a data set extending to 2.5 Å resolution has been collected (Su, X.D., Agango, E.G., Ramponi, G., Taddei, N., Stefani, M. and Nordlund, P. (1994) FEBS Lett.). A crystallographic study of the low Mr PTPase provides important information on the three-dimensional structure of this enzyme and on the structural basis for substrate recognition and for the catalytic mechanism (Su, X.D., Taddei, N., Stefani, M., Ramponi, G. and Nordlund, P., data sent for publication).

Catalytic mechanism

A number of studies (41, 47-49) on the catalytic mechanism of the bovine heart low Mr PTPase using pre-steady-state and steady-state kinetics led to

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