Purinergic Signalling (2009) 5:273–275
DOI 10.1007/s11302-009-9157-z

BRIEF COMMUNICATION

Prostatic acid phosphatase, a neglected ectonucleotidase

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Received: 10 February 2009 / Accepted: 11 March 2009 / Published online: 26 March 2009

Abstract Two recent papers reveal that the soluble and secreted prostatic acid phosphatase, an enzyme that has long served as a diagnostic marker for prostate cancer, has a membrane-bound splice variant. This enzyme exhibits ecto-5′-nucleotidase activity, is widely distributed, and implicated in the formation of chronic pain. While prostatic acid phosphatase hydrolyzes phosphomonoesters other than 5′-nucleoside monophosphates these novel data suggest that, in addition to ecto-5′-nucleotidase and the alkaline phosphatases, prostatic acid phosphatase must be taken into account in future studies on extracellular adenosine production.

Keywords Ecto-5′-nucleotidase · Prostatic acid phosphatase · Alkaline phosphatase · Pain

Controlling the availability of extracellular nucleotides or of adenosine is a major means of modulating the activity of purinergic receptors (P2 nucleotide receptors and P1 adenosine receptors [1]). Work of the past two decades resulted in the molecular and functional characterization of apparently all types of ectonucleotidases, including the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase), ectonucleotide pyrophosphatase/phosphodiesterase (E-NPP), and alkaline phosphatase (ALP) protein families, the soluble calcium-activated nucleotidase (SCAN) [2] and ecto-5′-nucleotidase. In addition evidence has been provided for cell-surface-located mitochondrial F_1F_o ATP synthase/F_1 ATPase and for ATPase activity associated with the neural cell adhesion molecule (NCAM) and the sarcolemmal α-sarcoglycan. Additional enzymes such as ectonucleoside diphosphate kinase and ectoadenylate kinase are capable of interconverting extracellular nucleotides [3–5].

The cell-surface-located members of the E-NTPDase family NTPDase1–3 and NTPDase8 hydrolyze extracellular nucleoside triphosphates and to varying extent nucleoside diphosphates. In contrast, NTPDase5 and NTPDase6, two largely intracellularly located nucleotidases that can be shed and released into the extracellular medium, hydrolyze only select nucleoside diphosphates. Similarly, the nucleoside diphosphate-hydrolyzing human ectonucleotiase SCAN is cleaved and released from cells. Physiological substrates of the three nucleotide-hydrolyzing members of the E-NPP family (NPP1–3) include ATP, NAD^+, nucleotide sugars and dinucleoside polyphosphates. None of these enzymes can hydrolyze nucleoside monophosphates. In contrast, alkaline phosphatases degrade nucleoside 5′-tri-, -di-, and -monophosphates and thus produce the nucleoside as final hydrolysis product. Ecto-5′-nucleotidase is peculiar as its substrate specificity (at least in mammals) is restricted to nucleoside monophosphates. Thus, the only two ectonucleotidases considered to form extracellular adenosine from AMP were ecto-5′-nucleotidase and the ALPs (Fig. 1).

In a recent study, Zylka et al. demonstrate that prostatic acid phosphatase (PAP, EC 3.1.3.2) functions as an additional ectonucleotidase capable of producing adenosine from extracellular AMP [6]. This followed the demonstration of a membrane-bound splice variant (*T*rans *M*embrane-PAP, TM-PAP) of the soluble and secreted prostate-specific enzyme PAP by Quintero et al. [7]. The secretory isoform of PAP had long served as a diagnostic marker for prostate cancer and is considered to function as a tumor suppressor. The open reading frame of TM-PAP encodes 417 amino acid residues and reveals a cytosolic C-terminus and a large N-terminal extracellular domain (type I transmembrane protein; Fig. 1). When transfected into cells it is targeted

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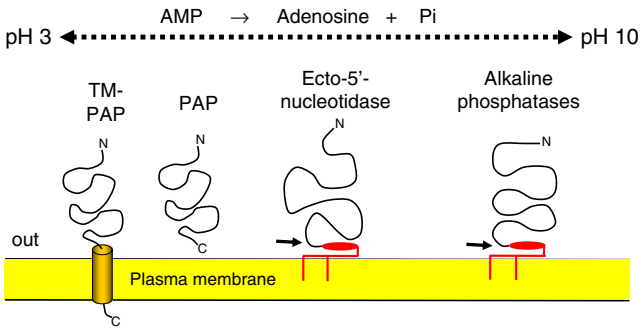

Fig. 1 Membrane topology of enzymes capable of hydrolyzing extracellular AMP to adenosine. The splice variant (TM-PAP) of the soluble and secreted enzyme PAP is a type I transmembrane protein whereas ecto-5′-nucleotidase and the alkaline phosphatases are glycosylphosphatidyl inositol (GPI)-anchored. The anchors of both proteins can be cleaved (arrows) resulting in the formation of soluble enzymes. The pH optima of the three enzymes differ, PAP is very active at acid pH, ecto-5′-nucleotidase around neutral pH and alkaline phosphatases at highly alkaline pH. Whereas ecto-5′-nucleotidase selectively hydrolyzes nucleoside 5′-monophosphates, prostatic acid phosphatase and alkaline phosphatases hydrolyze a large variety of additional phosphomonoesters

to the plasma membrane but also to lysosomes—presumably via its lysosomal targeting sequence and the indirect endosomal pathway. TM-PAP is shown to be widely expressed in mouse tissues (prostate lobes, salivary gland, thymus, lung, kidney, brain, spleen, and thyroid) as well as in a number of human cells. The data further suggest that the secreted form of PAP is not prostate-specific and similarly widely distributed as TM-PAP.

Zylka et al. [6] add a surprising new twist to the story. It had been known for 50 years that many small-diameter neurons of the dorsal root ganglia express a histochemically identifiable acid phosphatase (AP). The reaction was found to be resistant to fluoride inhibition; hence, the name "fluoride-resistant AP" (FRAP; for reference, see [8]). Thiamine monophosphate (TMP) was employed as a standard substrate for the enzyme histochemical reaction and—lacking better substrate information—the responsible enzyme was also referred to as TMPase. The apparently identical enzyme revealed in addition a typical curved histochemical staining in cross sections of the dorsal horn of the spinal cord (lamina II). Moreover, enzyme histochemistry at the ultrastructural level had demonstrated an association of FRAP specifically with the plasma membranes of synaptic glomeruli of the rat dorsal horn substantia gelatinosa [9]. Whilst previous studies had already recognized considerable overlap in the properties of PAP and FRAP/TMPase [8], Zylka et al. [6] demonstrate identity of TM-PAP and FRAP/TMPase. In PAP knockout mice ($PAP^{-/-}$), the enzyme histochemical staining in the nociceptice circuit had entirely disappeared. The heterologously expressed TM-PAP hydrolyzed AMP (to adenosine) and to a minor extent ADP but not ATP [6]. In the soma of small-diameter neurons of dorsal root ganglia TM-PAP is the predominant ecto-5′-nucleotidase and the secretory form is hardly detectable.

The authors thus investigated the possibility of a metabolic dysfunction in the nociceptive pathway of the knockout mice. Indeed, $PAP^{-/-}$ mice revealed increased thermal hyperalgesia and mechanical allodynia in animal models of pain. In line with this, injection into the lumbar spinal cord of the recombinant soluble (secreted form) of human PAP protein had potent antinociceptive, antihyperalgesic, and antiallodynic effects that lasted for several days. Additional experiments implied that injected PAP dephosphorylates endogenous extracellular AMP to adenosine and exerts its function via activation of A1 adenosine receptors, thus functioning as an ecto-5′-nucleotidase. Difficult to explain in this context is the observation that intrathecal injection of bovine ALP (that should also have produced adenosine from AMP) had no effect on noxious thermal or mechanical sensitivity. Taken together, the data identify TM-PAP as an AMP-hydrolyzing ectonucleotidase and implicate its involvement in chronic pain.

Previous studies had indicated that the enzyme reveals very broad substrate specificity. The secreted form of PAP was found to dephosphorylate β-glycerophosphate, lysophosphatidic acid, phospho-amino acids and 5′-nucleotides (literature in [6]). Similarly, in (TM-PAP-containing) spinal cord sections of several mammalian species, substrates other than TMP had been found to reveal the same enzyme histochemical staining pattern [8]. These include a large variety of phosphomonoesters; notably AMP, CMP, GMP, IMP, UMP, and XMP but also phospotyrosine, phosphoserine, β-glycerophosphate, α-naphthylphosphate, or glucose-6-phosphate (but not ATP or 3′,5′-cAMP) [8, 10]. The enzyme reaction could be inhibited by 5 mM L-(+)-tartrate and in part by NaF. While active at surprisingly acid pH, PAP is also active at physiological extracellular pH. The human prostatic enzyme was shown to exhibit a remarkably flat plateau between pH 3 and pH 8 [11]. A direct comparative analysis of recombinant PAP and TM-PAP is still missing but the available data infer similar catalytic properties.

This broad substrate specificity raises the question of the physiological substrate(s) of PAP. Its substrate specificity is very similar to that of ALPs that are non-specific phosphomonoesterases [12]. But, in contrast to ALPs, PAP does not hydrolyze ATP. While hydrolysis of extracellular nucleotides is a physiologically relevant function of ALPs, these enzymes are also capable of hydrolyzing a large number of additional substrates including the dephosphorylation of proteins or the detoxification the bacterial toxin lipopolysaccharide by dephosphorylation of lipid A [13]. In contrast to PAP, ALPs reveal a highly alkaline pH optimum but they are also active at neutral pH [14]. Similarly, autotaxin, a splice variant of NPP2, hydrolyzes not only nucleotides. It also has lysophospholipase D activity. By producing lysophosphatidic

acid (LPA), autotaxin is highly relevant for multiple LPA receptor-mediated cellular functions [15]. Possibly, one of the functional roles of PAP concerns the dephosphorylation of this lipid ligand [16].

Another acid phosphatase (EC 3.1.3.2) is now becoming recognized as an ectonucleotidase of potential physiological significance. Mammalian *t*artrate-*r*esistant *a*cid *p*hosphatase (TRAP), a member of the purple acid phosphatase protein family [17] is of widespread occurrence in osteoclasts, macrophages, dendritic cells, a number of additional cell types, and in serum [18]. It is targeted to lysosomes via its mannose-6-phosphate lysosomal targeting sequence but it also can exist in secreted form. Similar to ALPs, TRAP hydrolyzes a wide range of phosphate monoesters and anhydrides. Substrates include *p*-nitrophenylphosphate, pyrophosphate, phosphoproteins, and nucleotides such as ATP, ADP, and (to a minor extent) AMP. It occurs as a single-chain polypeptide and a dimeric nicked form arising from post-translational cleavage of the single-chain enzyme. Interestingly, proteolytic activation of the enzyme greatly increases ATPase activity, in particular, at more alkaline pH [19]. TRAP is highly expressed by osteoclasts and secreted into the acidic bone resorptive space where it plays an active role in the process of bone remodeling, possibly also involving its ectonucleotidase activity [20]. While the functional role of the ectonucleotidase activity of TRAP in bone and immune cells requires further investigation, these examples demonstrate that extracellular nucleotide hydrolysis can involve enzymes other than the nucleotide substrate-specific ectonucleotidases.

Taken together, the ecto-5′-nucleotidase activity of TM-PAP must be taken into account in future studies on extracellular adenosine production, in particular also regarding the analysis of ecto-5′-nucleotidase (CD73) knockout animals [4]. Remaining AMPase activity may not solely be due to ALPs but also due to PAPs. While the tissue and, in particular, the cellular distribution of the secreted PAP and of TM-PAP deserves further analysis, the RT-PCR data [7] imply a particularly strong expression of TM-PAP in thymus, lung, kidney, and spleen. A weak mRNA signal is also obtained for the brain. It will be fascinating to learn more about the physiological implications of both PAP and TM-PAP.

Acknowledgment This work was supported by the Deutsche Forschungsgemeinschaft (140/17-3)

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