

Is pantetheinase the actual identity of mouse and human vanin-1 proteins?

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Abstract Pantetheinase is an amidohydrolase involved in the dissimilative pathway of CoA, allowing the turnover of the pantothenate moiety. We have determined the N-terminal sequence as well as the sequences of a number of tryptic and chymotryptic peptides of the protein isolated from pig kidney. These sequence stretches were used as probes to search in the SwissProt database and significant similarities were found with a GPI-anchored protein (mouse vanin-1, with a suggested role in lymphocyte migration), with two putative proteins encoded by human cDNAs (VNN1 and VNN2) and with human biotinidase. On the basis of sequence similarity, we propose that vanin-1 and VNN1 should be identified as pantetheinase.

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Key words: Amidohydrolase; Pantetheinase; GPI-anchored; Vanin-1; Biotinidase

1. Introduction

Pantetheinase (EC 3.5.1.-, pantetheine hydrolase) hydrolyzes specifically one of the carboamide linkages in D-pantetheine, with the formation of D-pantothenate and cysteamine [1–6]. The specificity of the enzyme is very high for the pantothenate moiety [7], whereas the structure of the cysteamine-containing part is much less stringent and a thioether group may substitute for the sulfhydryl group in the substrate [5,8]. Cofactors are not required and a reducing agent (such as mercaptoethanol or dithiothreitol) must be present for full activity in vitro.

This hydrolytic activity has been found in many tissues, such as kidney, liver, heart and muscle of mammals, such as horse, pig, cattle, rat and cow. High enzyme activity was also found in the liver of pigeon [9] and other birds [10]. Intracellular distribution studies in rat liver showed its presence exclusively in the microsomal-lysosomal fraction [5], whereas in kidney the activity was found also in the soluble fraction [11].

The pig kidney enzyme has been purified and enzyme preparations, ranging in activity between 3 and 14 units/mg protein, show the presence of only one protein band on non-denaturing and SDS-PAGE and microheterogeneity on isoelectrofocusing, possibly due to the presence of carbohydrates

[6]. Data on molecular weight, isoelectric point, amino acid and carbohydrate content and other properties of proteins of different origin have been published [2,5]. Details on kinetic and catalytic features of the horse kidney enzyme [2,3], on inhibition patterns by disulfides [12,13], on the stability of the molecule and the flexibility of the active site of the pig kidney enzyme [14,15] were also reported.

The enzyme has a role in the dissimilative pathway of CoA and acyl carrier proteins, allowing the turnover of the pantothenate moiety. It seems to be also involved in metabolic pathways leading to taurine. In connection with pantothenoyl-4'-phosphate-L-cysteine decarboxylase (EC 4.1.1.36), pantetheinase has a role in the metabolic route that converts cysteine, with cysteamine and hypotaurine as intermediates, to taurine in those tissues (as heart or brain) where the main route, involving cysteine sulfinic acid decarboxylase (EC 4.1.1.29), is less efficient or absent [16,17]. A role in the biosynthesis of S-aminoethyl-L-cysteine and S-aminoethyl-L-cysteine ketimine has also been demonstrated in vitro [18,19].

Recently, 'prebiotic' synthesis of pantetheine has been demonstrated to occur in vitro under mild conditions, from a mixture of β-alanine, pantoyl lactone and cysteamine; all these reagents are supposed to be prebiotic compounds [20]. It has been suggested that pantetheine could have been important in the earliest metabolic systems for the so-called 'thioester world', which could have preceded the RNA world. In this context, pantetheinase could be considered as a pace-maker enzyme dictating the turnover rate of pantetheine, its activity being of great importance for maintaining a suitable concentration of an indispensable coenzyme. Thus, it would not be surprising if the sequence of this enzyme, as observed for other important proteins, would be largely conserved throughout living organisms. Furthermore, it is expected that an early enzyme has a large diffusion and possess a rather stable tertiary structure. In fact, pig kidney pantetheinase shows a thermal resistance unusual for mammalian enzymes [15], the overall structure seems to be formed by a very resistant external cage and a more flexible internal core where the active site is located [14].

The human enzyme has never been isolated, although pantetheinase activity has been observed in human cells (leukocytes, fibroblasts, intestine cells) and in plasma [10,21,22]. A protein cross-reacting with an anti-pantetheinase antibody has been detected in human plasma (G. Pitari, personal communication).

In this paper we report the partial sequence of the pig kidney enzyme, as determined following isolation and sequencing of a number of tryptic and chymotryptic peptides. Furthermore, on the basis of sequence similarities, we propose that mouse vanin-1 [23] and human VNN1 [24] could be iden-

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Abbreviations: GPI, glycosylphosphatidyl-inositol; EST, expressed sequence tags

tified as pantetheinase and suggest to the Enzyme Commission the assignment of a specific EC number to this enzyme.

2. Materials and methods

2.1. Chemicals

Trypsin and chymotrypsin were from Worthington; iodo-[^{14}C]acetate from The Radiochemical Centre, Amersham, UK; guanidinium chloride (recrystallized from methanol) from Merck. HPLC-grade solvents were from Carlo Erba; sequence-grade chemicals from Perkin-Elmer.

2.2. Purification of pantetheinase

Pig kidney pantetheinase was purified according to [5]. Pantetheinase activity was determined according to [25] with pantetheine *S*-pyruvate as substrate, after reduction of the enzyme with mercaptoethanol and filtration through Sephadex G-25 to remove the excess. Protein concentration was determined using $E_{1\%}^{1\text{cm}} = 11.3$ (280 nm) as extinction coefficient [5]. The purified protein was subjected to SDS-PAGE by the Laemmli method [26] at 10% acrylamide (Miniprotean, Bio-Rad) and stained both with Coomassie blue and silver stain and to isoelectric focusing on precasted Ampholine PAG plates, pH 3.5–9.5 (Pharmacia LKB Biotechnology), following the manufacturer's instructions; proteins were located by silver staining.

2.3. Enzymatic cleavage of pantetheinase

The purified protein (1 mg) was carboxymethylated with iodo-[^{14}C]acetate, dialyzed against water, lyophilized, suspended in 0.5 ml of 0.1 M ammonium bicarbonate and incubated at 37°C for 3 h after addition of 30 μg trypsin. A second sample (0.5 mg) was treated as before and digested with 10 μg chymotrypsin in the same conditions.

2.4. Peptide purification and analytical techniques

Tryptic and chymotryptic peptides were purified using a Beckman System Gold chromatograph on a reversed-phase column (Vydac C8, 4.6 \times 250 mm, 7 μm). Peptides were eluted with a linear gradient of 0 to 50% acetonitrile in 0.2% (v/v) trifluoroacetic acid at a flow rate of 1.0 ml/min. Elution of peptides was monitored using a diode array detector (Beckman model 168) at 220 and 280 nm.

The amino acid sequence of the carboxymethylated protein and proteolytic fragments was determined by automated Edman degradation using a Perkin-Elmer model AB476A sequencer. Samples (0.1–0.3 nmol) were loaded onto a polyvinylidene difluoride membrane (Problott, Perkin-Elmer) coated with 2 μl polybrene (20 mg/ml of 70% methanol).

2.5. Database searches

Similarity searches in the SwissProt database as well as searches in the expressed sequence tags database (dbEST) were performed using the Basic Local Alignment Search Tool (BLAST) algorithm from the National Center of Biotechnology Information (NCBI). Sequence alignment was performed by the MULTALIN program [27]. All other tools were from the ExPASy Molecular Biology Server.

3. Results and discussion

Pantetheinase purified from pig kidney, with a specific activity of about 3 U/mg, was homogeneous on SDS-PAGE giving a single band at about 58 kDa. Isoelectrofocusing in the pH range 3–11 shows some microheterogeneity, which disappears after treatment with neuroaminidase. This behav-

ior has been observed with many glycoproteins and may depend on different hydrolysis stages of the carbohydrate moiety of the protein. In fact, the sugar content of pantetheinase has been reported to be about 10% [6].

From the tryptic and chymotryptic digests of pig pantetheinase, several peptides were isolated and sequenced. The sequences of these peptides as well as the N-terminal sequence of the native protein were used as probes to search in the SwissProt database. Significant similarities were found with the putative proteins encoded by two human cDNAs, called VNN1 and VNN2 [24], and with a related mouse protein, vanin-1 [23]. The latter has been isolated from thymic pericytes and seems to be involved in lymphocyte migration [23]. VNN1 and VNN2 are located on chromosome 6q23-q24, whose mutations are involved in invasivity of metastasis in human cancer [24]. Recently, a glycosylphosphatidylinositol (GPI)-anchored protein from human leukocytes (termed GPI-80) has been described with a suggested role in the regulation of neutrophil adherence and migration [28]. This sequence is identical to that of VNN2.

The peptides isolated and characterized from the pig enzyme presumably account for about 64% of the whole protein sequence, assuming a length similar to that of vanins. We judged that this sequence information was sufficient to support the conclusions presented in this paper. Therefore, no effort was spent to complete the primary structure of the enzyme.

The peptides could be easily aligned to the corresponding regions of both mouse and human vanins (Fig. 1). Pairwise comparison of the sequences reported in Fig. 1 shows different levels of similarity (Table 1). The degree of similarity of pantetheinase to mouse vanin-1 or human VNN1 is higher (79.7 or 83.2% identity, respectively) than to VNN2 (65.2%). The percentage identity between mouse vanin-1 and human VNN1 is 79.1%, whereas the similarity between the two and VNN2 ranges around 63% identity.

As shown in Table 1, human and murine vanin proteins share 40% sequence identities with human biotinidase [29]. The latter enzyme catalyzes the hydrolysis of biocytin, the degradation product of biotin-dependent carboxylases, leading to free biotin and lysine, a reaction similar to that catalyzed by pantetheinase. Although no biotinidase activity was found associated to vanin-1 [23], the authors suggest that all these proteins belong to a new family of related molecules. The comparison between pig pantetheinase and human biotinidase shows 46.5% identity. Although these are only partial data, it is possible to speculate that pantetheinase and vanin-1/VNN1, that share the greatest similarity, are highly related proteins, both different from VNN2. In this view, mouse and human vanin-1 can be actually identified as pantetheinase, while human VNN2 and biotinidase can be considered related proteins belonging to the same amidohydrolase family with different substrate specificity.

Table 1
Sequence identities among amidohydrolases

	Mouse vanin-1	Human VNN1	Human VNN2	Human biotinidase
	%			
Pig pantetheinase	79.7	83.2	65.2	46.5
Mouse vanin-1		79.1	63.6	42.0
Human VNN1			63.7	42.7
Human VNN2				39.1

										70
BTD	HEAEYVAAV	YEHPISLSLN	PLALISRQEA	LELMNQNLDI	YEQQVMTAAQ	KDVQIIVFPE	DGIHGFNFTR			
VNN2	QDSFIAAV	YEHAVILPNK	TETPVSQEDA	LNLMNENIDI	LETAIKQAA	EGARIIVTPE	DALYGWKFTR			
VNN1	QDTFIAAV	YEHAAILPNA	TLTPVSREEA	LALMNRNLDI	LEGATISAAD	QGAHIIIVTPE	DALYGWVFNFR			
V-1	LDTFLAAV	YEHAVILPKD	TLLPVSHGEA	LALMNQNLDL	LEGATVSAAK	QGAHIIIVTPE	DGIYGVRFTR			
Pant	LDTFIAAV	YEHAVILPDA	PLTPVSHHEA	LALMNRNLDL	LEGAIMSAAK	QGAHIIIVTPE	DGVYGFFFSR			
		*** ** *	* *	* ** *	*	**	*** ** *	* *	* ** *	
										140
BTD	TSIYPFLDFM	PSPQVVRWNP	CLEPHRFNDT	EVLQRLSCMA	IRGDMFLVAN	LGTKEPCHSS	DPRCPKDGRY			
VNN2	ETVFPYLEDI	PDPQV.NWIP	CQDPHRFGHT	PVQARLSCLA	KDNSIYVLAN	LGDKKPCNSR	DSTCPPNGYF			
VNN1	DSLYPYLEDI	PDPEV.NWIP	CNNRNRFGQT	PVQERLSCLA	KNNSIYVVAN	IGDKKPCDTS	DPQCPDGRY			
V-1	DTIYPYLEEI	PDPQV.NWIP	CDNPKRFGST	PVQERLSCLA	KNNSIYVVAN	MGDKKPCNTS	DSHCPPDGRF			
Pant	ESIYSYLEDI	PDPHV.NWIP	CTNPNRFGHT	PVQ.KLSCLA	R		Y			
	*	* * * * *	* * * * *	* * * * *	*	*** *				
										210
BTD	QFNTNVVFSN	NGTLVDYRKY	HNLY.FEAAF	DVPLKVDLIT	FDTPFAGRFG	IFTCFDILFF	DPAIRVLRDY			
VNN2	QYNTNVVYNT	EGKLVARYHK	YHLY.SEPQF	NVPEKPELVT	FNTAF.GRFG	IFTCFDIFFY	YPGVTLVKDF			
VNN1	QYNTDVVFDI	QGKLVARYHK	QNLFMGENQF	NVPKEPEIVT	FNTTF.GSFG	IFTCFDILFH	DPVATLVKDF			
V-1	QYNTDVVFDI	QGKLVARYHK	QNLFMGEDQF	NVPMEPEFVT	FDTPF.GKFG	VFTCFDILFH	DPVATLVTEF			
Pant	QYNTDVVFDI	BGR	QNLFLGEDQF	DAPK		FH	DPVATL EF			
	* * * * *	*		* * * *	*		* *			
										280
BTD	KVKHVYPTA	WMNQLPLLA	IEIQKAFAVA	FGINVLAAV	HHPVLGMTGS	GIHTPLESFW	YH.DMENPKS			
VNN2	HVDTILFPTA	WMNVPLPLTA	IEFHSAWAMG	MGVNLVAVNT	HHVSLNMTGS	GIYAPNGPKV	YHYDMKTGEL			
VNN1	HVDTIVFPTA	WMNVPLPLSA	VEFHSAWAMG	MRVNFASANI	HYPSKMTGS	GIYAPNSSRA	FHYDMKTGEEG			
V-1	QVDTILFPTA	WMDVPLPLAA	IEFHSAWAMG	MGVNFALAANL	HNPSRRMTGS	GIYAPDSPRV	FHYDRKTQEG			
Pant	QVDTILFPTA	WMNPLA		VNFLATNI	HFPLR MTGS	GIYAPDSPPRA	FHYDMK			
	*	*** ** *		* * * *	*	**** ** *	** *			
										350
BTD	HLIIAQVAKN	PVGLIGAENA	TGETDPHSHK	FLKILSGDPY	CEKDAQEVHC	DEATKWNVNA	PPTFHSEMMY			
VNN2	KLLLSQVDSH	PLSSLAYPTA	VNWN..AYAT	TIKPPF....VQ	KNTFRGPISR				
VNN1	KLLLSQVDSH	PSHS...AV	NWNT..SYAS	SIEALS....SG	NKEFKGTVFF				
V-1	KLLFAQLKSH	PIHS...PV	.NWT..SYAS	SVESTP....TK	TQEPQIVFF				
Pant	LLLSQVDSH	P.HR...PA	VYWT..SY							
	*	*								
										420
BTD	DNFTLVVWVG	KEGYLHVCSN	GLCCYLLYER	.PTLSKELYA	LGVFDGLHTV	HGTYYIQVCA	LVRCGGLGFD			
VNN2	DGFNTELEFE	NAGNLTVQCQ	ELCCHLSYRM	LQKEENEVYV	LGAFGLHGR	RRREYVQVCT	MLKCKTNTLT			
VNN1	DEFTFVKLTG	VAGNYTVCQK	DLCCCHLSYKM	SENIPNEVYA	LGAFDGLHTV	EGRYYLQICT	LLKCKTNTLN			
V-1	DEFTFVLEKQ	IKGNYTVCQN	DLCCCHLSYQM	SEKRADEVYA	FGAFDGLHTV	EGQYYLQICI	LLKCKTNTLR			
Pant		G VAGGYTVCQK	DLCCQLSYR	EDEVYA	LGAFDGLHTV	EGSYYLQIC				
		* **	*** ** *	* * *	* * * * *	* * * *				
										490
BTD	TCGQEITEAT	GIFE.FHLWG	NFSTSYIFPL	FLTSGMTLEV	PDQLGWENDH	YFLRKSRLSS	GLVTAALYGR			
VNN2	TCGRPVETAS	TRFEMFSLSG	TFGTQYVFPPE	VLLTEIHL.S	PGKFEVLKDG	RLVKNKNGSSG	PILTVSLFGR			
VNN1	TCGDSAEATAS	TRFEMFSLSG	TFGTQYVFPPE	VLLSENQLA.	PGEFQVSTDG	RLVSLKPTSG	PVLTVTLFGR			
V-1	TCGSSVDTAF	TRFEMFSLSG	TFGTQYVFPPE	VLLSEVKLA.	PGEFQVSSDG	RLVSLKPTSG	PVLTVTLFGR			
Pant		FEMFSLSG	TFGTQYVFPPE	VLLSDIQLA.	PGEFQVSSDG	RLVSLKPPSG	PVLTVTLFGR			
		** * * *	* * * * *	*	*	*	**			
										530
BTD	LYERD									
VNN2	WYTKDSLYSS	CGTSNSAITY	LLIFILLMII	ALQNIIVML						
VNN1	LYEKD....	.WASNASSGL	TAQARIIMLI	VIAIVCSLSW						
V-1	LYGKD....	.WASNASSDF	IAHSLIIMLI	VTPIIHYLC						
Pant										

Fig. 1. Amino acid sequence comparison among amidohydrolases. BTD, human biotinidase [29]; VNN1 and VNN2, putative proteins deduced from human cDNAs [24]; V-1, mouse vanin-1 [23]; pant, pig kidney pantetheinase (this paper). Gaps (.) have been inserted to maximize the identities among the four sequences (marked by the asterisks). The first 10 residues of biotinidase are not included. The numbering is given starting from the first compared amino acid position. VNN2 is identical to GPI-80 [28].

To confirm this hypothesis, the sequence of human pantetheinase should be determined. However, by exploiting available sequence information, further evidence in favor of the proposed identification may be presented.

In fact, when the sequences of the peptides of the pig protein were translated, using the algorithm available among the Swiss-Prot tools, into the corresponding nucleotide sequences, and the latter were used to search in the EST database, some human and mouse cDNAs were retrieved with significant scores. The sequences of the retrieved human clones were then translated according to the correct reading frame and this provided more extended sequence information. These amino acid sequences in all cases matched only the sequences

of vanin-1/VNN1 and VNN2, indicating that no other related proteins are present in the database. This further supports the proposed identity between VNN1 and pantetheinase.

The N-terminal sequence of the pig enzyme was directly determined by automatic Edman degradation of the native protein and it is similar to that found for vanin-1 and GPI-80 (VNN2). Because of the similarities between the mammalian protein precursors, we assumed also for VNN1 a similar processing leading to the removal of the signal sequence. Therefore, this extension is not reported in Fig. 1. For biotinidase, the first 10 residues of the putative protein [29] are not reported in Fig. 1

Both vanin-1 and GPI-80 (VNN2) are GPI-anchored pro-

teins [23,28], sharing with VNN1 the C-terminus consensus GPI cleavage site (position 504–508 in Fig. 1). In this region we lack sequence information. However, some properties of pig kidney pantetheinase indicate that it is a hydrophobic protein, being extracted with butanol and purified on octyl-Sepharose. Furthermore, preliminary experiments on human leukocytes show that pantetheinase activity is detected in supernatants of plasma membranes only after treatment with phospholipase C. A specific activity of about 2.0 nmol of pantetheine/min/mg protein was calculated.

Another feature shared between pantetheinase and vanin-1 is the presence of carbohydrates, as detected following neuroaminidase digestion [23] (see above).

It is worth noting that using either VNN1 or VNN2 sequences as probes no significant matches with other known proteins in the database can be retrieved, despite the fact that complete genomes are now available. This is somehow unexpected, since pantetheinase should be a very ancient protein and pantetheine is considered an old cofactor shared by almost all known organisms.

The data so far available do not allow any obvious correlation between lymphocyte migration and pantetheine metabolism. Further studies will provide insights into the relationship among these proteins.

During the publication processing of this manuscript, a paper on Immunogenetics became available on PubMed at the National Center for Biotechnology Information [30]. The authors present results obtained by computational analysis of EST sequence database resources, which are available at the web site <http://tagc.univ-mrs.fr/pub/vanin/> and suggest that vanin molecules might bear an enzymatic activity.

The evidence we present in this paper may be crucial in the assignment of a function to this protein family.

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