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Visions & Reflections (Minireview)

Antiquitin, a relatively unexplored member in the superfamily of aldehyde dehydrogenases with diversified physiological functions

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Abstract. Antiquitin is a member of the aldehyde dehydrogenase superfamily. Sequence analyses indicate that the protein is highly conserved from plants to animals. The plant antiquitins are generally believed to play a role in osmoregulation and/or detoxification. The physiological functions of animal antiquitins remain largely elusive, their involvement in a number of human diseases has been implicated.

Keywords. Antiquitin, aldehyde dehydrogenase, detoxification, osmoregulation, alpha-aminoadipic semialdehyde.

The superfamily of aldehyde dehydrogenases

Aldehyde dehydrogenases (ALDH) form a superfamily of enzymes that catalyze the $NAD(P)^+$-dependent conversion of aldehydes (derived from both endogenous and exogenous sources) into their corresponding carboxylic acids. They carry out a diversity of metabolic functions including (i) detoxification, such as the removal of acetaldehyde from alcohol metabolism and 4-hydroxy-2-nonenal/malondialdehyde from lipid peroxidation [1], (ii) participation in intermediary metabolism, such as amino acid and retinoic acid metabolism, (iii) protection from osmotic stress by generating osmoprotectants, such as glycine betaine [2], and (iv) generation of NAD(P)H [3]. In addition, some ALDHs serve as structural proteins such as those found in the eye lens of cephalopod [4] and elephant shrew [5].

Up to now, approximately 1000 different ALDHs have been identified at the nucleotide level. In eukaryotes, these ALDHs can be categorized into 21 families [6] according to their sequence homologies. Protein sequences sharing ≥40% identity are considered to belong to the same family, while those sharing ≥60% are considered belonging to the same subfamily. Apart from their sequence identities, members of the ALDH superfamily also share similar three-dimensional conformation. X-ray crystallographic studies of ALDH1 [7], ALDH2 [8], ALDH3 [9], ALDH9 (betaine aldehyde dehydrogenase) [10] and ALDH11 (nonphosphorylating glyceraldehyde-3-phosphate dehydrogenase) [11] show the presence of three distinct domains in each monomer, *i.e.* catalytic domain, NAD^+-binding domain and oligomerization domain.

Antiquitin, with its sequence sharing approximately 30% identity to a number of ALDHs (*e.g.* ALDH1, ALDH2 and ALDH9), is classified as Family 7 in the ALDH superfamily [12]. This family of ALDH can be further differentiated into three subfamilies, *i.e.* ALDH7A, ALDH7B and ALDH7C (Table 1). ALDH 7A includes members that are found in animals, while ALDH7B are those found in plants. ALDH7C, with *Drosophila melanogaster* antiquitin as the only member identified so far, represents another subfamily. Amino acid sequence comparison between green garden pea ALDH7B1 and human ALDH7A1 shows an exceptionally high percentage (~60%) of identity [13]. Because of the long evolutionary

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distance between the two kingdoms, such a high percentage of sequence identity is unusual and is suggestive of an essential cellular function of antiquitin. The protein was in fact given the name 'antiquitin' to reflect its antique nature [13]. Although antiquitin has been identified in a variety of species, most reports concern merely sequence information from the respective genome projects. Relatively little information is available on the biochemical and physiological functions of the protein. Nevertheless, studies up to now suggest that the functions of plant and animal antiquitins may not be the same.

Antiquitin in plants

Antiquitin was first discovered in garden pea [19]. It was described as turgor responsive because the level of expression increased when the plant was dehydrated. In addition to garden pea ALDH7B1, induction of gene expression could also be observed in rapeseed ALDH7B3 [21] under similar conditions. Microarray analyses have also identified ALDH7B4 in *Arabidopsis thaliana* [22] and antiquitin in *Sorghum bicolor* [20] among the dehydration-inducible genes. These results strongly suggest that osmoprotection is a major function of antiquitin.

When cells are dehydrated, osmotic stress is one of the major challenges that they have to overcome for survival. Depletion of water results in an osmotic pressure difference across the cell membrane, thus driving water out of the cells. One way to act against such osmotic stress is by the generation of osmoprotectants [25]. Osmoprotectants are small molecules that can be accumulated at high concentrations inside a cell without affecting cellular functions [26]. It is possible that antiquitin can oxidize some aldehyde precursors to generate the carboxylate-containing osmoprotectants. A similar osmoregulatory role has been reported for ALDH9. ALDH9 oxidizes betaine aldehyde to form the common osmoprotectant glycine betaine [27]. In fact, plants overexpressing ALDH9 have been reported to have significant improvement in dehydration tolerance [2].

In addition to osmotic stress, cells are also subject to oxidative stress upon dehydration. Under such conditions, toxic molecules including aldehydes are generated. These aldehydes will lead to the production of reactive oxygen species, resulting in cell damage. To cope with this, effec-

Table 1. Antiquitins in different animal and plant species.

Species	Gene name (if assigned)	Protein database	Reference	Putative function
Homo sapiens	ALDH7A1	P49419	13, 14	
	ALDH7AP1	Pseudogene	15	
	ALDH7AP2	Pseudogene	15	
	ALDH7AP3	Pseudogene	15	
Rattus norvegicus	ALDH7A1	Q64057	13	
Caenorhabditis elegans	ALDH7A2	P46562		
Acanthopagrus schlegeli		AAX54912	16, 17	
Ctenopharyngodon idella		P84463	18	
Bos taurus		XP_584088		
Canis familiaris		XP_538607		
Danio rerio		NP_997889		
Euphorbia characias		AAX09646		
Gallus gallus		XP_424422		
Mus musculus		XP_911728		
Pisum sativum	ALDH7B1	P25795	19	Osmoprotection
Sorghum bicolor	ALDH7B2	P93684	20	
Brassica napus	ALDH7B3	Q41247	21	Osmoprotection
Arabidopsis thaliana	ALDH7B4	CAE48164	22	Osmoprotection
Malus × *domestica*	ALDH7B5	Q9ZPB7	23	Osmoprotection
Tortula ruralis	ALDH7B6		24	
Oryza sativa	ALDH7B6	Q9FPK6		Osmoprotection
Dictyostelium discoideum		P83401		
Glycine max		AAP02957		Detoxification
Drosophila melanogaster	ALDH7C1	NP 649099		

tive systems for the clearance of these toxic aldehydes are needed. The induced expression of antiquitin may contribute towards the removal of such aldehydes. This suggestion came from the recent demonstration that transgenic plants overexpressing soybean antiquitin exhibit higher tolerance against oxidative stress [28]. Other members of the ALDH superfamily have also been reported to play such anti-oxidative roles. For example, ALDH3 exhibits high activity towards 4-hydroxy-2-nonenal/malondialdehyde and is thus believed to play a role in detoxification [1].

The expression of antiquitin in plants has also been investigated under other conditions, such as abscisic acid treatment, salt treatment, heat shock and cold shock. Unfortunately, the results are inconsistent and appear to vary from species to species [19–21, 24]. Indeed, not all antiquitins are inducible. In the moss *Tortula ruralis*, the expression level of ALDH7B6 remains constant under various conditions including dehydration and salinity changes [24]. The constitutive expression of ALDH7B6 may function to maintain the essential dehydration-tolerance protection system in moss [29]. Phylogenetically, the moss ALDH7B6 is distinctly separated from all the other plant antiquitins and stands out as a discrete evolutionary group [24], raising the possibility that it might not be a functional homolog of plant antiquitins [22].

Antiquitin in animals

Because of the high sequence identity with its plant counterparts, human antiquitin has been assumed to play a role in osmoregulation and/or detoxification. This assumption, however, has not been experimentally substantiated. Tissue distribution studies of antiquitin gene expression indicate that it is highly expressed in the fetal cochlea, among other human tissues [15]. Such observation is consistent with the presumptive role of ALDH7A1 in osmoregulation as cochlea is a fluid-filled organ and its function depends on a proper osmotic balance. Because of its possible involvement in the control of fluid balance, antiquitin has been studied as a candidate gene in the hearing disorder Menière's disease in which patients suffer from an accumulation of endolymph in the inner ear. However, the results do not seem to support a direct relationship between antiquitin and the disease [30].

Despite intensive studies, animal antiquitins, unlike its plant counterparts, do not exhibit any inducible response to many different types of stress. Treatments such as dehydration, heat shock, ionizing irradiation and challenge with iron, *t*-butylhydroperoxide as well as glucocorticoids all fail to alter the mRNA level of ALDH7A1 in cultured human hepatocarcinoma HepG2 cells [13]. Similar treatments also fail to change its mRNA level in cultured human embryonic kidney HEK293 cells [31]. Besides the *in vitro* cell culture experiments, *in vivo* studies on the inducibility of antiquitin using black seabream (*Acanthopagrus schlegeli*) as the animal model have also been performed. No significant change was observed in the expression level of antiquitin when the fish are exposed to hyper- or hypo-osmotic environments, or injected with different hormones including thyroxine, growth hormone, prolactin, testosterone, estradiol and cortisol [unpublished data]. These observations raise the possibility that the function and regulation of antiquitin in animals might not be the same as in plants.

Most of the previous studies on antiquitin have been confined to the nucleotide level. At the protein level, only two antiquitins, one from black seabream [16, 17] and the other from grass carp (*Ctenopharyngodon idella*) [18], have been purified and characterized thus far. These two antiquitins are acidic tetrameric proteins. Like most members in the ALDH superfamily (except octopus ALDH1C1, Ω-crystallin [4]), these antiquitins possess acetaldehyde-oxidizing activity and prefer NAD^+ as the coenzyme. However, up to now the physiological substrate for the fish enzyme has not been identified. Recombinant seabream antiquitin failed to oxidize 4-hydroxy-2-nonenal, malondialdehyde, succinic semialdehyde, betaine aldehyde and *all-trans* retinal as substrates [17].

Future perspective

Antiquitin was first discovered in 1990 and remains a largely unexplored member of the ALDH superfamily. Studies in plants suggest that antiquitin is inducible and functions in osmoregulation and/or detoxification. However, in animals, experimental evidence on the inducibility of antiquitin is lacking and there is no evidence to support an osmoregulatory or detoxification role. Thus, although antiquitin is structurally conserved from plants to animals, identical physiological functions in the two systems have not been identified. However, it is possible that antiquitin may share a common, yet hitherto unidentified, role in the two kingdoms. One direction which deserves further pursuit is the possible involvement of antiquitin in growth and development. During the maturation of pig oocytes, there is an elevated expression level of antiquitin, indicating its involvement in primary oocytes to undergo successful fertilization and to initiate zygotic development [32]. Human cells with a mutated *PIG-A* (phosphatidylinositol glycan class A) gene do not express antiquitin and exhibit an enhanced rate of cell proliferation [33]. In apple, the expression of ALDH7B5 is up-regulated during fruit development [23]. Dehydration and other stresses, which are known to affect antiquitin in plants, also inhibit plant growth and development [34]. Based on these observations, it is tempting to speculate that antiquitin might play a role in cell proliferation.

However, whether the enzymatic properties of antiquitin are related to its putative role in cell proliferation remains to be investigated. In this regard, another member of the ALDH superfamily, human ALDH3A1 has recently been shown to be a negative cell cycle regulator [35], in addition to its detoxification function. The human corneal epithelial cell line (HCE) stably expressing ALDH3A1 exhibits a prolonged cell cycle with reduced DNA synthesis. These effects are associated with changes in the level of several different cyclins and cyclin-dependent kinases that regulate the cell cycle [35].

Unlike animal antiquitins, no plant antiquitins have been purified or expressed so far. It would be important to develop an efficient expression system for plant antiquitins to allow a detailed comparison of the protein and enzymatic characteristics between the animal and plant antiquitins. The demonstration of enzymatic activities towards osmoprotectant precursors and toxic aldehydes by plant antiquitins would constitute direct evidence for their purported osmoregulatory and detoxification functions. The plant and animal antiquitins appear to differ greatly in their propensity to induction. The underlying mechanism for the difference is not known but this could be related to their gene promoter activities. The identification of potential transcription factor binding sites in their promoter regions may provide hints on the putative element(s) which is(are) essential to regulate their expression. Further studies along this line are highly warranted.

The recent discovery that children with pyridoxine-dependent seizures have mutations in the antiquitin gene [14] opens up new avenues in understanding the physiological significance of this protein. Alpha-aminoadipic semialdehyde was identified as a physiological substrate of the human enzyme. The relationship of this enzyme with pyridoxal phosphate metabolism in human is thought provocative. In view of the essential role of pyridoxal phosphate in many enzymatic reactions, including those involved in neurotransmitter metabolism and amino acid metabolism, antiquitin is probably involved in further physiological processes than previously thought. More exciting and interesting findings are yet to come.

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