TYPE 4 PLANT METALLOTHIONEINS (MT4): AN OVERVIEW OF HORDEUM VULGARE

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Abstract— Metallothioneins are usually low molecular weight metal binding proteins which were first discovered in the late 1950’s following an intensive study of yeast, mammals and plants. Among the four subfamilies or classes of plant metallothioneins, Type 4 plant metallothioneins (PMT’s), metallothioneins 4 (MT4) or early cysteine as popularly known, stands out to be the most intensively studied group in vitro, perhaps this is attributed to their peculiar structure and function coupled with ease of isolation and purification when compared to other metallothioneins (MT’s). In a recent study on barley (Hordeum vulgare) a member of this group revealed that MT4 genes of the plant show local zinc binding in the grain aleuronic layer and act in putative storage. However, structural information and understanding of metal binding dynamics of these MT’s are scanty, with early cysteine metallothioneins (MT) from wheat as the only PMT with crystal 3D structure currently available. This paper reviews the state of researches on the structure and functions of Type 4 plant metallothioneins and considering their proposed function which includes provision of Zn (II) to the relevant processes during seed germination, it will be interesting to find out about its ability to transfer Zn (II) ions to other molecules.

Index Terms— Metallothioneins, metal binding proteins, Type 4 PMTs, metal binding dynamics.

I. INTRODUCTION

Currently, there are 298,900 known classified plant species and over 263,900 awaiting classification. A single characteristic unique to these plants are their requirements for macro and micronutrients such as calcium, potassium, magnesium and chloride, copper, zinc respectively, the deficiency of any of the above elements may hinder plants transition to life cycle [1, 2]. The mechanisms adopted by plant species to regulate the required amount of all essential elements when environmental conditions become unfavorable poses a question of great concern, exploring the ability for the control of bio-accumulation of highly nutritious and essential elements gives an opportunity and advantage for human nutrition and world food security at large [3-6], therefore, over dependence on the lithosphere for micronutrients also raises a concern and expose us to hazards[2] Metal homeostasis and tolerance, membrane transport proteins and heavy metals transporting proteins families proves to be of great significance [6, 7], however, one family of cytosolic proteins were found to efficiently functions in metal homeostasis and host of other stress responses not only confined to metal ions and these proteins are called metallothioneins (MT’s) [8].

Metallothioneins are usually low molecular weight metal binding proteins which were first discovered in the late 1950’s following an intensive study on yeast, mammals and plants [9]. They were found to functions in cytosolic zinc (Zn) and copper (Cu) binding by the formation of coordinate complexes between metal ions and thiol groups of multiple cysteine residues in the protein [10]. Additionally, several metallothioneins were reported to protect against reactive oxygen species in plants [11, 12]. Structurally, they differ with vertebrate MTs because they contain Cysteine rich domains in their N and C termini separated by different spacer regions with no or few metal binding residues. MTs are classified into 15 families based on their sequence similarity and evolutionary relationship which are further categorized in to 4 sub families (Type1-4) differentiated by the number Cysteine regions and their distribution [11, 13] and different MTs genes from different plant species have different expression patterns which are regulated onto-genetically [14, 15].

A recent study on barley (Hordeum vulgare) metallothioneins reveals that MT3 and MT4 genes of the plant show local differential zinc binding in the grain aleuronic layer, while MT3 act as Zn and Cu housekeeping protein there by maintaining metal homeostasis, MT4 act in putative storage [1, 16]. However, structural information and data both experimental and in silico on plant metallothioneins including MT4 is generally scanty, the only available experimental structure currently is for pioneer MT4 protein from wheat [8].

II. TYPE 4 PLANT METALLOTHIONEINS OR WHEAT EC1

Among the four sub families or classes of plant metallothioneins, Type 4 pMT, MT4 or early cysteine as popularly known, stands out to be the most intensively studied group in vitro, perhaps this is attributed to their peculiar structure and function coupled with ease of isolation and purification when compared to other MTs [8]. For instance, of the 79 amino acid residues in the primary structure of wheat MT (Methionine residue is not present in the N terminal in the native structure isolated from wheat germ) as such, numbering scheme have to start with Glycine as residue number one [17], this contains 17 cysteine residues which constitutes 21.5% of the total amino acids which lies between mammals and cyanobacteria MT’s. In flowering plants, two histidine residues were found to be conserved and function in determining unique metal binding properties of Type 4 plant metallothioneins [8].

THE BARLEY (Hordeum vulgare) MT4

As of today, only a few native plant MTs are isolated. The pioneer cysteine labelled protein (Ec), which is often used interchangeably as wheat MT4 was initially purified from mature wheat embryos[18] and was found to be a zinc binding MT [19]. In Arabidopsis, MT2 and MT3 were purified with the use of sequential size-exclusion, Cu and thiol-affinity chromatography even though they were not chemically characterized [20].
As stated earlier, in a recent study to address the physiological function of MTs in cereal grains, barley (*Hordeum vulgare*) have been functionally characterized and were found to be a diverse family of 10 MTs among which includes MT4 [1]. In a recent study, speciation analysis with the using electro spray ionization time of flight mass spectrometry and size exclusion chromatography were adopted to obtain a heterologous expression of MT3 and MT4 in yeast and E. coli to quantify each of the MTs specificity and metal binding capacity. A phylogenetic analysis of structural relationship among the various MTs showed that barley MTs were distributed among all the four subgroups of plant MTs which were identified according to the Institute for Genomic Research’s barley Gene Indices [21].

### III. Structure and Function of Type 4 Plant Metallothioneins

Although there has been an increased level of activity in plant metallothioneins protein biochemistry recently, the early cysteine MT from wheat is the only one with 3D structure information currently available [8], they normally forms two metal binding domains, residues 1-20 forming domain 1 in the N terminal and residues 32 to 79 forming domain 2 as depicted in figure 1 [11].

![Figure 1: Structures of the two domains and their metal binding sites of Type 4 pMT from wheat (pdb: 2126 and 1kak). Metal binding ligand were confirmed by experiment and are shown in straight lines in the lower part of the figure. Also indicated are the 9 cysteine residues forming M4_Cys9 cluster in domain 2 and are highlighted in black boxes in the sequence.](image)

Residues 21-31 and 49-62 form the two Cysteine poor regions with the former providing a link between the two domains while the latter is an integral part of the second domain. The two domain structure is corroborated by a proteolytic digestion experiment which forms two separate regions of residues 1-25 and 30-80 [22]. Additionally, the two domains were characterized irrespective of one another and each Nuclear Magnetic Resonance (NMR) spectrum was found to be similar to its respective region in the whole protein length [11, 23]. The structural characterization has revealed that six cysteine residues in the first domain can accommodate two Zinc (II) or Cadmium (II) ions to form a binuclear cluster of M(H)2Cys6 [23]. Even though, there has been some ambiguity in the thiolate bridges, this type of cluster was the first of its kind in the MT super family but also found in fungal transcription factors GAL4 [24]. In the second domain, eleven cysteine and two histidine binds to four zinc ions forming an exceptional mononuclear ZnCysHis site and a Zn3Cys9 cluster.

IV. Metal Binding Dynamics

In terms of metal affinity, pH(1/2) stood at 4.53 of Type 4 pMT’s measures at a low ionic strength of (ca. 10Mm) was found to be similar to that of zinc (II) bound mammalian MTs [25]. In contrast, competition with the chelator 5F-BAAFTA determined the stability constant which also indicated that MT4 binds Zn (II) with more lower affinity which stood at (log K = 10.7 and an ionic strength of 4mM) than found in mammals and bacterium MTs [17]. Specifically, low affinity of (log K = 8.6) observed at a higher ionic strength of (CA. 105 mM) which is physiologically relevant. The visible differences between the above measures may be limited to varied experimental conditions and the metal removal mechanisms but may also be linked to the presence of binding sites with different affinities, but the most logical conclusion may be the shape of the pH titration curves and the various metal binding protein structures [8].

Considering the proposed function of Type 4 pMT’s which includes provision of Zn (II) to the relevant processes during seed germination, it will be interesting to find out about its ability to transfer Zn (II) ions to other molecules.

V. Conclusion

Binding in the grain aleuronic layer and can act in putative storage as shown in barley MT4, which coordinates zinc and has a putative storage function, this was immunologically confirmed to be associated with embryo and aleuronic layer of developing grains. However, structural information and understanding of metal binding dynamics of these pMT’s are scanty, with early cysteine MT of wheat as the only pMT with crystal 3D structure currently available. Also, it was suggested that, MT4 qualifies this barley MT as an obvious potential candidate for future biofortification approaches aimed at increasing zinc food and seed concentration.

### REFERENCES


