



Comparative Analysis of Some Fungal Ferritins Belonging to Microsporidia & Ascomycota

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Abstract:

Iron is an extremely important element whose function is involved in the oxygen transport and it participates in the electron transfer reactions and various redox potential reactions. Ferritin, the globular cytoplasmic protein is highly conserved and is involved in the regulation of the flow of iron into and out of the cell. Ferritins play a key role in preventing Fe toxicity because of their ability to sequester several thousand Fe atoms in their central cavity in soluble, nontoxic bioavailable form. The structural integrity of the protein makes it convenient to store 45,000 molecules of iron in it. In this paper we have worked on the analysis of ferritin from different species of Fungi using bioinformatics tools. By these tools it was found that the myco ferritin of fungi belonging to the same species was closely related and is highly conserved.

Key words: Iron, Ferritin, Electron transfer reactions, Redox Potential, MycoFerritin.

Introduction:

Iron is a key element for the growth and development of any cell or organism. Eukaryotic cells, entering the S-phase of the cell cycle, up regulate transferring receptor-1 expression to obtain iron from the extracellular environment. Iron is a co-factor ribonucleotidreductase, which is necessary for DNA synthesis, and of oxygen transporters in mitochondria, and activates the cyclin/cyclin-dependent kinase complexes, thus regulating the progression from the G1- phase to the S-phase of the cell cycle^[1]

Anytime iron exceeds the metabolic needs of the cell it may form a low molecular weight pool, referred to as the Labile Iron Pool (LIP), which catalyzes the conversion of normal by- products of cell respiration, like superoxide anion (O₂⁻) and hydrogenperoxide (H₂O₂), into highly damaging hydroxyl radical(HO.)^[2].

The highly reactive hydroxyl radical (HO*) generated from an interaction between superoxide (O₂⁻) and hydrogen peroxide (H₂O₂) was proposed (with

Joseph Weiss) in Professor Haber's final paper.

[Haber-Weiss reaction (commonly referred to as the iron-catalysed Haber-Weiss reaction)]^[3]



Iron is toxic in uncontained situations because it catalyzes the production of free radical. Thus iron in the cell is stored in "FERRITIN".

Ferritin, the iron storage protein (450 kDa), plays a dynamic role in iron metabolism by sequestering toxic free iron^[4] Structurally, it consists of a central ferric hydroxy phosphate core surrounded by an outer protective protein shell called Apo ferritin. The iron content of the core ranges from 0 to 4500 iron atoms per molecule and is a reflection of somatic iron reserves^[5] Ferritin, the globular cytoplasmic iron storage protein is highly conserved. Ferritin is a protein of 24 peptide subunits assembled into a hollow shell of molecular weight 4,74,000g/mol (Briatet al., 1999). In iron rich conditions ferritin acts as iron sequestering protein, protecting cells against iron toxicity and at low iron conditions it acts as a source of iron ions necessary for iron-containing protein synthesis. However, the physiological mechanism of iron release from ferritin remains obscure

Apo ferritin part of Ferritin binds to the free ferrous iron and stores it in the ferric state i.e. catalyzes the oxidation of Fe²⁺ to Fe³⁺ state. The central core of the ferritin has a ferroxidase activity. Oxidation at specific site on the protein is involved in the initial phase of core formation, it is generally accepted that once a critical nucleus of Fe³⁺ ions has been formed, and has begun to hydrolyze, it will then act in an autocatalytic manner to promote crystal growth on the surface of the initial biomineral core.

Among different members of Fungi 3 different types of Ferritin's were found. And the three types of ferritins that have been described were

- (i) Mycoferritin, which resembles mammalian ferritins;^[6]
- (ii) Zygoferferritin, a unique form of ferritin found only in the zygomycetes;^[7]
- (iii) A Bacterioferritin found in Absidia spinosa^[8]

Under conditions of extreme iron stress, fungi produce low molecular-weight (*Molwt*, 1,500) ferric iron chelators known collectively as siderophores. Most of the fungal siderophores are hydroxamates. However, the zygomycetes form iron-regulated polycarboxylates and these are well documented.

Materials and Methodology:

In our paper we have worked on comparing the ferritin protein sequences among the 5 different species of the Fungi. And they are

- 1) *Encephalitozoon cuniculi* GB-M1 with the accession no. NP_585894.1,
- 2) *Enterocytozoon bieneusi* H348 with the accession no. XP_001828079.1,
- 3) *Penicillium marneffeii* ATCC 18224 with the accession no. XP_002144995.1, all these sequences are available at NCBI <http://www.ncbi.nlm.nih.gov/>
- 4) *Encephalitozoon intestinalis* ATCC 50506 with the accession no. XP_003073099.1,
- 5) *Talaromyces stipitatus* ATCC 10500 with the Accession no. xp_002340386.1

The comparative analysis was done by the use of the online tools like

1. ProtParam:

The primary structure analysis of proteins was done with the help of the ProtParam. ProtParam is a tool which computes various physico-chemical properties like molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

2. GOR:

The secondary structure analysis was done using the tool GOR. The GOR method is based on information theory and was developed by J.Garnier, D.Osguthorpe and B.Robson (*J.Mol.Biol.*120,97, 1978). The program gives two outputs, one eye-friendly giving the sequence and the predicted secondary structure in rows, H=helix, E=extended or beta strand and C=coil; the second gives the probability values for each secondary structure at each amino acid position. The predicted secondary structure is the one of highest probability compatible with a predicted helix segment of at least four

residues and a predicted extended segment of at least two residues.

3. Prosite:

For the identification of domains we have used Prosite PROSITE currently contains patterns and profiles specific for more than a thousand protein families or domains.

4. Nuclear export signals:

Nuclear export signals (NES) are extremely important regulators of the subcellular location of proteins. This regulation has an impact on transcription and other nuclear processes, which are fundamental to the viability of the cells. The NES are calculated by considering the Hidden Markoff Model and Neural Networks for each residue to the given sequence.

5. YLOC:

YLoc (Yeast Localisation) is an interpretable prediction system for protein subcellular localization prediction. In addition to the predicted location, YLoc gives a reasoning why this prediction was made and which biological properties of the protein sequence lead to this prediction. Moreover, a confidence estimate helps users to rate predictions as trustworthy. YLoc+ is able to predict the location of multiple-targeted proteins with high accuracy

Results:

By the use of the protparam for above 5 ferritin sequences it was found that leucine has the highest frequency of occurrence compared to other amino acids. The *Enterocytozoon bieneusi* H348 has a low frequency of leucine when compared to the other species. Theoretical PI, isoelectric point (pH at which a protein carries no net charge) was found to be high in *Penicillium marneffeii* ATCC 18224 and in *Talaromyces stipitatus* ATCC 10500. After analyzing with GOR all the amino acids that participate in the formation of Alpha Helix were identified

Table1: Summary of the results of PROTPARAM & GOR.

No	Accession Number	Total no. of A.Acids	Helices	Extended strand	Random coil	High % of A.A	Theoretical PI	No of - vely charged A.A (Asp +Glu)	No of +vely charged A.A (Arg +Lys)	Instability index
1	NP_585894.1 Encephalitozoon cuniculi GB-M1	176	52.27%	8.52%	39.20%	Ala(A)18 10.2% Leu(L)18 10.2% Ser(S)18 10.2%	5.11	24	19	41.09 unstable protein
2	XP_001828079.1 Enterocytozoon bienewisi H348	166	57.83%	10.24%	31.93%	Ile(I)18 10.80% Lys(K)17 10.2%	5.63%	24	18	22.01 stable protein
3	XP_003073099.1 Encephalitozoon intestinalis ATCC 50506	176	51.70%	15.91%	32.39%	Glu(E)21 11.90% Leu(L)19 10.8%	4.59%	31	17	43.05 unstable protein
4	XP_002144995.1 Penicillium marneffei ATCC 18224	244	31.97%	18.85%	49.18%	Leu(L)29 11.09%	6.97	32	31	44.20 unstable protein
5	XP_002340386.1 Talaromyces stipitatus ATCC 10500	244	48.77%	12.70%	38.52%	Leu(L)30 12.3%	6.7	30	28	50.34 unstable protein

The 2 analyses namely Protparam and GOR have revealed that the species Encephalitozoon cuniculi GB-M1, Enterocytozoon bienewisi H348, Encephalitozoon intestinalis ATCC 50506 which belongs to Microsporidia phylum are closely related than the Penicillium marneffei ATCC 18224, Talaromyces stipitatus ATCC10500 which belongs to Ascomycota phylum With the use of the tool Prosite it was found that the initial pattern of the domain region in all the 5 sequences was similar and it was found to be of same length and almost all the domains showed GLUTAMIC ACID as the amino acid which was involved in the functional activity of the protein.

Figure1: Result obtained by PROSITE

NP_585894.1| [Encephalitozoon cuniculi GB-M1]
MDPKTKQASSDEWNVEAGLLSSQLLLEYN
AAYFYSA CA AHFSRSDVCLKGLASFFRKKS
LDENAQAQKIIGFMNMRELDIEFRAIEAPDI
KKYGKTSGLKACKEFEQTVLSNISTISEIA
RKAGDGAIVQFLEDFIAEQVRSIS
ELSDLCANCARCGDGGVGLFLFDQSLISQH

307

XP_001828079.1| [Enterocytozoon bienewisi H348]
MTSHNNTIIKLLNDAIKLEHSAWYFYNYVSS
IFYKYAYKHL SKKFQNEASEELEHAQKVIKF
MNQLNYHVEF
FQIDNISVDLYSSNQLIHKVFEHA VALEITVLD
HYKKIKYEA EKINNF EISNFVDEFIELQIGEI
KNFKDNLEN AKRCCDTQLGEFIFDNSFK

XP_002144995.1| [Penicillium marneffei ATCC 18224]
MGESARAISEVSGHDLDKWLRTSTFTNELEESI
RGHIHQELTSWLFYRKLASDCSRSNISLHGFS
MLWE
RSAWECLKDMNWLEKYL VTRGGRSKPTNIE
APKFEWPDSPVEPVGPCREAMTVQRKLFDD
LQRLCTLAEKHQDTALVTALETQYLRKHAK
HLKNLGDLLQQVARASKQQGLGLYVLDKELR
HNSGCIPWQSMNDPDSHHER
VSVVTKKIGEGLLLHPHAAHHEGGCHEGKIPSH
XP_003073099.1| [Encephalitozoon intestinalis ATCC 50506]

MNPETREASTDEWKMEAGLLSSQLLLEYNA
 FYFYTACAAHFEDPNVCLKGLAGFFKKSLE
 ENEHAQKIIDFMNMRGLNIEFRTEAPDIKKY
 EKTSDILKASKEFEQTVLDNLTISEIAAKAGD
 ETIVQFLEDFIAEQVESIS
 EFNDLYVNCTRCGEGEVGLFLFDQSLKRH

RGHIHQELTSWLFYRKLAADCSRNLALHGF
 AMLWERSAWECLLDMNWLEKYLVTGGRS
 KPTNIEAPKIDFPDSPVEPVGPCREAFVQKK
 LFEDLQRLVTLAEKQODTALVTAIESRYLRK
 HAKHLKNLGDVQVQVARVSKQHGLGLYLLDS
 ELRRHNGVIPWMSLNDPDSHFE
 SVSVLTKKINEGLALDAHAAHQGGAHGAKGSG
 H

XP_002340386.1 [Talaromyces stipitatus ATCC
 10500]

MGESAGSIISDVSHQNIDTWLRPSTFTNELEESV

Table2: NES results:

S no	Organism	Seq Pos residue	ANN	HMM	NES	Prediction
1.	Encephalitozoon cuniculi GB-M1	Sequence 27-L	0.612	0.498	0.837	Yes
2.	Enterocytozoon bienewisi H348	Sequence- 135-I Sequence- 118-I	0.592	0.498	0.745	Yes
3.	Encephalitozoon intestinalis ATCC 50506	Sequence- 25-L	0.608	0.302	0.540	Yes
4.	Penicillium marneffei ATCC 18224	Sequence- 133-R Sequence- 134-L	0.523	0.470	0.784	Yes
5.	Talaromyces Stipitatus ATCC 10500	Sequence-133-R Sequence-225-L	0.420	0.426	0.612	Yes
			0.499	0.427	0.698	Yes
			0.604	0.294	0.531	Yes
			0.716	0.484	0.760	Yes

By the submission to the NES it was found that the amino acids Leucine and Isoleucine which are at the positions 130-135 will participated in the protein export

Table 3 Location of the Ferritin obtained by YLOC:

S.no	Organism	Cytoplasm	Nucleus	Mitochondrion	Confidence
1.	NP_585894.1[[Encephalitozoon cuniculi GB-M1]	81.3%	15.9%	1.6%	0.14
2.	XP_001828079.1[Enterocytozoon bienewisi H348]	70.6%	29.2%	0.2%	0.33
3.	XP_003073099.1Encephalitozoon intestinalis ATCC 50506]	58.2%	41.6%	0.2%	0.39
4.	XP_002144995.1[[Penicillium marneffei ATCC 18224]	58.1%	41.6%	0.3%	0.42
5.	XP_002340386.1[Talaromyces stipitatus ATCC 10500]	69%	29.4%	0.7%	0.51

From the above table it can be inferred that: Ferritin in cytoplasm was low in Penicillium marneffei ATCC18224 and Penicillium marneffei ATCC 18224 and as high as 81% of the protein was concentrated in cytoplasm of Encephalitozoon cuniculi GB-

Conclusion:

Ferritin is a molecule which acts as buffer in controlling the flow of Iron in the cell. In case of Fungi the, MycoFerritin plays an important role in the normal growth of the cells and for its pathogenicity. It has been found that Myco Ferritins are highly localized in cytoplasm and a little amount of it is present in the mitochondria (refer table 2). The domain region in all the above selected MycoFerritinis was found to start with similar amino acid and was found to be of similar length with respect to domain. The iron storing capacity of the MycoFerritin is because of its structural integrity. The alpha helix conformation of the protein is central criteria for the molecule to store 24000 iron molecules in it. It was found that the mycoferritin is highly conserved among the species of the same phylum i.e. the fungi of Ascomycota as compared to the Mycoferritin of species of Microsporidia. Thus by this we can say that the ferritin is functionally conserved among and between the phyla of fungi.

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