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Computer-Assisted analysis of subcellular localization signals and post-translational modifications of human prion proteins

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ABSTRACT

In the present work, computational analyses were applied to study the subcellular localization and posttranslational modifications of human prion proteins (PrPs). The tentative location of prion protein was determined to be in the nucleolus inside the nucleus by the following bioinformatics tools: Hum-PLoc, Euk-PLoc and Nuc-PLoc. Based on our results signal peptides with average of 22 base pairs in N-terminal were identified in human PrPs. This theoretical study demonstrates that PrP is post-translationally modified by: 1) attachment of two N-linked complex carbohydrate moieties (N181 and N197), 2) attachmet of glycosylphosphatidylinositol (GPI) at serine 230 and 3) formation of two disulfide bonds between "6–22" and "179–214" cysteines. Furthermore, ten protein kinase phosphorylation sites were predicted in human PrP. The above-noted phosphorylation was carried out by PKC and CK2. By using bioinformatics tools, we have shown that computationally human PrPs locate particularly into the nucleolus.

Keywords: Prion protein; Subcellular localization; Signal peptides; Post-translational Modifications; Bioinformatics

1. INTRODUCTION

Prion diseases or transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative conditions in humans and animals that originate spontaneously, genetically or by infection [1]. Human TSE diseases include sporadic, genetic, iatrogenic and variant Creutzfeldt-Jakob disease (CJD) and sporadic or familial fatal insomnia. Animal counterparts are scrapie in sheep and goats, bovine spongiform encephalopathy, and chronic wasting disease of mule deer and elk [2,3]. The critical pathogenetic events in TSE diseases are conformational changes of the physiological host prion protein (PrPc) into an insoluble form (PrPsc) [4].

Prions are devoid of nucleic acid and seem to be composed exclusively of protein. The normal, cellular PrP is converted into PrPsc through a process whereby a portion of its α-helical and coil structure is refolded into β-sheet [5,6]. This structural transition is accompanied by profound changes in the physicochemical properties of the PrP [7]. At the molecular level, PrP is a sialoglycoprotein of 253 amino acids in human [8]. The C-terminal end is a signal peptide allowing the anchoring of the protein to the plasma membrane via a glycosylphosphatidylinositol residue in the early steps of maturation in the endoplasmic reticulum [9]. The ultimate destination is then the plasma membrane where PrP can be released by phospholipase or protease treatment [10]. Finally, PrP cycles between the plasma membrane and the endocytic pathway. During infection, PrPsc is thought to derive from PrPc after exposure to the plasma membrane [11]. It has been suggested that PrPc can bind a putative ''protein X'' that may function as a molecular chaperone in the formation of PrPsc [12].

The cellular role of the normal host protein PrP is still unknown. Oesch has characterized membrane associated proteins that interact with PrP [13,14]. Immunocytochemical studies reveal that PrPsc and PrPc are present, not only at the plasma membrane or in cytoplasmic compartments [15,16,17], but also in the nuclear compartment, particularly into the nucleolus [18]. The inconsistency in the nuclear localization of PrP between non-infected and infected cells may account for important pathogenetical mechanisms [19].

The posttranslational modification of amino acids expands the range of functions of the protein by attaching it to other biochemical functional groups such as acetate, phosphate lipids and carbohydrates. This occurs by altering the chemical nature of an amino acid or by making structural changes, like the formation of disulfide bridges. The molecular mechanism by which the PrPsc is formed and causes infectivity or neurodegeneration is not known. In an emerging view, post-translational modifications play roles in the transformation of PrPc to PrPsc [20]. Moreover, Post-translational modification of the scrapie prion protein is thought to account for the unusual fea-

tures of this protein [21].

All eukaryotic cells are compartmentalized into separate membrane-bound organelles and require tightly regulated transport of proteins and lipids between these compartments. The function of a protein is closely correlated with its subcellular location. With the rapid increase in new protein sequences entering into data banks, we are confronted with a challenge. Proteins are classified, according to their subcellular locations, into the following 18 groups: cell wall, centriole, chloroplast, cyanelle, cytoplasm, cytoskeleton, endoplasmic reticulum, extracell, Golgi apparatus, hydrogenosome, lysosome, mitochondria, nucleus, peroxisome, plasma membrane, plastid, spindle pole body, and vacuole [22].

Determination of subcellular location of a protein is essential for understanding its biochemical function. These data are hard to obtain experimentally but have become especially significant since many protein sequences are still lacking detailed functional information. To address this rarity of data, many computational analysis methods have been developed. However, these methods have varying levels of accuracy and perform differently based on the sequences that are presented to the underlying algorithm. Giving the huge number of uncharacterized protein sequences, computer-aided analysis of posttranslational modifications and translocation signals from amino acid sequence becomes a necessity.

In this study, we have analyzed subcellular localization, signals peptides and posttranslational modifications of human PrPs.

2. MATERIALS AND METHODS

2.1. Amino Acids Sequence

The sequences of human PrPs were obtained from http://www.ncbi.nlm.nih.gov/sites/Entrez and http//beta.uniprot.org. Accession numbers of human PrPs are shown in **Table 1**.

2.2. Consensus Sequence and Percentage of Different Amino Acids

The consensus sequence was achieved by using Multalin 5.4.1 server and the percentage of different amino acids was calculated by expasy server.

Table 1. Accession numbers of human PrPs

AAD46098	AAG21693	AAO83636	AAC05365
AAC78725	AAV38303	AAB59442	AAB59443
AAA60182	AAO83635	A2A2V1	AAR21603
AAH12844	AAH22532	AAS80162	AAA19664
A1YVW6	ABM85428	ABD63004	ABM82244
ABL75508	BAA00011	CAG46836	CAD62016
CAB75503	CAM27320	CAA56283	CAI19053
CAI19053	CAA58442	CAG46869	EAX10449
EAX10450	NP_000302	NP_001073592	NP_001073591
NP_898902	NP_001073590	O75942	P04156
P23907	Q6FGR8	Q5QPB4	Q53YK7
Q6FGN5	Q540C4	Q6SES1	Q5U0K3
Q86XR1			

2.3. Prediction of Signal Peptides

Signal-CF server was employed to study the signal peptides. The web interface to the Signal-CF tool was accessible at http://www.chou.med.harvard.edu/shen. This server is called Signal-CF, where C stands for "coupling" and F for "fusion", meaning that Signal-CF is formed by incorporating the subsite coupling effects along a protein sequence and by fusing the results derived from many width-different scaled windows through a voting system. Signal-CF is featured by high success prediction rates with short computational time, and hence is particularly useful for the analysis of large-scale datasets [23].

2.4. Prediction of Subcellular Localization

Several computational tools for predicting the subcellular localization of a protein are available. In this study, Hum-PLoc, Euk-PLoc and Nuc-PLoc have been utilized to study the localization of prion protein sequences.

Hum-PLoc is a server that analyzes the subcellular localization of human proteins among the following 12 locations: centriole, cytoplasm, cytoskeleton, endoplasmic reticulum, extracell, Golgi apparatus, lysosome, microsome, mitochondrion, nucleus, peroxisome, and plasma membrane [24]. The web interface to this tool is present at http://www URL: http://www.chou.med.harvard.edu/shen.

Euk-PLoc is available as a web-server at http://202.120.37.186/bioinf/euk. A new benchmark dataset is constructed that covers the following 18 localizations: cell wall, centriole, chloroplast, cyanelle, cytoplasm, cytoskeleton, endoplasmic reticulum, extracell, Golgi apparatus, hydrogenosome, lysosome, mitochondria, nucleus, peroxisome, plasma membrane, plastid, spindle pole body, and vacuole [25].

A new classifier, called Nuc-PLoc, has been developed that can be exploited to recognize nuclear proteins among the following nine subnuclear locations: chromatin, heterochromatin, nuclear envelope, nuclear matrix, nuclear pore complex, nuclear speckle, nucleolus, nucleoplasm and nuclear promyelocytic leukemia (PML) body. As a user-friendly web-server, Nuc-PLoc is accessible at http://chou.med.harvard.edu/ bioinf/Nuc-PLoc [25].

2.5. Analysis of Posttranslational Modifications

N-myristoylation, N-glycosylation, protein kinase C, casein kinase II and Serine, threonine, tyrosine phosphorylation sites were predicted. Expasy which is available at www.expasy.ch/tools was applied for this purpose. Big-PI server was utilized to study the glycosylphosphatidylinositol (GPI) anchor signal [26]. The web server http://clavius.bc.edu/~clotelab/DiANNA was chosen for prediction of disulfide bonds [27].

3. RESULTS

3.1. Sequences and Signal Peptides

Number of amino acids and molecular weight of human consensus sequence of prion protein were 253 and 27661.1 respectively. The consensus sequence of human PrPs is shown in **Figure 1**.

```
          10         20         30         40         50         60         70
MANLGCWMLV LFVATWSDLG LCKKRPKPGG WNTGGSRYPG QGSPGGNRYP PQGGGGWGQP HGGGWGQPHG
          80         90        100        110        120        130        140
GGWGQPHGGG WGQPHGGGWG QGGGTHSQWN KPSKPKTNMK HMAGAAAAGA VVGGLGGYML GSAMSRPIIH
         150        160        170        180        190        200        210
FGSDYEDRYY RENMHRYPNQ VYYRPMDEYS NQNNFVHDCV NITIKQHTVT TTTKGENFTE TDVKMMERVV
         220        230        240        250
EQMCITQYERES QAYYQRGS SMVLFSSPPVILLISFLIFL IVG
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Figure 1. Consensus sequence of the human prion protein

Percentage of different amino acids in the protein was calculated (**Table 2**). The most prevalent amino acid was glycine (45 residues), and the least one was cysteine (4 residues).

To identify functional signal peptides in the human PrP, 49 FASTA format sequences of prion protein input in Signal-CF sever. Signal peptide sequences of human PrPs were sorted in 4 groups based on their length (**Table 3**).

3.2. Prediction of Subcellular Localization

The subcellular distribution of PrP proteins was verified by Euk-Ploc, Hum-Ploc and Nuc-ploc. These results showed a strong tendency of the protein to nucleus and especially to nucleolus.

3.3. Prediction of Post-translation Modifications

It is interesting that the post-translational modifications alone, or in combination with amino acid changes, play dominant roles in the pathogenic transformation of PrP(C) to PrP (SC). According to our analysis 2 asparagines in positions 181 and 197 were predicted to be glycosylated. Results also showed that threonine in positions 107, 183, 190, 191, 192, and 193 and serine in position 132 were predicted to be kinase C phosphorylated. Serine in position 143, and threonines in positions 201 and 206 were expected to be casein kinase II phosphorylated and no glycine was predicted to be myristoylated. Disulfide bridges in cysteine residues at positions 6–22 and 179–214 were predicted.

GPI anchors, which allow the attachment of proteins to the extracellular leaflet of the plasma membrane, were also analyzed. Glycosylphosphoatidylinositol (GPI) lipid anchoring is a common posttranslational modification known mainly in extracellular eukaryotic proteins. Attachment of the GPI moiety to the carboxyl terminus (omega site) of a polypeptide happens following proteolytic cleavage of a C-terminal propeptide (**Figure 2**).

The best predicted site was G229 and the second best was S230 (underlined). Furthermore, potential phosphorylation sites of serine, threonine and tyrosine in the human PrPs were determined (**Table 4**).

Table 2. Residue composition for consensus sequence of human PrPs

A	%4	10	C	%1.6	4	D	%2.4	6	E	%3.6	9	F	%2.8	7
G	%17.8	45	H	%4.0	10	I	%3.6	9	K	%4.0	10	L	%4.7	12
M	%4.7	12	N	%4.7	12	P	%6.7	17	Q	%5.9	15	R	%4.3	11
S	%5.9	15	T	%5.1	13	V	%5.5	14	W	%3.6	9	Y	%5.1	13

Table 3. Categories of signal peptides of human PrPs

Position of signal peptides	1-14	1-15	1-22	1-24
Number of signal peptides	1	8	38	2

Table 4. Predicted phosphorylation sites of consensus sequence of human PrPs

Amino acid	Phosphorylation position			
Ser	43	143	23	231
Thr	191	192	163	-
Tyr	145	149	169	225

MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGG
GGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKP
KTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRENMHRYPN
QVYYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQM
CITQYERESQAYYQRGS SMVLFSSPPV ILLISFLIFL IVG

Figure 2. GPI lipid anchoring signals sequence

4. DISCUSION

The goal of this investigation was to apply bioinformatics methods to study the subcellular localizations, signal peptides and posttranslational modifications of human PrPs.

4.1. Identification of Signal Peptides in PrP

Based on our results, there were signal peptides with average 22 bp in N-terminal of human PrPs. According to a survey, conducted by Alexandre and his colleagues, PrP does not contain a nuclear localization signal and that, in normal conditions, PrP cannot be released in the cytosolic compartment remaining membrane bound till its degradation and in infected cells, PrP can interact with a molecule to form a complex able to be released in the cytosol and then targeted enters the nucleus [18]. However in another investigation, the presence of two independent nuclear localization signals in the N-terminal region of PrP was observed. The first signal included residues 23–28, and the second one included residues 101–106 of PrP [29].

Protein signals have become crucial tools for researchers to construct new drugs which are expected to enter a particular organelle to correct a specific defect. For example, by adding a specific tag to a desired protein, one can tag it for excretion, making it much easier to harvest. To use such a tool successfully, first one has to identify the signal sequences. Since the number of nascent protein sequences entering databanks is rapidly increasing, it is time consuming and expensive to identify the signal peptides entirely by experiments [28].

4.2. Determinants of Subcellular localization of PrP

Protein subcellular localization prediction has been widely studied (reviewed in [30,31]). Available servers differ in many aspects including the computational method, the type and diversity of protein characteristics, the localization coverage, the target organism(s) and the reliability. Servers can be grouped into 4 general classes based upon the protein characteristics that are considered: amino acid composition and order based predictors [32,33,34], sorting signal predictors [35,36], homology based predictors [37,38] and hybrid methods that integrate several sources of information to predict localization [39,40]. Nowadays, the importance of developing a powerful high-throughput tool to predict protein subcellular location has become obvious [41].

In the present study, the tentative location of prion protein was determined to be in the nucleolus inside the nucleus by bioinformatics tools, Hum-PLoc, Euk-PLoc and Nuc-PLoc. There are different opinions regarding the subcellular localization of PrP. Stahl and his colleagues considered a signal peptide at the C-terminus of prion protein allowing the anchoring of the protein to the plasma membrane via a glycosylphosphatidylinositol residue in the early steps of maturation in the endoplasmic reticulum [8]. Oesch has characterized membrane-associated proteins that interact with PrP [12,13]. More recently, it has been shown that the 37-kDA laminin receptor interacts with PrP [42].

A number of cellular proteins, among them the nuclear lectin CBP35, was identified that bound to the predicted RNA stem-loop structure of PrP RNA. CBP35 could also be detected in purified infections prions, [43]. Moreover, the presence of PrP in the nucleus and its subnuclear location in the nucleolus has been reported [17,44].

In addition, Gu and his coworkers demonstrated that nuclear accumulation of PrP fragments was mediated by nuclear localization signals in the N-terminal domain of PrP that became functional under certain conditions and might contribute to the pathogenesis of certain prion disorders [29].

4.3. Post-translational Modifications of Consensus Sequence of PrP

Our analysis shows that PrP is post-translationally modified by the attachment of two N-linked complex carbohydrate moieties (N181 and N197) and a GPI anchor at serine 230 as well as by the formation of a disulfide bond between 6−22 and 179−214 cysteins.

Glycosylation is one of the most complex and ubiquitous post-translational modifications of proteins in eukaryotic cells. It is a dynamic enzymatic process in which saccharides are attached to proteins or lipoproteins, usually on serine (S), threonine, asparagine, and tryptophan residues. Glycosylation, like phosphorylation, is clinically important because of its role in a wide variety of cellular, developmental and immunological processes, including protein folding, protein trafficking and localization, cell-cell interactions, and epitope recognition [45,46,47,48,49,50]. The number of glycosylation sites in our work is in agreement with the results obtained by molecular cloning of a PrP cDNA [20]. It has already been shown also that addition of one or two N-glycans causes retention of the N-terminal PrP fragment in the endoplasmic reticulum in a partially aggregated form, and a small amount is secreted into the medium. Presence of two glycans in the N-terminal fragment is more conducive to proper folding and secretion into the medium than one glycan, which largely remains in the ER [29].

In GPI anchors, a hydrophobic phosphatidylinositol group is linked to a residue at or near the C-terminus of a protein through a carbohydrate-containing linker. GPI anchor addition is both structurally and functionally related to another important post-translational modification, prenylation, in which hydrophobic farnesyl or geranylgeranyl moieties are added to C-terminal cysteine residues of target proteins. Additionally, GPI anchors proteins to the cell membrane [51]. Although we determined the nucleus as the tentative location for prion proteins, this fact also should be taken in mind that according to a previous study GPI anchor and the N-glycans function in a complicated way to reduce the tendency of PrP for localization in nucleus [30].

In our study, 10 protein kinases phosphorylation sites were predicted in the human PrPs. The addition of a phosphate molecule to a polar R group of an amino acid residue can turn a hydrophobic portion of a protein into a polar and extremely hydrophilic part. In this way, it can introduce a conformational change in the structure via interaction with other hydrophobic and hydrophilic residues in the protein. Moreover, phosphorylation may modulate PrP biological activity. Regarding the bonding states of cysteine also, it has been found out that it plays important functional and structural roles in proteins. Particularly, disulfide bond formation is one of the most important factors influencing the three-dimensional fold of proteins [52].

In conclusion, this study can help in better understanding of signal peptides of prion proteins. Generally, our results indicate the role that bioinformatics can play in analysis of proteins modification and localization.

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