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Unfoldin, A Novel Tool for the Analysis of Protein Misfolding or Neurodegenerative Diseases

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Abstract

The achievement of accurate protein folding in living cells is not easy. Many newly synthesized proteins fail to adopt the correct conformation, and such misfolded proteins are more likely to form aggregates. These aggregates lead to the collapse of cellular proteostasis and the formation of inclusion bodies inside or outside the cell membrane, thus exhibiting toxicity to neurons, in particular. This phenomenon has been a major causative factor of neurodegenerative diseases, including protein-misfolding/aggregation diseases.

The solubilization of aggregated proteins remains an unsolved problem for protein analysis, because only solubilized proteins can be subjected to biochemical analyses. Therefore, we are all so still in limited supply for developed LC-MS/MS. The solubilization of highly aggregated proteins requires highly concentrated denaturants or detergents; however, these chemicals also effective to the second step for the biochemical analysis. This issue is particularly concerning when the problem of aggregates arises during LC-MS/MS analysis.

In this mini review, we introduced a novel oligomeric protein with solubilization activity from yeast cells, and called it Unfoldin. Unfoldin exhibited an ATP-dependent activity, and it had no substrate specificity in vitro. This protein oligomer serves as a powerful tool for developing a new strategy for processing aggregation-prone proteins for biochemical analyses.

Keywords: Protein misfolding disease; Neurodegenerative disease; Proteostasis; Aggregation; Biochemical analysis; LC-MS/MS; Unfoldin

Introduction

The accurate synthesis, folding, and degradation of proteins are extremely important to life; otherwise, it could cause various protein-misfolding diseases. However, genetic mutations alter the folding of proteins because of changes in amino acid sequences, and some proteins, such as prions, undergo spontaneous changes in conformation. Consequently, these conformational changes lead to protein-misfolding diseases [1,2], such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), and Creutzfeldt-Jakob disease (CJD) [3]. Defective proteins tend to accumulate in neurons, because these cells do not undergo cell division. Inclusion bodies, which are implicated in diseases, serve as important clues to investigate disease etiology. The recent development of LC-MS/MS has helped us in analyzing disease-related proteins [4-7]; however, it is usually difficult to obtain optimal results with aggregation-prone proteins [8,9]. The dissolution of such proteins is difficult, and certain quantities of the insoluble proteins remain as residues. Such residues hinder biochemical analyses, and this could be one of the reasons for the limited number of preventive and therapeutic approaches to protein-misfolding diseases.

Here, we introduce our novel molecular chaperone, unfoldin, which has helped in providing an alternative approach to the analysis of aggregation-prone proteins.

Collapse of Proteostasis

Patients with neurodegenerative diseases present with neurological and psychological symptoms and undergo progressive degeneration and loss of neurons. Recently, it has been considered that these diseases are caused by the collapse of proteostasis [10]. "Proteostasis" refers to the delicate balance between cellular systems that regulate the synthesis, folding, localization, and degradation of proteins. The maintenance of proteostasis is important when cells are exposed to various conditions of stress, and thus, this concept is named based on homeostasis [11-15].

Once proteostasis collapses, aggregation-prone proteins escape the degradation pathways, such as the ubiquity in-proteasome [16-19] and autophagy-lysosome pathways [20-23]. Upon oligomerization, proteins sometimes exhibit neurotoxicity [24], and their accumulation inside or outside cells may result in the formation of inclusion bodies; these phenomena gradually cause cells to be affected by protein-misfolding diseases.

In most patients, protein-misfolding or neurodegenerative diseases exhibit sporadicity. This implies that the disease-related proteins undergo spontaneous changes in their structure, and these proteins provide us with clues for investigating the disease pathogenesis. Nonetheless, these proteins exhibit highly aggregated structures, and therefore, the insolubility of these proteins continues to pose challenges to biochemical analyses.

Protein-Unfolding Factor, Unfoldin

Unfoldin, the gene product of YDL178W, was first identified from the yeast S. cerevisiae as actin-interacting protein2 [25], and then as D-lactate dehydrogenase2 [26]. Next, we purified it using a protease sensitivity assay with trypsin [27]. The purified sample exhibited strong protein-unfolding activity in an ATP-dependent manner, and exhibited no substrate specificity in vitro. Based on this activity, we named this protein as Unfoldin. Interestingly, the molecular weight of Unfoldin was determined to be 59KDa by SDS-PAGE; however, using gel-chromatography, it was found to be ~700KDa. This implied that Unfoldin forms an oligomer that consists of 10-12 monomeric proteins. Electron microscopy revealed that Unfoldin forms a grapple-like structure with a diameter of ~10nm and a central cavity of ~2nm [28]. Unfoldin adopts two states: an open state in the presence of ATP and a closed state in the absence of ATP. The oligomerization of Unfoldin appears to be dependent on the C-terminal coiled-coil region of this monomeric protein, since a deletion mutant that lacked this region did not exhibit the oligomeric form. The robust protein-unfolding activity of Unfoldin increased the sensitivity of the proteins that are highly prone to aggregation (such as Aβ-1-42, α-synuclein and prion protein) to protease in-vitro, and these proteins could easily be solubilized with trypsin at a low concentration of 200ng/ml [29].

Recently, we found that YDL178W encodes two gene products. One of them is the L-form, whose molecular weight is 59KDa, which was the same as that of the structural component of the monomeric form of Unfoldin [30]. Another product is the S-form, whose molecular weight is 55KDa, and is shorter than the L-form of the protein. The N-terminal of the S-form is cleaved at the residue Y36/S37, which is recognized by the mitochondrial processing protease, Icp55 [31]. This result showed that the S-form is the mitochondrial protein, Dld2. On the other hand, the N-terminal of the L-form of the protein is not processed, and the full-length protein is localized on the contractile ring during the log phase of cell growth. The physiological role of Unfoldin is not elucidated yet; however, the protein exhibits moonlighting, by which it can perform multiple functions in different locations within the cell [32-35].

A Novel Approach to the Solubilization of Aggregated Proteins Using Unfoldin

In almost all the techniques of biochemical analysis, such as column chromatography, SDS-PAGE and mass spectrometry, solubilization is the most critical step. Recently, LC-MS/MS has revealed a large quantity of information for samples even at the fem to molar order; however, access of aggregated proteins to proteases is still poor in some cases, and thus, it difficult to obtain satisfactory results. However, when the concentration of the denaturant or detergent for solubilization is increased, their elimination would be difficult. In case of LC-MS/MS analysis, next step of protein digestion also disturbed because of deactivation of proteases by the high concentrated denaturant or detergent. Proteins that are implicated in protein-misfolding or neurodegenerative diseases tend to exhibit this phenomenon.

Unfoldin could a ray of light to this need to be solved dilemma, because robust protein-unfolding activity of Unfoldin caused from protein. This advantage does not disturb next step of protein analysis, because “Protein solubilized proteins”. In fact, we solubilized 500 Pick bodies of Pick’s disease, which were isolated by laser micro-dissection [36] using only Unfoldin in the presence of ATP; we detected a novel candidate biomarker, Calmodulin-like skin protein (CLSP) except phosphorylated tau protein [37,38]. Moreover, with the solubilization of Pick bodies using Unfoldin, the sensitivity of western was blotting increased by 100-fold [39].

Thus far, the oligomeric structure of Unfoldin has been found to be unstable, and hence, the maintenance of its structure is difficult; however, unknown co-factors or stabilizing factors could be discovered after the physiological function of Unfoldin is studied.

Conclusion

Although there is a remarkable increase in life expectancy, the number of patients with age-related diseases, such as AD and PD, has increased. Protein-misfolding or neurodegenerative diseases also belong to this category of diseases. Unfortunately, we do not have effective methods for the treatment and prevention of such diseases, because of the challenges to the analysis of aggregation-prone proteins. Unfoldin, which was first found to be encoded by YDL178W in yeast, forms oligomers, and exhibits robust protein-unfolding activity in vitro. This activity could help in further analysis of protein-misfolding diseases, and be useful for basic biochemical studies in the future.

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Conflict of Interest

The authors declare no competing financial interests.

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