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Spontaneous Determinants of Protein Aging
Mini Review

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Contents:

Abbreviations
Advanced glycation endproducts (AGPs).
Amino acids (AAs).
Deoxyribonucleic acid (DNA).
Heat shock proteins (HSPs).
Local minimums of Gibbs free energy (E^{min}).
Post-translational modification (PTM).
Unfolded protein response (UPR)

Key words: protein aging**,** spontaneous post-translational modification, racemization, glycation, glycosylation.

There is no conflict of interest.

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Abstract

The universal chirality is the commonly accepted view of nature. Biological chirality is the distinct part of the more general phenomena. Following this view, all living organisms are characterized by the non-equilibrium state of their molecular constituents. From the thermodynamic perspective, the non-equilibrium state of biomolecular ensemble holds inevitable consequences being the substrate of spontaneous reactions directed to equilibrium (not associated with life) state.

At the protein level, spontaneous biological reactions represent the natural part of proteins' post-translational modifications (PTMs). The essential contribution to the origin and maintenance of the non-equilibrium state belongs to prevalent bio-molecular chirality. Correspondently, spontaneous PTMs such as racemization and glycation, working against life-supporting prevalent chirality, are known as the significant determinants of protein misfolding, dysfunctions, and aggregation. Accumulation of aberrant protein during life-span allows consideration of time-dependent spontaneous racemization and glycation as protein aging. Spontaneous PTMs of proteins is occurring in the interaction with other forms of enzymatic and non-enzymatic PTMs.

In this review, we are considering the contribution of spontaneous racemization and non-enzymatic glycosylation to protein aging.

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Introduction

The prevalent molecular chirality plays a causal role in neuronal polarity and motility, which in turn, are the cellular determinant of the brain bilaterality and the asymmetry of perceptual, cognitive, and behavioral functions [1,2,3]. Generally speaking, brain-body mediated interaction of the internal bio-molecular chirality with the external environment's chiral factor occurs due to the hierarchical chain of chirality transfer [1] associated with the hierarchical organization of living systems [4]. Aging, identified as a time-dependent process leading to the loss of biological functions and the onset of age-related diseases, should be explored across the matrix of hierarchical domains. The multi-modal determinants of lifetime involve complex bio-chiral events, most of which are not entirely understood. The hierarchical relationship of molecular, phenotypic, and functional aging domains recently gains attention [5]. The development and longevity of organisms are linked through adaptation to the environment [6,7]. Such evolutionary adaptation occurred through the hierarchical levels of biological complexity: molecular [10], cellular, organ, and organism [8,9].

At the organism level, aging is characterized by the progressive loss of physiological and perceptual functions and terminated by death. A species-specific lifetime duration makes it plausible that the aging of molecular constituencies, cells, and the organism is programmed in genes [11] and mediated by the epigenetic impact [12].

At the molecular level, aging is influenced by the transcriptional and post-transcriptional regulatory mechanisms involving crosstalk of DNA and protein pathways [13,14,15]. In all animal species, the biological clock of molecular aging is associated with epigenetic changes involving DNA methylation and post-translational modification (PTM) of proteins [16]. Recent development reveals that protein turnover and pathological protein aging are mediated by the set of enzymatic and spontaneous PTMs (see Fig. 1) [14, 17].

Proteins Aging: Spontaneous Age-dependent PTMs

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Various theories of molecular aging have been proposed. As the most abundant and structurally diverse components, proteins represent a suitable platform for analyzing age-associated molecular mechanisms. Most studied mechanisms are linked to spontaneous non-enzymatic PTMs [18] * such as oxidation [19], nitration [20], racemization [14] and glycation [21]. Non-enzymatic PTMs are recognized as an integral part of cellular metabolism [22]. In the network of PTMs, seemingly distinct biological reactions such as phosphorylation and racemization [13] or racemization, glycation, and glycosylation, [19] share many common chemical, metabolic and thermodynamic attributes. Spontaneous racemization and accumulation of advanced glycation end-products (AGPs) have been most intensively studied concerning protein aging, aggregation, and dysfunctions in the fields of neuroscience [1-3] and forensic applications [15]. In our review, we are considering the contribution of spontaneous racemization [13, 23] and non-enzymatic glycosylation [24 - 26] to protein aging.

Racemization

The spontaneous transformation of L-AAs to D-AAs (mirror images of each other) in peptides and proteins is associated with the dramatic changes in the three-dimensional (3-D) orientation of the molecular orbitals. Therefore, racemization can significantly alter not only primary, but secondary and even higher-order protein structures, thereby leading to alterations in corresponding cellular functions (the example of an exception is leucine) [27]. The racemization may also play a role in protein proteolytic stability as L-enzymes (including proteases) would not work on D-substrates. Since racemization is a time-dependent phenomenon, it can be used to determine the relative age and turnover rates of long-lived proteins [20]). Furthermore, in some diseases (e.g., musculoskeletal diseases [20] and likely in the normal aging, turnover rates can be altered, generating pathologically racemized proteins. Due to the

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* *The non-enzymatic and enzymatic reactions obey the same thermodynamic principles, As a result, every enzymatic reaction can occur in principle also non-enzymatically [18].*

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advances in chiral proteomics, it has become known that protein misfolding and malfunction, associated with the snowballing and accelerated accumulation of aberrant proteins, correlate with the irreversibility of age-related and disease pathology [28, 29].

Glycation

Glycation has been studied for over 100 years. However, its importance in biology, the science of aging, medicine, food and nutrition, pharmacology and toxicology, and technological processing remains intriguingly undisclosed [29]. Understanding the aging mechanism requires paying attention to the crosstalk between different pathways leading to the degradation of cellular homeostasis. Non-enzymatic glycosylation (glycation) is a spontaneous reaction among reducing sugars (such as glucose), proteins, lipids, or nucleic acids leading to the age-associated accumulation of advanced glycation end products (AGEs) [30]. Glycation of proteins occurs by a complex series of sequential and parallel PTM reactions called collectively the Maillard reaction named after the leading pioneer of glycation research, Louis Camille Maillard (1878–1936) [31]. This Millard process is a non-enzymatic reaction between ketones or aldehydes, the amino groups of proteins, lipids, and nucleic acids eventually leading to the appearance of AGEs [32]. The interaction of AGEs with their receptors (RAGEs, which is a multiligand cell surface protein from the immunoglobulin superfamily capable of interaction with various partners, such as AGEs, amphoterin, amyloid fibrils, and S100/calgranulins [33]) is implicated in factors enhancing promoting protein racemization and aggregation (such as oxidative stress [34] and phosphorylation [13]). AGEs were experimentally observed in the seen in the skin [21], heart [35], lung [36], brain [37], and alcohol-mediated tissue injury [38]. AGEs were linked to the pathogenesis of numerous autoimmune, metabolic, and neurological disorders [39, 40] .
They have been identified during the progression of various aging-related diseases, such as diabetes [41], cardiovascular complications [42], kidney malfunctions [43,44], liver disorders [45, 46], lung injury [36], osteoporosis [47], atherosclerosis [48], skin cell melanogenesis [49], cancer [50], alcohol-

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mediated tissue injury [36]. arthritis [51], lupus [52], macular degeneration, cataract [42],and various neurodegenerative diseases linked to the abnormal accumulation of aggregated proteins [53, - 55] (e.g., Alzheimer's disease [56, 57] , Parkinson's disease [58], Huntington's disease [59], multiple sclerosis [60], and amyotrophic lateral sclerosis [61]).

Conclusions

Amino acid racemization and AGE protein formation represent convolution of multistep reactions involving reversible and irreversible modifications of protein-specific amino acids (AAs) [21, 30]. Being spontaneous, age-dependent PTM glycation and racemization target many proteins and peptides {including amyloid-beta (A-β) [62], collagen [63 - 67], and chaperone heat shock proteins (HSPs) [65]} and different cell type (including neurons). In turn, age-related decreases in neurogenesis are the primary causal events contributing to the cognitive decline. The crosstalk between the different forms of spontaneous PTMs is evident in the amyloid aggregates, which are insoluble proteinaceous entities composed of linear unbranched fibrils formed from the misfolded proteins [68, 69]. Proteostasis is a dynamic ensemble of cellular processes and pathways dedicated to the regulation of a balanced and functional proteome. Among the principal constituents of proteostasis are the common cellular pathways for the degradation of misfolded proteins. An important site for the degradation of misfolded proteins and dysfunctional protein aggregates is the lysosomes. Misfolded proteins and protein aggregates are trafficked to lysosomes by the macro-autophagy mechanisms, chaperone-mediated autophagy, and endocytosis [69]. In eukaryotic cell, many damaged, misfolded, or oxidized proteins may also be degraded via the ubiquitin proteasome system (UPS) that conducts the non-lysosomal degradation of such targets. However, the amyloids represents the class of misfolded proteins resistant to proteasomal degradation, in part, due to their modification via the spontaneous non-enzymatic reactions. The representative example is β-amyloid (Aβ) peptides, which are the substrate for spontaneous non-enzymatic age-dependent PTMs, including glycation [70] and

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racemization [13, 14]. It is in logical agreement with the facts that glycated Aβ exhibits prolonged half-life [71], time-dependent isomerization/racemization of several AAs residues [72], misfolding, aggregation [73], and neurotoxicity [74].

Finally, we want to emphasize the fact that practically all glycation-prone proteins contain racemization-prone AA residues, providing the ground for the interaction of two distinct spontaneous determinants of protein aging, thereby defining the link between metabolic and epigenetics pathways of aging [12, 66, 67 - 77].

Afterword

The accumulation of unfolded/misfolded proteins activate a series of cell-specific stress responses, including unfolded protein response (UPR) [78, 79]. In neuronal cells, the aggregates of unfolded [80] and misfolded [81] proteins lead to re-establishing cell homeostasis, dysfunctions of sub-cellular organelles, neurodegeneration, and cell death [82]. The complex of aforementioned events is associate with multiple spontaneous reactions [83, 84]. However, the specific role of non-enzymatic spontaneous PTMs in cellular stress pathways remain mostly unclear [85].

Figure legend

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Fig. 1. Schematic representation of funnel-shaped energy landscapes (entropy as a function of Gibbs free energy) for A-β folding (enzymatic, reversible, non-amyloidogenic PTMs, and aggregation (spontaneous, irreversible, amyloidogenic PTMs). Conformational space for enzymatic and spontaneous phase transitions of protein between distinct protein folding states.

ATP-fueled enzymatic PTMs of proteins drive their substrates out of the thermodynamic equilibrium (toward the metastable native state) [56]. Spontaneous nonenzymatic PTMs drive the entire population of proteins toward thermodynamic equilibrium (to un-native state).

The local minimums of Gibbs free energy (E min) in conformational space provide relative nonequilibrium (NE) stabilization of native proteins (physiological conditions) and irreversible stabilization for misfolded proteins [56]. For misfolded states of proteins, E min can be two distinct categories. The discriminant between them is the condition whether the corresponding local minimum (min) of free energy (E^{min}) is above (I) or below (II) the E^{min} of native (functional b or physiological) state. The first categories (I) (relative E^{min}) are characterized as reversible and dynamic (physiological), while the second (II) (absolute $E^{min)}$ as irreversible, pathological.

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a. The native state (NS) can contain several E^{min} [73].

b Proteins kinetically trapped in the local E^{min} have a prolonged lifetime which increases the probability of the spontaneous PTMs [1,2,13,14]

Fragments are adopted with the alterations from [71, 72, 73].

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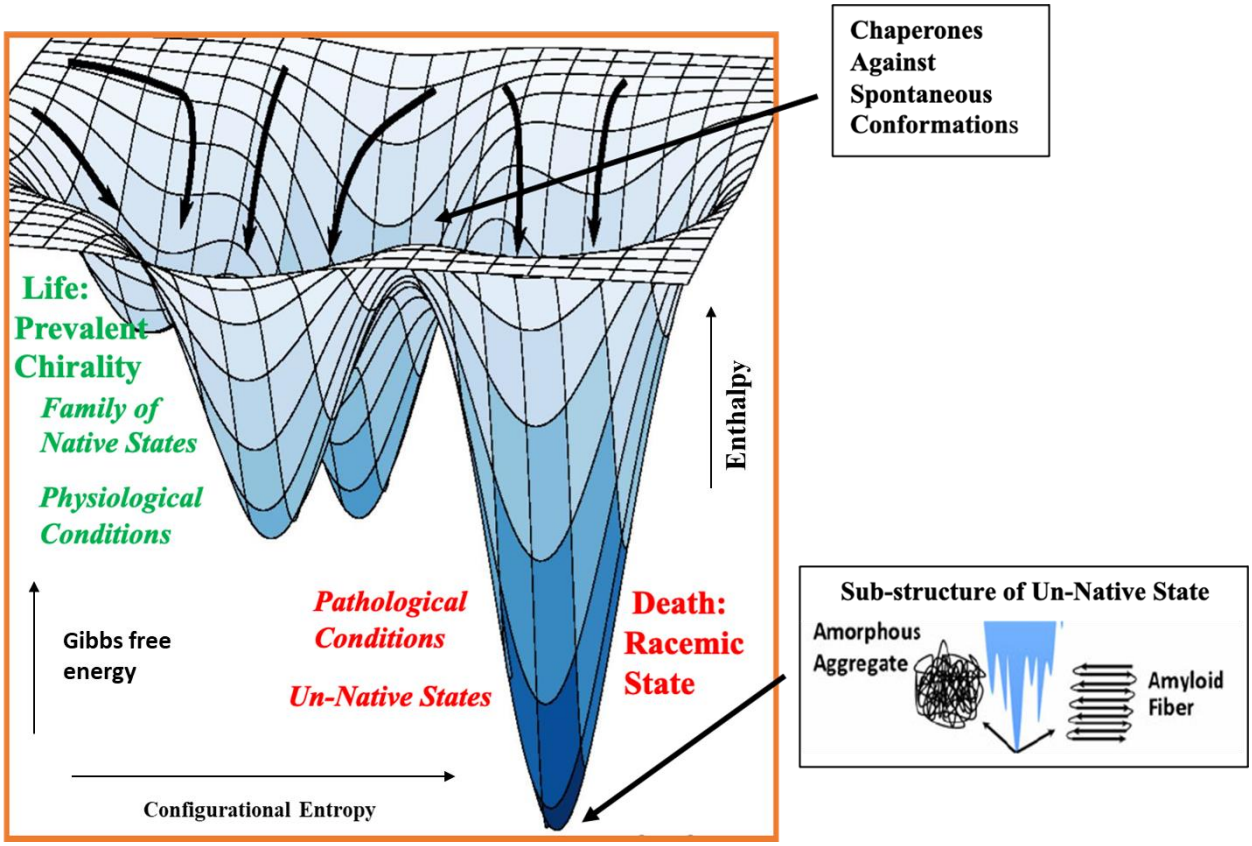

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