Computers in Biology and Medicine 53 (2014) 179–189

Contents lists available at ScienceDirect

Computers in Biology and Medicine

journal homepage: www.elsevier.com/locate/cbm

A content and structural assessment of oxidative motifs across a diverse set of life forms

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ARTICLE INFO

Article history:
Received 25 February 2014
Accepted 16 July 2014

Keywords:
Carbonylation
Sequence comparison
Composition
Mitochondrial protein
Oxidative damage

ABSTRACT

Exposure to weightlessness (microgravity) or other protein stresses are detrimental to animal and human protein tissue health. Protein damage has been associated with stress and is linked to aging and the onset of diseases such as Alzheimer's, Parkinson's, sepsis, and others. Protein stresses may cause alterations to physical protein structure, altering its functional identity. Alterations from stresses such as microgravity may be responsible for forms of muscle atrophy (as noted in returning astronauts), however, protein stresses come from other sources as well.

Oxidative carbonylation is a protein stress which is a driving force behind protein decay and is attracted to protein segments enriched in R, K, P, T, E and S residues. Since mitochondria apply oxidative processes to produce ATP, their proteins may be placed in the same danger as those that are exposed to stresses. However, they do not appear to be impacted in the same way.

Across 14 diverse organisms, we evaluate the coverage of motifs which are high in the amino acids thought to be affected by protein stresses such as oxidation. For this study, we study RKPT and PEST motifs which are both responsible for attracting forms of oxidation across mitochondrial and non-mitochondrial proteins. We show that mitochondrial proteins have fewer of these oxidative sites compared to non-mitochondrial proteins. Additionally, we analyze the oxidative regions to determine that their motifs preferentially tend to make up the connection points between the four kinds of structures of folded proteins (helices, turns, sheets, and coils).

1. Introduction

1.1. The effects of weightlessness on mitochondrial function

The effects of exposure to microgravity or weightlessness for extended periods of time have proven to have negative impacts on mitochondrial protein function. For instance, in Philpott et al. [19] it was found that morphological changes were observed in the left ventricle of rat hearts after space flight for 12.5 days. After this short time in weightlessness aboard the Cosmos 1887 bio-satellite, many of the rats in the experiment acquired damaged and irregular-shaped mitochondria and generalized myofibrillar edema which contributed to heart failures and death. Mitochondria, which are unable to orient themselves in the cell, have been studied [4] where these dynamics were linked to several major neurodegenerative diseases – including Alzheimer's, Huntington's, Parkinson's and other diseases. The animals also exhibited myofibrils (rod-like units of muscles) which were abnormal after this short time of exposure. In addition, the rats in the study by Philpott et al. [19] exhibited loss of filament protofibrils (e.g., actin and myosin). The literature notes that protofibrils may be responsible for cell death in the organism, as noted in Caughey and Lansbury [3] and may have been implicated as the toxic species responsible for cell dysfunction and neuronal loss such as in Alzheimer's disease and other protein aggregation diseases, explored in Haass and Steiner [11].

Oxidative stresses on Earth may be very similar to those noted during space flight due to naturally created free radicals and reactive oxygen species, as noted by Nikawa et al. [17]. In their study it was discovered that altered gravity conditions may be responsible for the onset of skeletal muscle atrophy in rat models, where rats were subjected to two forms of simulated weightlessness and also to actual space-flight conditions. Their study concluded that the distribution of muscular mitochondria had become diminished as a consequence of the damage to muscle fibers in all three conditions. They suggested that the muscular atrophy could be traced down to the interactions of free radicals and reactive oxygen species as a result of space-flight stresses.

Since stresses and their accompanying free radical and reactive oxygen species damage also exist on Earth, the study of their interaction sites in protein may provide insight into how similar damage may be incurred in space and on Earth. In this paper,

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http://dx.doi.org/10.1016/j.compbiomed.2014.07.008
0010-4825/

we show that there are generally less oxidative motifs in mitochondrial proteins (from our data set of enzymatic and non-enzymatic proteins) than in non-mitochondrial proteins (for the same two sub groups of protein). We draw our evidence from the reduced occurrence of carbonylation motif *hot-spots* which were defined by Maisonneuve et al. [15]. We contrast the scarcity of mitochondrial protein oxidative sites by showing that nuclear protein code contains many motifs which were probably not lethal to the cell since they continued to exist, or were embedded in folded protein at locations where they were allowed to prevail.

We analyze the functional make-up of the existing regions of oxidation in mitochondrial and non-mitochondrial proteins to determine that the oxidative sites tend to be located in the connection points between two structural events in folded proteins (helices, turns, sheets, and coils). We determine that these functional regions hold some protein structural importance which may explain why they still exist in mitochondrial protein which produces high levels of dangerous oxidative activity.

1.2. Carbonylation and PEST protein regulation mechanics

RKPT sequences and general carbonylation: Carbonyl derivatives are the result of direct metal-catalyzed oxidation interactions with the carbonylatable amino-acid side chains of arginine (R), lysine (K), threonine (T) and proline (P) residues and were explored in Maisonneuve et al. [15]. Carbonyl derivatives of cysteine, histidine, and lysine may also be formed by the adduction of reactive aldehydes which are derived from the metal-catalyzed oxidation of polyunsaturated fatty acids. In Dalle-Donne et al. [6], it was noted that the residues of lysine carbonyl derivatives may be formed by secondary reactions with reactive carbonyl compounds on carbohydrates and advanced glycation/lipoxidation end products.

Proteolysis is the process of naturally removing proteins that are non-functional due to the stresses of aging and related kinds of natural damage. Here, a region of protein sequence signals a natural removal by cellular processes. The literature suggests that the age of the protein may not always be the needed trigger for protein carbonylation [14]. The same authors also studied insulin resistance (e.g., a symptom of protein degeneration) in mouse models in which they discovered that mice, having an over-expression of the human catalase gene to mitochondria, are protected from an age-induced decrease in muscle mitochondrial function and muscle insulin resistance. Furthermore, the study suggested that age-associated reductions in mitochondrial function are due to organelle-generated reactive oxygen species production, contributing to the pathogenesis of age-associated muscle insulin resistance (protein degeneration). We note that insulin-resistance, and perhaps the above-mentioned diseases associated with aging, may be avoided by therapies that reduce mitochondrial oxidative damage.

Previously mentioned, aging or non-functional proteins are marked for destruction to avoid risks of failing protein in tissues. Oxidative carbonylation may not always be beneficial when it is due to environmental stresses that could create ailments such as Alzheimer's, cancer, cataractogenesis, diabetes, sepsis and others. Carbonylation may be central to these misfortunes since they all exhibit marked protein structures for degradation.

PEST sequences: PEST sequences are hydrophilic, at least 12 amino acids in length and are rich in proline (P), glutamic acid (E), serine (S), and threonine (T). As in the case of carbonylation motifs, these regions also contain proline and threonine which may be attractors of protein degradation due to an associated short intracellular half-life. In Rechsteiner and Rogers [21], it was noted that PEST sequences are involved in proteolytic signaling for rapid protein degradation by cellular regulation and its associated control systems. The PEST sequences typically signal the protein which contains the motif(s) for quick proteolytic degradation by the 26S ubiquitin proteasome system. It was also noted that this mechanism is active after the ubiquitination at the lysine residues within the PEST sequence. Rechsteiner and Rogers [21] maintained that the PEST sequence generally acts as a signal peptide since its phosphorylation is likely necessary for protein degradation noted in Salmerón et al. [25]. These sequences have also been noted to be a stabilizing factor for L-type calcium channel proteins explored in Rogers et al. [23].

PEST sequences are involved in the regulation of proteins in plants [13]. Dehydration responsive element binding is an important transcription factor that regulates environmental (abiotic) stress tolerance in plants. It was noted in Sakuma et al. [24] that a central region of the DREB2A transcription factor in *Arabidopsis*, acting as a negative regulatory domain, and when deleted, activates its protein under stress conditions and also allows for the up-regulation of genes associated with salt or heat-stress responsive genes. Furthermore, the authors have suggested that this mechanism involves a PEST sequence acting as a negative regulatory domain that contains phosphorylation target sites for protein kinases such as PKC and CK2.

1.3. Mitochondria

Mitochondria play a part in cellular signaling, cellular differentiation and are able to initiate cellular death. Because they are important to the cellular house-keeping and the general health of the cell, any alterations to prevent normal function in mitochondria may be lethal to the cell. The host is also at risk in the event of the dysfunction of the organelle – functional mitochondrial respiration and energy homeostasis are critical for normal heart function and skeletal muscle maintenance [12]. General muscular atrophy is also a result of impaired mitochondria [16]. Interruptions to normal mitochondrial function are often associated to other ailments and disorders such as myopathies and cardiomyopathies, diabetes mellitus, neurodegenerative illnesses such as Alzheimer's disease, diabetes [14] and aging, [5,7,27,28].

Mitochondria are also responsible for the energy production of eukaryotic cells. In their absence, the cell would depend entirely on the anaerobic glycolysis as a source of ATP (adenosine triphosphate). When glucose is converted to pyruvate by glycolysis, only a marginal quantity of total free energy is released from the glucose which makes this an inefficient process for energy production. In the metabolism of sugars by mitochondria, the pyruvate is imported into the organelle where it is oxidized by molecular oxygen to carbon dioxide and water. The release of free energy from this operation makes an efficient process: 30 molecules of ATP are produced for each molecule of oxidized glucose, where as, only two molecules are released by glycolysis in the absence of the energy-making organelles. Mitochondria are mobile, able to change shape in the cytoplasm, and are able to drift around the cell while apparently associated with the microtubules. In some cells, they have been observed to anchor themselves to cellular locations where large amounts of ATP are necessary, such as in-between the myofibrils in a cardiac muscle cells or at the base of the flagellum of sperm cells.

In muscle cells, much ATP energy is required for function which is provided by the mitochondrial matrix enzymes of inner membrane along the respiratory chain. Since the mitochondria produce these sizable amounts of energy by oxidation processes, it is likely that proteins (or regions along the proteins) which attract oxidative carbonylation would not provide an evolutionary advantage and may be removed due to evolutionary pressures. Furthermore, since mitochondria are able to merge with other like-organelles, a failure in energy production may be introduced to the unified pair