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Are Ancient Proteins Responsible for the Age-Related Decline in Health and Fitness?

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Abstract

There are a number of sites in the body where proteins are present for decades and sometimes for all of our lives. Over a period of many years, such proteins are subject to two types of modifications. The first results from the intrinsic instability of certain amino acid residues and leads to deamidation, racemization, and truncation. The second type can be traced to relentless covalent modification of such ancient proteins by reactive biochemicals produced during cellular metabolism.The accumulation of both types of posttranslational modifications over time may have important consequences for the properties of tissues that contain such proteins. It is proposed that the age-related decline in function of organs such as the eye, heart, brain, and lung, as well as skeletal components, comes about, in part, from the posttranslational modification of these long-lived proteins. Examples are provided in which this may be an important factor in the etiology of age-related conditions. As the properties of these proteins alter inexorably over time, the molecular changes contribute to a gradual decline in the function of individual organs and also tissues such as joints. This cumulative degeneration of old proteins at multiple sites in the body may also constrain the ultimate life span of the individual. The human lens may be particularly useful for discovering which reactive metabolites in the body are of most importance for posttranslational modification of long-lived proteins.

Introduction

Biochemical renewal of macromolecules is crucial for the maintenance of cellular integrity in most tissues of the body. Indeed, much has been discovered about the elaborate machinery that cells possess to target macromolecules for degradation. Proteins are a major focus for rejuvenation, and well-known pathways of catabolism involve ubiquitination and proteosomal degradation.[1,2] The ubiquitous presence of heat shock proteins, which can bind denatured polypeptides,[3] is an indicator of the importance of monitoring protein quality within cells.

Therefore, it may be surprising to discover that some tissues contain macromolecules that do not turn over.[4] One example is the human lens, which contains the highest concentration of protein of any tissue in the body, yet there is no protein turnover. For crystallin proteins within lens cells, it is a life sentence. Recent data suggest that there may be major consequences of this molecular longevity in terms of the properties of the human lens.[5]

The remarkable longevity of proteins arises as a direct consequence of the growth pattern of the lens. During our lives, new fiber cells are layered over the lens that was present at birth, and differentiation into fiber cells is accompanied by the complete loss of subcellular organelles that would otherwise scatter light. In the absence of the machinery necessary for protein synthesis, it is unsurprising that there is no turnover.

The lens may not be unique in containing proteins that last for our entire lives. At other sites, some proteins may be lifelong and others very long-lived. One factor that has constrained our knowledge in this area is the difficulty in determining exactly how old a protein really is. One approach involves measurement of racemized aspartic acid residues. Proteins such as dentin in human teeth, accumulate D-aspartic acid (D-Asp) at a constant rate.[6] In a more intricate approach, it is also possible to take advantage of the aboveground testing of nuclear weapons that took place in the 1950s.[7–9] Atmospheric levels of ^{14}C increased substantially in this period and then fell exponentially following the nuclear test ban treaty in 1963. This global pulse-chase experiment meant that $^{14}CO_2$ became incorporated into foodstuffs worldwide and ultimately into the amino acids used for synthesis of proteins. Because it is possible to quantify the amount of ^{14}C in a sample using sensitive accelerator mass spectrometry procedures (see, e.g., ref. 10) one can carbon date any given

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protein. The question in its simplest form is: "Does the ^{14}C level in an isolated protein correspond to the time of birth of the individual?" If so, then that protein has not been subject to turnover.

Hypothesis

Several human tissues contain lifelong proteins and others contain proteins that turn over very slowly. It is proposed that progressive damage to these polypeptides over decades may contribute to the age-related decline in function and that the cumulative denaturation of such proteins may ultimately limit human life span.

Mechanisms of damage

What damages old proteins in the body?

The modification of lifelong, as well as other old, proteins arises from two separate sources. One is the intrinsic instability of some amino acid residues in proteins, and the second is inadvertent posttranslational modification by reactive metabolites.

Intrinsic instability of amino acids. Three amino acids in proteins seem to be particularly vulnerable—asparagine, glutamine, and aspartic acid. Over time, these asparagine (Asn) and glutamine (Gln) amide-containing amino acid residues can be hydrolyzed to their corresponding carboxylic acids, aspartic acid (Asp) and glutamic acid (Glu). Deamidation of Asn residues is generally more rapid[11] and is governed by the sequence of adjacent amino acids, particularly on the carboxy-terminal side. The rate will also be governed by secondary and tertiary structure,[12] as well as by the local environment. One immediate result of deamidation is that a negative charge is introduced where the precursor amino acid side chain was uncharged. This is likely to destabilize the three-dimensional structure,[13] and the more residues that deamidate, the greater will be the degree of protein denaturation.[14] Most deamidation is thought to arise via a cyclic intermediate, and hydrolysis of this intermediate can lead to several outcomes, one being cleavage of the peptide bond on the carboxyl terminus of the Asn/Gln residue. Such hydrolysis will yield a truncated version of the original protein. An example of cleavage of the peptide bond on the carboxy-terminal side of an Asn residue is illustrated at two sites in the cytoplasmic tail of the major integral membrane protein in the lens, aquaporin 0.[15] Because this carboxy-terminal portion binds calmodulin and cytoskeletal elements, such truncation may alter the ability of the cell, and the lens itself, to regulate water flow.

Another product arising from the cyclic intermediate is isomerization to D-Asp. In addition, the cyclic succinimide intermediate can decompose via an alternate pathway yielding isoaspartate (isoAsp). Conversion of Asn in the polypeptide chain to this non–alpha amino acid disrupts the regular backbone peptide bond sequence and protein structure. Analagous processes to those summarized here for Asn can generate L- and D-Glu from Gln residues.

Asp residues are prone to isomerization, resulting in the formation of D-Asp.[16] Because this is a time-dependent process, the amount of D-Asp in a protein can be used as an indicator of its age.[17] In proteins, L- and D-isoAsp can also form via a cyclic intermediate and this process too will disrupt protein structure. Other amino acid residues undergo racemization, although the extent is less than that for Asp.

Covalent modification by reactive molecules. This subject is large, and only one aspect will be canvassed. Proteins and other macromolecules within the body exist in a chemical "soup." Many intermediates in biochemical pathways are chemically reactive. Compounds such as aldehydes and ketones can covalently modify the amino groups of proteins. For example, quantification of glycohemoglobin, a modified version of hemoglobin that results from the addition of glucose to the amino-terminal group, is used clinically as a measure of the control of blood sugar in diabetics.[18] Aldehydes such as malondialdehyde or nonenals, which arise from the breakdown of unsaturated fatty acids, can also react with Lys, His, and Arg residues in proteins.[19] Arg residues are particularly susceptible to covalent modification by diketones.[20]

It is likely that small ketones and aldehydes, which are formed from decomposition of the unstable oxidized form of vitamin C (dehydroascorbate),[21] and monosaccharides are responsible for significant levels of protein posttranslational modification. The literature on glycation is vast (e.g., see ref. 22). For many proteins in the body, these modifications may be of little consequence because the lifetimes of these proteins are relatively short and the modified polypeptides will be degraded. This option is clearly not available to a lifelong protein. The degree to which this group of proteins is covalently modified will depend to some degree on the concentration of the reactive chemicals, but possibly more importantly on the concentration of low-molecular-weight scavenger compounds, the most important of which is the antioxidant glutathione (GSH).[23] GSH acts in two ways to protect proteins. It can reduce the more chemically reactive oxidized form of some molecules (e.g., dehydroascorbate to ascorbate)[24] that may otherwise modify proteins. In addition, GSH is a potent nucleophile and can intercept small reactive molecules by reacting with them before they can bind to protein.[25] The GSH adducts can simply diffuse away and be excreted.[26] Significant protein modification[27] is observed if levels of GSH fall significantly.[28]

Disturbances in metabolism, as well as ingestion of some drugs and/or other dietary chemicals that either react with GSH or alter the balance from a reducing to an oxidative environment, may exacerbate the underlying process of chemical modification of lifelong proteins.

Oxidative modification, due to exposure of proteins to reactive oxygen species (ROS) is widely recognized as a major source of cellular damage (e.g., refs. 29 and 30). The literature is vast and there is insufficient space to cover the field adequately here.

Particular amino acid residues are susceptible to damage as a result of prolonged exposure to the biological milieu, particularly if the environment becomes oxidative.[31] For example, methionine (Met) is readily converted to the sulfoxide, cysteine to cystine; histidine, tyrosine, and tryptophan can also be oxidized. Redox-active metals play an important role in ROS damage[32,33] because they tend to promote hydroxyl radical formation and the hydroxyl radical is the most reactive of the ROS. Enzymes such as catalase and those that use GSH, such as glutathione peroxidase and glutathione-*S*-transferase, or regenerate reduced GSH (glutathione reductase) are vitally

important for minimizing cellular damage in cells exposed to oxidants.

For polypeptides in the body that are exposed to ultraviolet (UV) light, photooxidation can also be an important factor promoting protein oxidation. In the case of the human lens, protection is afforded by endogenous UV filter compounds; however, the levels diminish linearly with age[34] so our lens proteins are more prone to damage[35] as we age. This feature has been reviewed.[36]

Repair Systems

The importance of ameliorating amino acid damage within proteins is illustrated by the widespread occurrence of enzyme repair systems. In the case of Met, enzymes such as methionine sulfoxide reductases[37] can reverse the oxidation. Other enzymes such as L-isoaspartyl methyltransferase, act to repair L-isoaspartate residues.[38] Analyses of tissues from methyltransferase knockout mice revealed an accumulation of protein substrates for this enzyme in brain, heart, liver, and erythrocytes. The knockout mice showed significant growth retardation and succumbed to fatal seizures early in life.[39] These results suggest that the ability of mice to repair L-isoaspartyl– and D-aspartyl–containing proteins is essential for normal growth and central nervous system function. It should be emphasized that these data were derived from an animal with a short lifespan.

For enzymatic repair processes to work properly the substrates must be soluble and the enzymes active and available. Indeed, some authors have proposed that age changes result from entropic factors where the accumulation of deleterious changes to macromolecules slowly overwhelms the maintenance systems of the body.[40] This may well be the case within cells. For extracellular proteins, there appear to be no comparable enzymatic recovery options. Such polypeptides, particularly if they are insoluble or insulated by the local environment (for example, by a myelin sheath), may well accumulate posttranslational modifications that would otherwise be repaired if such macromolecules were intracellular. Membrane proteins may be another example of proteins that are less easily repaired by enzyme-mediated processes.

Implications of the Hypothesis for Selected Tissues

To date, few proteins have been investigated via ^{14}C analysis to determine if they are lifelong macromolecules, although there is more information based on measurement of Asp racemization. Only in the lung have ^{14}C and D-Asp measurements for the same protein (elastin) been compared directly. Because most longevity studies to date rely on measurement of D-Asp, it is important to recognize that time is not the only factor that determines racemization of Asp. Nuclear cataract lens proteins have higher levels of D-Asp than age-matched normal lenses,[41] a finding that we have recently verified (unpublished). Some tissues, and their proteins, are discussed briefly below with a particular emphasis on ocular tissues.

Lung

The elastic properties of the lung are due mostly to the presence of elastic fibers in the extracellular space. These elastic fibers are composed of elastin, together with other glycoproteins, which make up microfibrils. Measurement of ^{14}C in purified parenchymal elastin was found to correspond with the age of the subject from whom it had been isolated.[42] Racemization of aspartate in the elastin was also measured as an indicator of turnover, and the D-Asp levels supported the findings of the radiocarbon analyses showing that the proteins in the microfibrils and elastic fibers are also long-lived.

A decline in normal lung elastic recoil becomes apparent in the fifth to sixth decade of life and this is associated with diminished lung function. It is possible that age-related modification to the proteins of elastin fibers may be responsible. Lung disorders such as chronic obstructive pulmonary disease and ideopathic pulmonary fibrosis also become evident in elderly persons. Lifestyle factors (e.g., smoking) may exacerbate the age-related changes to the proteins.

The eye

The lens. Because the lens grows continuously throughout life, and preexisting cells are encapsulated by layers of more recently differentiated fiber cells, all proteins, including soluble, cytoskeletal, and integral membrane polypeptides, are likely to be lifelong. ^{14}C measurement of the central regions of human lenses support this conclusion.[4]

Proteomic approaches have provided evidence of the consequences of this protein longevity. For example, the amount of free α-crystallin (a small heat shock protein) in the lens center decreases with time until none remains by age 40.[43] It is very likely that our allocation of α-crystallin at birth has been used for chaperoning other crystallins as they denature over time, forming high-molecular-weight complexes.[44] The amount of deamidation,[45,46] racemization,[47,48] and truncation[49,50] of crystallins also increases as a function of age, with the result that by middle age half of the formerly soluble crystallins become insoluble.[51] The content of deamidated residues within the insoluble proteins of the lens is greater than that from the same proteins that remain soluble.[14]

Data for cytoskeletal and intrinsic membrane proteins of the human lens are less available. It is clear that truncation of the most abundant intrinsic membrane protein in the lens, aquaporin 0,[15] occurs progressively over time.[52] Two significant cleavages of the cytosolic carboxyl terminus have been documented, each of which is adjacent to an Asn residue.[53] Such a pattern is exactly what would be predicted from a truncation that is nonenzymatic and arises due to the formation, and subsequent cleavage, of the succinimide intermediate of Asn.[12] The consequences of this inexorable truncation for aquaporin 0, or other proteins, are yet to be fully characterized.

By middle age, a large proportion of the proteins within the center of the lens have become insoluble, and the stiffness of the lens increases almost 1000-fold.[54] It is very likely that this inflexibility of the lens is the primary cause[5,55] of presbyopia, the inability to focus on nearby objects that affects people by age 40–50. Another consequence of lifelong proteins in the lens may be age-related nuclear cataract. It has been proposed that protein aggregation, due to the accumulation of age-related changes within the crystallins, and their consequent denaturation, could lead to the occlusion of membrane pores, such as aquaporin 0 and connexons, as the protein aggregates bind to the inner surfaces of the fiber cell membranes.[5] This

lens barrier becomes detectable at middle age and leaves crystallins in the center of the lens in older individuals susceptible to oxidation and posttranslational modification due to the impairment of diffusion of vital antioxidants from the outside of the lens. Oxidative changes to proteins in the lens center is the hallmark of age-related nuclear cataract.[56] When viewed in this way, both presbyopia and age-related nuclear cataract can be thought of as being direct outcomes of the denaturation of lifelong proteins in the lens.[57]

The vitreous humor. The vitreous humor is a transparent gel that fills the major internal space of the eye. It is composed chiefly of collagen fibrils and proteoglycan molecules.[58] For this tissue, direct measurement of ^{14}C levels is not yet available; however, it is likely that once synthesized most, if not all, of the proteins of the vitreous do not turn over. The evidence is circumstantial and is based on two observations: First that gene expression of the mRNA for the collagens is switched off around the time of birth[59] and second that if the vitreous is removed during surgery, it is not replaced.

The vitreous humor becomes more liquid with age and this change in physical properties may well contribute to an increase in the incidence of retinal detachment in older people.[60] It has been proposed that increased liquification of the vitreous may also lead to an increased exposure of the lens to oxygen and an increased risk of developing nuclear cataract.[61] It is known that once vitrectomy is performed on a patient, nuclear cataract will follow shortly afterward as an inevitable consequence. It is not yet clear if progressive age-related changes to the proteins in the vitreous humor, or other factors, are chiefly responsible for these changes in physical properties.

Brain

Cellular turnover in the major areas of the human neocortex was examined recently.[62] Neurons were birth-dated by measuring the level of ^{14}C, and, in addition, the brains of individuals who received bromodeoxyuridine (BrdU) were also analyzed. Neocortical neurons had ^{14}C levels corresponding to the atmospheric levels at the time of birth of the individual. The conclusion reached was that whereas non-neuronal cells turn over, neurons in the neocortex are formed perinatally. These experiments demonstrated that DNA in the neuronal cortex does not turn over; however, there is less known about the proteins associated with the DNA, such as histones. Studies of the racemization of Asp within human myelin basic protein indicate that there is a high content of D-Asp at some sites, supporting longevity of this abundant polypeptide.[63]

It has, for example, been proposed that accumulation of oxidative damage to proteins, and possibly nucleic acids particularly those of brain mitochondria, may lead to neuronal and cognitive dysfunction.[64] Feeding aged rats acetylcarnitine and lipoic acid restored mitochondrial function and delayed mitochondrial decay and aging.[65]

Several neurological conditions (e.g., dementias) are known to occur predominantly in older people, and some, such as Parkinson and Alzheimer disease, involve protein aggregation.[66,67] The role of old brain proteins in such conditions is largely unknown, although neurofibrillary tangles do contain high D-Asp.[68]

The heart and blood vessels

The aging myocardium becomes stiffer and loses its ability to adapt to physiological stresses. Its color alters as lipofuscin is deposited and calcification of the valves and muscles becomes evident. It is likely that modifications to long-lived proteins in the heart and also the blood vessels may be a contributing factor. For example, the content of D-Asp in elastin purified from the aorta was found to be 3% of total Asp in youth, but accounted for 13% in people in their mid 80s.[69] The data were consistent with the view that mature elastin is not synthesized in the adult aorta.

Age-related changes to the circulatory system are well recognized. In the case of the heart, there is a gradual decrease in cardiac output while at rest, and the maximal heart rate attainable by a healthy adult falls by approximately one beat per year. Each heart beat pumps out less blood than the previous year. A loss of cardiac muscle cells with age is another important factor in this age-related decline. Fewer than 0.5% of cardiomyocytes turnover annually by age 75.[70] Others have proposed a similar hypothesis, in which age-related changes to elastin in the cardio-respiratory system could potentially limit lifespan.[71]

Skeletal and dermal tissues

In this section, data for one major extracellular protein component, collagen, will be summarized briefly. Radiocarbon dating has not so far been attempted on collagen isolated from human organs; however, several studies have been published using the more traditional methods based on alterations to Asp residues.[72] Using both racemization of Asp, and its isomerization to isoAsp, it has been demonstrated that the apparent turnover rate for Type I collagen in the dermis is low and comparable to that of bone. Quantitatively this extent of denaturation is impressive, because it has been estimated that there is approximately 4–5 kg of Type I collagen in the skin of an adult.[72] Using the same indicators, these researchers found that the apparent turnover of collagen in tendons and liver was also low, with slightly higher rates for ligament and lung. Progressively higher turnover was indicated for collagen in kidney, intestine, skeletal muscle, arteries, and heart. Recent data on collagen from intervertebral discs gave half-lives of between 95 and 215 years.[73]

The skin is the largest organ in the body and intervertebral discs comprise the largest avascular cartilaginous structure, responsible for providing flexibility to the spine. Age-related declines in appearance and function are well known.

Relative Importance of These Changes for Health and Life Span

It is clear that the impact of the deleterious changes that have been outlined in this article will depend on the degree of change and the site of alteration. It is much more likely that the accumulation of altered polypeptides in the heart, cardiovascular system, brain, and lungs will directly affect life span. Diminished function of tendons, ligaments, and musculoskeletal sites in our current society is more likely to influence the quality of life rather than life span. This may not have been the case during our evolution.

Because in most cases it will be difficult, or impossible, to repair the modified proteins, the possibility for amelioration

appears remote. Lifestyle and diet may diminish the rate of damage from some sources, e.g., chemical addition. In some cases, replacement of the aged organ or tissue can be contemplated. This is already commonplace for some body parts. For example, cataract lenses containing modified proteins can be replaced with plastic lens implants[74] and joint repair is commonplace.

It is not straightforward to test the relative importance of the ancient proteins on human life span. It is clear that the vast majority of animal models will not be applicable because the protein changes discussed herein take place over decades. One means could involve replacement of certain organs, e.g., heart, lungs, etc., with donor organs from younger individuals; however, the impact of the trauma associated with the surgery, infections, and tissue rejection interventions would need to be taken into account. Another possibility would be to analyze the levels of posttranslational modification of selected proteins within target organs such as the heart and lung and to correlate them with function. This would, however, entail proteomic procedures that are currently beyond our capabilities. We understand very little about the spectrum of posttranslational modifications that are present in old proteins, nor do we yet know which are the most common or have the most impact on function. The lens appears to be an ideal tissue for identifying such age-related posttranslational modifications.

Summary

The hypothesis outlined here postulates that the modification of ancient proteins plays a key role in aging of the human body and its deterioration. As more tissues are studied, it will become more clear which ones contain long-lived proteins. Recent results suggest that this may be more common than previously imagined. For example, ^{14}C measurements have revealed that adipocytes, which are formed during adolescence and early adulthood, turn over very slowly with approximately 10% of cells turned over annually.[75]

Protein deterioration is clearly not the only factor that influences aging. Other, more well-known, contributors such as oxidative stress, genomic instability, and mitochondrial dysfunction are likely to play a part (e.g., see refs. 30, 76, 77). Although it may be possible to intervene pharmacologically in some of these latter factors, with the aim of increasing human life span, there seems little prospect of doing so in the case of ancient proteins. Replacement by bioengineered substitutes may be the only available option, and this may not be available for all tissues.

Human lenses of different ages are readily available from eye banks, can be extracted easily, and contain a very high concentration of proteins that were made *in utero* as well as every year since. Because the crystallins are soluble, they may be ideal for studying the types of posttranslational modifications outlined in this article. In addition, because the center of the lens is largely devoid of active enzymes, it is a unique environment where one can focus on the outcome of covalent changes in the absence of enzymatic involvement in macromolecular turnover or amelioration. Therefore, lenses may be useful for determining which of the many reactive metabolites in the body are quantitatively of most importance for protein modification. In other tissues, a proportion, even of insoluble proteins such as elastin, is degraded.

Conclusion

It is hypothesized that persistent proteins within the body, whose most extreme manifestation is the family of lifelong polypeptides, may be of considerable significance in governing the age-related decline in the capacity and function of organs and tissues. This is particularly true for animals, such as humans, who typically live for many decades. Interestingly, recent data suggest that protein stability, together with resilience of tissues to oxidative flux, may be the key factors in determining longevity of animals.[78] Age-related deterioration of long-lived proteins may be responsible to a significant degree for the deterioration of individual organs, and, as a consequence, whole body function that occurs as we age. Such cumulative changes may also confer a limitation to human life span.

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