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Mini Review

Standardization and quality control in quantifying non-enzymatic oxidative protein modifications in relation to ageing and disease: Why is it important and why is it hard?

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ABSTRACT

Post-translational modifications (PTM) of proteins determine the activity, stability, specificity, transportability and lifespan of a protein. Some PTM are highly specific and regulated involving various enzymatic pathways, but there are other non-enzymatic PTM (nePTM), which occur stochastically, depend on the ternary structure of proteins and can be damaging. It is often observed that inactive and abnormal proteins accumulate in old cells and tissues. The nature, site and extent of nePTM give rise to a population of that specific protein with alterations in structure and function ranging from being fully active to totally inactive molecules. Determination of the type and the amount (abundance) of nePTM is essential for establishing connection between specific protein structure and specific biological role. This article summarizes analytical demands for reliable quantification of nePTM, including requirements for the assay performance, standardization and quality control, and points to the difficulties, uncertainties and un-resolved issues.

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Introduction

Common to all biological processes are molecular interactions, which include binding, followed by a cascade of reactions leading to specific physiological events. A cascade may consist of many

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binding interactions that obey a certain hierarchy such that the first event induces an ordered series of happenings that determines the so-called normal healthy state of a biological system. However, the biochemical processes that sustain life are prone to alterations owing to intrinsic and extrinsic factors and these changes underlie the emergence of ageing and diseases. Although most physiological macromolecules fit into a general binding-propagating pathway, this article deals with proteins, their interactions, effects of post-translational structural modifications, and the importance of standardization and quality control in the detection and measurement of molecular forms in physiological samples. Before analyzing difficulties in quantification of specific protein forms, we recapitulate some structural and binding properties of proteins, as follows.

Folding and binding properties of proteins

All proteins exist as a population of many conformational states, known as conformers. Multiple conformers are derived from molecular motions that do not demand significant energy input, resulting in molecular states of similar stability [1]. Motions are predominantly at the level of side-chains, but the polypeptide backbone may also exert some flexibility. Although native protein conformation is dependent primarily on the amino-acid sequence, the surrounding environment contributes as well. The larger the molecule, the more conformers are likely to exist. Distribution of conformers is not even, as some are present in greater proportion and others in much smaller quantities. This dynamic population equilibrium shifts in the presence of a binding partner (ligand), which will "select" the favorable conformer (the one to interact with), causing redistribution of the conformer set. According to the concept of a pre-existing population of conformers [2], the ligand "chooses" the most suitable one from the "library" of offered molecules. The binding process further stabilizes the complex by optimizing protein folding and enabling new non-covalent interactions both within the protein and with the ligand. Along the cascade of interactions, each additional step induces conformational adjustments leading to multi-molecular complexes that will trigger specific physiological events.

Researchers use different words to express their understanding of molecular flexibility, introducing concepts such as intrinsic disorder, controlled chaos, conformational breathing or structural plasticity [3]. Proteins with a greater number of conformers exhibit more flexible ligand binding capacity, enabling interactions with a range of ligands [2]. These ligands may have homologous structure, but even dissimilar ligands may bind to the same site. The most typical examples are proteases and their substrates, which possess appropriate short amino-acid sequence regardless of the rest of the molecule [4]. Other examples are proteins involved in the innate immune response, where they recognize certain motives within a range of native molecules [5]. Antibodies bind epitopes which fit conformationally into Fab pockets, regardless of the appearance of the remaining molecule [6]. Computer modeling analysis has provided the information that proteins with a greater degree of conformational change utilize more interfaces for interactions [7].

Post-translational modifications of proteins

Accurate translation of mRNA, followed by appropriate modifications of the polypeptide chain, is essential for normal folding, targeting and specificity. Misregulation in any of these steps can have far reaching biological consequences, including effects on cell growth, division and survival. Many post-translational modifications (PTM) of proteins have been described that determine their activity, stability, specificity, transportability and lifespan [8]. There are two distinct types of protein modification, one initiated (programmed) and catalyzed in the presence of specific enzymes (enzymatic modifications), and the other which occurs in the presence of compounds chemically reactive with proteins (e.g. reactive oxygen- and nitrogen-species) or is due to physical modification (e.g. irradiation). The second type of modification is known as non-enzymatic. Enzyme catalyzed PTM include phosphorylation, acetylation, glycosylation, ribosylation, methylation and some oxidation reactions such as formation of disulfide bonds and nitrosylation. Non-enzymatic post-translational modifications (nePTM) occur stochastically, depend on the ternary structure and can be physiologically damaging [9]. Such nePTM include oxidation, glycation, deamidation, racemization and isomerization.

Different reactive species are responsible for the formation of a range of oxidative products, while different amino-acid side chains can undergo modification contributing to an additional variety of derivatives. Some of these end-products are unstable, present in very low quantities and decompose quickly. The physiological response of an organism to nePTM proteins can be repair or degradation. Lysosomal or proteasomal degradation systems are primarily involved in the removal of damaged proteins [10], but cell surface receptors participate as well [11]. Other modified proteins acquire stability by further structural adjustments, which may end with misfolding. A misfolded protein has an increased chance of self-aggregation into insoluble fibrillar structures, which accumulate in cells and tissues. Fibrils display common cross-β sheets, regardless of the original protein structure. A strategy to reduce fibrillogenesis involves stabilization of the native structure, for example by binding an appropriate ligand [3]. That event would favor non-covalent inter-molecular interactions which do not encourage self-assembly [12]. Formation of amyloid fibrils underlines neurodegenerative disorders associated with ageing [13].

Proteins are not equally vulnerable to non-enzymatic modifications. In general, abundant and long living proteins have the most easily observed nePTM. Half-lives of soluble proteins are generally shorter than those of structural proteins, so the effects of modification may be limited and relatively small. If, however, conditions under which nePTM form last for a long period (i.e. permanent exposure to modifying agents), the consequences of even short-living derivatives may be serious. Besides half-life and concentration, the sequence and conformation of the protein contribute to its overall susceptibility to modification. Oxidation of fibrinogen, for example, occurs more readily than oxidation of albumin, immunoglobulins or transferrin [14]. Modifications of intracellular proteins induce functional metabolic changes. Proteins in mitochondria are subjected to oxidation more intensively than those in other compartments [15]. Oxidative changes of endothelial proteins are also common, underlying the pathogenesis of cardiovascular diseases [16]. Specific examples of modified proteins will be given further in the text, where appropriate.

Accumulation of abnormal proteins during ageing

It is often observed that inactive and abnormal proteins accumulate in old cells and tissues [17,18]. This increased amount of debris in the cytoplasm can be inhibitory for cell growth and normal metabolism, and thus contributes towards failure of maintenance. One reason for inactivation of an enzyme can be oxidative modification by oxygen free radicals, by mixed-function oxidation (MFO) systems or by metal catalyzed oxidation (MCO) systems. Since some amino-acid residues, particularly Pro, Arg, Thr and Lys, are oxidized to carbonyl derivatives, the carbonyl content