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Review

Detection of oxidized and glycated proteins in clinical samples using mass spectrometry — A user's perspective☆

Paul J. Thornalley [a,b,*], Naila Rabbani [a,b]

[a] Clinical Sciences Research Laboratories, Warwick Medical School, University of Warwick, University Hospital, Coventry CV2 2DX, UK
[b] Warwick Systems Biology Centre, Coventry House, University of Warwick, Coventry CV4 7AL, UK

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ABSTRACT

Background: Proteins in human tissues and body fluids continually undergo spontaneous oxidation and glycation reactions forming low levels of oxidation and glycation adduct residues. Proteolysis of oxidised and glycated proteins releases oxidised and glycated amino acids which, if they cannot be repaired, are excreted in urine.
Scope of Review: In this review we give a brief background to the classification, formation and processing of oxidised and glycated proteins in the clinical setting. We then describe the application of stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry (LC-MS/MS) for measurement of oxidative and glycation damage to proteins in clinical studies, sources of error in pre-analytic processing, corroboration with other techniques – including how this may be improved – and a systems approach to protein damage analysis for improved surety of analyte estimations.
Major conclusions: Stable isotopic dilution analysis LC-MS/MS provides a robust reference method for measurement of protein oxidation and glycation adducts. Optimised pre-analytic processing of samples and LC-MS/MS analysis procedures are required to achieve this.
General significance: Quantitative measurement of protein oxidation and glycation adducts provides information on level of exposure to potentially damaging protein modifications, protein inactivation in ageing and disease, metabolic control, protein turnover, renal function and other aspects of body function. Reliable and clinically assessable analysis is required for translation of measurement to clinical diagnostic use. Stable isotopic dilution analysis LC-MS/MS provides a "gold standard" approach and reference methodology to which other higher throughput methods such as immunoassay and indirect methods are preferably corroborated by researchers and those commercialising diagnostic kits and reagents. This article is part of a Special Issue entitled Current methods to study reactive oxygen species - pros and cons and biophysics of membrane proteins. Guest Editor: Christine Winterbourn.

1. Introduction

Proteins in human tissues and body fluids continually suffer spontaneous modifications by reactions with oxidants, saccharides and saccharide derivatives. This forms oxidised and glycated amino acid residues within proteins [1–3].

1.1. Classification of glycated and oxidised proteins

Glycation adducts are classified into two groups: early glycation adducts – Schiff's base and Amadori products or fructosamine residues formed at early stages of glycation processes – also called the Maillard reaction; and advanced glycation endproducts (AGEs) — other glycation adducts initially considered to be formed in later or advanced stages of the Maillard reaction but are rather formed in both early and advanced stages [4]. Major glycation adducts in physiological systems, N_{ε}-fructosyl-lysine (FL) residues and methylglyoxal derived hydroimidazolone N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine (MG-H1), are formed by non-oxidative processes [5,6]. Some AGEs are formed by oxidative

Abbreviations: AASA, aminoadipic semialdehyde; AGE, advanced glycation endproduct; AOPP, advanced oxidation protein product; AQC, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate; BUL, bilateral ureteral ligation; BNX, bilateral nephrectomy; CEL, Nε-(1-carboxyethyl)lysine; CML, N_{ε}-carboxymethyl-lysine; CRF, chronic renal failure; 3DG-H, 3DG-H1 (N_{δ}-[5-(2,3,4-trihydroxybutyl)-5-hydro-4-imidazolon-2-yl]ornithine) and related structural isomers 3DG-H2 and 3DG-H3 see [1]; ESI, electrospray ionisation; FCR, fractional clearance rate; FL, N_{ε}-fructosyl-lysine; GSA, glutamic semialdehyde; FN3K, fructosamine-3-kinase; FN3KRP, fructosamine-3-kinase related protein; G-H1, N_{δ}-(5-hydro-4-imidazolon-2-yl)ornithine; GOLD, glyoxal-derived lysine dimer, 1,3-di(N^{ε}-lysino)imidazolium salt; HD, hemodialysis; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LDL, low density lipoprotein; MALDI, matrix assisted laser desorption ionisation; MetSO, methionine sulfoxide; MG, methylglyoxal; MG-H1, N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)ornithine; MOLD, methylglyoxal-derived lysine dimer, 1,3-di(N^{ε}-lysino)-4-methyl-imidazolium salt; MRM, multiple reaction monitoring; MSR, methionine sulfoxide reductase; NFK, N-formylkynurenine; 3-NT, 3-nitrotyrosine; PD, peritoneal dialysis; SAF, skin autofluorescence; TFA, trifluoroacetic acid; UPLC, ultra high performance liquid chromatography

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* Corresponding author at: Clinical Sciences Research Laboratories, Warwick Medical School, University of Warwick, University Hospital, Coventry CV2 2DX, UK.
E-mail address: P.J.Thornalley@warwick.ac.uk (P.J. Thornalley).

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processes and are called glycoxidation products [4]. Examples are N_{ε}-carboxymethyl-lysine (CML) [7] and pentosidine [8].

A classification in protein oxidation research that has been used is the term "advanced oxidation protein products" (AOPPs). AOPP measurement is an indirect measure of protein oxidation: a measure of the ability of oxidised proteins to oxidise iodide to iodine, with measurement of iodide formation by quantitation of the tri-iodide ion I_3^- by spectrophotometric measurement at 340 nm [9–11]. This is a mechanism of formation related definition where the molecular nature of AOPPs remains unclear. This is thought to be related to N-chloramines derivatives formed by oxidation of protein with hypochlorite generated by myeloperoxidase [12]. Dityrosine and pentosidine have bene claimed to contribute to the AOPP response but independent measurements show that their low levels in plasma cannot account for the absorbance at 340 nm found in the AOPP measurement [13]. The molecular species contributing to the AOPP response are most readily formed by the hydrogen peroxide/myeloperoxidase/ chloride which forms hypochlorite [12]. A recent study has claimed that AOPP activity in plasma is due to oxidised fibrinogen on the basis of AOPP activity correlating to fibrinogen concentration but confirmation of molecular nature of the oxidant is required [14]. Molecular characterisation of AOPP(s) of fibrinogen and possibly other proteins remain to be disclosed.

Oxidation and glycation of proteins form oxidation and glycation adducts, respectively, which are part of the peptide backbone of the protein and are appropriately called oxidation and glycation adduct residues. Measurement of these is often normalised to the amount of related unmodified amino acid in the protein sample. For example, estimates of methionine sulfoxide (MetSO) residues are often reported as mmol/mol methionine and estimates of FL residues as mmol/mol lysine [1]. In some clinical research areas – particularly studies of renal failure – oxidation and glycation adduct residues have been called "protein-bound" adducts. This nomenclature is not strictly correct as part of the oxidation and glycation adduct is a residue of the protein precursor. Nomenclature and collective terms commonly used in protein oxidation and glycation research are given in Table 1. Important oxidised and glycated amino acid residues are given in Fig. 1.

1.2. Endogenous repair of oxidised and glycated proteins

It was initially thought that protein oxidation and glycation gives rise to adducts that remain in organs and body fluids throughout life and accumulate therein. This is only correct for oxidation and glycation adducts that are chemically stable, not repaired *in situ* and formed on lived-lived proteins. Examples are dityrosine and CML residues on articular cartilage in good skeletal health [19]. For most oxidised and glycated proteins, however, this is not the case. Oxidation and glycation adducts are repaired *in situ* or the oxidised and glycated proteins are degraded and replaced by *de novo* protein synthesis. MetSO residues are repaired by reduction to methionine residues by methionine sulfoxide reductases (MSRs) [20] and FL residues are repaired to lysine residues by fructosamine-3-phosphokinase [21]. This is of current clinical importance and impact as the universally used marker of glycemic control, glycated hemoglobin HbA_{1c}, is hemoglobin modified mainly on β-val-1 and α-lys-66 with 1-deoxyfructosyl residues [22]. The extent of glycation of hemoglobin is a balance between rate of glycation by glucose exposure in the 6–8 weeks prior to blood sampling and the rate of de-glycation by fructosamine-3-phosphokinase [23]; and also influenced by red blood cell turnover [24]. Where there is slow dynamic reversibility of adduct formation – such as for MG-H1 formation from methylglyoxal [25] – sustained decrease of the modifying agent – as may likely be achieved by induction of expression of glyoxalase 1 which metabolises methylglyoxal [26] – will provide for *in situ* repair of the modified amino acid residue. Examples of repair of oxidation and glycation adducts are given in Table 2.

1.3. Turnover of oxidised and glycation proteins and measurement of protein oxidation and glycation in the steady-state

Oxidised and glycated proteins that are not repaired are usually degraded and replaced. Measurement of oxidised and glycated proteins, therefore, is an estimate of the steady-state level dependent on the rate of formation, rate of repair or further modification and rate of turnover or clearance of the protein substrate. Proteolytic degradation of oxidised and glycated proteins by cellular proteolysis gives rise to release and excretion of oxidised and glycated amino acids, also called oxidation and glycation free adducts [1]. Oxidised and glycated proteins are often predicted to have distorted or damaged structures [6,29,30] and therefore may be targeted for proteolysis by the proteasome [31–33]. Lysosomal proteolysis is also important for degradation of long-lived cellular proteins, endocytosed extracellular proteins [34] and chaperone-mediated autophagy of cellular proteins [35]. Release of glycation and oxidation free adduct from cells and tissues [1] and decrease of oxidised and glycated proteins with increased cellular 20S proteasome activity [31,32] is consistent with targeting of proteins damaged in this way for proteasomal degradation. Oxidation and glycation free adducts are also formed by direct oxidation and glycation of amino acids and are also absorbed from the gastrointestinal tract from digested damaged proteins in ingested foods [16]. They are the major form by which oxidation and glycation adducts are excreted from the body [1,36,37]. A scheme of multi-compartment formation and gastrointestinal tract absorption of oxidized and glycated proteins and their proteolytic processing in the body and renal excretion of free adducts is given in Fig. 2.

Estimation of protein oxidation and glycation adduct content of proteins in the steady-state suggests that the extent of modification is influenced by both changes in rate of oxidation and glycation and also rates of protein turnover. In the case of plasma protein, the level of

Table 1
Classification terms of protein oxidation and glycation research.

Term	Definition	Comment	Reference
Oxidation/glycation adduct residues	Product of oxidation/glycation of precursor amino acid residues in a protein or peptide	Also termed "protein bound" oxidation/glycation adducts	[1,4,15]
Oxidation/glycation free adducts	Oxidised/glycated amino acids formed by release of oxidation/ glycation adduct residues from proteins and direct oxidation and glycation of amino acids.	Also absorbed from food and are the major form of oxidation/ glycation free adduct excretion in urine.	[1,4,15]
Oxidised/glycated peptides	Peptides (molecular mass <10 kDa)[a] containing oxidation/ glycation adduct residue(s)	Initially mistaken for oxidation/ glycation free adducts	[16,17]
Early glycation adducts	Schiff's base and Amadori products formed in early stages of the Maillard reaction		[4]
Advanced glycation endproducts (AGEs)	Non-early glycation adducts formed in early and late, advanced stages of the Maillard reaction.	Initially defined as "brown fluorescent pigments which crosslink proteins"	[4,18]
Advanced oxidation protein products (AOPPs)	Measure of ability of protein oxidation products to oxidise iodide to iodine – thought to be activity of N-chloramine derivatives	Molecular components of AOPPs are unknown.	[11,12]

[a] Molecular mass cut-off is arbitrary.