2-Alkenal modification of hemoglobin: Identification of a novel hemoglobin-specific alkanoic acid-histidine adduct

Jun Yoshitake\textsuperscript{a}, Takahiro Shibata\textsuperscript{a,b}, Chihiro Shimayama\textsuperscript{b}, Koji Uchida\textsuperscript{a,d,*}

\textsuperscript{a} Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, Nagoya 464-8601, Japan
\textsuperscript{b} Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan
\textsuperscript{c} Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan
\textsuperscript{d} Japan Agency for Medical Research and Development, CREST, Tokyo, Japan

\textbf{ABSTRACT}

\textbf{Keywords:} Lipid peroxidation, Aldehyde, Hemoglobin, Protein chemical modification, Red blood cells

\textbf{1. Introduction}

The lipid peroxidation reaction represents a decomposition process in the body arising from the production and propagation of free radical reactions primarily involving membrane polyunsaturated fatty acids (PUFAs), such as linoleic acid and arachidonic acid. This reaction has been implicated in the pathogenesis of various diseases, such as cancer, diabetes, atherosclerosis, as well as aging [1]. The peroxidative degradation of PUFAs leads to the generation of a broad array of different molecules with diverse chemical and biological properties [2]. In the presence of transition metals, the lipid hydroperoxides, which are the primary products of the peroxidation reaction, can undergo C–C bond cleavage via alkoxyl radicals to generate unesterified aldehydes of 3–9 carbons in length, and core-aldehydes (aldehydes still esterified to the parent lipid). Due to the electrophilic properties, these reactive aldehyde molecules readily react with cellular macromolecules, including proteins, to form covalent adducts, leading to the disruption of important biological functions. Among them, \(\alpha,\beta\)-unsaturated aldehydes, such as 2-alkenals, and 4-hydroxy-2-alkenals, are important agents to form the covalent modification of proteins [3,4]. 2-Alkenals represent one of the groups of highly-reactive lipid aldehydes possessing two electrophilic centers, carbon positions 1 and 3. It has been suggested that 2-alkenals primarily react with the nucleophilic amino acid residues, such as lysine, cysteine, and histidine, in the proteins [3,5]. The modification of histidine by 2-alkenals primarily consists of the Michael addition reaction of the nitrogen atom in the imidazole moiety to the \(\alpha,\beta\)-unsaturated bond. On the other hand, the modification of lysine by 2-alkenals is known to be quite diverse compared to the histidine modification. 2-Alkenals can undergo a nucleophilic addition reaction of the \(\epsilon\)-amino group of the lysine residue at the carbon position 1 (aldehyde moiety) and the carbon position 3 (double bond) to form pyridinium adducts via Schiff base adducts, and

Abbreviations: LC-ESI-MS/MS, liquid chromatography with electrospray ionization tandem mass spectrometry; PUFA, polyunsaturated fatty acids; HSA, human serum albumin; PP IX, protoporphyrin IX; RBCs, red blood cells; SRM, Selected reaction monitoring; m/z, mass-to-charge ratio; CHH, \(N^2\)-(1-carboxyheptan-2-yl)-histidine; LDL, low-density lipoproteins

* Corresponding author. Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan.

E-mail address: a-uchida@mail.ecc.u-tokyo.ac.jp (K. Uchida).
Michael adducts including the β-substituted and 3-formyl-3,4-dehydropiperidino adduct, respectively [6]. The reactions of protein with 2-alkenals have been mainly studied with acrolein, crotonaldehyde, and 2-nonenal. Pocker and Janjic [7] identified the formylethylated histidine derivative as the predominant histidine adduct using the N\(^\varepsilon\)-(3-formyl-3,4-dehydropiperidino)lysine, which requires attachment of two acrolein molecules to one lysine side chain, using 1H and 13C NMR and LC-MS [8]. This and the fact that crotonaldehyde also forms a similar adduct, N\(^\varepsilon\)-(2,5-dimethyl-3-formyl-3,4-dehydropiperidino)lysine [9], suggest that this type of condensation reaction is characteristic of the reaction of 2-alkenals with primary amines. In addition, Furuhata et al. [10] characterized the acrolein modification of a model peptide (the oxidized B chain of insulin) by LC-ESI-MS/MS and established a novel acrolein-lysine condensation reaction. Based on the analysis of both acrolein-modified peptide and lysine derivatives, they identified the pyridinium-type lysine adduct, N\(^\varepsilon\)-(3-methylpyridinium)lysine, as the novel acrolein-lysine adduct. Moreover, Ishino et al. identified cis- and trans-N\(^\varepsilon\)-[(hept-1-eny1)-4-hexylpyridinium]lysine as major 2-nonalen-lysine adducts [11]. They also demonstrated that these adducts were indeed formed during the lipid peroxidation-mediated modification of protein in vivo and in vitro using the stable isotope dilution-based LC-ESI-MS/MS and immunohistochemical techniques with the specific antibody.

In the present study, to gain further structural insight into covalent modification of the proteins by lipid peroxidation products, we carried out a comprehensive analysis of the histidine adducts in the 2-alkenal-modified proteins and identified an adduct specifically generated in the 2-alkenal-modified heme proteins.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA) and human hemoglobin was purchased from Sigma. According to the supplier, because the hemoglobin protein was exposed to air during purification and packaging, it would be predominantly methemoglobin. 2-alkenals (acrolein, crotonaldehyde, 2-pentenal, 2-hexenal, 2-heptenal, 2-octenal, 2-nonenal, 2-decanal, 2-undecanal, 2-dodecanal) were purchased from Tokyo Chemical Industry (Tokyo, Japan) and Wako Pure Chemical Industry (Osaka, Japan).

2.2. Preparation of 2-alkenal-treated proteins and acidic hydrolysis

Human hemoglobin or HSA (1 mg/ml) was treated with 1 mM 2-alkenals at 37 °C for 24 h in PBS. The reaction mixture was reduced by the addition of 100 mM NaBH₄ at 4 °C overnight and then treated with a quarter volume of 50% trichloroacetic acid on ice for 1 h following neutralization by HCl. After centrifugation, the proteins were washed with cold acetone, then hydrolyzed under acidic conditions for 24 h at 110 °C, and dried under vacuum conditions in a desiccator. The dried sample was dissolved in ethanol and subjected to analysis.

2.3. Preparation of red blood cells from human blood

Blood from a healthy human volunteer was immediately treated with EDTA and fractionated the plasma and red blood cells by centrifugation (1200 × g, 4 °C, 10 min). The RBC fraction (precipitate) was washed three times with equal volumes of PBS.

2.4. Adductome analysis of protein using LC-ESI-MS/MS

The hydrolyzed samples were subjected to the adductome analysis using a TQD triple stage quadrupole mass spectrometer (Waters) equipped with an ACQUITY ultra-performance LC system (Waters) on a reverse-phase column (Develosil HB-C30-UG 3-µm column (100 × 2.0 mm), Nomura Chemical). Elution was performed using mobile phase A (0.1% formic acid) and B (methanol) at the flow rate of 0.3 ml/min with a discontinuous gradient as follows: 1% B at 0 min, 1% B at 1 min, 99% B at 15 min, and 99% B at 20 min. Selected reaction monitoring (SRM) was performed in the positive ion mode using nitrogen as the nebulizing gas under the following conditions: ion source temperature, 120 °C; desolvation temperature, 350 °C; cone voltage, 25 V; collision energy, 25 eV; desolvation gas flow rate, 700 L/h; cone gas flow rate, 50 L/h; collision gas, argon. The strategy was designed to detect the product ion of m/z 110.0 from the positively ionized histidine adducts by monitoring the sample transmitting their [M + H]⁺ > 110.0 transitions. The MS data could be visualized as a two-dimensional image, in which the x axis represents the retention time (min), y axis represents the mass-to-charge ratio (m/z) for the individual detected adducts. The adductome maps are shown with a size of circle encoding the relative abundance.

2.5. Preparation of N\(^\varepsilon\)-(1-carboxyheptan-2-yl)-histidine (CHH) and its stable isotope

N\(^\varepsilon\)-(1-Carboxyheptan-2-yl)-histidine (CHH) and its stable isotope-labeled sample were prepared by a previously described method [12]. Briefly, the 10 mM histidine or [\(^{13}\text{C}_6\)] histidine stable isotope was reacted with 1 mM 2-octenal in PBS. After incubation for 6 h at 37 °C, the reaction mixtures were treated with NaClO₂ and the oxidized product, CHH, was purified by reverse-phase HPLC with a Develosil HB C30-UG-5 column (100 mm × 8.0 mm, Nomura Chemical, Japan), eluted with a linear gradient of water containing 0.1% trifluoroacetic acid (solvent A)-acetoni trite containing 0.1% trifluoroacetic acid (solvent B) (time = 0 min, 0% B; 40 min, 100% B) at a flow rate of 2.0 ml/min.

2.6. LC-ESI-MS/MS analysis of 2-alkenal-histidine Michael adduct and 2-alkanoic acid histidine adduct

A mass spectrometric analysis was performed using the ACQUITY TQD system (Waters) equipped with an ESI probe and interfaced with a UPLC system (Waters). The sample injection volume of 10 μl each was separated on a Develosil HB C30-UG-3 (100 mm × 2.0 mm, Nomura Chemical, Japan) at the flow rate of 0.3 ml/min under the following elution conditions: mobile phase A (0.1% formic acid) and B (methanol) with a linear gradient from 1% to 99% mobile phase B in 6 min. A mass spectrometric analysis in the positive ion mode was performed with the SRM mode (cone potential 40 eV/collision energy 30 eV) and product ion scan mode (precursor ion; m/z 298, cone potential 30 eV/collision energy 30 eV). The monitored SRM transitions were as follows: [\(^{13}\text{C}_6\)]-CHH, m/z 304 > 116, and CHH, m/z 298 > 110. The quantification of CHH was performed by the ratio of the peak area of the target adduct and of the stable isotope labeled CHH.

2.7. Quantification of CHH in 2-ocetal or H₂O₂ treated red blood cells (RBCs)

The RBCs were treated with 1 mM 2-octenal or 10 mM H₂O₂ containing 100 μM sodium nitrite at 37 °C in 10 mM sodium phosphate buffer (pH 7.4) supplemented with 152 mM NaCl. After 24 h of incubation, the RBCs were reduced under alkaline conditions by 100 mM NaBH₄ at 4 °C overnight, then treated with a quarter volume of 50% trichloroacetic acid on ice for 1 h following neutralization by HCl. After centrifugation, the proteins were washed with cold acetone and hydrolyzed under acidic conditions for 24 h at 110 °C, then dried under vacuum conditions in the desiccator. The dried sample was dissolved in ethanol containing a stable isotope labeled CHH as the internal standard and subjected to a quantitative analysis. The quantitative analysis
was repeated for at least three different preparations.

2.8. Statistical analysis

All experiments for quantification were repeated for at least three different preparations. All data are expressed as means ± S.D. Statistically significant differences were determined using the unpaired t-test.

3. Results

3.1. Comprehensive analysis of histidine adducts in the 2-alkenals-modified proteins

Glycated hemoglobins have been widely used as a useful biomarker for diabetic complications [13,14]. Based on our speculation that hemoglobin might undergo covalent modification by lipid peroxidation products, we attempted to identify a hemoglobin-specific adduct using the adductome approach [15]. Both HSA and hemoglobin were incubated with 1 mM 2-alkenals (2-octenal, 2-nonenal, and 2-decenal) at 37 °C for 24 h in PBS. After reduction with NaBH₄, they were hydrolyzed under the conventional acidic conditions, then subjected to the adductome analysis against the histidine residue using LC-ESI-MS/MS (Fig. 1). As shown in Fig. 2, most of the peaks, corresponding to the 2-alkenal-histidine Michael adducts, namely 2-octenal-histidine (m/z 284), 2-nonenal-histidine (m/z 298) and 2-decenal-histidine (m/z 312), were detected in both modified proteins. However, the adducts X₈ (m/z 284), X₉ (m/z 298), and X₁₀ (m/z 312), corresponding to the reduced form of the 2-alkenal-histidine Michael adducts plus 14 mass unit, were detected only in the 2-alkenal-modified hemoglobin, but not in the modified HSA. The data suggested the formation of histidine adducts unique to the 2-alkenal-modification of hemoglobin.

3.2. Identification of a histidine adduct unique to the 2-alkenal-modified hemoglobin

When the kinetics for the formations of the 2-octenal-histidine Michael adduct and X₈ were determined by LC-ESI-MS/MS, the formation of X₈ steadily increased up to 24 h, whereas the 2-octenal-histidine Michael adduct increased in the early phase and thereafter decreased (Fig. 3A). The data suggest a possibility that the 2-octenal-histidine Michael adduct may be a precursor of X₈. In addition, the high resolution ESI-MS of X₈ showed a molecular ion peak at m/z 298.1765, [M+H]⁺, corresponding to the molecular formula of C₁₄H₂₅N₃O₄. These and the fact that the molecular formula of the reduced form of the 2-octenal-histidine Michael adduct is C₁₄H₂₃N₃O₃ suggested that the aldehyde group of the 2-octenal-histidine Michael adduct might be oxidized to form the corresponding carboxylic acid. Thus, it was speculated that the adduct might be an oxidized form of the 2-octenal-histidine Michael adduct, N°-(1-carboxyheptan-2-yl)-histidine (CHH) (Fig. 3B). To prove this hypothesis, CHH was prepared by the oxidation of the 2-octenal-histidine Michael adduct with sodium chlorite (NaClO₂) and the product was analyzed by LC-ESI-MS/MS. Fig. 3C shows that X₈ was indistinguishable from the oxidized form of the 2-octenal-histidine Michael adduct. The chemical structure of adduct was elucidated by the comparison of fragment ion pattern of authentic standard with that of X₈ from 2-octenal-treated hemoglobin. As shown in Fig. 3D, the fragment pattern of X₈ was in agreement with that of authentic CHH. In a manner similar to X₈, X₉ and X₁₀ were also identified as N°-(1-carboxyoctan-2-yl)-histidine and N°-(1-carboxynonan-2-yl)-histidine, respectively. Other 2-alkenals also generated similar alkanoic acid-histidine adducts upon the reaction with hemoglobin (Fig. 4). These results suggest that 2-alkenals may ubiquitously generate the oxidized forms of the 2-alkenal-histidine Michael adducts, namely, alkanoic acid-histidine adducts, upon the reaction with hemoglobin.

3.3. Involvement of heme iron in the formation of the alkanoic acid-histidine adducts

The finding that the alkanoic acid-histidine adducts were formed in the 2-alkenal-treated hemoglobin, but not in the 2-alkenal-treated HSA (Fig. 2), suggests the involvement of iron in the formation of the adducts. Hence, we investigated if the alkanoic acid-histidine adducts could be formed in non-heme iron proteins upon incubation with 2-alkenal. A representative non-heme iron protein, transferrin, and three heme iron proteins, namely hemoglobin, cytochrome C, and myoglobin, were treated with 2-octenal for 24 h at 37 °C. CHH was detected in the 2-octenal-treated hemoglobin and myoglobin, which directly bind to an oxygen molecule via the ferrous ion of heme (Fig. 5A), whereas the adduct was barely observed in cytochrome C, a heme-containing but not O₂-binding protein, and transferrin, an iron-binding non-heme protein (Fig. 5A). These results suggested that the heme-oxygen complex may contribute to the formation of the alkanoic acid-histidine adducts. To elucidate the possible involvement of iron ion in the generation of alkanoic acid-histidine adducts, we examined the effect of the heme derivatives on the CHH formation in HSA. As shown Fig. 5B, CHH was formed in the presence of hemin, whereas protoporphyrin IX did not enhance the formation of CHH, suggesting that a heme iron may play a role in the oxidation of the histidine adducts in the proteins.

3.4. Formation of CHH in red blood cells

We then examined the formation of the alkanoic acid-histidine adducts in the RBCs treated with the 2-alkenals. As shown in Fig. 6, the alkanoic acid-histidine adducts were detected upon the reaction of the RBCs with the 2-alkenals, whereas the adducts were not detected in the plasma fractions treated with the 2-alkenals. To quantify CHH, we established a quantification method using a stable isotope dilution
method. The amount of CHH was quantified by the ratio of the peak area of the target and of the stable isotope-labeled internal standard octanoic acid-\[ ^{13}\text{C}_6\] histidine. The limit of the quantification of the CHH is ∼200 fmol. Using this method, we quantified the CHH formed in the 2-octenal-treated RBCs. The isolated human RBCs (5 × 10^8 cells/ml) were incubated with 2-octenal (1 mM) at 37 °C, and subjected to an LC-ESI-MS/MS analysis following reduction with NaBH4 and hydrolyzed under the conventional acidic conditions. As shown in Fig. 7A, CHH was detected in 2-octenal-treated RBCs. The formation of CHH was also confirmed by the fragment ion pattern of CHH formed in 2-octenal-treated RBC (Fig. 7B). The amount of CHH reached at ∼300 pmol/mg RBC protein (Fig. 7C).

Finally, we examined the formation of CHH in RBCs exposed to oxidative stress. After H_2O_2 (10 mM) treatment in the presence of nitrite (100 μM) for 24 h, the RBCs were lysed with RIPA buffer, and subjected to an LC-ESI-MS/MS analysis. The CHH levels in the H_2O_2-treated RBCs were significantly higher than those of the control RBCs. The amount of CHH in the H_2O_2-treated RBCs was about 6 pmol/mg protein (Fig. 7D). These results suggest that the alkanoic acid-histidine adducts could be formed in the RBCs under oxidative stress.

4. Discussion

Protein modifications are generally catalyzed by a specific enzyme,
Fig. 4. Formation of alkanoic acid-histidine adducts in hemoglobin treated with 2-alkenals. The 2-alkenal-treated hemoglobins were reduced with NaBH₄, acid-hydrolyzed, and analyzed by LC-ESI-MS/MS in the SRM mode.

Fig. 5. Heme-dependent formation of CHH. (A) 2-octenal-treated iron-containing proteins (hemoglobin, myoglobin, cytochrome c and transferrin) were analyzed by LC-ESI-MS/MS in the SRM mode following NaBH₄ reduction and acid hydrolysis. (B) HSA was treated with 2-octenal in the presence of protoporphyrin IX (PP IX) or hemin, then analyzed by LC-ESI-MS/MS in the SRM mode following NaBH₄ reduction and acid hydrolysis.
but can also progress through non-enzymatic mechanisms, such as the reaction between lipid metabolites and nucleophilic amino acids like histidine and lysine [16]. These modifications modulate biological functions such as gene expression, protein activity and stability, intracellular localization of proteins and protein-protein interactions [17–19]. Based on these and the fact that post-translational modifications increase the complexity of the eukaryotic proteome, the identification and comprehensive evaluation of the covalent modifications in proteins are important to gain an understanding of various cellular functions. Taking advantage of the fact that the histidine and lysine adducts produced specific fragment ions that were observed at m/z 110 and 84, respectively, we performed a comprehensive analysis of the histidine and lysine adducts using LC-ESI-MS/MS and identified Nε-(8-carboxyoctanyl)lysine as the most abundant lysine adduct in the oxidized LDL [15]. In the present study, to gain further structural insight into the covalent modification of proteins by lipid peroxidation products, we comprehensively analyzed the histidine adducts in the 2-alkenal-treated hemoglobin and unexpectedly identified an adduct specifically generated in the 2-alkenal-modified heme proteins. We also observed that the alkanoic acid-histidine adducts could be formed in (i) the oxygen-binding heme proteins (hemoglobin and myoglobin) treated with 2-alkenal (Fig. 5A), and (ii) the 2-alkenal-treated HSA in the presence of hemin (Fig. 5B). Based on these findings, we propose a possible mechanism for the formation of the alkanoic acid-histidine adducts as follows: (i) The 2-alkenal-histidine Michael adduct is formed upon the reaction of heme proteins with the 2-alkenal. (ii) The Michael adduct is oxidized by Fe3+ to form an acyl radical, then the acyl radical reacts with molecular oxygen to afford a peracid radical. (iii) When the peracid radical is reduced by Fe2+ in the presence of H+, a peracid is generated. (iv) The alkanoic acid-histidine adduct is formed via the reaction between the peracid and aldehyde (Michael adduct) or via a Fe3+-catalyzed conversion of the peracid to the carboxylic acid (alkanoic acid-histidine adduct). This iron ion and oxygen-dependent mechanism is also suggested by our preliminary data that His59 in the α chain of hemoglobin, which is involved in the molecular oxygen binding with an iron ion in heme [20,21] (Fig. S1), has been identified as one of the target histidine residues in hemoglobin (Yoshitake, Shibata, Shimayama, & Uchida, unpublished observation). These findings also speculated that hemoglobin modification by 2-alkenals may contribute to the oxidative-stress-dependent dysfunction of hemoglobin.

RBCs transport respiratory gases between the lungs and tissues via hemoglobin, which constitutes about 90% of the dry weight of the RBCs. Various previous studies showed the chemical modifications of hemoglobin with endogenous and exogenous electrophiles [22,23]. Hemoglobin is readily covalently modified by reduced glucose at the N-terminal valine of the beta chain to form glycated hemoglobin (hemoglobin A1c) [24]. In the clinical scenes, the hemoglobin A1c is a useful indicator for the long-term average glucose level and the risk of diabetic complications [25]. In addition, Stevens and his co-workers reported that acetaldehyde, a reactive metabolite of ethanol, reacts with valine, lysine and tyrosine residues of hemoglobin to form acetaldehyde-hemoglobin adducts. They also demonstrated that the amount of acetaldehyde-hemoglobin adducts from alcoholic patients was significantly elevated compared to normal subjects [26]. Moreover, Carlsson et al. established the novel Edman degradation-based method for the LC-MS/MS analysis of N-terminal valine adducts in hemoglobin detached as fluorescein thiohydantoin derivatives. Using this methods, they detected some known adducts, such as the methyl adduct and methyl vinyl ketone adduct, and many unknown adducts in RBCs from smokers [27]. In the present study, we detected CHH in the H2O2-
treated RBCs in the presence of nitrite, which can convert hemoglobin to methemoglobin (Fig. 7D). On the other hand, CHH was not detected in the H2O2-treated RBCs in the absence of nitrite (Yoshitake, Shibata, Shimayama, & Uchida, unpublished observation), suggesting that CHH could be preferentially formed in methemoglobin. These results suggest a possibility that the alkanoic acid-histidine adduct could be a candidate biomarker for methemoglobinemia, a condition caused by elevated levels of methemoglobin in the blood [28].

In summary, we identified the alkanoic acid-histidine adducts as novel adducts specifically generated in the 2-alkenal-modified hemoglobin. The adducts were suggested to be formed by the oxidation of 2-alkenal-histidine Michael adducts by the iron ion and oxygen. In addition, the alkanoic acid-histidine adducts were also detected in the RBCs treated with H2O2. These findings suggest the connection between the heme-dependent modification of proteins by 2-alkenals and human pathogenesis. Further studies are required to understand the biological consequences of the production of alkanoic acid-histidine adducts.

Conflicts of interest

The authors declare no conflicts of interest in this work.

Acknowledgments

This work was supported in part by Grant-in-aid for Scientific Research (A) 26252018 (to K. U.), Grant-in-aid for Scientific Research on Innovative Areas “Oxygen Biology: a new criterion for integrated understanding of life” 26110111 (to K. U.), the Japan Science and Technology Agency PRESTO program (JPMJPR1334 to T. S.), and the Center of Innovation Program of the Ministry of Education, Sciences, Sports, Technology (MEXT), Japan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2019.101115.

References

[1] J.M. Gutteridge, B. Halliwell, The measurement and mechanism of lipid peroxidation in biological system, Trends Biochem. Sci. 15 (1990) 129–135.
[2] G. Witz, Biological interactions of α,β-unsaturated aldehydes, Free Radic. Biol. Med. 7 (1989) 333–349.
[3] K. Uchida, Role of reactive aldehyde in cardiovascular disease, Free Radic. Biol. Med. 28 (2000) 1685–1696.
[4] L.J. Marnett, J.N. Riggins, J.D. West, Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein, J. Clin. Invest. 111 (2003) 583593.
[5] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radic. Biol. Med. 11 (1991) 81–128.
[6] K. Uchida, Histidine and lysine as targets of oxidative modification, Amino Acids 25 (2003) 249–257.
[7] Y. Pocker, N. Jankić, Differential modification of specificity in carbonic anhydrase catalysis, J. Biol. Chem. 263 (1988) 6169–6176.

[8] K. Uchida, M. Kanematsu, K. Sakai, M. Matsuda, N. Hattori, Y. Mizuno, D. Suzuki, T. Miyata, N. Noguchi, E. Niki, T. Osawa, Protein-bound acrolein: potential markers for oxidative stress, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 4882–4887.

[9] K. Ichihashi, T. Osawa, S. Toyokuni, K. Uchida, Endogenous formation of protein adducts with carcinogenic aldehydes: implication for oxidative stress, J. Biol. Chem. 276 (2001) 23903–23913.

[10] A. Furuhata, T. Ishii, S. Kumazawa, T. Yamada, T. Nakayama, K. Uchida, Nε-[(3-Methylpyridinium)lysine, a major antigenic adduct generated in acrolein-modified protein, J. Biol. Chem. 278 (2003) 48658–48665.

[11] K. Ichihashi, C. Wakah, T. Shibata, S. Toyokuni, S. Machida, S. Matsuda, T. Matsuda, K. Uchida, Lipid peroxidation generate body odor component trans-2-nonenal covalently bound to protein in vivo, J. Biol. Chem. 285 (2010) 15302–15313.

[12] E. Dalcanale, F. Montanari, Selective oxidation of aldehydes to carboxylic acids with sodium chloride-hydrogen peroxide, J. Org. Chem. 51 (1986) 567–569.

[13] S. Rahbae, O. Blumenfeld, H.M. Ranney, Studies of unusual hemoglobin in patients with diabetes mellitus, Biochem. Biophys. Res. Commun. 36 (1969) 838–843.

[14] E. Selvin, M.W. Steffes, H. Zhu, K. Matsushita, I. Wagenskrecht, J. Pankow, J. Coreb, F.L. Brancati, Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults, N. Engl. J. Med. 362 (2010) 800–811.

[15] T. Shibata, K. Shimizu, K. Hirano, F. Nakashima, R. Kikuchi, T. Matsushita, K. Uchida, Adductome-based identification of biomarkers for lipid peroxidation, J. Biol. Chem. 292 (2017) 8223–8235.

[16] W.N. Beavers, K.L. Rose, J.J. Galligan, M.M. Mitchener, C.A. Rouzer, K.A. Tallman, C.R. Lamberson, X. Wang, S. Hill, P.T. Ivanova, H.A. Brown, B. Zhang, N.A. Porter, L.J. Narnett, Protein modification by endogenously generated lipid electrophiles: mitochondria as the source and target, ASC Chem. Biol. 12 (2017) 2062–2069.

[17] R.E. Moeller, B.F. Cravatt, Functional lysine modification by an intrinsically reactive primary glycotic metabolite, Science 341 (2013) 549–553.

[18] M. Nishida, T. Sawa, N. Kitajima, K. Ono, H. Inoue, H. Bara, H. Motohashi, M. Yamamoto, M. Suenatsu, H. Kuruse, A. van der Vliet, B.A. Freeman, T. Shibata, K. Uchida, Y. Kumagai, T. Akaike, Hydrogen sulfide anion regulates redox signaling via electrophile sulfhydration, Nat. Chem. Biol. 8 (2012) 714–724.

[19] S. Fuji, T. Sawa, H. Ibara, K.I. Tong, T. Ida, T. Okamoto, A.K. Abinten, Y. Ishima, H. Motohashi, M. Yamamoto, T. Akaike, The critical role of nitric oxide signaling, via protein S-guanylation and nitrated cyclic GMP, in the antioxidant adaptive response, J. Biol. Chem. 285 (2010) 23970–23984.

[20] C. Poyart, H. Wajcman, J. Kister, Molecular adaptation of hemoglobin function in mammals, Respir. Physiol. 90 (1992) 3–17.

[21] E.V. Phillips Simon, P. Schoenborn Benno, Neutron diffraction reveals oxygen-histidine hydrogen bound in oxymyoglobin, Nature 292 (1981) 81–82.

[22] M. Törnqvist, C. Fred, J. Haghund, H. Helleberg, B. Paulsson, P. Rydberg, Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications, J. Chromatogr. B 778 (2002) 279–308.

[23] E. Bergmark, C.J. Callman, F. He, L.G. Costa, Determination of hemoglobin adducts in humans occupationally exposed to acrylamide, Toxicol. Appl. Pharmacol. 120 (1993) 45–54.

[24] H.F. Bunn, D.N. Hansey, S. Kamin, K.H. Gabbay, P.M. Gallop, The biosynthesis of human hemoglobin A1c, Slow glycosylation of hemoglobin in vivo, J. Clin. Invest. 57 (1976) 1652–1659.

[25] E.J. Gallagher, D.L.E. Roith, Z. Bloomgarden, Review of hemoglobin A1c in the management of diabetes, J. Diabetes 1 (2009) 9–17.

[26] V.J. Stevens, W.J. Fantl, C.B. Newman, R.V. Sims, A. Cerami, C.M. Peterson, Acetaldehyde adducts with hemoglobin, J. Clin. Invest. 67 (1981) 361–369.

[27] H. Carlsson, H. von Stedingk, U. Nilsson, M. Törnqvist, LC-MS/MS screening strategy for unknown adducts to N-terminal valine in hemoglobin applied to smokers and nonsmokers, Chem. Res. Toxicol. 27 (2014) 2062–2070.

[28] T.S. do Nascimento, R.O.L. Pereira, H.L.D. de Mello, J. Costa, Methemoglobinemia: from diagnosis to treatment, Rev. Bras. Anestesiol. 58 (2008) 651–664.