International Journal of Biological Macromolecules xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Role of peroxynitrite induced structural changes on H2B histone by physicochemical method

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ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 26 October 2015
Accepted 27 October 2015
Available online xxx

Keywords:
Peroxynitrite
Histone H2B
Nitrotyrosine
Dityrosine

ABSTRACT

Histones are small highly conserved cationic proteins which bind DNA and remain confined in the nucleus. These histones are quite vulnerable to oxidizing and nitrating agents. Peroxynitrite is a powerful oxidant and nitrating agent present in the biological system. In this study, peroxynitrite-induced nitration and oxidation of H2B was assessed by various physicochemical techniques. The carbonyl content and dityrosine were markedly elevated in peroxynitrite-modified H2B histone as compared to the native histone. Cross-linking of H2B was evident on polyacrylamide gel electrophoresis. 3-Nitrotyrosine was present only in peroxynitrite-modified H2B revealed by HPLC. The results showed that peroxynitrite-mediated nitration and oxidation in H2B histone exhibited hyperchromicity, decrease of tyrosine fluorescence accompanied by increase in ANS-binding specific fluorescence, loss of β-sheet structure, appearance of new peak in FT-IR, increase in melting temperature and also loss of α-helix to produce a partially folded structure in comparison to intrinsically disordered structure of native H2B histone. We concluded that the H2B histone, a constituent of core histones, is highly sensitive to peroxynitrite and can adopt different structures under nitrosative and oxidative stress in order to protect the packaged DNA from the deleterious insult of peroxynitrite.

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1. Introduction

Peroxynitrite ($ONOO^-$) is a relatively long lived potent oxidant formed from the reaction between superoxide and nitric oxide (NO) [1]. Its biological effects are due to its reactivity toward biomolecules including lipids, proteins and nucleic acids causing oxidative as well as nitrosative damage and other free radical-induced chain reactions. These reactions trigger cellular responses such as cellular signaling, oxidative injury and committing cells to necrosis or apoptosis. It is also involved in tissue damage in a number of pathophysiological conditions such as neurodegenerative diseases, cardiovascular disorders and autoimmune diseases [2,3]. Peroxynitrite exhibits unique chemical reactivities such as protein nitration, DNA strand breakage, base modification, which may have cytotoxic effects and also lead to mutagenesis. It nitrates free or protein-bound tyrosine to form the stable product, 3-nitrotyrosine (3-NT), by addition of a nitro group to the 3-position adjacent to the hydroxyl group of tyrosine. The nitrated product, 3-NT, is found in many pathophysiological conditions such as chronic inflammation, myocardial infarction, chronic heart failure, diabetes, neurodegenerative disorders, cancer, atherosclerosis, autoimmune diseases and acute lung injury [4,5]. The formation of nitrotyrosine represents a specific peroxynitrite-mediated protein modification; thus, detection of nitrotyrosine in proteins is considered as a biomarker for endogenous peroxynitrite activity [6]. The most often used marker of protein oxidation is protein carbonyls, and such structures are formed due to oxidative modification of the side chains of lysine, proline, arginine and threonine [7]. As peroxynitrite reaction involves free radical intermediates, it may favor cross-linking and aggregation during nitration. Formation of tyrosyl radical by peroxynitrite and its reaction with another tyrosyl radical may generate O,O′-dityrosine covalent cross-links. Peroxynitrite induces an array of modifications in histones structure, like tyrosine nitration, protein carbonyl, dityrosine and cross-linking. Such gross structural changes might favor polymerization of native epitopes of histone into potent immunogenic neoepitopes [8].

Histones are small highly conserved cationic proteins and week immunogenic which bind DNA and are major components of chromatin and remain confined to nucleus. Both chromatin and histone proteins are components of the nucleosome [9]. The N-termini of histones are enriched in lysine and arginine residues, which protrude out of the nucleosome and are therefore accessible to

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http://dx.doi.org/10.1016/j.ijbiomac.2015.10.085
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dynamic post-translational modifications. Lysine residues can be acetylated, mono-, di-, and tri-methylated, while arginine is mono- and dimethylated (symmetric and asymmetric) [10].

Histones are highly α-helical proteins with 65–70% of helix content. Only 3% of residues can be assigned to short parallel β-sheets, and the remainder is not ordered [11]. The sequence of a given type of histone is highly conserved from yeast to mammals, but there is 4–6% sequence identity. These modifications that are found in histones include phosphorylation of serine and threonine residues, and ubiquitination and sumoylation of lysine [12,13]. Once DNA is packaged into chromatin, its function is controlled by the ordered recruitment of diverse enzymatic complexes that chemically modify nucleosomes, remodel their structure, or change their position relative to the DNA [14].

The type and combination of histone modifications influence gene regulation and they act synergistically to alter chromatin structure and regulate transcription [15]. Thus, histone modifications may establish a code that is "read" by other proteins and that directs the function of chromatin-remodeling machines and transcription factors [16]. Chromatin remodeling is important for genome integrity, epigenetic inheritance, and in genome defense as well as during development [17]. Although the existence of a histone code is still controversial, there is increasing evidence to support this concept. However, the information on histone modifications is not complete, especially concerning the possible combinations of modifications and their function. Immunoblotting using antibodies that detect specific modified histone amino acids have now provided valuable insights into the association of histone modifications with gene activity. But this approach has limitations, since it does not allow detection of new or multiple modification sites and it cannot distinguish between different histone variants.

Fluorescence spectroscopy, CD, FTIR and HPLC provide an excellent alternative approach to identify post-translational modifications, and some histones have already been characterized by fluorescence, CD, FTIR and HPLC techniques [18]. In this communication, we report the peroxynitrite-induced structural changes in H2B histone by quantitative and qualitative methods.

2. Material and methods

2.1. Materials

H2B histone, 3-nitrotyrosine, diethylenetriaminepenta-acetic acid (DTPA), 2,4-dinitrophenyl hydrazine (DNPH), 0.45 μm filter units and 1-anilinonaphthalene-8-sulfonic acid (ANS) were obtained from Sigma Chemical Company, St. Louis, USA. Sodium nitrite, hydrogen peroxide, silver nitrate, guanidinium chloride, sodium hydroxide and ammonium persulphate were obtained from Qualigens, India. All other reagents were of highest analytical grade available.

2.2. Peroxynitrite-modification of H2B

Peroxynitrite was synthesized in laboratory by rapid quenched flow method [19] using sodium nitrite and acidified hydrogen peroxide. The yellow color peroxynitrite solution showed maximum absorbance at 302 nm and was stored in 1.2 M NaOH at −20 °C. Concentration of stored peroxynitrite was determined before each use by absorbance measurement at 302 nm using molar extinction coefficient of 1670 $M^{-1}cm^{-1}$. The modification was carried out by incubating 25 μM H2B histone in a buffer (10 mM sodium phosphate buffer, pH 7.4 containing 150 mM NaCl and 100 μM DTPA) with peroxynitrite (50, 100 and 200 μM) at 37 °C for 30 min. The approximate pH of the incubation mixture was in the range of 10–11. Unmodified H2B histone served as control.

2.3. UV spectroscopy

The absorption profile of native and peroxynitrite-modified H2B histone was recorded on spectrophotometer (Shimadzu, model UV 1700) in the wavelength range of 250–500 nm in a quartz cuvette of 1 cm path length.

2.4. Intrinsic and extrinsic fluorescence studies

Emission profile was recorded on spectrofluorometer (Shimadzu, model RF 5301 PC). Tyrosine fluorescence of native and peroxynitrite-modified H2B histone was monitored in the wavelength range of 275–400 nm after excitation at 275 nm. Loss in emission intensity was computed using the following formula:

$$\text{Percent loss in emission} = \frac{\text{Emission of native H2B} - \text{Emission of peroxynitrite-modified H2B}}{\text{Emission of native H2B}} \times 100$$

ANS binding with native and peroxynitrite-modified H2B histone was evaluated in terms of fluorescence. ANS fluorescence is affected by exposure or masking of hydrophobic groups/patch of proteins. The molar ratio between ANS and H2B histone was kept at 1:5 and emission spectra were recorded in the wavelength range of 400–600 nm after excitation at 380 nm. Increase in fluorescence intensity (FI) was calculated as follows:

$$\text{Percent increase in FI} = \frac{\text{FI of peroxynitrite-modified H2B} - \text{FI of native H2B}}{\text{FI of peroxynitrite-modified H2B}} \times 100$$

2.5. SDS-polyacrylamide gel electrophoresis

Native and modified H2B histone samples were analyzed by non-reducing SDS polyacrylamide gel electrophoresis as described previously [20]. Samples (25 μg) were mixed with one-fourth volume of sample buffer (10% glycerol, 0.5% SDS, 0.002% bromophenol blue and 0.5 M Tris–HCl, pH 6.8) and the protein bands were visualized by silver staining.

2.6. Quantitation of protein nitrotyrosine by HPLC

Pronase digested samples (native H2B as well as those modified by different doses of peroxynitrite) were subjected to HPLC analysis for nitrotyrosine. The retention time of standard 3-nitrotyrosine on HPLC was taken as reference [8]. Briefly, the pronase protease was first dialysed against 0.1 M sodium acetate buffer, pH 7.2 and then added to native and modified histone samples (12.5 μM) kept in 20 mM Tris buffer, pH 7.4. The pronase:histone ratio was 1:5 (w/w). The assay tubes were then heated to 50 °C for 16 h to ensure complete hydrolysis of the proteins. The hydrolysed samples were then subjected to ultrafiltration through 0.45 μM filter units. The filtered samples were diluted 1:2 with eluant buffer (0.5 M potassium phosphate, pH 3.0 containing 10% methanol) before placing on a C-18 reversed phase column (25 cm × 4.6 mm; Vydac, USA).

2.7. Estimation of dityrosine

The dityrosine content of peroxynitrite-modified H2B was determined spectrophotometrically using a molar extinction coefficient of 4000 M^{-1} cm^{-1} at 330 nm [21].

Please cite this article in press as: M.A. Khan, et al., Int. J. Biol. Macromol. (2015), http://dx.doi.org/10.1016/j.ijbiomac.2015.10.085

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