

Scanning Electron Microscopic Analysis of Glycated Histone H2B



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Abstract

Glycation is a widely reported modification of proteins. It leads to the compromise in the structural integrity of the proteins and have been widely implicated in pathological problems. While a large number of protein structures have been analysed for post glycation effects, no work reports changes in histone H2B upon non enzymatic glycation. The work presents the scanning electron micrographic study on the structure of histone H2B derived from calf thymus after its modification by an oxo-aldehyde named methylglyoxal. The results reveal a complete different look of H2B post modification under SEM, and the images point towards the amorphous aggregate adduct generation. While, many protein modifications may have little significance, the modifications of H2B can have severe impact on the gene expression and epigenetics as it is a part of core histone octamer. The work needs to be taken forward for further analysis.

Introduction

Glycation is a multi-step series of events wherein nucleophilic part of a proteins reacts with the sugars or their aldehyde and ketone derivatives leading to the generation of irreversible cross-linked products, described as advanced glycation end products (AGEs) [1]. The research has revealed that α -dicarbonyls or oxoaldehydes are the most potent glycating agents that damage the structural integrity of proteins [2]. Glycation and its associated carbonyl and oxidative stress have clinical implications in multiple pathological conditions including diabetes and cancer [3-5]. Giovannucci et al. [6] have pointed towards a possibility wherein hyperglycemia in diabetes conditions may confer a growth advantage to the tumors and have also suggested not-to overlook even glucose as a potentially relevant mediator. It may be noted that hyperglycemia leads to the accumulation of AGEs. In the context, the glycation induced modification of proteins and the structural and conformational changes thereof have emerged as an important field of study. In our earlier works, we have established the role of glycation in histone H1 and H2A modification [5,7]. In this study we present the scanning electron microscopic analysis of glycation of histone H2B. Histone H2B is a 14 kDa protein that consists of 126 amino acid residues with abundance of lysine and arginine residues. The later make the protein susceptible to glycation. Its N-terminal tails are the most accessible regions of its peptide as they protrude from the nucleosome and they have been reported to be prone to various post-translational modifications (PTMs).

H2B is a basic protein that is part of the histone octamer and is found associated with DNA in cell nuclei of eukaryotic cells [8].

H2B glycation

The concentration of H2B was ascertained by using molar absorption coefficient (280 nm) of 6400 M⁻¹ cm⁻¹ and the molecular weight of 13774 Dalton for calf thymus H2B. Earlier published protocol was adopted wherein H2B (50 μ M) was glycated by 7.5mM of methylglyoxal in phosphate buffer saline (10 mM sodium phosphate buffer, pH 7.4 containing 150mM NaCl) and incubated overnight [5].

Scanning electron microscopy (SEM)

The unmodified and the glycated samples of H2B protein were subjected to scanning electron microscopic analysis to obtain the alterations in its structural architecture. Samples were air dries and adsorbed upon the cellulose ultra filtration membrane that was coated with gold. The sample was mounted on a carbon tape that had coated stainless steel grids working at 15kV. The instrument employed was a JSM-6510LV (JEOL JAPAN) scanning electron microscope running. The detailed protocol has been presented earlier [9].

Results

The images obtained under SEM have been provided as Figure 1 for native H2B and Figure 2 for glycated H2B. The results reveal a dramatic change in the structural integrity of the protein.

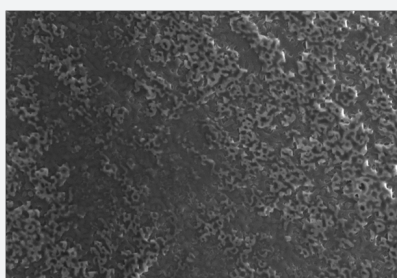


Figure 1: Scanning electron micrograph of native histone H2B (scale bar of 5µm and viewed at 5000×).

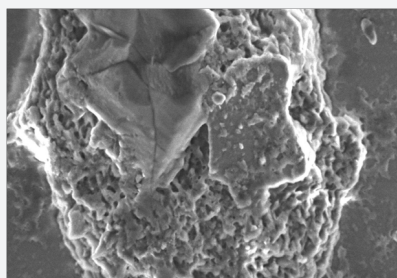


Figure 2: Scanning electron micrograph of methylglyoxal modified histone H2B (scale bar of 5µm and viewed at 5000×).

Discussion

Glycation induced structural modifications have been presented by wide number of workers [1-14]. However, there is no study on the SEM studies of histone H2B in the published literature. Our experimental analysis of H2B glycation reveals severe damage of protein integrity as is evident from the morphological featuring of the glycated H2B. Visibly, the native protein appears to show no aggregation. Incubation with methylglyoxal has changed altogether in shape and its presents an amorphous type aggregate adduct. The formation of in proteins upon sugar mediated modifications can be traced from earlier papers as well [9-11]. Aggregation of glycated proteins has been observed with varied shapes and sizes and many have reported clustered granular precipitates and large branched chain shapes in the modified proteins as well [12]. The aggregation of proteins has been attributed with cytotoxic implications in the earlier literature [10-13]. This study and its related works confirm the diversity of aggregates formed in proteins upon glycation. To conclude, it may be argued that histone glycation is a well reported phenomenon and this study extends the work to H2B histone, wherein glycation led to the generation of amorphous aggregates [14-15].

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