Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 163 (2016) 60–67

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Advanced glycation end products induce differential structural modifications and fibrillation of albumin

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 11 March 2016
Accepted 20 March 2016
Available online 22 March 2016

Keywords:
Glycation
Amyloid
Fibrillation
Carboxymethyllysine
Carboxyethyllysine
Argpyrimidine
Albumin

ABSTRACT

Glycation induced amyloid fibrillation is fundamental to the development of many neurodegenerative and cardiovascular complications. Excessive non-enzymatic glycation in conditions such as hyperglycaemia results in the increased accumulation of advanced glycation end products (AGEs). AGEs are highly reactive pro-oxidants, which can lead to the activation of inflammatory pathways and development of oxidative stress. Recently, the effect of non-enzymatic glycation on protein structure has been the major research area, but the role of specific AGEs in such structural alteration and induction of fibrillation remains undefined. In this study, we determined the specific AGEs mediated structural modifications in albumin mainly considering carboxymethyllysine (CML), carboxyethyllysine (CEL), and argpyrimidine (Arg-P) which are the major AGEs formed in the body. We studied the secondary structural changes based on circular dichroism (CD) and spectroscopic analysis. The AGEs induced fibrillation was determined by Congo red binding and examination of scanning and transmission electron micrographs. The amyloidogenic regions in the sequence of BSA were determined using FoldAmyloid. It was observed that CEL modification of BSA leads to the development of fibrillar structures, which was evident from both secondary structure changes and TEM analysis.

1. Introduction

There is an alarming increase in the incidence of type 2 diabetes, which has directly been linked to the severe complications such as nephropathy, neuropathy, retinopathy and cardiovascular complications [1]. Non-enzymatic glycation of proteins leads to the formation of early and advanced glycation end products (AGEs) which have been identified as the fundamental cause of the pathology of diabetic complications [2–4]. CML and CEL are the major AGEs reported by both *in vitro* and *in vivo* studies [5–8]. Methylglyoxal (MG), which is produced as an intermediate from the metabolism of sugar, amino acids, and lipid, plays a major role in the development of diabetic complications as it reacts with the free arginine residues in proteins leading to the formation of argpyrimidine (Arg-P) [9–11]. Arg-P is a major AGEs observed in conditions of familial amyloidotic polyneuropathy (FAP) which is characterized by the localized deposition of amyloids [12]. CML is the most dominant AGEs formed in the body and also based on the analysis of different age groups it has been observed that the physiological level of CML in infants goes up to 1500 ng/ml [13]. The physiological accumulation of Arg-P also has been found to be highly increased under pathological conditions such as cataract, familial amyloidotic polyneuropathy (FAP) where Arg-P level was found to be very high and valued 162 pmol/ml of the protein [14].

Protein aggregation and amyloid deposition have been recognized as a fundamental to the development of many fatal pathogenic conditions, which includes Alzheimer's and prion disease [15,16]. With the increasing incidences signifying the role of glycated proteins in the progression of many cardiovascular and neurodegenerative disorders, the research in the recent have been focused to determine the role of glycation in fibrillation and aggregation of proteins. Several studies have been carried out considering both structural and functional modifications in proteins to understand the consequences of glycation mediated changes in conditions of high sugars, and its intermediates such as di-carbonyl compounds [17–20].

Albumin is the most abundant plasma protein, which plays a key role in the pharmacokinetics of many drugs. Albumin contains seven fatty acids and two drug binding sites (Sudlow's site I, and II). Bovine serum albumin (BSA) has been frequently used as a model protein due to its high sequence and structural identity with human serum albumin. Both Human serum albumin (HSA) and BSA have a typical heart shaped tertiary structure at the physiological pH with three

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http://dx.doi.org/10.1016/j.saa.2016.03.023
1386-1425/

domains (I, II, III) subdivided into two sub-domains (A and B). Besides its high affinity for both endogenous and exogenous ligands, it is highly prone to the glycation induced structural modification [21–24]. The glycated form of albumin has been observed to exhibit impaired drug binding and antioxidant activity [25]. Lysine and arginine residues have been recognized as the hot spot of non-enzymatic glycation for albumin by at different stage of Maillard reaction based on mass spectroscopic studies [26]. The amyloid form of glycated BSA has been observed to have high cytotoxicity mediated by induction of signaling pathways leading to the development of oxidative stress. Conformational transition in BSA has been suggested to be fundamental during formation of amyloid like fibrils and also glycation has been found to increase the toxicity of β-amyloid in neuronal cells [27,28].

Although glycation has been proposed to induce the conformational change in albumin from globular native state to the fibrillar amyloid state, the role of specific AGEs in such conformational transition remains undefined. With this motivation, the present study was intended to determine the specific AGEs mediated structural modifications and fibrillation of albumin. The three most pre-dominant AGEs (CML, CEL, and Arg-P) modifications were used due to their physiological relevance. BSA was used for experiments involving *in vitro* AGEs modifications and characterization was done to determine the structural changes based on spectroscopic studies involving circular dichroism for secondary structural changes. The AGEs mediates fibrillation of BSA was determined based on the amyloid specific dye *i.e.* congo red binding and electron microscopy. Both Field Emission-Scanning Electron Microscope (FE-SEM) and Field Emission-Transmission Electron Microscope (FE-TEM) imaging were carried out to observe the AGEs induced fibrillar state of BSA.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), glyoxylic acid (GA), pyruvic acid (PA), methylglyoxal (MG), 2,4,6-trinitrobenzene sulfonic acid (TNBSA), and 9,10-phenanthrenequinone and Congo red (CR) were purchased from Sigma, USA. Sodium phosphate buffer (50 mM, pH-7.4) was used for the preparation of all the solutions. All other chemicals of analytical grade were obtained from Merck.

2.2. AGE modification of BSA

Formation of CML, CEL and Arg-P modified BSA was carried out as explained earlier [29]. Breifly, GA and PA were used for the preparation of CML and CEL modification respectively. Methylglyoxal was used for the preparation of Arg-P modified BSA. Sodium cyanoborohydride was used as a reducing agent for CML and CEL. Seven different concentrations (2.5, 5, 10, 15, 20, 25, 50 mM) of GA, PA and MG were used for AGEs modification of BSA (10 mg/ml). After incubations, the modified BSA was dialyzed against PBS (pH-7.4) for 24 h and protein concentration was determined. Gel electrophoresis for the AGEs modified BSA was carried out by using agarose gel. Staining was done using 1% (w/v) coomassie blue followed by overnight de-staining. Gel images were taken with UVi-Tec gel documentation system.

2.3. Determination of glycation potential for lysine residues

NetGlycate 1.0 [30] was used to predict the glycation potential of lysine residues in BSA. NetGlycate predicts the glycation of ε amino groups of lysine in proteins with high accuracy and provides the best

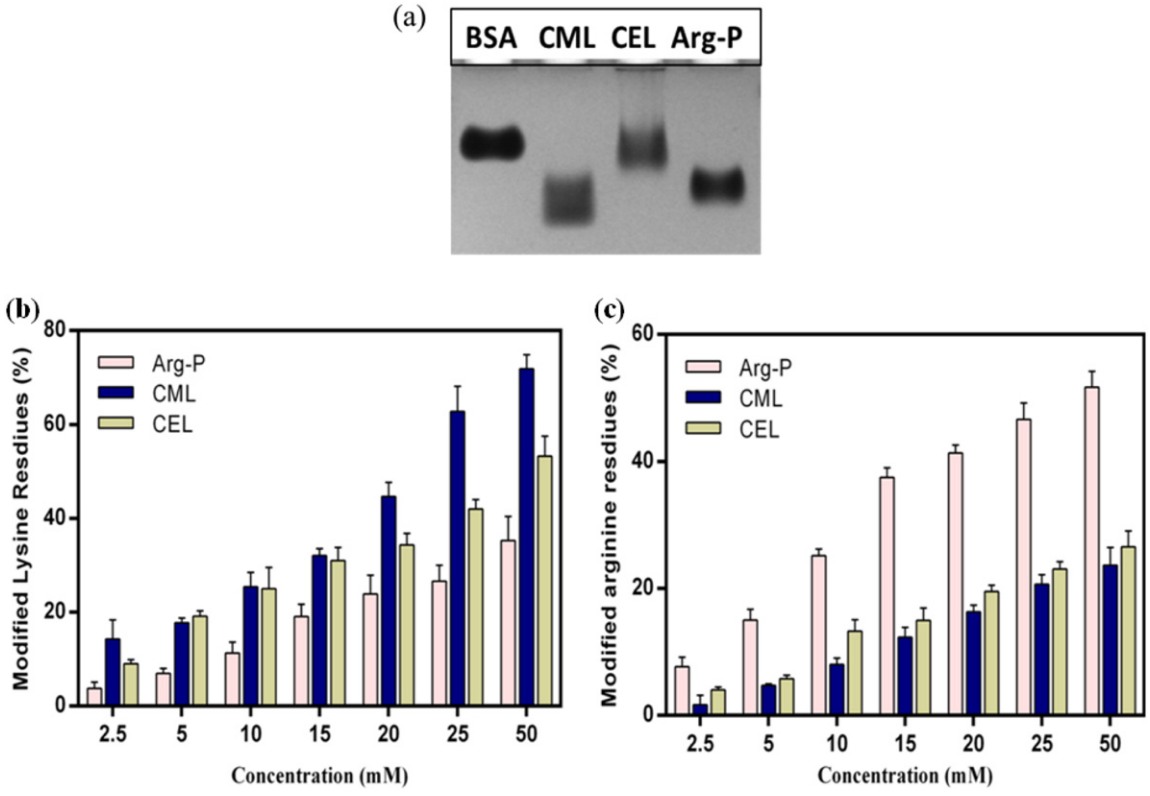

Fig. 1. Gel electrophoresis and quantification of lysine and arginine modification for AGE modified BSA. (a) Agarose gel for AGEs modified forms of BSA (b) Amount of lysine modification as determined using TNBSA (c) Amount of arginine modification as determined using 9, 10-phenanthroquinone.