EXTENDED ABSTRACT

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The comparative study of potassium sorbate and sodium benzoate upon treated with human serum albumin concerning Maillard reaction and amyloid formation

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Abstract

In general, Maillard reaction is known as a process which usually can be triggered by reducing sugars. However, it has been noticed that the reaction products are involved in many diseases. Along with reducing sugars, some preservatives can also interfere with Maillard reaction. Among them, potassium sorbate (PS) and sodium benzoate (SB) are widely used and daily intake through daily diets, drugs and cosmetics by consumers. Here we compare the effect of PS and SB on human serum albumin (HSA) concerning Maillard reaction and amyloid formation using biophysical techniques such as fluorescence spectroscopy and atomic force microscopy. The spectroscopic results showed that both of PS and SB can trigger Maillard reaction but with obvious difference in advanced glycation end products formation and their components. Moreover, the quantity of the obtained amyloid fibrils by PS was quietly larger than it by SB and also the appearance of the obtained fibrils were different. The formation of high amounts of α-helical structures in treated HSA by SB and high amounts of β-sheet structure in treated HSA by PS due to different amounts of AGEs formation seems to be the reason of various HSA intermediates creation.

Introduction

Glycation is a widespread and non-scheduled process that impacts on the structure and function of biomacromolecules, especially proteins. The cascade nature of Maillard reaction and its consequences including formation of various molecular species, as the origin and driving force of glycation, are quite similar with free radicals chain reactions [1]. The covalent binding of reducing sugars to protein's free amino groups is considered as initiation of Maillard reaction [2]. All of these phenomena are highly involved to result in abnormal structures and aggregation formation which are the main causative factor to develop amyloidopathy [3]. During recent years, it is shown that other oxidative agents like some preservatives can promote Maillard reaction and its consequent events in the absence of sugars [4]. Sodium benzoate and potassium sorbate are two preservatives, widely used in the food, pharmaceutical, cosmetic and health industries. The comparative effect of potassium sorbate (PS) and sodium benzoate (SB) upon treating on HSA respect to Maillard

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reaction and amyloid formation is our aim of this study.

Materials and Methods

HSA, sodium benzoate, potassium sorbate were from Sigma Aldrich (USA) and β-d-(+) glucose was from Santa Cruz Biotechnology (USA). The incubation of HSA (40 mg/ml) at 37 ºC with SB (7 mM) was performed in the absence or presence of glucose (16.5 mM) for 35 and 120 days) in sealed-recipients. Samples were dialysed for 48h against phosphate buffer 50 mM, pH 7.4, before use. Then, fluorescence spectrophotometery and AFM analysis were carried out.

Results

The formation of AGEs in treated HSA with PS or SB in the presence or absence of glucose was followed via fluorescence spectroscopy to detect fluorescing AGE-species in which samples were excited at 322, 335, 365, 375 or 380 nm and emission spectra were collected in the wavelength range of 300-600 nm. The maximum fluorescence intensities for desired treatments is tabulated at Table 1. The results showed the difference between AGEs formation by SB and PS.

Table1. Various AGEs formation after 35 days of incubation.

	Maximum Fluorescence intensity(a.u.)				
Samples	**Ex: 322nm**	**Ex: 335nm**	**Ex: 365nm**	**Ex: 375nm**	**Ex: 380nm**
HSA+Glc	200±11.1	230±8.03	170±12	140±10.58	125±8.4
HSA+PS	175±6.5	165±7.2	130±9.5	125±7.5	100±8.3
HSA+ SB	66±5.3	72±6.8	43±3.5	61±4.2	55±4.9
HSA+PS+Glc	410±8.7	520±10.5	435±9.3	270±11.4	230±9.8
HSA+SB+Glc	90±4.6	101±7.6	66±6.1	55±4.7	42±3.9

AGEs has an important role in protein aggregation and amyloid fibril formation [5]. So, AFM was used to observe amyloid fibrils. The size and diameter of particle or fibril formation were tabulated at Table 2. As depicted, there are significant differences between the size of particles or fibrils formed by SB and PS in the presence or absence of Glc.

Table 2. The size of particles and fibrils after 120 days of incubation.

Samples	**Particle**	**Fibril diameter**	**Fibril Length**
HSA+Glc	105.5 ±7.9nm [4]	---	---
HSA+ SB	45±7.3nm	58±5.6nm	375±25nm
HSA+SB+Glc	1±0.2 µm	---	---
HSA+PS	Width:1.46±0.3µm Length:216±0.7µm[4]	188 ±11.5nm[4]	---
HSA+PS+Glc	---	212.4±18nm[4]	2±0.3 µm[4]

Discussion

PS and SB are two oxidative preservatives with different chemical structures which can influence on HSA structure differently. AGEs as complicated molecules are produced due to oxidation, dehydration, oxidative cleavage, polymerization and cross linking of Maillard products. These products cause the biomacromolecular cross linking, then cellular malfunction. The AGEs detection in studied wavelengths revealed meaningful differences in AGEs formation in treated HSA with PS or SB in the presence or absence of Glc (Table 1). Based on results, SB can produce AGEs in very low amounts than PS. Also, there is a difference in the maximum AGEs production between SB and PS in the presence or absence of Glc.

Thioflavin T (ThT) binds to bundle of beta sheets as amyloid structures. We have already reported the intensive amyloidogenic effect of PS and even more intensive effect in the presence of Glc on HSA in 120 days of incubation [4]. In the present research, the ThT results of treated HSA with SB, also implied the formation of amyloid structures (data are not shown). Also, there was a difference between amyloidogenic effect of SB and PS as follows:

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HSA+PS+Glc>HSA+PS>HSA+SB+Glc>HSA +SB>HSA+Glc

The amyloidogenic effect can be related to AGEs formation as oxidative components of Maillard reaction [2]. During with 120 days of incubation, the aggregation and fibril formation took place, due to the intra- or extra-molecular cross linking in which AGE species are directly involved. As mentioned, the amount of AGEs in modified HSA with PS was much more than SB.

Regards to the dependence between amyloid fibril formation and protein secondary structural changes, the comparison between treated HSA by PS [4], SB [6] and Glc after 35 days of incubation was performed by circular dichroism (CD). The comparative results showed that both of PS and SB altered HSA secondary structure compared with control HSA but in different manner: Modified HSA with PS (α- Helix; 58.2±0.4; Antiparallel:3.7±0.03; Parallel:4.6±0.05; β-Turn:14.5±0.02; Random-coil:19±0.04) had increased β-sheet, random coils and decreased α-helix structures, while SB had controversial effect. SB altered HSA secondary structures as (α-Helix:71.51±2.9; Antiparallel:1.06±0.3; Parallel:4.04±0.2; β-Turn:9.53±1.3; Random-coil:13.76±1.4) with increased α-helix, reduced β sheet and random coil structures compared with its control HSA. It is worth mentioning that, previous researches reported that many fibril formations are initiated by β-sheet and the less numbers are initiated by α -helical precursors through dipole – dipole interaction [7]. So, our results can imply that SB stimulated amyloid fibril formation by α-helical and PS by β-sheet precursors. For more information and direct observation of amyloid structures, the samples were checked by AFM. The comparative results demonstrated HSA+SB with a very thin fibrils (375±25nm in length) plus some particles(45±7.3nm in diameter); HSA+SB+Glc with large particles(1±0.2 µm in diameter); HSA+PS with the dense large fibrillar particles (1.46±0.3 µm) and HSA+PS+Glc with a branched fibrillar texture (2±0.3 µm). So, SB and PS had different characters in HSA fibril formation.

Conclusion

SB and PS as oxidative preservatives were approved to role differently as amyloidogenic agents. Both agents exert their effects through AGEs production. PS is not only more potent in AGEs production than SB but also produced AGEs components in different amounts than SB. So, these differences in maximum AGEs production (because of their specific chemical structure and different intra or extra-molecular cross linking ability) induced different HSA secondary structure by SB (α-helix) and PS (β-sheet). This alteration can be followed by different amyloid fibril formation by these two industrial preservatives. In this way, SB and PS can produce different toxicity and diseases.

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