〈Original Article〉

Reactivity of rare sugar D-allose during glycation of human serum albumin

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Summary A rare sugar, D-allose (All), exhibits antioxidant activity, and its application in the fields of medicine and food chemistry can be expected. Glycation of proteins has been thought to evoke an oxidative stress state in vivo, but the detailed process involved is unclear. We estimated the reactivity of All during the glycation of human serum albumin (HSA), which was carried out in the presence of All, D-glucose (Glc), D-fructose (Frc) and D-psicose (Psi). The glycation conditions were at pH 7.4 and pH 9.0, 37℃ for 7 days and 50℃ for 48 hours, respectively. The amount of glycation was measured using a glycated albumin kit (Lucica GA-L), fructosamine assay, MALDI-TOF MS, etc,. All reactivities were 1.93-2.38-fold, 1.83-2.40-fold, and 1.63-2.78-fold higher than Glc in glycated albumin, fructosamine, and molecular mass, respectively. Molecular mass increases were larger at pH 9.0 than at pH 7.4. Our results also revealed that alkaline conditions promoted glycation.

Key words: D-Allose, Glycation, Human serum albumin, D-Glucose, MALDI-TOF MS

1. Introduction

Nonenzymatic glycation of proteins, or the Maillard reaction, has been suggested to play an important role in the development of diabetic complications[1)]. In the early stage of the Maillard reaction, reducing sugar as D-glucose (Glc) reacts with free amino groups on proteins to form a Schiff base, which is stabilized by an Amadori rearrangement. The Amadori (glycated) products, hemoglobin A1c (HbA1c) and glycated albumin, are recognized as indicators for glycemic control. In an advanced Maillard reaction, the Amadori rearrangement products undergo oxidation, dehydration, and condensation to form advanced glycation end products (AGEs). The formation of AGEs leads to browning, fluorescence-formation, and the cross-linking of proteins. AGEs induce oxidative stress by the generation of reactive oxygen species (ROS) from Amadori products. Maillard reaction products (MRPs) are well known to exhibit antioxidant activities through the formation of hydroxyl radical scavenging activity[2)].

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Rare sugars are expected to be useful in the fields of medicine and the food industry. D-Allose (All; the C-3 epimer of Glc, Fig. 1) is a rare sugar whose biological functions remain unclear. Recent studies have shown that All exerts neuroprotective effects against ischemia-reperfusion injury and a significant inhibitory effect on cancer cell proliferation[3)-6)]. All inhibits production of ROS, for which it shows a weak scavenging activity. This mechanism is postulated to depend on the antioxidant activities of All[7)]. Glycation of proteins is thought to be a condition of oxidative stress. If All exhibiting antioxidant activities suppresses glycation, a reduction of diabetic complications may be expected.

We then estimated the reactivity of rare sugar All during the glycation of human serum albumin (HSA).

2. Materials and methods

1. Materials

D-Allose (All, Lot No. KAI004-46-048) and D-psicose (Psi, Lot No. FDP040 622.056) were obtained from the Kagawa Rare Sugar Research Center (Kagawa, Japan). Human serum albumin (HSA, essentially fatty acid free, Lot No. 018K7540), D-glucose (Glc) and D-fructose (Frc) were purchased from Sigma-Aldrich Co., and phosphate buffered saline (PBS), sodium hydrogen carbonate and sodium carbonate were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals used were of guaranteed reagent grade.

2. Glycation of HSA

Glycation of HSA was carried out with two aldohexoses (All and Glc) and two ketohexoses (Frc and Psi). HSA was dissolved in PBS (pH 7.4) or 10 mmol/L carbonate buffer solution (CBS, pH 9.0) at a protein concentration of 5% (w/v). HSA solutions were added to a 1/9 volume of sugar solutions dissolved in deionized water (final concentration; 1,000 mg/L = 55.5 mmol/L). The solutions were filter-sterilized through Millex-GV 0.22 μm filters (Millipore, Carrigtwohill, Co. Cork, Ireland). The mixed samples were incubated at 37℃ for 7 days in an electric incubator (KF-60D, Kayagaki-irika-kogyo Co., Tokyo, Japan) or at 50℃ for 48 hours in an aluminium block incubator (Dry Thermo Unit DTU-2C, Taitec Co., Saitama, Japan). Control experiments were carried out with no added sugars.

3. Glycated albumin assay

Glycated HSA was measured with reagents from Asahi Kasei Pharma (Lucica GA-L Glycated Albumin Assay Kit; Tokyo, Japan)[9), 10)]. The test was based on an enzymatic method that uses liquid reagents.

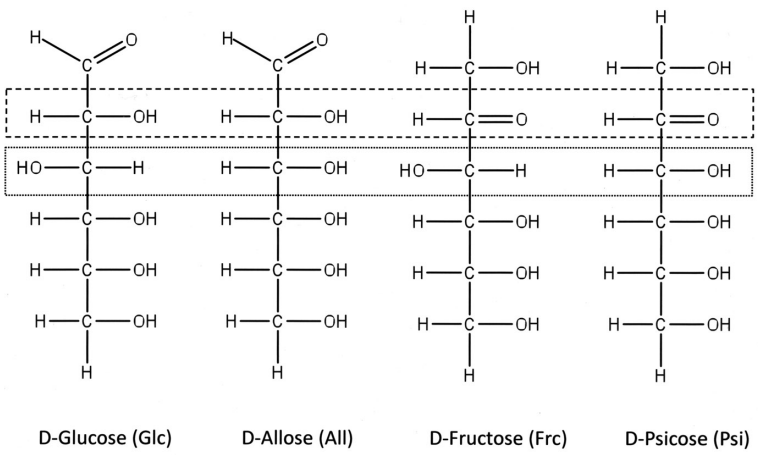

Fig. 1 The stereochemical relationships of the D-hexose. D-allose (All) is the C-3 epimer of D-glucose (Glc). D-psicose is the C-3 epimer of D-fructose (Frc).

Glycated albumin assay

Reagent-1 (R-1): Endogenous glycated amino acids were eliminated. Reagent-2 (R-2): Glycated albumin was hydrolyzed to glycated amino acids by proteinase digestion, and glycated amino acids were quantitatively measured.

Albumin assay

Reagent-1 (R-1): Mercaptalbumin was converted into nonmercaptalbumin. Reagent-2 (R-2): Albumin was measured by the new BCP (bromocresol purple) method.

The automated procedure developed using the TBA-200 FR Neo system (Toshiba Medical Systems, Tokyo, Japan) was as follows:

To determine the glycated albumin concentration, 140 μL of R-1 was incubated at 37℃ with 3.5 μL of samples for 5 min. The absorbance at 548/700 nm was measured. The ketoamine oxidase reaction was then initiated by the addition of 35 μL of R-2, and the absorbance at 548/700 nm was measured. The difference between the measurements made before and 5 min after the start of the ketoamine oxidase reaction was calculated (point 31-33, End up mode).

In the assay of albumin concentration, 160 μL of R-1 was incubated at 37℃ for 5 min with 2.0 μL of samples. The absorbance at 604/660 nm was measured. A dye-binding reaction was then initiated by the addition of 35 μL of R-2, and the absorbance at 604/660 nm was measured. The difference between the measurements made before and 5 min after the start of the dye-binding reaction was calculated (point 31-33, End up mode). In addition, the albumin (protein) concentration of samples was determined by the Biuret method (Aquaauto TP Ⅱ, Kainos Co., Tokyo, Japan).

4. Fructosamine assay

Fructosamine concentration was analyzed using a commercial kit (Liquitec Fructosamine, Roche, Basel, Switzerland)[10)-12)]. The automated determinations were performed with the TBA-80FR Neo system (Toshiba Medical Systems, Tokyo, Japan). 140 μL of reagent was incubated with 14.0 μL of samples for 10min, and the absorbance at 458/700 nm was measured. The difference between 7 min and 10 min was calculated (point 86-136, Rate up mode).

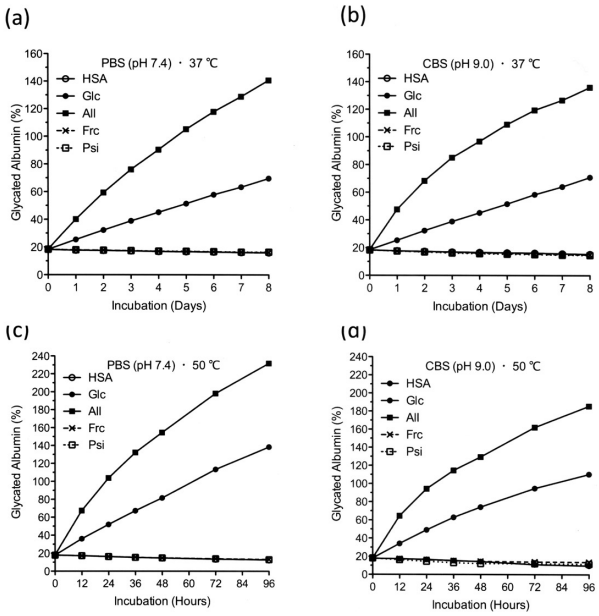

Fig. 2 Time course of glycated albumin formation. (a) PBS (pH 7.4) at 37 ℃, (b) CBS (pH 9.0) at 37 ℃, (c) PBS (pH 7.4) at 50 ℃, (D) CBS (pH 9.0) at 50 ℃. HSA (HSA alone), Glc (HSA + D-glucose), All (HSA + D-allose), Frc (HSA + D-fructose), Psi (HSA + D-psicose).

5. MALDI-TOF Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis was performed using a Voyager System 4314 (Applied Biosystems). The samples were diluted 10-fold with diluent (0.1% trifluoacetic acid, 30% acetonitrile). 5 μL of diluted samples were mixed in the diluent with 5 μL of the matrix saturated sinapinic acid. 1 μL of the sample/matrix mixture was spotted onto a sample plate, air-dried, and subjected to mass determination.

All mass spectra were obtained in a positive ion linear mode, acquisition mass range: 30,000 to 80,000 dalton (Da). External mass calibration was performed using the Sequazyme BSA Test Standard Kit (Applied Biosystems).

6. Absorption spectra

Browning indices of the samples were measured by their absorbance at 420 nm on a UV-2500PC spectrophotometer (Shimadzu Co., Kyoto, Japan), the samples were diluted with each buffer. Absorption curves in the region from 300 to 500 nm were drawn.

7. Fluorescence spectra

Fluorescence spectra were recorded with a Shimadzu RF-540 fluorescence spectrometer. The fluorescence derived from advanced glycation end products (AGEs), termed a glycophore, was monitored with excitation at 350 nm and emission at 430 nm, using a sample 30-fold dilution with each buffer. The fluorescence of the tryptophan residue in HSA was monitored with the excitation at 295 nm and the emission measured at 340 nm, using a sample 300-fold dilution with each buffer.

8. Statistical analysis

Values were expressed as mean±SD. In order to assess the significance of their differences, the Mann-Whitney U test was used. All statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc., version 5.0).

3. Results

1. Glycation of HSA

The effects of pH (7.4 and 9.0) and temperature (37℃ and 50℃) on the glycation of HSA were

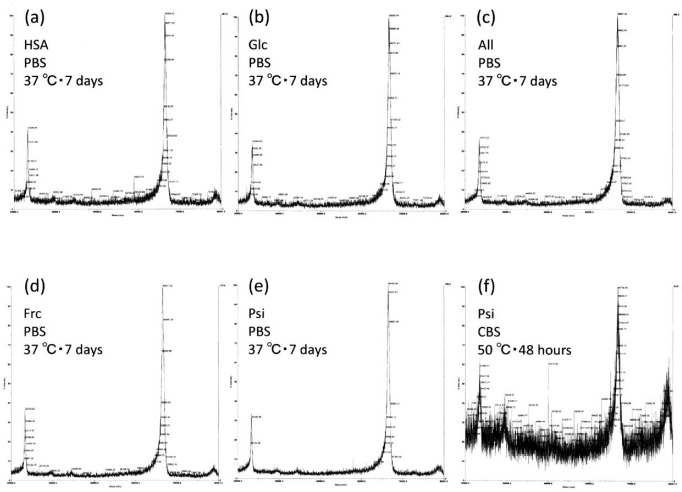

Fig. 3 Typical MALDI-TOF MS spectra of glycated HSA. Acquisition mass range 30,000 -- 80,000 Da (dalton). MALDI- TOF MS yielded mass spectra with two peaks of mass/charge ratios identifiable as mono-charged molecular ions (molecular mass approx. 66,425 - 66,540 Da) and double-charged molecular ions (molecular mass approx. 33,250 - 33,270 Da) of HSA. (a) - (e) : PBS (pH 7.4) 37 ℃ for 7 days. HSA (a), Glc (b), All (c), Frc (d), Psi (e). (f): Psi - CBS (pH 9.0) 50 ℃ for 48 hours.

Table 1 Comparison among glycated albumin (GA), fructosamine (FRA) and MALDI-TOF MS · molecular mass.
A/G: D-allose/ D-glucose ratio. H/G: D-hexose (D-allose, D-fructose, D-Psicose) ratio.

Incubation	Glycated Albumin (GA,%)					Fructosamine (FRA, μ mol/L)					MALDI-TOF MS・Molecular mass (m/z)				
	n	Mean ± SD	n	Mean ± SD	ΔGA	A/G	n	Mean ± SD	n	Mean ± SD	ΔFRA	A/G	n	Mean ± SD	Δmass	H/G
PBS・37℃	0 day		7days				0 day		7days				7days			
HSA alone	3	18.23 ± 0.12	3	16.13 ± 0.15	—	—	3	214.87 ± 0.53	3	185.63 ± 0.59	—	—	4	66453.625 ± 26.241	—	—
HSA+Glucose	3	18.33 ± 0.15	3	63.40 ± 0.44	47.27	—	3	236.88 ± 0.47	3	603.47 ± 4.30	417.84	—	4	66588.750 ± 43.361	135.125	—
HSA+Allose	3	18.59 ± 0.21	3	128.80 ± 0.53	112.67	2.38	3	268.25 ± 0.63	3	1190.00 ± 6.39	1004.37	2.40	4	66829.125 ± 30.503	375.500	2.78
HSA+Fructose	3	18.33 ± 0.15	3	16.33 ± 0.06	0.20		3	556.42 ± 1.72	3	571.70 ± 2.95	386.07		3	66510.690 ± 23.252	57.065	0.42
HSA+Psicose	3	18.37 ± 0.06	3	16.50 ± 0.10	0.37		3	1059.80 ± 6.15	3	1065.70 ± 8.42	880.07		3	66518.943 ± 40.300	65.318	0.48
CBS・37℃	0 day		7days				0 day		7days				7days			
HSA alone	3	18.40 ± 0.00	3	15.77 ± 0.06	—	—	3	218.29 ± 1.35	3	170.17 ± 1.35	—	—	3	66472.453 ± 46.589	—	—
HSA+Glucose	3	18.50 ± 0.20	3	64.10 ± 0.44	48.33	—	3	239.59 ± 0.64	3	679.10 ± 5.46	508.93	—	3	66774.177 ± 24.397	301.724	—
HSA+Allose	3	18.67 ± 0.15	3	126.67 ± 0.31	110.90	2.29	3	277.00 ± 0.58	3	1260.70 ± 7.90	1090.53	2.14	3	67052.760 ± 37.475	580.307	1.92
HSA+Fructose	3	18.30 ± 0.10	3	15.13 ± 0.12	−0.64		3	563.32 ± 0.67	3	604.57 ± 6.52	434.40		3	66536.680 ± 37.773	64.227	0.21
HSA+Psicose	3	18.30 ± 0.10	3	14.80 ± 0.17	−0.97		3	1055.10 ± 2.86	3	1038.20 ± 6.75	868.03		3	66585.433 ± 52.868	112.980	0.37
PBS・50℃	0 hour		48 hours				0 hour		48 hours				48 hours			
HSA alone	3	17.93 ± 0.15	3	14.80 ± 0.10	—	—	3	214.99 ± 1.44	3	180.97 ± 1.33	—	—	3	66468.820 ± 15.923	—	—
HSA+Glucose	3	17.93 ± 0.06	3	81.57 ± 0.21	66.77	—	3	238.48 ± 1.46	3	786.03 ± 3.52	605.06	—	3	66658.587 ± 55.168	189.767	—
HSA+Allose	3	18.20 ± 0.10	3	154.57 ± 0.25	139.77	2.09	3	263.13 ± 0.52	3	1396.90 ± 1.41	1215.93	2.01	3	66983.290 ± 79.123	514.470	2.71
HSA+Fructose	3	17.97 ± 0.15	3	14.90 ± 0.10	0.10		3	560.19 ± 2.09	3	590.73 ± 1.90	409.76		3	66529.870 ± 16.189	61.050	0.32
HSA+Psicose	3	17.87 ± 0.06	3	14.87 ± 0.15	0.07		3	1050.70 ± 4.45	3	1078.50 ± 0.99	897.53		3	66580.183 ± 63.141	111.363	0.59
CBS・50℃	0 hour		48 hours				0 hour		48 hours				48 hours			
HSA alone	3	17.87 ± 0.06	3	14.40 ± 0.10	—	—	3	208.63 ± 1.42	3	198.10 ± 0.70	—	—	3	66490.077 ± 44.772	—	—
HSA+Glucose	3	18.03 ± 0.15	3	74.13 ± 0.25	59.73	—	3	235.45 ± 0.81	3	840.50 ± 2.59	642.40	—	3	66903.260 ± 3.197	413.183	—
HSA+Allose	3	18.23 ± 0.21	3	129.40 ± 1.31	115.00	1.93	3	261.50 ± 0.89	3	1374.40 ± 3.72	1176.30	1.83	3	67162.100 ± 3.855	672.023	1.63
HSA+Fructose	3	17.87 ± 0.06	3	14.47 ± 0.12	0.07		3	554.62 ± 0.92	3	672.53 ± 3.84	474.43		3	66653.157 ± 71.059	163.080	0.39
HSA+Psicose	3	18.07 ± 0.12	3	12.03 ± 0.06	−2.37		3	1060.30 ± 4.80	3	1039.80 ± 6.08	841.70		6	66792.733 ± 112.633	302.656	0.73

*,**,*** : $p < 0.05$; Mann-Whitney test

estimated with a Lucica GA-L kit (Fig. 2). Increases of glycated albumin values were observed not only in Glc but also in All. The results for glycated albumin values at 37℃ for 7 days and 50℃ for 48 hours are shown in the Table 1. All exhibited 1.93 - 2.38 fold higher reactivities than Glc in a glycated albumin assay. In controls (HSA alone), Frc, and Psi, the glycated albumin values were decreased during incubation. In the CBS (pH 9.0) 50℃ condition, the albumin values measured by the BCP method were also decreased during incubation, and were then corrected by the Biuret method for estimating proteins.

2. Fructosamine

Glycated HSA was estimated by measuring fructosamine, as an index of glycated protein. The results are shown in Table 1. Increases in fructosamine values were observed in both Glc and All during incubation. All showed 1.83 - 2.40 fold higher reactivities than Glc in the fructosamine assay.

The interfering effects of sugars were observed without incubation. In the PBS (pH 7.4) 37℃ case: control (HSA alone). those effects were 214.87±0.53 μmol /L (Mean±SD, n=3), Glc 236.88±0.47 μmol/L (Δ22.01 μmol/L), All 268.25 ±0.63 μmol/L (Δ53.38 μmol/L), Frc 556.42±1.72 μmol/L (Δ341.55 μmol/L), Psi: 1,059.80±6.15 μmol/L (Δ844.93 μmol/L).

3. MALDI-TOF Mass Spectrometry

Typical MALDI-TOF mass spectra of glycated HSA are shown in Fig. 3. MALDI- TOF MS yielded mass spectra with two peaks of mass/charge ratios identifiable as mono-charged molecular ions (molecular mass approx. 66,425 - 66,540 Da) and double-charged molecular ions (molecular mass approx. 33,250 - 33,270 Da) of HSA. In an acquisition mass range of 30,000 - 80,000 Da, the separation of glycated HSA from non-glycated HSA proved difficult, a data evaluation of the main peak was performed (Table 1). We investigated the molecular mass changes of HSA with and without sugars. Mean ± SD molecular mass increases (Δ) were as follows:

PBS･37℃･7 days: controls 66,453.625±26.241

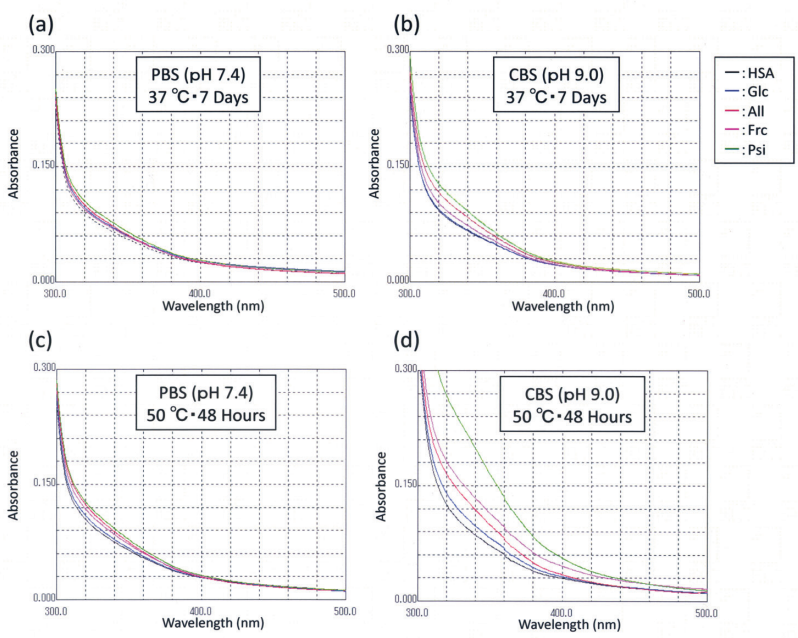

Fig. 4 Absorption spectra of glycated HSA. (a) PBS (pH 7.4) at 37 ℃ for 7 days, (b) CBS (pH 9.0) at 37 ℃ for 7 days, (c) PBS (pH 7.4) at 50 ℃ for 48 hours, (D) CBS (pH 9.0) at 50 ℃ for 48 hours. HSA (black), Glc (blue), All (red), Frc (pink), Psi (green).

Da (n=4), Glc 66,588.750±43.361 Da (Δ135.125 Da, n=4), All 66,829.125±30.503 Da (Δ375.500 Da, n=4), Frc 66,510.690±23.252 Da (Δ57.065 Da, n=3), and Psi 66,518.943±40.300 Da (Δ65.318 Da, n=3). Glc and All increased significantly with respect to controls (Mann-Whitney U test). All reactivities were 2.78- fold higher than Glc in molecular mass.

CBS·37℃·7 days: controls were comprised of 66,472.453±46.589 Da (n=3), Glc 66,774.177±24.397 Da (Δ301.724 Da, n=3), and All 67,052.760±37.475 Da (Δ580.307 Da, n=3).

PBS·50℃·48 hours: controls were 66,4468.820±15.923 Da (n=3), Glc 66,658.587±55.168 Da (Δ189.767 Da, n=3), and All 66,983.290±79.123 Da (Δ514.470 Da, n=3).

CBS·50℃·48 hours: controls were 66,4490.077±44.772 Da (n=3), Glc 66,903.260±3.197 Da (Δ413.183 Da, n=3), and All 67, 162.100±3.855 Da (Δ672.023 Da, n=3).

Under CBS·50℃·48 hour conditions, a sub-peak molecular mass of approx. 78,000-79,000 Da was observed. Baseline noises were increased, especially in Psi.

4. Absorption spectra

The absorption spectra of between 300-500 nm for glycated HSA are shown in Fig. 4. No changes in absorbance at 420 nm (browning indicators) were observed. The absorbance between 300-400 nm, however, increased in the following order: PBS (pH 7.4) < CBS (pH 9.0), 37℃·7 days< 50℃·48 hours, Glc<All and Frc<Psi. Under CBS·50℃·48 hour conditions, Psi showed a large increase in absorbance and visibly exhibited a brown color .

5. Fluorescence spectra

Fig. 5 shows the fluorescence spectra of glycated HSA with excitation at 350 nm, and emission at 300 - 500 nm. Fluorescence intensities at 430 nm were in the following order: PBS (pH 7.4) < CBS (pH 9.0), 37℃·7 days< 50℃·48 hours, Glc<All and Frc<Psi.

Fig. 6 shows the fluorescence excitation at 295 nm and emission at 300-400 nm. Fluorescence intensity at 340 nm was decreased under CBS·50℃·48 hour

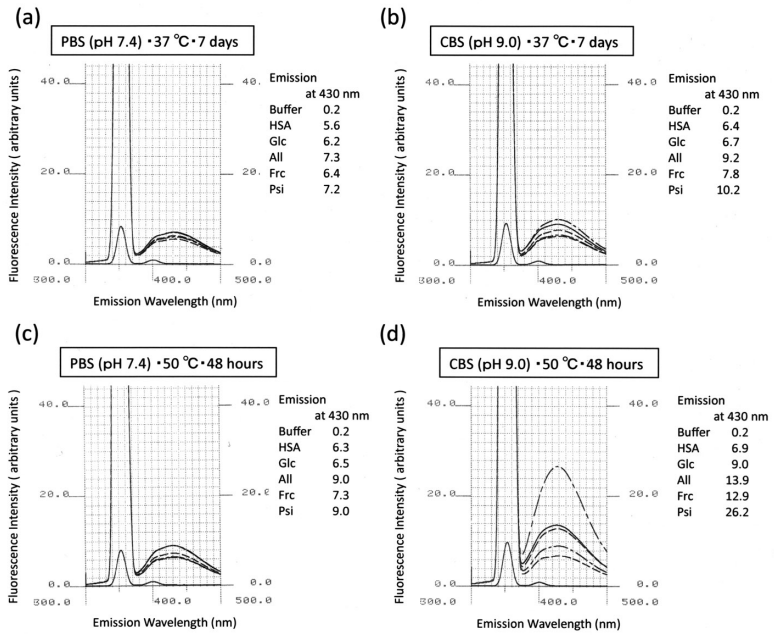

Fig. 5 Fluorescence spectra of glycated HSA exited at 350 nm. (a) PBS (pH 7.4) at 37 ℃ for 7 days, (b) CBS (pH 9.0) at 37 ℃ for 7 days, (c) PBS (pH 7.4) at 50 ℃ for 48 hours, (D) CBS (pH 9.0) at 50 ℃ for 48 hours.

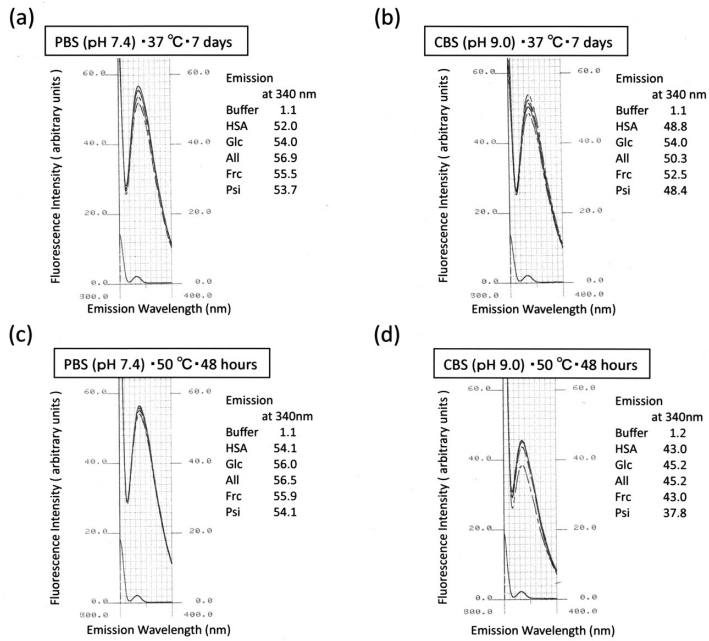

Fig. 6 Fluorescence spectra of glycated HSA exited at 295 nm. (a) PBS (pH 7.4) at 37 ℃ for 7 days, (b) CBS (pH 9.0) at 37 ℃ for 7 days, (c) PBS (pH 7.4) at 50 ℃ for 48 hours, (D) CBS (pH 9.0) at 50 ℃ for 48 hours.

conditions, especially in Psi.

4. Discussion

The concentrations of glycated HSA have been measured by thiobarbituric acid assay[16] and the high-performance liquid-chromatographic (HPLC) method[17]. However, several operations were complex, and performances were low. In the present study, we used a Lucica GA-L kit for the measurement of glycated albumin by an automated biochemical analyzer.

We examined the effects of pH (pH 7.4 and pH 9.0) and temperature (37℃ and 50℃). In the food technology field, glycation (Maillard reaction) is performed under alkaline conditions and high-temperature[18].

Fig. 2 shows the time course of glycated albumin formation. Reactivities of All were higher than those of Glc. The glycated albumin assay kit is based on an enzymatic method using albumin-specific proteinase and ketoamine oxidase with high specificity for N ε -fructosyl-lysine[9, 10]. Glycation with Glc and All could be detected by the enzymatic method, but not with Frc or Psi. In addition, protein glycation with All showed additive effects on glycation with Glc (unpublished data). Under CBS (pH9.0)・50℃ conditions, albumin values by the BCP method were diminished. BCP is more specific for albumin than BCG (bromocresol green), which does not bind to globulins. Under these conditions, the denaturation of albumin might have occurred, thus decreasing the binding activity of BCP for albumin.

The All/Glc ratios in fructosamine values (1.83-2.40) were similar to glycated albumin values (1.93-2.38). Additional sugars (1,000 mg/dL = 55.5 mmol/L) interfered with fructosamine testing: Glc Δ 22.01 μ mol/L, All Δ 53.38 μ mol/L, Frc Δ 341.55 μ mol/L, Psi Δ 844.93 μ mol/L. Fructosamine values in Frc and Psi were considerably elevated without glycation.

Ahmed et al.[19] reported that the serum fructosamine method implied that Frc causes only about 5% of the glycation induced by Glc.; Frc is as

much as 10-fold more efficient at forming AGEs (glycation)[20]: Glycation was performed by the incubation of bovine serum albumin (10 g/dL) in a sugar solution (0.5 mol/L) at pH 7.4 and 37℃ for 16 days. The results are as follows: The respective fructosamine values after 5 days of incubation were Glc 3.9 mmol/L (3.900 μ mol/L), and Frc 0.3 mmol/L (300 μ mol/L), after 10 days were Glc 5.8 mmol/L (5.800 μ mol/L) and Frc 0.2 mmol/L (200 μ mol/L); and after 16 days were Glc 7.9 mmol/L (7.900 μ mol/L); and Frc 0.4mmol/L (400 μ mol/L). There was only a Frc reaction of 5% to Glc after 16days. Glycation with Glc, but not with Frc, is time-dependen,.

Glycation of HSA producing early glycation adducts (fructosamine derivatives) such as N ε -fructosyl-lysine and AGEs occurs mainly on lysine residues. When Glc binds to lysine residues of HSA and N ε -fructosyl-lysine is formed, the molecular mass of HSA increases to 162.0528 Da[21]. Our MALDI-TOF MS results indicate the glycation (%) as follows:

PBS・37℃/7 days was Glc 83.38% (Δ135.125 Da), and All 231.71% (Δ375.500 Da); CBS・37℃/7 days was Glc 186.19% (Δ301.724 Da), and All 358.10% (Δ580.307 Da); PBS・50℃/48 hours was Glc 117.10% (Δ189.767 Da), and All 317.47% (Δ514.470 Da); CBS・50℃/48 hours was Glc 254.97% (Δ413.183 Da), and All : 414.69% (Δ672.023 Da). In any case, molecular mass results show clearly that PBS<CBS, 37℃・7 days<50℃・48 hours, and alkaline conditions and high temperature promote glycation, whereas measurement of glycated albumin and fructosamine are not so clear as the assay.

While no absorptional changes in browning at 412 nm were not observed, absorbance (300 - 400 nm) increases (probably caused by glycation) were noted, as Monnier et al. demonstrated[22]. In CBS・50℃・48 hours of Psi, a major increase in absorbance shows that denaturation and modification have occurred.

The order of fluorescence intensity (glycophore) was PBS (pH 7.4) < CBS (pH 9.0), 37℃・7 days< 50℃・48 hours, Glc<All and Frc<Psi. All (the C-3 epimer of Glc) and Psi (the C-3 epimer of Frc) showed higher reactivities than Glc and Frc, and ketohexose was stronger than aldohexoses. The tryptophan fluorescence intensity decreased in CBS・50℃・48 hour conditions, especially in Psi, which was to be expected from the MALDI-TOF MS spectra (baseline-noise).

Although the MALDI-TOF MS results indicated that the amounts of glycation with Frc and Psi are less than half those with Glc and All, the fluorescence intensity of Frc and Psi is more than that of Glc and All. It is not appropriate to estimate glycation between aldohexoses and ketohexose by fluorescence intensity.

The reactivity of All during the glycation of HSA is higher than that of Glc. All is expected to be utilized in medicines and functional foods to reduce oxidative stress under the hyperglycemic state. Contrary to the hypothesis that All, having antioxidant activities, suppress glycation, it does not reduce Glc-mediated HSA glycation. Glycated albumin is the early stage of Maillard reaction products (MRPs).

It is well known that MRPs produced in heat-treated food processing systems have antioxidant activity, which is related to the production of late-stage MRPs. In a recent study, the reactivity of seven aldohexoses with ovalbumin at 55℃ was investigated[23]. The order of reactivity was: D-altrose /All > D-talose /D-galactose > D-gulose > D-mannose/Glc. It was concluded that the configuration of OH groups around C-3 and C-4 might be very important for the formation of MRPs and their antioxidant behaviors.

These findings (late-stage MRPs) and our results (early stage MRPs) share the same order of reactivity : Glc<All. Thoush All has a high reactivity for glycation, its antioxidative mechanism is not clear. Additional studies need to determing human applications.

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