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Analyses of the in vitro non-enzymatic glycation of peptides/proteins by matrix-assisted laser desorption/ionization mass spectrometry

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Abstract

Non-enzymatic glycation of proteins with the reducing agent glucose is implicated to be responsible for diabetes-derived complications, food browning, and aging. However, the non-enzymatic glycation process of peptides/proteins is not well understood and further research is needed to gain an understanding of the underlying principles involved in diabetes-related complications. In this study, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is used to analyze the in vitro glycation of peptides/proteins. In addition to the physiological conditions, harsh conditions (higher concentration of glucose, higher or lower pH, and higher temperature) are also used in this study. Peptides/proteins are reacted with glucose for up to 120 h at 4 °C, 37 °C, or 65 °C. Single and/or multiple glycations are observed using broad pH conditions (from 10% TFA with pK_a of 0.5 to pH 10) at various glucose concentrations (from 0.01 M to 1 M). Data suggest that glucose reacts readily with both peptides and proteins, and the efficiency of the glycation increases with higher temperature, higher pH, higher glucose concentration, or longer incubation time. However, influence of the buffer pH on the efficiency of the glycation of peptides is less pronounced compared to that of proteins. This effect could result from denaturation of proteins at higher pH and the resultant exposure of potential glycation sites. This data could lead to the inference that the glycation process of peptides/proteins would occur but proceed very slowly under the diabetes conditions in vivo (37 °C, ∼neutral pH, ∼0.007 M glucose). Postsource decay and MS/MS results of singly glycated angiotensin I, $P_{14}R$ (PPPPPPPPPPPPPR), and human adrenocorticotropic hormone (ATCH) fragments 1–13 indicate that glucose reacts with the amino group of the N-terminal of ATCH 1–13 and the guanidino group of the arginine residue of both angiotensin I and $P_{14}R$.

Keywords: In vitro non-enzymatic glycation; Peptide/protein; Glucose; MALDI-TOF MS; FT-ICR MS

1. Introduction

Diabetes is one of the leading causes of death in USA [1]. Diabetes-derived complications are many and their links to the glycation of proteins have been indicated [2,3]. The reducing agent glucose is reported to react with the amino groups of circulating proteins such as hemoglobin, albumin, and IgG, as well as the tissue proteins such as collagen [4–13]. Mechanistically, the aldehyde group of glucose first forms a reversible Schiff's base linkage with the α-amino or ε-amino groups of the protein. This bond then undergoes an Amadori rearrangement to form a stable ketoamine derivative [14]. Subsequent rearrangement, dehydration, and degradation (Maillard reaction) of the ketoamine derivatives produce yellow-brown, fluorescent products, which are the advanced glycosylation end products (AGEs) [14]. The majority of previous studies have focused on the measurement of AGEs. AGEs are also implicated in the aging and browning reactions in food science. These findings suggest that much of the characteristic pathology of diabetes results primarily from increase in the non-enzymatic glycation of proteins, induced by hyperglycemia. Despite the importance of this subject and many previous studies [14–18], detailed information on the process of glycation of peptides/proteins is lacking. The majority of previous studies were performed at 37 °C in physiological conditions on proteins; and only the ε-amino groups of lysine and N-treminal residue were considered the possible glycation sites. In this report we describe a detailed investigation on the state of the glycation of peptides/proteins using a simple, fast and informative MALDI-TOF MS technique. Human angiotensin I, $P_{14}R$ (PPPPPPPPPPPPPR), human adrenocorticotropic hormone (ATCH) fragments 1–13, bovine insulin, human hemoglobin A, hen egg white lysozyme, soybean trypsin inhibitor, and bovine serum albumin are used as the testing

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cases. Besides commercially available in high purity, these peptides/proteins are chosen to represent a wide range of M.W. Non-physiological conditions are also used to investigate on the reaction kinetics (different temperatures) or to unfold the proteins (pH 10). Since increased glucose concentration increases the glycation rate of proteins/peptides, we choose glucose concentration of 1 M for the glycation reaction to speed up and maximize the glycation.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma (Saint Louis, MO). Highest purity grade peptides and proteins were also purchased from Sigma and used without further purification. Hemoglobin was prepared from blood specimens for its high quality.

2.2. Preparation of hemoglobin samples

Blood specimens from normal subjects were collected in heparin after informed consent from donors. Hemolysates were prepared by lysis of the washed erythrocytes with three–five volumes of cold water and gassing with carbon monoxide, followed by centrifugation for 20 min at 3000 × *g*. The clear hemolysate was stored at −85 °C. Purified hemoglobin was prepared from the hemolysate by diethylaminoethyl Sephadex anion exchanger chromatography and/or carboxymethyl Sephadex cation exchanger chromatography and dialyzed against cold deionized water.

2.3. In vitro glycation of peptides/proteins

Ten microliters of peptide/protein solution (1 μg/μl) containing 0.01–1 M glucose was incubated at 4 °C, 37 °C, or 65 °C for up to 4 days. PBS buffer (0.01 M; 0.318 M NaCl; 0.027 M KCl; pH 7.4), 50 mM Na_2CO_3 aqueous solution at pH 10, or 10% TFA (pK_a of 0.5) aqueous solution was used as the solvent. Ten microliters of peptide/protein solution (1 μg/μl) without added glucose was incubated as above and used as control. All control samples showed normal non-glucosylated peptide/protein mass spectra. One microliter aliquots were drawn at desired periods and diluted with 10-fold volumes of 0.1% TFA aqueous solution for mass spectrometric analyses.

2.4. MALDI-TOF and hybrid linear trap/FT-ICR mass spectrometric analyses

Zip-Tips (Millipore, Billerica, MA) packed with C18 or C4 resin were used to prepare the solution for MS analysis of peptides and proteins, respectively. Cyano-4-hydroxycinnamic acid (CHCA) and sinapinic acid (SA) were used as the matrix for peptides and proteins, respectively. Aliquots (1.3 μl) of the matrix solution (3–10 mg CHCA or SA in 1 ml aqueous solution of 50% acetonitrile containing 0.1% TFA) were used to elute the peptide/protein from Zip-Tips and spotted onto a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) target. A Voyager-DE PRO mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a 337 nm pulsed nitrogen laser was used to analyze the samples. Peptide/protein mass was measured using the positive-ion linear mode. External mass calibration was performed using the peaks of a mixture of bradykinin fragments 1–7 at *m*/*z* 758, angiotensin II (human) at *m*/*z* 1047, $P_{14}R$ (synthetic peptide) at *m*/*z* 1534, adrenocorticotropic hormone fragments 18–39 (human) at *m*/*z* 2467, insulin oxidized B (bovine) at *m*/*z* 3497, insulin (bovine) at *m*/*z* 5735, cytochrome *c* (equine) at *m*/*z* 12362, apomyoglobin (equine) at *m*/*z* 16952, adolase (rabbit muscle) at *m*/*z* 39212, and albumin (bovine serum) at *m*/*z* 66430.

The glycated angiotensin at *m*/*z* 1460, $P_{14}R$ at *m*/*z* 1697, and ATCH 1–13 at *m*/*z* 1788 were selected as precursor ions using an ion gate at a resolving power of 100 for positive MALDI-TOF postsource decay (PSD) analysis. PSD was used with 15 kV accelerating potential, 100 laser pulses averaged for each reflector voltage segment. A composite mass spectrum was produced by stitching 15 different mass spectra, each spectrum containing a portion of the entire mass range produced with a different reflector potential. This is required to observe the entire mass spectrum of fragment ions containing the desired sequence information.

The electrospray ionization Fourier transform (FT) MS and MS/MS were obtained using a LTQ-FT hybrid linear trap 7 T actively shielded superconducting magnet FT-ion cyclotron resonance (FT-ICR) mass spectrometer (Thermo Electron, Waltham, MA) equipped with an open-end cylindrical cell. The Xcalibur software package enables detection in both the linear trap and the ICR cell simultaneously, which increase the duty cycle of the instrument. In this study, the experiment utilized the parallel data acquisition of MS and MS/MS by using both the ICR cell and the linear trap. Simultaneous detection is accomplished by filling the ICR cell using automatic gain control for the full scan MS with a resolution setting of 50,000, and the precursor mass are queried by the linear trap so that the MS/MS spectra can be obtained in the time required for transient detection to be complete. Collision-induced dissociation (CID) was performed on singly glycated angiotensin at *m*/*z* 1460, $P_{14}R$ at *m*/*z* 1697, and ATCH 1–13 at *m*/*z* 1788 in the linear trap using helium as the target gas with 25% normalized collision energy and an activation time of 30 ms.

3. Results and discussion

The molecular ions of human angiotensin I, $P_{14}R$ (PPPPPPPPPPPPPPR), human adrenocorticotropic hormone fragments 1–13, and bovine insulin as well as their glycated ions are exhibited in Fig. 1. Molecular masses of glycated peptides produced by the in vitro incubation with glucose and the number of glucose molecules attached to the peptides are compiled in Table 1. The molecular ions are observed at *m*/*z* 1297 (angiotensin I), at *m*/*z* 1534 ($P_{14}R$), at *m*/*z* 1625 (ACTH 1–13), and at *m*/*z* 5735 (insulin). Their corresponding glycated ions are detected at *m*/*z* 1460 (singly glucosylated), *m*/*z* 1623 (doubly glucosylated), and *m*/*z* 1786 (triply glucosylated) in

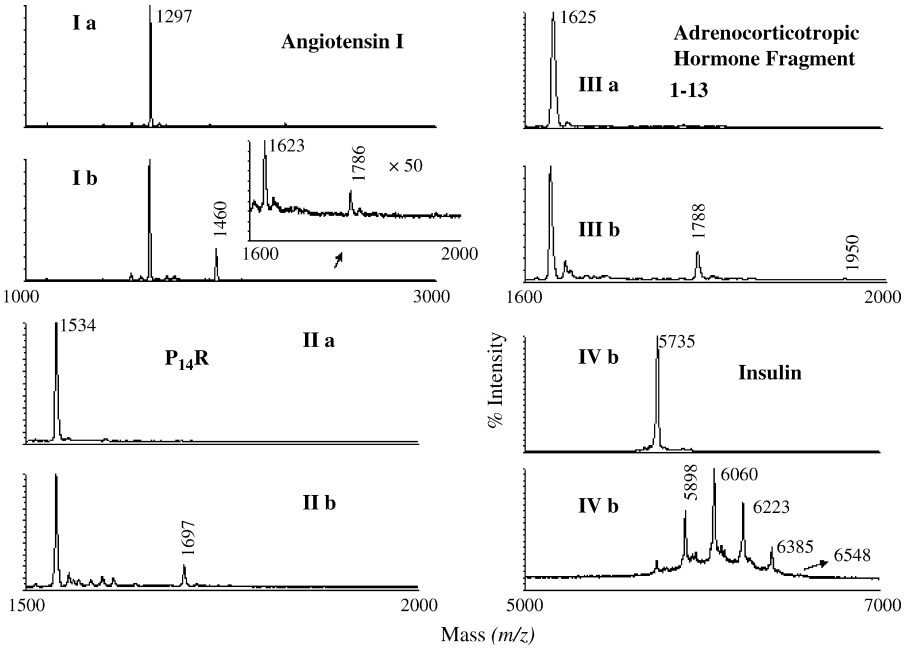

Fig. 1. The MALDI-TOF spectra of peptides and their reactions with glucose: (I a) angiotensin I at 65 °C for 19 h in PBS without glucose; (I b) angiotensin I at 65 °C for 19 h in PBS with 1 M glucose; (II a) $P_{14}R$ at 65 °C for 96 h in PBS without glucose; (II b) $P_{14}R$ I at 65 °C for 96 h in PBS with 1 M glucose; (III a) ACTH fragments 1–13 at 65 °C for 19 h in PBS without glucose; (III b) ACTH fragments 1–13 at 65 °C for 19 h in PBS with 1 M glucose; (IV a) insulin at 65 °C for 96 h in PBS without glucose; (IV b) insulin at 65 °C for 96 h in PBS with 1 M glucose. Peptide solution (1 μg/μl) was processed with Zip-Tip packed with C18 resin before MALDI-TOF MS. CHCA was used as the matrix.

the case of angiotensin I, at *m/z* 1697 (singly glcosylated) in the case of $P_{14}R$, at *m/z* 1788 (singly glucosylated) and *m/z* 1950 (doubly glucosylated) in the case of ACTH 1–13, and at *m/z* 5898 (singly glucosylated), *m/z* 6060 (doubly glucosylated), *m/z* 6223 (triply glucosylated), *m/z* 6385 (four glycations), and *m/z* 6548 (five glycations) in the case of insulin. Since there are up to three glycations in the case of angiotensin I and the sequence of angiotensin I (DRVYIHPFHL) contains no lysine residue (K), other parts of the peptide should be glycated. Similarly, since there are up to two glycations sites in the case of ACTH 1–13 and the sequence of ACTH fragments 1–13 (SYSMEHFRWGKPV) contains only one lysine residue, other parts of the peptide besides ε-amino group of the lysine residue must be glycated. In the synthetic peptide $P_{14}R$ only one glycation is detected, indicating that the glycation must occur in either guanidino group of the arginine residue or α-amino group of N-terminal. Previous studies on reaction of glucose with valylhistidine [19] and powder insulin [20] seem to suggest that the α-amino group of N-terminal is more reactive than the ε-amino group. Nevertheless, our MALDI-TOF postsource decay (PSD) and FT-ICR MS collision-induced dissociation results (described later) suggest that glucose can react with the amino group of the N-terminal of ATCH 1–13 and guanidino group of the arginine residue of both angiotensin I and $P_{14}R$. Insulin (GIVEQCCASVCSLYQL-ENYCNFVNQHL-CGSHLVEALYLVCGERGFFYTPKA) contains only one lysine residue but is found to be glycated at five sites. This observation provides another example that amino acid residues other than lysine residue and N-terminal can be glycated. This data agrees with previous study on the browning reaction of powder insulin with glucose for 37 days at 55 °C [21] which had proposed six potential reactive sites (two N-terminals, two histidines, one arginine, and one lysine). It is noted that only two glycations are reported for the in vitro non-enzymatic glycation of insulin with 0.22 M at 37 °C in a recent study [22]. The glycation of these peptides may change their functions and turn over

Table 1
Molecular mass of glucosylated peptides/proteins produced by in vitro incubated with glucose and number of glucose molecules attached to the peptides/proteins

Proteins/peptides	Incubation conditions	Mass (*m/z*)	Number of Glc attached
Angiotensin I	65 °C, 19 h, PBS, 1 M Glc	1460, 1623, 1786	1, 2, 3
$P_{14}R$	65 °C, 96 h, PBS, 1 M Glc	1697	1
ACTH fragments 1–13	65 °C, 19 h, PBS, 1 M Glc	1788, 1950	1, 2
Insulin	65 °C, 96 h, PBS, 1 M Glc	5898, 6060, 6223, 6385, 6548	1, 2, 3, 4, 5
Lysozyme	65 °C, 19 h, PBS, 1 M Glc	15118	5
Hb A (α-chain)	37 °C, 120 h, PBS, 0.6 M Glc	15289, 15451, 15614	1, 2, 3
Hb A (β-chain)	37 °C, 120 h, PBS, 0.6 M Glc	16030, 16193, 16355	1, 2, 3
SBTI	65 °C, 19 h, PBS, 1 M Glc	21055, 21228, 21364	6.5, 7.6, 8.4
BSA	65 °C, 24 h, PBS, 1 M Glc	68705, 72280, 75773	14, 36, 57.5

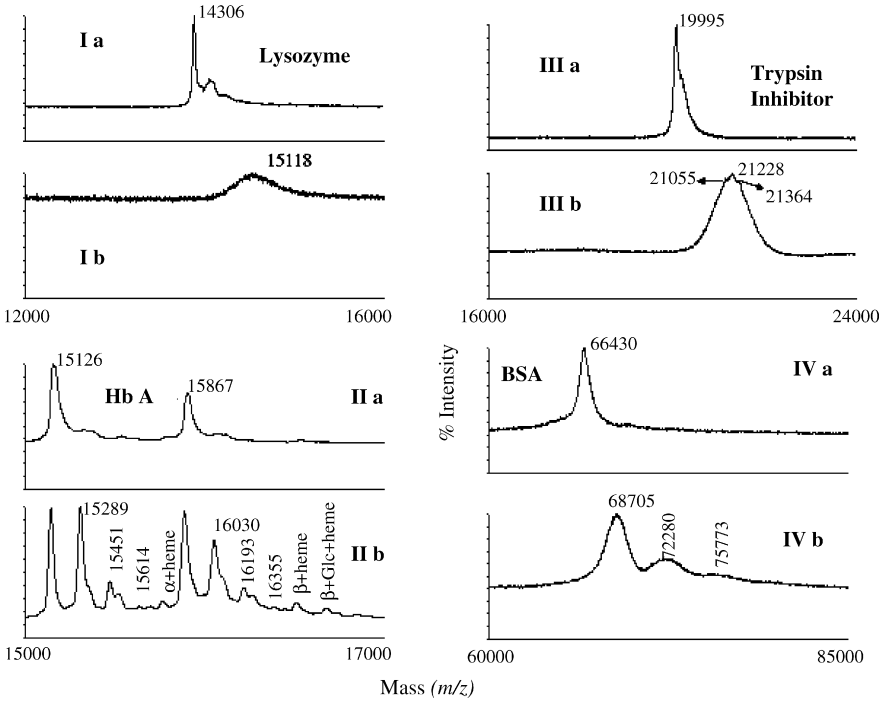

Fig. 2. The MALDI-TOF spectra of proteins and their reactions with glucose: (I a) lysozyme at 65 °C for 19 h in PBS without glucose; (I b) lysozyme at 65 °C for 19 h in PBS with 1 M glucose; (II a) Hb A at 37 °C for 120 h in PBS without glucose; (II b) Hb A at 37 °C for 120 h in PBS with 0.6 M glucose; (III a) trypsin inhibitor at 65 °C for 19 h in PBS without glucose; (III b) trypsin inhibitor at 65 °C for 19 h in PBS with 1 M glucose; (IV a) BSA at 65 °C for 24 h in PBS without glucose; (IV b) BSA at 65 °C for 24 h in PBS with 1 M glucose. Protein solution (1 μg/μl) was processed with Zip-Tip packed with C4 resin before MALDI-TOF MS. SA was used as the matrix.

rates in vivo, and further work is needed to fully understand the effects.

The molecular ions of human hemoglobin A (Hb A), hen egg white lysozyme, soybean trypsin inhibitor (SBTI), and bovine serum albumin (BSA) as well as their glycated molecules are displayed in Fig. 2. Molecular masses of glycated proteins produced by the in vitro incubation with glucose and the number of glucose molecules attached to the proteins are listed in Table 1. The molecular ions are detected at *m/z* 14306 (lysozyme), at *m/z* 15126 (α-globin of Hb A) and *m/z* 15867 (β-globin of Hb A), at *m/z* 19995 (SBTI), and at *m/z* 66430 (BSA). Their corresponding glycated ions are detected respectively at *m/z* 15118 (average five glucosylated sites) in the case of lysozyme, at *m/z* 15289 (singly glucosylated), *m/z* 15451 (doubly glucosylated), and *m/z* 15614 (triply glucosylated) in the case of α-globin of the Hb A, at *m/z* 16030 (singly glucosylated), *m/z* 16193 (doubly glucosylated), and *m/z* 16355 (triply glucosylated) in the case of β-globin of the Hb A, at *m/z* 21055 (average 6.5 glycations), 21228 (average 7.6 glycations), and 21364 (average 8.4 glycations) in the case of SBTI, and *m/z* 68705 (average 14 glycations), *m/z* 72280 (average 36 glycations), and *m/z* 75773 (average 57.5 glycations) in the case of BSA. In general, due to the presence of many different glycated molecules, the bands corresponding to glycated proteins are broader than their non-glycated counterparts. In the case of lysozyme, the glycated proteins exhibit a band range from *m/z* 14698 (average 2.4 glucoses) to 15692 (average 8.5 glucoses) which centers at *m/z* 15118 (average 5 glucoses). This data together with the literature data at 37 °C (up to eight glycations) [8] of lysozyme suggests that most of the nine lysine residues in lysozyme could be glycated. Beside two major molecular ions of α-globin and β-globin of the Hb A, additional molecular ions of SA matrix adducts of these two globins (M + 207 and M + 225) are also observed (Fig. 2II a) [23]. Also, molecular ions of α-globin + heme, β-globin + heme, and β-globin + Glc + heme are observed at *m/z* 15742, 16483, and 16646, respectively. Minor ions from glycated Hb A's dehydration products are also observed. We have also detected triply glycated α-globin and β-globin of the Hb A that were never been reported before. Previous studies [7,24] indicate that glycations might occur on the terminal valine and the free ε-amino group of lysine at positions 40 and 61 of the α-globin and 8, 17, and 66 of the β-globin. It is noted that the majority of the 22 lysine residues in the Hb A are non-reactive. In addition, data on SBTI indicated that majority of the 10 lysine residues are reactive. Previous studies have demonstrated that BSA glycation under different glucose concentration and incubation times from this study at 37 °C reaches a steady state, corresponding to the glycation process occurring on all the proposed reactive 51 sites of the protein [4,10]. Nevertheless, data from this study on reaction between BSA and glucose (1 M) at 65 °C for 24 h in PBS suggests that up to 58 sites instead of 51 sites are glycated, indicating that at higher temperature probably more of the 60 lysine residues' ε-amino group or other residues of BSA are reactive. The glycation of these proteins may change their functions in vivo, e.g., glycated Hb A exhibits greater oxygen affinity then Hb A in the presence of 2,3-diphosphoglycerate and glycated albumin is reported to be taken up more avidity than native albumin by endothelial cells.

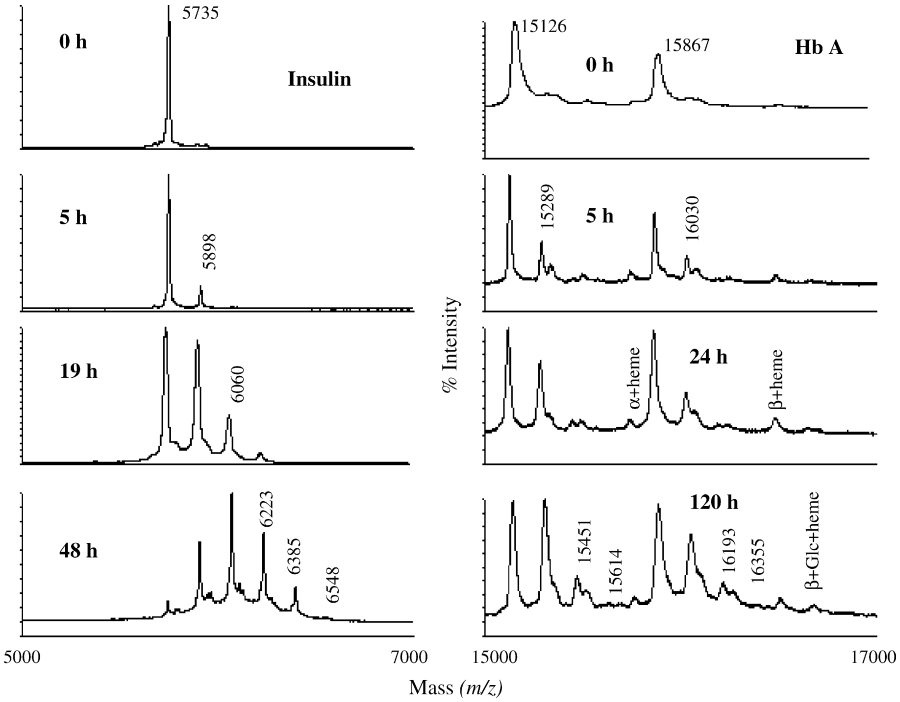

Fig. 3. Time courses of the glycation of insulin at 65 °C and Hb A at 37 °C with 1 M glucose in PBS by MALDI-TOF MS. Peptide/protein solution (1 μg/μl) was processed with Zip-Tip before MALDI-TOF MS.

Fig. 3 shows the time course studies of the in vitro glycation of insulin and Hb A with 1 M glucose in PBS at 65 °C and 37 °C, respectively. With 5 h of incubation, one glucose molecule is attached to both insulin and Hb A. As time progresses, more glucose molecules are attached to both insulin and Hb A. After 48 h, five glucose molecules had attached to insulin, however, only three glucoses are attached to Hb A. This data indicates that efficiency of the glycation process is time dependent but reaches a maximum when all the glycation sites are saturated. This general observation is in agreement with previously reported data [10]. It is noted that, increases in degradation products are detected for all the peptides and proteins study here.

Fig. 4 shows the effect of the glucose concentration on the in vitro non-enzymatic glycation of insulin and BSA. Different glucose concentrations (0.01 M, 0.1 M, or 1 M) are used to glycate insulin and BSA in PBS at 65 °C for 24 h. At glucose concentration of 0.01 M, there is one glycation for BSA but there is almost no observable glycation for insulin. At glucose concentration of 0.1 M, there are three glycations in the case of BSA and there is one observable glycation in the case of insulin. At glucose concentration of 1 M, there are up to 58 glycations in the case of BSA and there are up to 3 observable glycations in the case of insulin. This data suggests that glycation efficiency of peptides/proteins increases with at higher glucose concen-

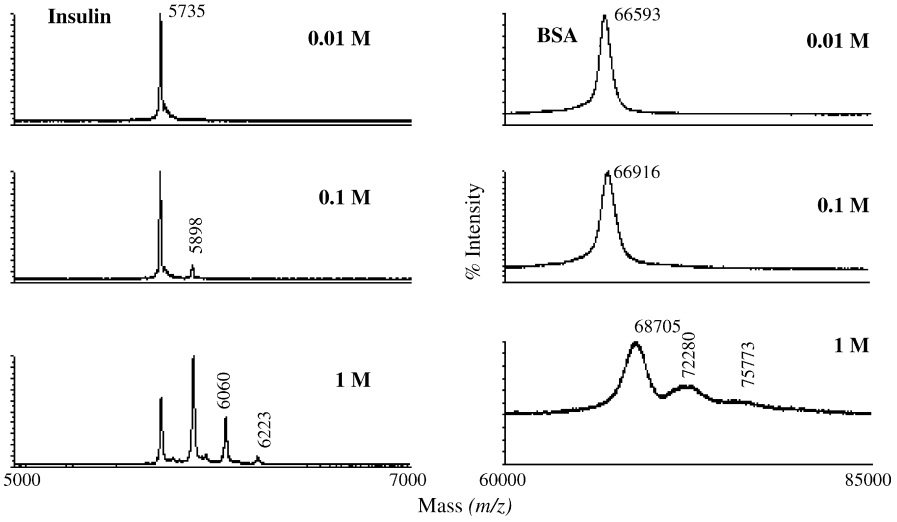

Fig. 4. The glycation of insulin and BSA with glucose at 65 °C and 24 h in PBS as a function of glucose concentration by MALDI-TOF MS. Peptide/protein solution (1 μg/μl) was processed with Zip-Tip before MALDI-TOF MS.

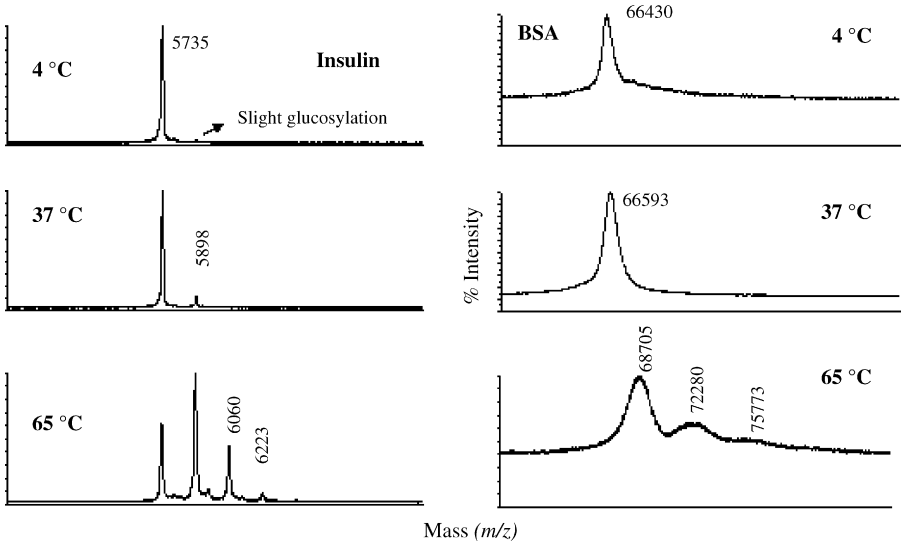

Fig. 5. The glycation of insulin and BSA with 1 M glucose for 24 h in PBS as a function of temperature by MALDI-TOF MS. Peptide/protein solution (1 μg/μl) was processed with Zip-Tip before MALDI-TOF MS.

tration, albeit the relationship is not linear. This data together with the results from the previous paragraph suggest that the non-enzymatic glycation of proteins may take time to reach a significant amount in diabetic patient. In addition, this data is most useful in food science which produces foods in various glucose concentrations.

Fig. 5 shows the effect of temperature on glycation of insulin and BSA with glucose concentration of 1 M in PBS for 24 h. At 4 °C, there is no observed glycation for BSA and there is one probable glycation for insulin. At 37 °C, there is one glycation observed for both BSA and insulin. At 65 °C, there are up to 58 glycations for BSA and up to 3 glycations for insulin. Data shows that higher temperature speeds up the glycation process but the relationship between temperature and rate of glycation is not linear. Since no glycation is observed for BSA at 4 °C but there is some glycation for insulin, and exactly the opposite effect is observed under a different reaction condition (glucose concentration of 0.01 M, 65 °C, 24 h), it appears that the glycation sites of BSA may not be readily accessible at lower temperatures due to perhaps the protein structure/folding. It is noted that all control samples show normal non-glucosylated peptide/protein mass spectra. This data is most useful in food science which produces foods in glucose at different temperature. Also, it is valuable information on glycation in thermophilic bacteria.

Fig. 6 shows the effect of the pH on glycation of insulin and BSA with glucose concentration of 1 M at 65 °C for 5 h. Using the 10% TFA (pK_a of 0.5) as the solvent, there is one glycation in the case of BSA and up to two glycations in the case of insulin.

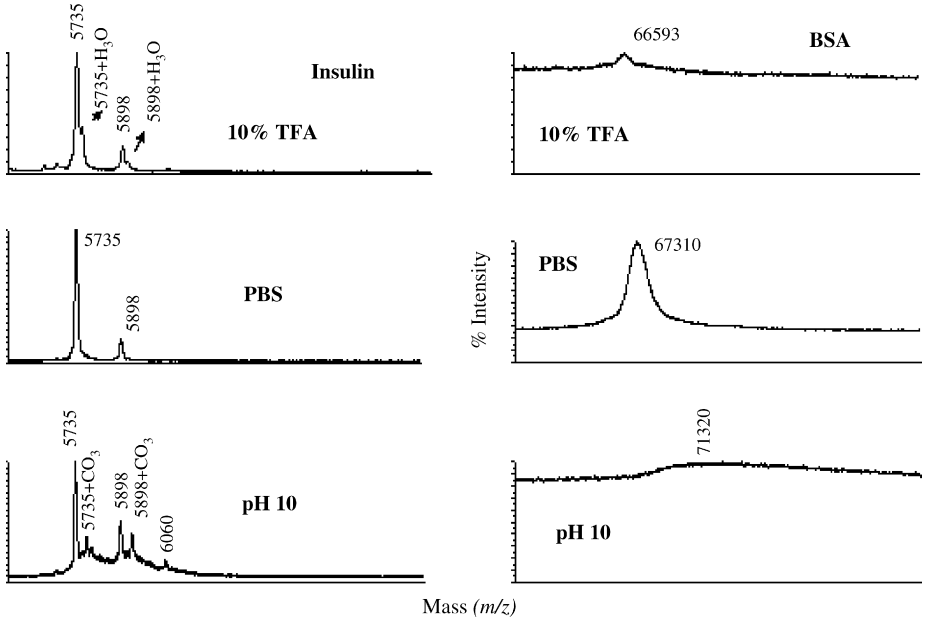

Fig. 6. Buffer pH dependence of the glycation of insulin and BSA with 1 M glucose at 65 °C and 5 h by MALDI-TOF MS. Peptide/protein solution (1 μg/μl) was processed with Zip-Tip before MALDI-TOF MS.

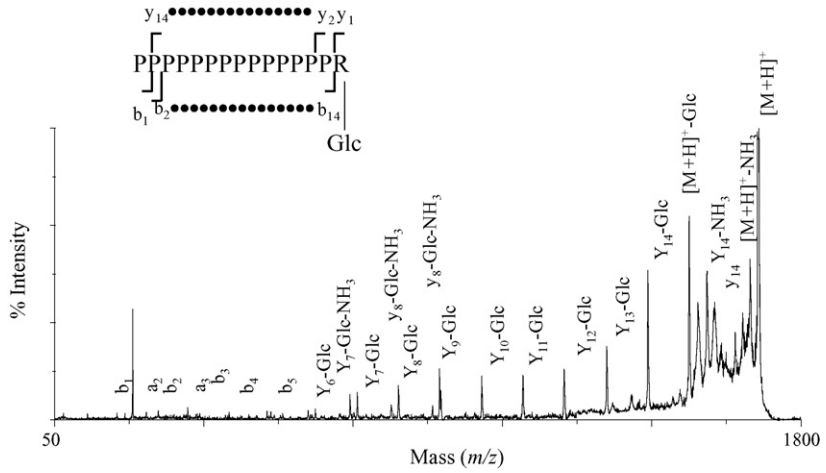

Fig. 7. The MALDI-PSD TOF spectrum of glucosylated $P_{14}R$. Glucosylated $P_{14}R$ was produced by reacting 1 M glucose with $P_{14}R$ at 65 °C for 24 h. Peptide/protein solution (1 μg/μl) was processed with Zip-Tip before MALDI-TOF MS.

Hydrated molecular ions are also detected. Using the PBS (pH 7.4) as the solvent, there are on the average 5.4 glycations in the case of BSA and up to two glycations in the case of insulin. Using the 50 mM Na_2CO_3 (pH 10) as the solvent, there are on the average 30 glycations in the case of BSA and up to 2 glycations in the case of insulin. In addition, molecular ions of CO_2 adduct are also observed. Data suggests the glycation efficiency increases at higher pH in the case of BSA but has a little effect on insulin. This effect could result from denaturation of BSA at higher pH and the resultant exposure of potential glycation sites. It is noted that all control samples show normal non-glycated peptide/protein mass spectra. This data is most useful in food science which produces foods in glucose at different pH. In addition, the information may have values on glycation in biological system at extreme pH conditions or for pH denatured proteins.

Fig. 7 shows the MALDI-TOF PSD spectrum of glycated $P_{14}R$ at *m/z* 1697. Data displays "y" (bond-cleavage fragments that retain C-terminal side of the peptide), "b" (bond-cleavage fragments that retain N-terminal side of the peptide), and "a" (b–CO ion) fragment ions. The observation that "b" fragment ion series (b_1–b_5) agrees with fragment without attached glucose, and y_{14} (*m/z* 1599) and y_{14}–NH_3 (*m/z* 1582) fragment ions agrees with fragments carrying a glucose, suggests that the glucose is attached to the arginine guanidino group at the C-terminal part of the peptide. Interestingly, y fragment ion series do not exhibit any attached glucose except y_{14}, indicating that glucose could be released from a majority of y fragment ions during the mass measurement. Postsource decay and MS/MS results (data not shown) of singly glycated angiotensin I and human adrenocorticotropic hormone fragments 1–13 indicate that glucose reacts with the amino group of the N-terminal of ATCH 1–13 and guanidino group of the arginine residue of both angiotensin I.

4. Conclusion

MALDI-TOF MS has been successfully used for the systematic study of the in vitro glycation of peptides/proteins. Results indicate that glycation occurs between peptides/proteins and the reducing glucose at different temperature, glucose concentration, incubation time, and pH; and peptides are glycated as efficiently as proteins. Human angiotensin I, human ACTH fragments 1–13, $P_{14}R$, bovine insulin, hen egg white lysozyme, human hemoglobin A, soybean trypsin inhibitor, and bovine serum albumin are used as test cases. The reaction rate increases with higher temperature, higher glucose concentration, and/or longer incubation time. All control samples (pure peptides/proteins) show normal non-glycated peptide/protein mass spectra. Postsource decay and MS/MS results of singly glycated angiotensin I, $P_{14}R$ (PPPPPPPPPPPPPPR), and human adrenocorticotropic hormone fragments 1–13 indicate that glucose reacts with the amino group of the N-terminal of ATCH 1–13 and the guanidino group of the arginine residue of both angiotensin I and $P_{14}R$. Contrary to proteins, pH has a little effect on the glycation efficiency of peptides. Glycation reaction at higher pH and longer incubation time produce more degradation products. This data could lead to the inference that the glycation process of peptides/proteins would occur but proceed very slowly under the diabetes conditions in vivo (37 °C, neutral pH, 0.007 M glucose). Further work on using trypsin proteolysis to yield more specific information about glycated sites of peptides/proteins by hybrid linear trap/FT-ICR mass spectrometric analyses is in progress.

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