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Detection and Quantitation of β-2-Microglobulin Glycosylated End Products in Human Serum by Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

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β-2-Microglobulin (β_2M) is a major protein component found in the amyloid deposits of dialysis-related amyloidosis (DRA) patients. Evidence has been shown that the advanced glycosylated end-products (AGEs) of β_2M present in sera were related to DRA. We demonstrated that matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a useful tool to investigate the nature of glycosylation of β_2M and detection of β_2M or β_2M-AGEs in human serum. The high-mass end of β_2M-AGE distribution was found to extend to the neighborhood of 12 868 Da, corresponding to condensations with seven glucose molecules. We also have shown that both β_2M and β_2M-AGEs can be detected at low picomole levels directly in bovine serum. Based on these findings, the sera of DRA patients were studied to determine whether β_2M-AGEs can be detected by MALDI-MS. In an attempt to investigate the possibility of quantitation with MALDI, human sera samples with different concentrations of β_2M-AGE were examined. We were able to correlate the concentration of β_2M-AGE with the number of detected AGE products, pointing to the feasibility of MALDI as a quantitative tool.

β-2-Microglobulin (β_2M) is a globular protein with 99 amino acids and is usually located on the membrane surface of a variety of mammalian cells. Levels of β_2M in the blood of hemodialysis patients can be as high as 60 times the levels observed in healthy individuals. Some patients in this category suffer from amyloid disease, which is characterized by protein deposits associated with a major complication of long-term hemodialysis, known as dialysis-related amyloidosis (DRA). These amyloid deposits are mainly found in the various joint structures of DRA patients, resulting in joint and bone destruction. Although there is no clear statistical correlation between the concentration of β_2M in serum and the occurrence of amyloid disease, β_2M should at least be considered as one of the factors involved, because different forms of this protein have been identified in amyloid deposits.[1−3]

Recently, advanced glycosylated end-products (AGEs) of β_2M have been linked pathogenically to the bone and joint destruction in DRA and were claimed to be the major components of amyloid fibrils from DRA patients.[2,3] β_2M-AGEs are produced from the nonenzymatic glycation of β_2M. The reaction arises between sugar aldehyde group and protein amino group to form a labile Schiff base, which may isomerize to form a more stable ketoamine via an Amadori rearrangement. After a series of further rearrangement and dehydration steps, the ketoamine is converted to AGEs that may accumulate in the joint structures to cause DRA.[4]

Because of the possible link between β_2M-AGEs in blood and DRA, it is important to detect the different forms of β_2M, the presence of β_2M-AGEs, and the nature of glycation of β_2M in the sera of DRA patients. If a correlation between β_2M-related species and DRA is found, rapid clinical measurements of these species in sera might be used to identify patients at risk for developing DRA.

A number of analytical methods have been developed to evaluate the presence of proteins including glycoproteins, such as ultraviolet spectrometry, radiotracer measurements, mass spectrometry, and some classical wet chemical methods. Among these techniques, mass spectrometry is especially powerful because of its high specificity. Fast atom bombardment mass spectrometry (FAB-MS) has been used to identify glycation sites on β_2M fragments both from dialysis patients and products of in vitro incubation with glucose.[4] However, due to the mass limitations of FAB-MS (<10 000 Da), enzymatic or chemical cleavage of the protein was necessary before analysis. In the same study, another powerful ionization method, electrospray ionization (ESI), was also combined with mass spectrometry to detect the glycated β_2M.[4] Due to the susceptibility of ESI-MS to impurities of the analyte solution, extensive purification was required prior to measurements. In addition, the presence of multiply charged analyte ions in ESI-MS leads to complex mass spectra.

Current developments in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) led to the ability to

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acquire simple mass spectra, even in the presence of salt contaminants, which in turn facilitated the detection of proteins in blood serum and other crude biological solutions.[5−8] Recently, MALDI-MS investigations of other protein glycation products have been reported in the literature.[9] Lapolla and co-workers used MALDI-MS to measure the number of glucose molecules that reacted in vitro with bovine serum albumin. At least 51 glucose units were found to be condensed onto the albumin structure. In addition, the nonenzymatic glycation of ribonuclease with glucose and fructose was investigated by the same group.[10] They concluded that glucose reacted faster than fructose in the early stage of the glycation, whereas rearrangement of the Amadori adducts was preferred with fructose.

A promising potential application of MALDI-MS is the quantitative analysis of proteins in crude biological solutions. Quantitation, if achieved, could help MALDI-MS to become a useful tool in routine clinical diagnostics. Jespersen and co-workers demonstrated the possibility of quantitative MALDI analysis for three plasma proteins in the mass range of 1000−12 000 Da using internal standards.[11] Simultaneous quantification of both cyclosporin A and its metabolite in blood had been attempted by MALDI-MS, and the results showed good agreement with the HPLC results.[12] A damped nonlinear least-squares curve-fitting method was applied to resolve the interference between the proteins and their alkali adducts. In a related study, fast evaporation in the sample preparation step was found to improve the quantification of protein.[13] However, the application of MALDI-MS in more challenging quantitative bioanalysis of clinically important biomolecules, such as glycosylated products, is still sporadic.

The detection of AGEs of these other proteins suggested the feasibility of measuring β_2M-AGE in serum by MALDI-MS. The objective of this report is to demonstrate the capability of MALDI-MS to measure β_2M and β_2M-AGEs directly in human serum. Initially, the extent of the condensations between glucose and β_2M in phosphate buffer was determined. It was also demonstrated that MALDI-MS was capable of detecting β_2M and β_2M-AGEs in both bovine and human sera at low picomole levels. In addition, the possibility of β_2M-AGE quantitation in real patient serum samples was evaluated.

EXPERIMENTAL SECTION

Instrumentation. The mass spectrometer used in the experiments was a home-built linear time-of-flight instrument (TOF-MS) equipped with a nitrogen laser at 337 nm (VSL-337ND, Laser Science Inc., Newton, MA). This system has been described in detail elsewhere.[14] In brief, in this investigation the laser beam was focused onto the probe tip at 45° with the power density of around 10^6 W/cm^2. The generated ions were extracted by 30.0 kV accelerating voltage into the 2.15 m field-free drift region of the TOF-MS. A dual-microchannel plate (Galileo Electrooptics Corp., Sturbridge, MA) biased to 1800 V was used for detection of the ions. After 10 times amplification, the signal was registered by a transient recorder (TR8828D, LeCroy, Albuquerque, NM) with 10 ns/channel resolution. Control of data acquisition parameters and transferring and averaging of spectra, as well as further data processing, were carried out by a custom-made data acquisition package (Tofware, Version 2.1, Ilys Software, Pittsburgh, PA) on a 486 PC. Ion current data generated by 40 laser shots were averaged to obtain the final spectra. For proteins, a mass resolution of 500 could be routinely achieved for standard insulin at m/z 5734.5.

Table 1. Concentrations of β_2M and β_2M-AGE in the Samples Present in Normal Human Serum

	concn (M)			concn (M)	
sample	β_2M	β_2M-AGE	sample	β_2M	β_2M-AGE
1	8.5×10^{-7}	0	6	4.25×10^{-6}	8.5×10^{-8}
2	8.5×10^{-7}	8.5×10^{-8}	7	4.25×10^{-6}	4.25×10^{-7}
3	8.5×10^{-7}	4.25×10^{-7}	8	4.25×10^{-6}	8.5×10^{-7}
4	8.5×10^{-7}	8.5×10^{-7}	9	8.5×10^{-7}	8.5×10^{-7}
5	4.25×10^{-6}	0			

Sample Preparation. In vitro glycation of pure β_2M was carried out with glucose according to a published procedure.[3] Briefly, β_2M (2.17 mg/ml, Cortex Biochem. Inc., San Leandro, CA) was incubated at 37 °C for 30 days with 0.1 M glucose in 0.1 M phosphate buffer (pH 7.4) containing 200 units/mL penicillin and 8 μg/mL gentamycin. The presence of AGEs was confirmed by both enzyme-linked immunosorbent assay (ELISA) and fluorescence measurements. Control β_2M samples were prepared without addition of glucose. To demonstrate the feasibility of detecting β_2M by itself and together with β_2M-AGE in serum, β_2M and β_2M-AGE were added exogenously to bovine serum at concentrations of 3.7×10^{-6} and $3.7 \times 10^{-6}/3.3 \times 10^{-6}$ M, respectively. Standards were also prepared using human serum from a symptom-free individual by addition of β_2M (8×10^{-7} M) and the mixture of β_2M (8×10^{-7} M) and β_2M-AGEs (8×10^{-7} M). In addition, three serum samples from dialysis patients were obtained from National Medical Care (NMC, a subsidiary of W. R. Grace & Co.-Conn.). The concentrations of β_2M in the serum samples, as determined by Lifechem Laboratories (division of NMC) using standard radioimmunoassay procedures, were 2.4×10^{-7}, 1.6×10^{-7}, and 4.2×10^{-6} M. For the quantitative studies, varying amounts of β_2M-AGE and β_2M were mixed with a normal human serum samples. The concentrations of β_2M-AGE and β_2M in these samples are listed in Table 1. After preparation, all bovine and human serum samples were stored in a freezer at −5 °C. No degradation of the analytes was found within 1 month. Before MALDI-MS analysis, the sera were filtered by Ultrafree-MC filter units (Millipore Co., Bedford, MA) with a 30 000 molecular weight cutoff. The filtrate was used directly for MALDI-MS measurements.

The matrices 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid or SA, Aldrich Chemical Co., Milwaukee, WI) and α-cyano-

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4-hydroxycinnamic acid (CHCA, Sigma Chemical Co., St. Louis, MO) were used without any purification. Saturated matrix solutions were prepared fresh in 7:3 (v/v) acetonitrile (HPLC grade)−deionized water solvent mixture on a daily basis. Samples for analysis were prepared by mixing 10 μL of matrix solution with 3 μL of the analyte on the probe tip, providing higher than 1000:1 matrix-to-analyte molar ratio. The sample layer was formed by drying the solutions in a stream of cold air. For all experiments, bovine insulin (MW = 5733.5) and bovine ubiquitin (MW = 8564.9) were used as external standards, providing mass accuracy values within ±0.1% for β_2M.

Data Evaluation. The mass resolution of our instrument did not allow for separate spectral representation of β_2M and β_2M-AGE peaks. Therefore, deconvolution of the composite peaks became necessary. The deconvolution was based on a three-step nonlinear regression analysis. We started out with the hypothesis that the β_2M−β_2M-AGE region in the spectra can be modeled by summing Lorentzian curves corresponding to β_2M and its glycation products of varying degree. After fixing the number of glycation products included in the analysis, nonlinear least-squares fit was performed using the Levernberg−Marquardt iterative procedure.[15] In the final step, the quality of the fit was assessed on the basis of the sum of squared residuals (χ^2) and visual inspection.

Initially, the β_2M peak alone was used in every case, followed by the incorporation of peaks resulting from stepwise glycation. During the analysis of β_2M-AGE spectra, χ^2 decreased rapidly with the incorporation of increasing number of glycated products. There was a point, however, where the drop in χ^2 became insignificant. The number of glycation products associated with this point was accepted as most likely for the particular spectrum. The possibility of interference between the singly glycated product and the matrix adduct peaks was carefully monitored throughout the study. Although we could not rule out the presence of matrix adducts completely, the fairly accurate position of the deconvoluted singly glycated product peak (<0.5% deviation in mass) made significant matrix adduct contributions unlikely.

To deconvolute the peak profiles of the individual components of β_2M-AGEs, a scientific graphics package (Microcal Origin, Version 3.5, Microcal Software Inc., Northampton, MA) was used. Spectra were saved in ASCII format and imported into Origin. The imported data contained the averaged ion current and the corresponding flight time, instead of *m/z*, because in this case the area under each peak represented the actual amount of ions collected by the MCP detector. After baseline correction, the β_2M-AGE-related peaks were ready to be deconvoluted. The previously discussed multiple Lorentzian fitting procedure was used.

In an attempt to study the possibility of quantitation by MALDI, the peak areas of β_2M-AGEs were correlated with the concentrations of these species in human serum standards. Pulse-to-pulse laser energy variations and sample inhomogeneity necessitated the use of internal standards for quantitative studies. Throughout the study, β_2M was used as an internal standard to normalize the β_2M-AGE signals.

RESULTS AND DISCUSSION

MALDI Monitoring of the in Vitro Glycation Process. One of the attractive features of MALDI-MS over other mass spectroscopic methods is its high tolerance to impurities and buffers in the analyte. This feature was utilized in the analysis of complete mixtures in this study. A typical MALDI spectrum is shown in Figure 1a for the control β_2M incubated in the absence of glucose at 37 °C for 1 month in 0.1 M phosphate buffer containing 200 units/mL antibiotic penicillin. Despite the presence of potassium salts from the buffer, an intense protonated ion $[M + H]^+$ peak of β_2M was detected, along with the less intense $[2M + H]^+$, $[M + 2H]^{2+}$, and $[M + 3H]^{3+}$ ion peaks. The detected mass of $[M + H]^+$ was 11 731 Da, in close agreement with a calculated value of 11 730 Da based on the average molecular mass of residues in β_2M.

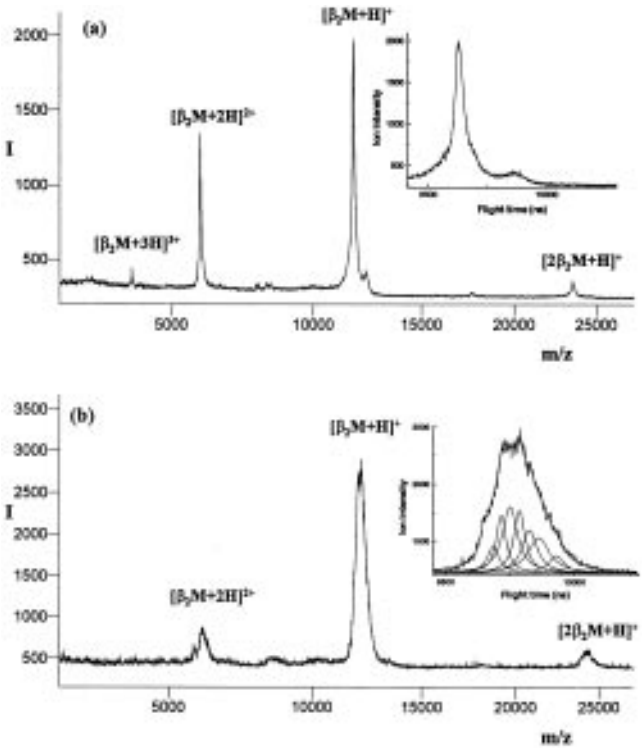

Figure 1. MALDI mass spectrum of β_2M incubated without (a) and with (b) glucose for 1 month at 37 °C. CHCA is used as matrix. The deconvoluted Gaussian peaks of β_2M-AGEs are shown by dotted lines in the inset of spectrum b.

The MALDI mass spectrum of β_2M incubated for 1 month with glucose is shown in Figure 1b. The β_2M-AGE peak was much broader than the β_2M peak, indicating the presence of a heterogeneous mixture of AGEs primarily as a consequence of glycation. In addition, the peak of pure β_2M was narrower than that of β_2M incubated in buffer solution (in Figure 1a). Alkali adducts, oxidation, and deaminidation of β_2M during the incubation may have attributed to the broadening. The center of the protein peak was found to shift to a mass value higher by 321 Da, corresponding to the condensation of two glucose molecules onto a β_2M molecule. The peak profile, after background substraction, was found to be asymmetrical, having a sharper slope on the lower mass side of the peak. The inset in Figure 1b illustrates the deconvoluted Lorentzian profile of the molecular ions of β_2M-AGEs. The β_2M-AGE peak appears to have 1−7 glucose units associated with one β_2M molecule. The value of seven glucose condensation units is in good agreement with the results reported by Miyata et al.[4] According to their results, seven amino groups could be glycated in the β_2M sequence, in which the α-amino group of Ile-1 was the major glycation site, and the ϵ-amino groups

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of Lys-19, Lys-41, Lys-48, Lys-58, Lys-91, and Lys-94 were the minor glycation sites. The ion abundance corresponding to the condensation of seven glucose units was low, possibly due to the metastable fragmentation of glycoproteins. The tendency for extensive fragmentation of glycoproteins has been indicated by other investigators in reflectron TOF-MS experiments.[16] However, we did not observe fragments exhibiting masses lower than that of the singly glycated β_2M in our linear instrument. Therefore, we can only estimate the total amount of β_2M-AGEs, but not the relative ratio of different β_2M-AGE products.

MALDI-MS Analysis of β_2M and β_2M-AGE in Bovine Serum. One of the aims of this investigation was to develop a rapid MALDI-MS screening procedure for measuring β_2M and β_2M-AGE in human serum. To demonstrate feasibility, bovine serum was used instead of human serum in the preliminary investigations. MALDI-MS analysis of bovine serum produces a simple mass spectrum (data not shown), with the most abundant peak centered at *m/z* 2189.7 and the less intensive peaks corresponding to the dimer and trimer of this protein. None of these ions interfered with detection of ions formed from β_2M or β_2M-AGE. Neither β_2M nor β_2M-AGE was detected in untreated bovine serum, even at the highest sensitivity available.

Ultrafiltration of serum samples through a 30 000 molecular weight cutoff microfilter was found to be particularly efficient in the preparation of serum-containing samples for analysis. Without the ultrafiltration, sample-specific signal was difficult to detect. The lack of homogeneous matrix crystal formation was observed during the preparation of serum-containing samples. This phenomenon was probably due to the disruption of matrix crystal structure by the relatively large amount of high-mass (>30 000 Da) proteins such as bovine serum albumin. The dilution of bovine serum did not alleviate the situation, since the initial concentrations of β_2M and/or β_2M-AGE were low ($\sim 5 \times 10^{-6}$ M). In addition, the presence of a large variety of other proteins in the serum introduced strong competition for protons during the ionization process, making detection of proteins at low concentrations difficult. Both signal suppression of analyte and disruption of crystallization process due to impurity are well-known effects.[17]

The MALDI mass spectrum of bovine serum to which β_2M was added to reach 3.7×10^{-6} M is shown in Figure 2a. The strong singly and doubly charged molecular ions of β_2M were readily identified. Both CHCA and SA matrices were used in the preparation of the bovine serum samples for MALDI-MS. Similar spectra were obtained in both matrices, although SA appeared to provide greater sensitivity for the detection of β_2M and β_2M-AGE. The amount of β_2M and β_2M-AGE used in a single sample preparation was around 7 pmol; however, the actual amount of analyte consumed in the MALDI experiment was much less. Matrix adducts of β_2M were absent in the mass spectrum when CHCA was used as matrix. Thus, the use of CHCA greatly reduced interferences in the region of the spectrum corresponding to β_2M-AGE. Therefore, CHCA was selected as the matrix for most of the experiments unless the concentrations of β_2M and β_2M-AGE were very low, in which case SA matrix was applied.

When 3.7×10^{-6} M β_2M and 3.3×10^{-6} M β_2M-AGEs were added into bovine serum and analyzed by MALDI-MS, the protonated β_2M ion peak was clearly present (Figure 2b).

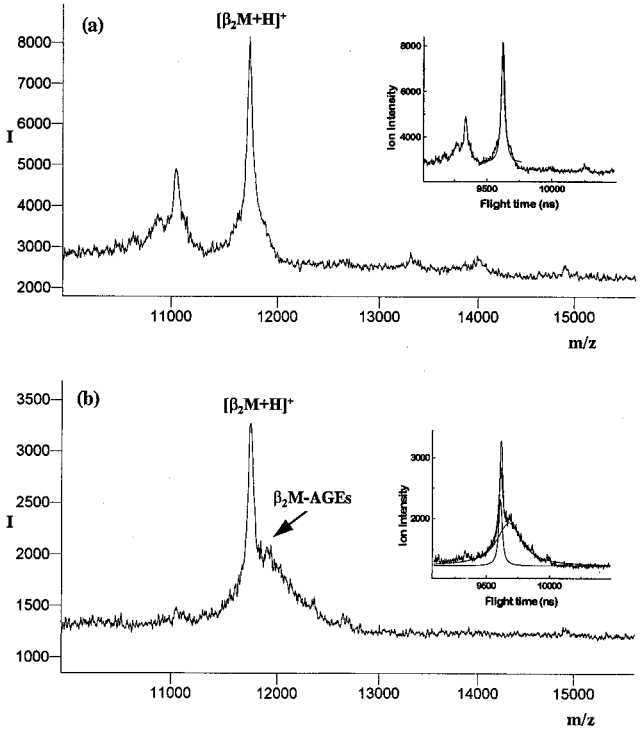

Figure 2. Comparison of MALDI spectra of (a) bovine serum containing β_2M (3.7×10^{-6} M) and (b) bovine serum containing a mixture of β_2M (3.7×10^{-6} M) and β_2M-AGE (3.3×10^{-6} M) using CHCA matrix.

Although the broad peak of β_2M-AGEs could not be completely separated from that of β_2M, a pronounced shoulder was observed with a peak centered at 12 050 Da. The peak height of β_2M appears to be about twice that of β_2M-AGEs, despite their similar concentrations in bovine serum. However, by integrating the area under the ion current peaks, the total charge associated with β_2M ions is only a factor of 1.2 higher than that of β_2M-AGE. This value is close to the 1.12:1 molar ratio of β_2M:β_2M-AGE. The lower signal intensity of β_2M-AGE may be partially due to the decreased protonation efficiency of β_2M-AGE. Glucose molecules react with the α-amino group of Ile-1 at the N-terminus of β_2M, which has been suggested as a major protonation site of proteins in MALDI-MS.[14]

MALDI-MS Analysis of β_2M and β_2M-AGE in Normal Human Serum. Both reversed-phase HPLC and anion exchange HPLC, combined with off-line MALDI-MS analysis, have recently been proposed to explore protein profiles in human blood plasma.[18] Using protein databases, more than 50 proteins, including β_2M, were identified in the spectra. About 26 of these proteins had molecular weights below 30 000.

Since the purpose of our study was the rapid detection of β_2M and β_2M-AGE in crude serum, no HPLC separation was performed. Due to the compositional complexity of human serum, the deterioration of MALDI-MS response was expected. Figure 3a shows the mass spectrum of human serum from a healthy subject with exogenously added β_2M at 8×10^{-7} M. SA matrix was chosen here, as no response would be expected from β_2M at such a low concentration using CHCA. In the spectrum, more than 15 proteins were observed, all below 15 000 Da. A relatively

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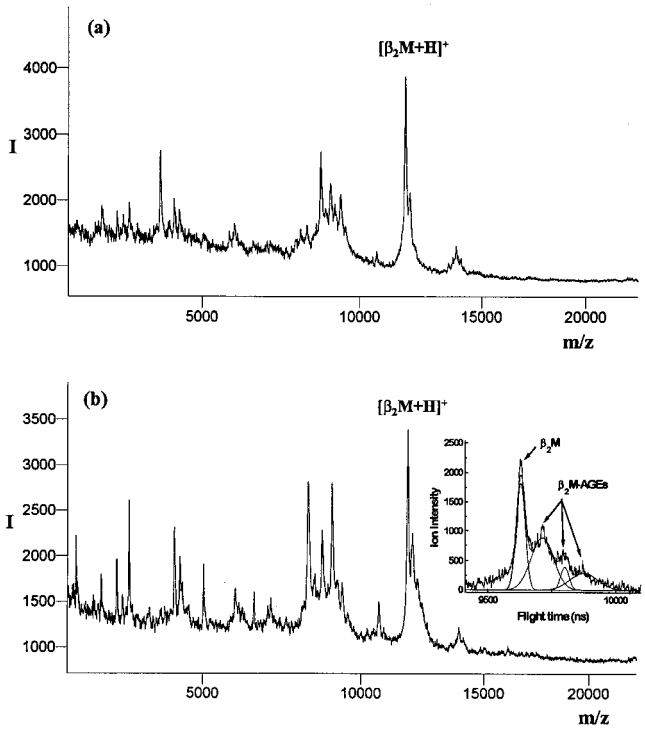

Figure 3. Mass spectra of human serum standards containing (a) β_2M (8×10^{-7} M) and (b) the mixture of β_2M (8×10^{-7} M) and β_2M-AGEs (8×10^{-7} M).

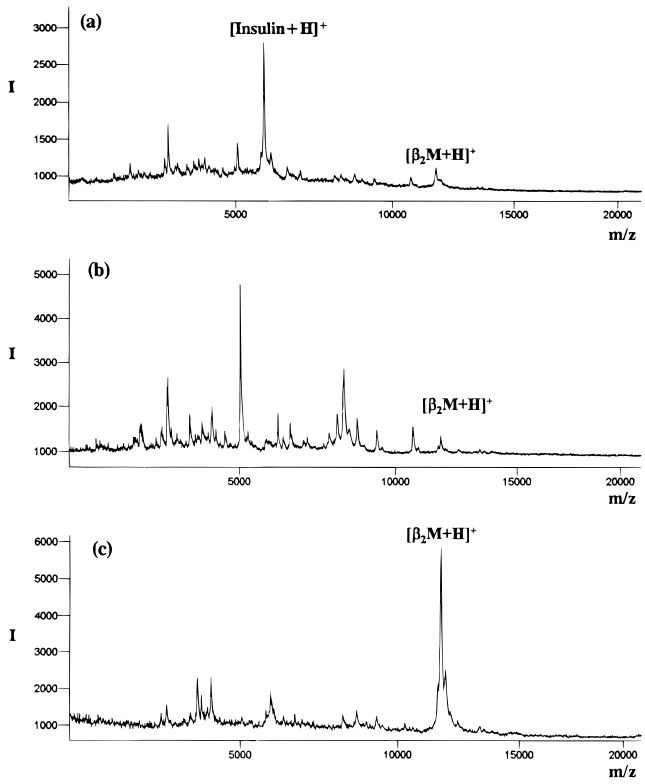

Figure 4. MALDI results of serum samples from three dialysis patients. Concentration of β_2M in each sample: (a) sample A, 2.4×10^{-7} M; (b) sample B, 1.6×10^{-7} M; and (c) sample C, 4.2×10^{-6} M. (See text for explanation.)

strong peak originating from the molecular ion of β_2M was obtained, noticeably broadened by its matrix adducts. The interference between β_2M−matrix adducts and the β_2M-AGE was negligible because of the substantial mass difference between matrix SA (224 Da) and two glucose molecules (324 Da). A spectrum of a β_2M (8×10^{-7} M)−β_2M-AGE (8×10^{-7} M) mixture in normal human serum is shown in Figure 3b. In addition to β_2M and its SA adduct (11 955 Da), a series of peaks was observed after deconvolution (see inset of Figure 3b), corresponding to β_2M-AGE with two, three, and four glucose units centered at 12 051, 12 211, and 12 371 Da, respectively. Although the β_2M-AGE peaks were not well resolved, the deconvolution analysis clearly confirmed the presence of those components. The other 15−17 peaks in the spectrum most likely correspond to other serum proteins. Since our objective was limited to β_2M and β_2M-AGE detection, we did not attempt the identification of the other components. Future diagnostic application of these peaks hinges on their assignment and the development of quantitation protocols.

MALDI-MS Analysis of Dialysis Patient Sera. Figure 4 shows mass spectra of three serum samples from end-stage renal disease patients on kidney dialysis. Several observations are noteworthy. First, the numbers of proteins observed in MALDI-MS were different in the three samples. A strong insulin ion peak (m/z 5750) was observed for sample A (Figure 4a), while no such signal was detected in the other two samples. The number of detected proteins in sample B (Figure 4b) was the largest among the three samples, and the intensities for most of these proteins were relatively strong. The lower relative intensities for other proteins may be due to the strong suppression effects of the large amounts of insulin in sample A (Figure 4a) and of β_2M in sample C (Figure 4c).

It was observed that the signal intensity of β_2M correlated quite well with the amount of β_2M in the sample. A comparison of the β_2M peak areas in Figure 4a−c indicates 1.0:1.0:23.2 ratio. These results were close to the concentration ratio of 1.5:1.0:26.3, as determined by radioimmunoassay in a clinical lab, indicating the utility of MALDI-MS for the quantitative analysis of β_2M in human serum. This possibility is further explored in the following section.

Quantitative Analysis of β_2M-AGE in Human Serum. For quantitation, β_2M was chosen as internal standard. Nine samples containing different concentrations (as shown in Table 1) of β_2M-AGE and β_2M were prepared and mixed with a single source of normal human serum. In an attempt to perform quantitative analysis, integrated peak areas were used to represent the amounts of analyte and internal standard.[14] The integration was achieved by deconvoluting the unresolved peaks of interest (β_2M-AGE and β_2M) and allowing the integration of each peak individually. This method was found to be more accurate than the one relying on peak heights.

In some of the spectra, a matrix adduct was also present. These peaks were not very intense and appeared mostly as a shoulder attached to the β_2M signal. The peaks with matrix adduct were excluded from the peak area calculations. In general, the AGE products seemed to be easily distinguishable. Usually they appeared as a series of peaks with a repeat unit of 162 due to glucose condensation and occasionally shifted higher by 39 due to cationization with potassium. In addition, they were relatively more intense in comparison to the matrix adduct signals.

To confirm the deconvolution results, we chose sample 4 in Table 1 (8.5×10^{-7} M for both β_2M-AGE and β_2M) as a characteristic sample. The calculated and measured differences in mass and time of flight between each peak of interest and β_2M were compared. The expected mass differences were calculated from the structure of the compounds. The calculated values were

Table 2. Comparison of the Expected and Measured Values for the Molecular Masses of Different Peaks Observed in One of the Serum Samples

	ΔM (amu)	
	calcd	measd
β_2M	0	0
β_2M-matrix	225	223
β_2M + 1G[a] + K	201	198
β_2M + 2G + K	363	370
β_2M + 3G + K	525	526

[a] G represents a glucose molecule condensed onto the β_2M molecule.

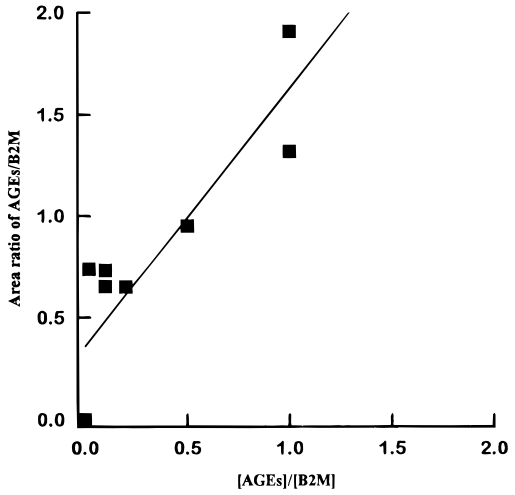

Figure 5. Ratio of β_2M-AGE and β_2M peak areas as a function of concentration ratios of the two compounds in the serum samples. Linear regression showed a regression coefficient of $R = 0.88$.

compared to the experimental ones obtained from the deconvolution of the spectra in Table 2. Good correlation was observed between the expected and calculated values, which points to the accuracy of the flight time calibration and the reliability of the deconvolution method.

The sum of the AGE-related peak areas over the β_2M peak area was calculated and plotted as a function of the concentration ratio of β_2M-AGE and β_2M (see Figure 5). The linear least-squares fit to these data shows a fairly good correlation for concentration ratios of β_2M-AGE/β_2M ranging from 0.01 to 1.0. The results indicated that the concentration of β_2M-AGE relative to β_2M was related to the corresponding ratio of ion yields with $R = 0.88$ regression coefficient.

The linear correlation between sample loading and peak area ratio was based on at least two assumptions: the homogeneous distribution of analyte in the matrix crystal and unchanged ionization efficiency of the analyte in the presence of other proteins. Therefore, careful sample preparation was required for the quantitation of β_2M-AGE. In our experiments, a fixed amount of matrix was applied, whereas the amount of sample was increased. The increased amount of sample, with a corresponding increase in protein content, disrupted the matrix crystallization process and resulted in lower signal intensity. Due to the different proton affinities of β_2M and β_2M-AGE, the ionization efficiency of β_2M-AGE may also change in the presence of different amounts of β_2M. Generally, increasing concentration of β_2M will suppress the signal of β_2M-AGE since β_2M is easier to protonate.

Based on the data analysis, we were able to correlate the concentration of β_2M-AGE to the number of detected AGE products. For example, samples 4 and 9, in which the content of β_2M-AGE was relatively higher, produced up to three AGE-related peaks. On the other hand samples 2, 3, 6, 7, and 8 produced mostly two or at least one AGE product. These signals were not easily resolved; therefore, these peaks were deconvoluted before analysis.

CONCLUSION

We demonstrated that MALDI-MS was a sensitive method to investigate the nature of glycosylation of β_2M. It was shown that β_2M and β_2M-AGEs can be detected directly in both bovine and human serum at low picomole levels—a typical level of β_2M in serum. No interfering proteins were observed. The profiles of β_2M-AGEs exhibited that there were seven glucose molecules condensed on one β_2M molecule. In addition, the study of quantitation with MALDI indicated that we were able to correlate the concentration of β_2M-AGE with the number of detected AGE products, pointing to the feasibility of MALDI as a quantitative tool.

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