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Imaging mass spectrometry reveals direct albumin fragmentation within the diabetic kidney

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Abstract

Albumin degradation in the renal tubules is impaired in diabetic nephropathy such that levels of the resulting albumin fragments increase with the degree of renal injury. However, the mechanism of albumin degradation is unknown. In particular, fragmentation of the endogenous native albumin has not been demonstrated in the kidney and the enzymes that may contribute to fragmentation have not been identified. To explore this we utilized matrix assisted laser desorption/ionization imaging mass spectrometry for molecular profiling of specific renal regions without disturbing distinct tissue morphology. Changes in protein expression were measured in kidney sections of eNOS^{-/-} *db/db* mice, a model of diabetic nephropathy, by high spatial resolution imaging allowing molecular localizations at the level of single glomeruli and tubules. Significant increases were found in the relative abundances of several albumin fragments in the kidney of the mice with diabetic nephropathy compared to control non-diabetic mice. The relative abundance of fragments detected correlated positively with the degree of nephropathy. Furthermore, specific albumin fragments accumulating in the lumen of diabetic renal tubules were identified and predicted the

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DISCLOSURE

All other authors declare no competing interests.

enzymatic action of cathepsin D based on cleavage specificity and *in vitro* digestions. Importantly, this was demonstrated directly in the renal tissue with the endogenous non-labeled murine albumin. Thus, our results provide molecular insights into the mechanism of albumin degradation in diabetic nephropathy.

Keywords

Chronic kidney disease; diabetic nephropathy; distal tubule; proximal tubule

INTRODUCTION

While diabetic nephropathy (DN) takes years to develop, it is largely irreversible and remains a major health complication for diabetic patients. The pathogenic mechanisms underlying this disease remain unclear. Uncovering molecular events that define disease progression can improve early detection and provide potential targets for drug therapy. Proteomic technologies have been used in this vein to discover proteins and pathways that contribute to the pathophysiology of the kidney. Previous proteomic studies have looked for biomarkers in the urine and blood.^{1–4} A number of studies have analyzed renal tissues directly using either total kidney lysates or laser capture micro-dissection technique.^{5–8} However, the ability to localize molecular changes in the morphologically intact renal tissue, at the site of disease, provides a more specific insight into disease mechanisms.^{9–12}

It is well established that processing of albumin is mishandled in the diabetic kidney. Detection of albumin in the urine indicates kidney damage and is in fact the main diagnostic tool used to assess early disease and disease progression.^{13, 14} In healthy kidneys, only a small amount of albumin passes through the glomerular filter. The majority of it is then reabsorbed by the proximal tubules and degraded, while the remainder is excreted in the urine.¹⁵ While intact urinary albumin is the main measure in the clinic, more studies are showing that albumin fragments increase as well and these are not always detected by traditional assays.^{16–18} The origin of albumin fragments is of interest but the mechanism of albumin fragmentation is unknown. Some studies suggest fragments are made in the urine by urine proteases,^{19, 20} while others found fragments present in the kidney as well.^{21–24} However, the fragmentation of the endogenous native albumin has not been demonstrated in the kidney and the enzymes that may contribute to fragmentation have not been identified.

In the present study, we used matrix assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) for molecular profiling of specific tissue regions without disturbing the distinct tissue morphology.²⁵ Briefly, IMS acquires mass spectra at discrete coordinates directly from an intact tissue coated in an organic matrix. The dataset can be queried to monitor the location of molecules spatially where the relative intensity of the molecular ion detected is represented in color as a heat map. A more detailed description of the MALDI IMS workflow is depicted in Fig. S1.²⁶ High spatial resolution IMS, sufficient to analyze individual renal glomeruli and tubules, was performed to determine albumin fragments in the kidney of eNOS^{−/−} db/db mouse, a DN model.^{27, 28} This is the first report showing localization of endogenous unlabeled albumin fragments in the kidney with the

identification of the fragments and the protease involved, cathepsin D, using specificity of the cleavage sites, immunohistochemistry (IHC) staining, and *in vitro* digestions.

RESULTS

Albumin excretion in eNOS^{-/-} db/db mice

As expected, eNOS^{-/-} db/db mice developed hyperglycemia and albuminuria by 12 wks of age, which significantly progressed by 22 wks of age (Fig. 1).

Detection and identification of albumin fragments in the kidney

We have used IMS to determine the presence and localization of albumin fragments in the kidney of diabetic mice and non-diabetic controls. Several albumin fragments were detected directly from the kidney section of a diabetic kidney (Fig. 2A). Based on accurate mass measurements and tandem mass spectrometry (MS/MS) experiments (Fig. 2B), 18 peaks were determined to originate from albumin (Table 1). Interestingly, all of the detected albumin fragments originated from three surface regions of the albumin molecule (Fig. S2 and S3).

Imaging albumin fragments with IMS

Kidneys from control, 12-wk DN and 22-wk DN mice were imaged at either 10 m or 25 m spatial resolution and albumin fragments were tracked. We found that degradation of albumin was increased in the DN kidney and was not detected in control tissue. Additionally, the relative abundance of fragments increased with DN progression (Fig. 3). Several selected fragments had specific localized distributions within the diabetic kidney (Fig. 3).

Immunofluorescence staining and tubular localization of albumin fragments

After determining the presence of albumin fragments in the DN kidney, we wanted to determine localization of these fragments to specific renal structures. First we found that albumin fragments were not detected in glomeruli by comparing IMS images with PAS staining of the same section (Fig. 4A) and confirmed tubular localization of the fragments using 10-m spatial resolution MALDI IMS (Fig. 4 B). Next, we compared IMS images with immunofluorescence tissue staining for tubular specific proteins (Fig. 4C and D). Albumin fragments identified by IMS co-localized with dolichos biflorus agglutinin (DBA), a distal tubule and collecting duct marker (Fig. 4C), and Lotus tetragonolobus lectin (LTL), a proximal tubule marker (Fig. 4D).

Major N-terminal albumin fragment in urine

We sought to determine if the fragments that we identified in the kidney were also present in urine. We found that the major albumin fragment in the kidney at *m/z* 2791 is also the major albumin fragment in the urine (Fig. 5). This was detected in the urine of 12-wk old and 22-wk old DN mice but not in the control urine (Fig. 5). Interestingly, a similar N-terminal albumin fragment has been reported in urine of humans with renal disease.^{29, 30} Other

albumin fragments were not detected in urine possibly because they were either further degraded or reabsorbed by the renal tubules and not excreted.

Albumin degradation by cathepsin D in the kidney

Since IMS technology provided information about the sequence of the albumin fragments (Table 1), we searched for possible proteolytic enzymes that could produce these specific albumin peptides. The analysis of cleavage sites using PICS software³¹ revealed that the residues L, E, V and Y were prominently featured at P1 and P1' positions of the cleavage site and the residues K and E at P2 and P2' positions as has been previously described for cathepsin D (CatD) and, with some broader specificity, for pepsin.³² The cleavage specificity was different from those of cathepsins B, L, and S, which preferred G at P1 and P1' and F, V and Y at P2.³¹ Since pepsin tissue distribution is restricted to gastrointestinal system, we focused our studies on the role of CatD in albumin fragmentation in diabetes.

To further investigate the potential involvement of CatD in the degradation of albumin observed in renal tissues of DN mice, *in vitro* digestion of mouse serum albumin with purified CatD was performed. Several albumin fragments found in the mouse kidney were also identified by proteomic analysis of the CatD digestion (Table 2 and Fig. S4-S11).

Detection of albumin fragments in renal tissues and urine of DN mice suggested that albumin degradation by CatD may take place in either kidney or blood. However, an extensive analysis of diabetic mouse serum using several high-resolution mass-spectrometry approaches did not detect any CatD-specific albumin peptides (Fig. S12-S15), consistent with the renal origin of albumin fragments.

Immunohistochemical staining of renal tissues using CatD antibody confirmed that the level of this protease was significantly increased in the tubule areas of DN kidneys compared with non-diabetic controls (Fig. 6). Furthermore, some of the identified albumin fragments localized to areas of stronger CatD staining in DN kidneys (Fig. 7).

DISCUSSION

Our data demonstrate that the levels of albumin fragments are significantly increased in the DN kidney (Fig. 3). We have detected these fragments specifically in tubular lumen regions suggesting that they may be formed during processing in the tubules through the proteolytic action of cathepsin D which is localized to tubular cells (Fig. 4, 6 and S16-17). Fragments likely localize in the lumen of the distal tubules and collecting ducts as a result of diffusion. These data are in agreement with previous reports, which suggested that after lysosomal degradation in the proximal tubule, albumin fragments are returned to the lumen and excreted.²¹⁻²⁴ This process can be compromised in diabetes thus affecting the amount of fragments in kidney and urine.³³ Even though our findings do not completely exclude prerenal albumin fragmentation,³⁴ the fact that the fragments were not detectable in serum and that the amount of fragments found in the kidney increased with extent of renal disease suggests a predominantly renal source. This notion is in agreement with other reports that failed to detect radiolabeled albumin fragments in the circulation suggesting albumin fragmentation does not occur prior to the kidney.^{35,36}

The label-free IMS technology used in this study has several advantages over previously employed methods to study albumin fragmentation. The radioactive isotope labeling or fluorescent methodology used in the previous studies did not allow for identification of the fragments. Therefore, mechanism(s) of fragmentation, which may include enzymatic degradation and non-enzymatic oxidative fragmentation,³⁷ have not been determined previously. Moreover, the fluorescent technology uses a bovine serum albumin conjugate labeled with BODIPY dyes, bulky three-ring molecules, to a very high degree in order to insure self-quenching of dye fluorescence.²⁴ In radioactive isotope labeling, albumin is subjected to either oxidation or reductive methylation.²² These labeling procedures are likely to affect albumin structure and, thus, its susceptibility to degradation.

We demonstrated that a major part of the albumin structure was degraded in the kidney enzymatically through the action of cathepsin D as determined based on cleavage specificity (Table 1) and *in vitro* digestion (Table 2 and Fig. S4-11). Importantly, albumin is a known substrate for cathepsin D.^{32, 38} Sun et al. created a cathepsin D-cleaved peptide database using liquid chromatography-tandem mass spectrometry to monitor the cleavage products of proteins, including albumin, after incubation with cathepsin D.³² The albumin peptide fragments found in our work overlap with cathepsin D derived albumin fragments reported in that database. Because the crystal structure for mouse serum albumin was not available, the 3D structures of rabbit serum albumin and human serum albumin were analyzed as there was greater than 70% sequence overlap with mouse serum albumin. The *in vivo* proteolytic peptide fragments identified here are derived from three albumin sites located at the surface of the parent albumin molecule and therefore accessible to the protease (Fig. S2). The additional analysis of the secondary structure of rabbit and human serum albumin revealed that the sequences of the peptides detected *in vivo* originate from surface regions containing either a turn or bend (Fig. S3). This may contribute to CatD's accessibility and the production of the observed peptides in mouse kidney tissues.

Significant increases in the levels of cathepsin D in the tubular areas (cellular localization) of DN kidneys compared to controls were confirmed using IHC with a CatD antibody (Fig. 6). Cat D and CatD-derived proteolytic albumin fragments were present in the same tubular areas of the kidney but the fragments were within tubular lumen (Fig. 7). This differential localization suggests potential movement of the fragments away from protease upon cleavage. Consistent with this notion, the proteolytic fragments had a diffused localization pattern.

The role of cathepsin D in renal disease is unclear. Contrary to previous reports of decreased renal CatD activity and renal protein degradation in STZ-diabetic animal models,³⁹⁻⁴² in the present study, CatD levels and CatD-specific albumin degradation was increased in kidneys of eNOS^{-/-} db/db mice compared to non-diabetic controls, consistent with importance of this protease in albumin fragmentation in DN. One possible explanation for this apparent discrepancy is that STZ-diabetic models, unlike the eNOS^{-/-} db/db model used in our study, do not develop significant DN lesions. On the other hand, in line with our findings, others have found an increase in cathepsin D activity⁴³ and stronger cathepsin D staining that correlates with renal disease.⁴⁴

It is important to note that the renal accumulation of albumen fragments may be a multifactorial process. Although we demonstrate the importance of cathepsin D mediated degradation, other factors such as reduced renal clearance may also contribute. Additionally, we cannot exclude contribution of other proteases in the degradation process, even though we have not found evidence of such protease activities based on cleavage specificity.

The extent of renal albumin fragmentation in our study correlated with the level of hyperglycemia and functional renal damage as characterized by albuminuria. There has been growing interest in finding biomarkers, particularly in the urine, that correlate with kidney disease progression^{45, 46} which has led to the development of the CKD273 classifier.⁴⁷ This proteomics classifier, based on 273 urinary peptides, has been used to detect and predict progression of CKD in humans.⁴⁸ In the classifier, urinary concentrations of specific albumin peptides were found to correlate negatively with renal function. We compared the albumin peptides detected in our study with the CKD273 classifier and found three N-terminal albumin peptides that matched the CKD273 database. The notion that renal albumin fragmentation is correlated with renal damage is further supported by inhibition of albumin fragmentation in eNOS^{-/-} db/db mice receiving pyridoxamine therapy (Fig. S18), which has been shown to ameliorate major DN lesions in this mouse model.⁹

In summary, we have demonstrated, using IMS technology in a DN model, that endogenous albumin fragmentation is significantly increased in renal tubules through the proteolytic action of cathepsin D and the abundance of the fragments correlates with the severity of renal disease.

METHODS AND MATERIALS

Mouse model of diabetic nephropathy

Animal experiments were performed at the AAALAC-accredited animal facilities at Vanderbilt University Medical Center according to institutional guidelines and IACUC-approved experimental protocol. In this study, we employed eNOS^{-/-} C57BLKS db/db mice, a mouse model of Type 2 DN.²⁷ Some mice were treated with pyridoxamine (PM) as previously described.⁹ Control group included wild type C57BLKS mice. Experiments continued until mice were sacrificed at 12 wks or 22 wks of age and kidneys were removed, flash frozen in liquid nitrogen and stored at -80°C. Glucose levels were measured in blood collected from the tail vein using OneTouch glucometer and Ultra test strips (LifeScan, Milpitas, CA). Albumin and creatinine excretion was determined in spot urine collected from individually caged mice using Albuwell-M kits (Exocell Inc, Philadelphia, PA).

Immunostaining

Fluorescein-labeled lotus tetragonolobus lectin (LTL) and fluorescein-labeled dolichos biflorus agglutinin (DBA) kits were from VectorLabs (Burlingame, CA). Immunostaining was performed as previously described.⁴⁹

Renal tissue preparation

Frozen kidneys were sectioned on a cryostat at 10 μ m thickness and thaw mounted on to ITO coated glass slides. Kidney tissue sections were washed by immersion in 70% ethanol for 30s, 100% ethanol for 30s, Carnoy's fluid for 2 min, 100% ethanol for 30s, water for 15s, and then 95% ethanol for 30s as described previously.⁵⁰ Slides were then stored in a desiccator until further processing on the same day.

Cathepsin D in vitro digestion of albumin

Cathepsin D human expressed protein (Novus Bio, Littleton, CO) was diluted in 50 μ L D_2O and immediately aliquoted into 5 μ L aliquots in low binding tubes and stored at -80°C to reduce freeze-thaw cycles. Albumin from mouse serum (Sigma Aldrich, St. Louis, MO) was reconstituted in acetate buffer (pH 3.6) to a final concentration of 1mg/mL. Mouse serum albumin (MSA) was denatured by adding 100 μ L of 1% formic acid to 100 μ L stock MSA and heating to 85°C for 5 minutes. Following the denaturing, 788 μ L acetate buffer (pH 3.6) and 12 μ L of 0.1 $\mu\text{g/mL}$ cathepsin D were added to MSA and subsequently incubated at 37°C for 5 hours. Digests were immediately placed in the -80°C freezer to stop digestion and then dried down in a speedvac prior to proteomics analysis.

High spatial resolution IMS

Sample preparation—MALDI matrix was applied using a custom built sublimation apparatus which uses reduced pressure and heat for vapor deposition of the MALDI matrix on to the sample slide resulting in a uniform MALDI matrix coating over the tissue.⁵¹ α -Cyano-4-hydroxycinnamic acid (CHCA) was sublimed at 135°C and 50 mTorr for 30 minutes. Matrix coated sample slides were then rehydrated in a Petri dish containing 1 mL of 10% acetic acid for 3.5 min at 85°C .

MALDI TOF IMS—Imaging experiments were performed in positive ion mode using a Bruker Ultraflexxtreme mass spectrometer in linear geometry which is capable of routinely detecting sub-picomolar amounts of peptide standards. Spectra were collected in the range of m/z 1000-20,000. Raster steps were taken in 25 μ m stage increments and the laser spot size was also 25 μ m in diameter as measured on a thin matrix coating. The ion images with 10 μ m spatial resolution were acquired using a Bruker Rapiflex MALDI Tissue Typer with a 10 μ m lateral step in positive reflectron mode. Each imaging experiment was run as a sample set containing three mice per experimental group where image regions were randomly selected within the cortex of the kidney. FlexImaging was used for image visualization.

MALDI FTICR MS—Imaging experiments were performed in positive ion mode using a 15 T SolariX MALDI Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics, Billerica, MA). Spectra were collected in the range of m/z of 800-10,000. Image resolution was set at 60 μ m. FlexImaging and DataAnalysis were used for image visualization and data analysis.

After all imaging experiments, the MALDI matrix was removed from the slides by immersion in 70% ethanol followed by 95% ethanol for 30 s each. Kidney sections were

then stained with PAS and renal tissue structures were matched to IMS data via image overlay.

LC-MS/MS—Proteomics experiments of the *in vitro* cathepsin D digestion of mouse serum albumin were performed on a Dionex UltiMate 3000 coupled to a Q-Exactive orbitrap (Thermo Scientific) mass spectrometer. A 3 μ L aliquot of the cathepsin D digest was injected onto the column and eluted during a 90 minute gradient of acetonitrile with 0.1% formic acid (solvent A) and water with 0.1% formic acid (solvent B). The starting flow rate of 1.25 μ L/min at 2% B was held for one minute followed by a flow rate of 0.4 μ L/min at 2% B for one minute, increased to 40% B over 62 minutes, increased to 95% B over 9 minutes, held at 95% B for one minute, and finally, decreased to 2% B in 1 minute. The column was re-equilibrated at 2% B for 14 minutes. To identify albumin peptide fragments from *in vitro* experiments, MS data were acquired with a resolving power of $\sim 55,000$ at m/z 400 and an AGC target of $1E^6$ in positive ion mode. Spectra were obtained across the mass range of 375-1800 m/z in profile mode with DDA fragmentation of the top 20 ions and a dynamic exclusion time of 15 s.

Protein Identification in vivo

For identification using image-directed on-tissue MS/MS, serial sections were cut and mounted on the IMS target with the same orientation. One section was imaged to determine the localization of signals of interest. The imaged tissue was used as a guide for regions to sample on the serial section for MS/MS of the peaks of interest. Peaks were isolated and fragmented using LIFT mode on a Bruker Ultraflexxtreme. MS/MS spectra were searched using MASCOT.

Protein Identification in vitro

LC-MS/MS DDA data acquired on the Q-Exactive orbitrap was processed through Skyline (v 3.7.0.10940, MacCoss Lab, University of Washington). The analysis of peptide chromatography and spectral isotopic distributions are reported in Figures S4-S9. Raw fragmentation spectra was viewed in Qual Browser (Thermo Scientific) and manually annotated against *in silico* fragmentation from the Peptide Sequence Fragmentation Modelling tool in the Molecular Weight Calculator (Pacific Northwest National Laboratory, Richland, WA) (Fig. S10-11).

Relative abundance of protein signals

For the IMS imaging datasets, ImageJ software (National Institutes of Health, Bethesda, MD) was used to measure the relative abundance of the protein signals of interest between experimental groups. Monochromatic TIFF images were exported from FlexImaging to ImageJ. Signal intensity was measured as the mean intensity per imaged area.

Statistical analysis

Data were expressed as means SEM and statistical analysis was performed using Student's *t* test for unpaired samples or one-way ANOVA followed by post-hoc Student-Newman-Keuls

comparisons. For the IMS imaging datasets, the mean and standard error were calculated for each MS peak of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CKD	chronic kidney disease
DN	diabetic nephropathy
FTICR	Fourier transform ion cyclotron resonance
IMS	imaging mass spectrometry
MALDI	matrix assisted laser desorption/ionization
TOF	time-of-flight

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Figure 1.

Hyperglycemia and albuminuria in diabetic mice. (A) Blood glucose levels were determined in non-diabetic WT mice and *eNOS*^{-/-} *db/db* mice as described under Methods. (B) Urinary albumin-to-creatinine ratio (ACR) was determined in non-diabetic WT mice and in *eNOS*^{-/-} *db/db* mice as described under Methods. Each bar graph represents the mean $\pm$ SEM (n=8). * $P < 0.05$ vs. non-diabetic; ** $P < 0.05$ vs. 12 wks old diabetic.

Figure 2.

Identification of albumin fragments detected in diabetic mouse kidneys. (A) MALDI FTICR spectrum from DN kidney. Albumin fragments marked by *. (B) MALDI TOF MS/MS spectrum of *m/z* 2791 measured directly from tissue surface identifying N-terminal sequence of albumin.

Figure 3.

IMS and relative abundance of albumin fragments. Imaging performed with MALDI TOF instrument at 25- μ m spatial resolution. (A-D) Albumin fragments had specific and unique localization patterns. (E-G) The box plots show the relative peptide abundance in non-diabetic control mice and diabetic *eNOS^{-/-} db/db* mice at 12 and 22 weeks of age. The relative abundance was determined by the average intensity per image area, $n=3$ mice per group. All IMS images were randomly selected within the cortex of the kidney.

Figure 4.

Examples of albumin fragment localization in diabetic renal tubules. PAS staining (right) and IMS (left) were performed on the same section; immunostaining and IMS were performed on serial sections. The resulting immunofluorescence and IMS images were co-registered. (A) Albumin fragments are not detected in glomeruli (marked by white-dashed circles). (B) Albumin fragments are localized to renal tubules. An example IMS ion image of the albumin peptide 25-48 acquired at 10 µm spatial resolution with corresponding H&E stain. After the tissue was imaged, the matrix was removed with a series of ethanol washes

and the tissue was H&E stained. A zoomed in region is displayed in the right panel for clarity. (C) Albumin fragments (Left) co-localize with Lotus tetragonolobus lectin (LTL), a proximal tubule marker (Right). (D) Dolichos biflorus agglutinin (DBA), a distal tubule and collecting duct marker (Right) co-localizes with albumin fragments (Left). Scale bars for panels A, C and D = 200 μm . Scale bars for panel B= 50 μm (top panel) and 20 μm (bottom panel).

Figure 5.

N-terminal albumin fragment also found in urine of diabetic mice. The major N-terminal fragment detected in DN kidneys was also detected in the urine of diabetic mice (middle and bottom panels) but not in non-diabetic controls (top panel).

Figure 6.

Increase in cathepsin D expression in diabetic mouse kidneys. Renal sections from non-diabetic control (A) and *eNOS*^{-/-} *db/db* mice at 22 wks of age (B) were stained using CatD antibody as described under Methods. (C) Cathepsin D positive staining area determined as a mean±SEM from 25 random areas in 6-8 mice per group. *p<0.05. Negative control staining shown for *eNOS*^{-/-} *db/db* kidney (D) was performed by omitting the primary antibody. Scale bar = 100 μm.

Figure 7.

Cathepsin D and major albumin fragment present in the same tubular region of the kidney. (A) Localization of major albumin fragment m/z 2039 within the area of CatD staining. (B) PAS-stained renal tissue is shown for spatial reference. (C) IHC staining for CatD in renal tissue of DN mouse. The IMS image and the PAS-stained image originate from the same tissue section while the IHC image is from a serial section. Scale bar = 100 μ m.

Table 1

Endogenous albumin fragments detected in diabetic mouse kidneys. Monoisotopic mass measured using MALDI FTICR MS. Sequence information was determined by accurate mass measurements and MS/MS experiments except for those peptide sequences shown in italics which were identified based on accurate mass alone. All measured masses were <1 ppm from the theoretical peptide mass.

Measured monoisotopic mass (<i>m/z</i>)	Albumin peptide sequence	Position within albumin primary structure
2409.169	<i>(R)EAHKSEIAHRYNDLGEQHF(K)</i>	25–44
2466.189	<i>(R)EAHKSEIAHRYNDLGEQHF(K) G(L)</i>	25–45
2791.427	(R)EAHKSEIAHRYNDLGEQHF(K)GLV(L)	25–48
2662.384	(E)AHKSEIAHRYNDLGEQHF(K)GLV(L)	26–48
1585.738	<i>(F)LYEYSRRHPDYS(V)</i>	355–366
1107.532	(L)YEYSRRHPDYS(V)	356–363
1472.656	(L)YEYSRRHPDYS(V)	356–366
1222.560	<i>(Y)EYSRRHPDYS(S)</i>	357–365
1093.517	<i>(E)YSRRHPDYS(S)</i>	358–365
1180.550	<i>(E)YSRRHPDYS(V)</i>	358–366
1017.485	(Y)SRRHPDYS(V)	359–366
2299.247	<i>(E)LVKHKPKATAEQLKTVMDDF(A)</i>	556–575
1236.706	(L)VKHKPKATAEQL(K)	557–567
1349.790	(L)VKHKPKATAEQL(K)	557–568
1477.885	(L)VKHKPKATAEQL(K)	557–569
1578.932	(L)VKHKPKATAEQL(K)	557–570
2039.096	(L)VKHKPKATAEQLKTVMDDF(F)	557–574
2186.163	<i>(L)VKHKPKATAEQLKTVMDDF(A)</i>	557–575

Table 2

A series of albumin peptides detected in both *in vitro* and *in vivo* experiments. *In vitro* peptides were products of a cathepsin D digestion of mouse serum albumin protein measured by LC-MS/MS. The measured *in vitro* monoisotopic mass from a Q-Exactive orbitrap MS along with respective charge states and mass accuracy (ppm) are reported where mass accuracies are <3.2 ppm from the theoretical peptide mass. Additional information such as isotopic distribution comparisons, retention time alignment and example fragmentation spectra from the *in vitro* peptides is reported in Figures S4-S11 of the supplemental materials. *In vivo* peptides were detected in diabetic mouse kidneys sections by MALDI imaging mass spectrometry. Measured *in vivo* monoisotopic masses from a MALDI FTICR MS were reported with mass accuracies of <1 ppm. Example *in vivo* spectrum and subsequent fragmentation are shown in Figure 2.

Albumin peptide sequence	Measured monoisotopic mass (m/z) <i>in vitro</i>	Mass accuracy (ppm)	Measured monoisotopic mass (m/z) <i>in vivo</i>	Mass accuracy (ppm)
(L)YEYSRRHPDYS(V)	736.834	-3.16	[M+H] ⁺	-0.46
(F)LYEYSRRHPDYS(V)	793.376	-2.77	[M+H] ⁺	0.87
(L)VKHKPKATAEQLKTVMDDF(A)	729.395	-1.99	[M+H] ⁺	0.37
(E)LVKHKPKATAEQLKTVMDDF(A)	1150.131	-2.48	[M+H] ⁺	0.38
(R)EAHKSEIAHRYNDLGEQHFKG(L)	1233.603	-3.03	[M+H] ⁺	0.77
(R)EAHKSEIAHRYNDLGEQHFKGLVL(I)	1396.222	-2.84	[M+H] ⁺	0.16