Supporting Information

for

Correlation between Glyoxal-Induced DNA Cross-Links and Hemoglobin Modifications in Human Blood Measured by Mass Spectrometry

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Table legend

Table S1. Characteristics of the study participants

Table S2. Multiple regression analysis of the extent of modification in the study subjects for variables of HbA1c, age, cigarettes smoked per day (cig/day), and BMI.

Figure legend

Figure S1. nanoLC-NSI/MS/MS chromatograms of SRM transitions of the modified peptides in blank injection.

Figure S2. Dose-dependent formation of the glyoxal-modified peptides in human hemoglobin (Sigma Chemical Co.) incubated with glyoxal at 37 ℃ for 48 h.

Figure S3. Dose-dependent formation of (A) TNV^{11}KAAWGK (α-chain) and (B) SAVTALWG^{17}KVNVDEVGGEALGR (β-chain) in the incubation mixture of the parent peptide with glyoxal at 37 ℃ for 1 h.

Figure S4. nanoLC-NSI/MS/MS chromatograms of (A) TNV^{11}KAAWGK (α-chain) and (B) SAVTALWG^{17}KVNVDEVGGEALGR (β-chain) in the incubation mixture of the parent peptide with glyoxal (0.1 μM) at 37 ℃ for 1 h. The extracted mass spectrum of the respective modified peptide is shown below.

Table S1. Characteristics of the study participants

	DM patients	Normal controls
	mean ± SD (range)	
age (years)	57.2 ± 12.7 (39 – 77)	31.0 ± 12.9 (22 – 60)
Gender		
Male	15	14
Female	9	5
BMI (kg/m^2)	27.3 ± 4.2 (18.6 – 34.6)	24.5 ± 2.9 (18.7 – 29.3)
HbA1c	9.5% ± 2.1% (7.0% – 15.1%)	5.1% ± 0.2% (5.0% – 5.6%)
smoking status		
cigarettes/day	40 ± 30 (n=6)	25 ± 1 (n=5)

Table S2. Multiple regression analysis of the extent of modification in the study subjects for variables of HbA1c, age, cigarettes smoked per day (cig/day), and BMI.

Multiple Regression Analysis (n = 43)	HbA1c[b]	age	cig/day
	p value		
$[\alpha\text{-}^{11}K^{gx}]$ = 1.573E-05 + 3.246E-06*[HbA1c] – 2.397E-07*[BMI] – 3.836E-08*[age] + 3.242E-07*[cig/day]	0.0090		
$[\alpha\text{-}^{92}R^{gx}]$ = – 3.981E-06 + 3.585E-07*[HbA1c] + 1.232E-07*[BMI] + 3.717E-08*[age] + 4.423E-08*[cig/day]	0.0219		0.0372
$[\beta\text{-}^{17}K^{gx}]$ = 8.866E-06 – 2.213E-07*[HbA1c] – 2.411E-07*[BMI] + 4.419E-07*[age] – 9.082E-10*[cig/day]		0.0004	

*Only the statistically significant results are listed.

Figure S1.

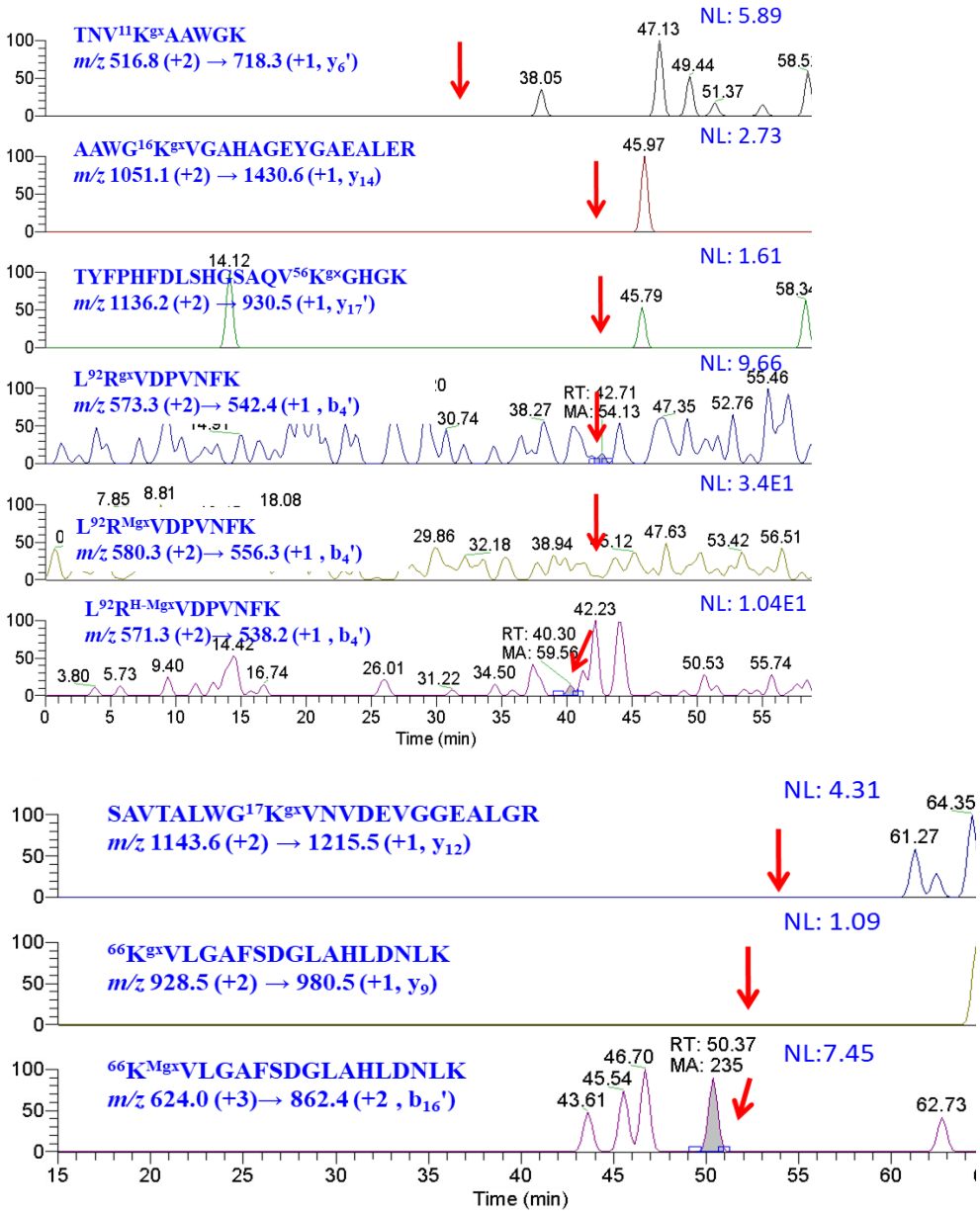

TNV[11]K[gx]AAWGK
m/z 516.8 (+2) → 718.3 (+1, y6')
NL: 5.89
AAWG[16]K[gx]VGAHAGEYGAEALER
m/z 1051.1 (+2) → 1430.6 (+1, y14)
NL: 2.73
TYFPHFDLSHGSAQV[56]K[gx]GHGK
m/z 1136.2 (+2) → 930.5 (+1, y17')
NL: 1.61
L[92]R[gx]VDPVNFK
m/z 573.3 (+2)→ 542.4 (+1 , b4')
RT: 42.71
MA: 54.13
NL: 9.66
L[92]R[Mgx]VDPVNFK
m/z 580.3 (+2)→ 556.3 (+1 , b4')
NL: 3.4E1
L[92]R[H-Mgx]VDPVNFK
m/z 571.3 (+2)→ 538.2 (+1 , b4')
RT: 40.30
MA: 59.56
NL: 1.04E1
Time (min)
SAVTALWG[17]K[gx]VNVDEVGGEALGR
m/z 1143.6 (+2) → 1215.5 (+1, y12)
NL: 4.31
[66]K[gx]VLGAFSDGLAHLDNLK
m/z 928.5 (+2) → 980.5 (+1, y9)
NL: 1.09
[66]K[Mgx]VLGAFSDGLAHLDNLK
m/z 624.0 (+3)→ 862.4 (+2 , b16')
RT: 50.37
MA: 235
NL:7.45
Time (min)

Figure S2.

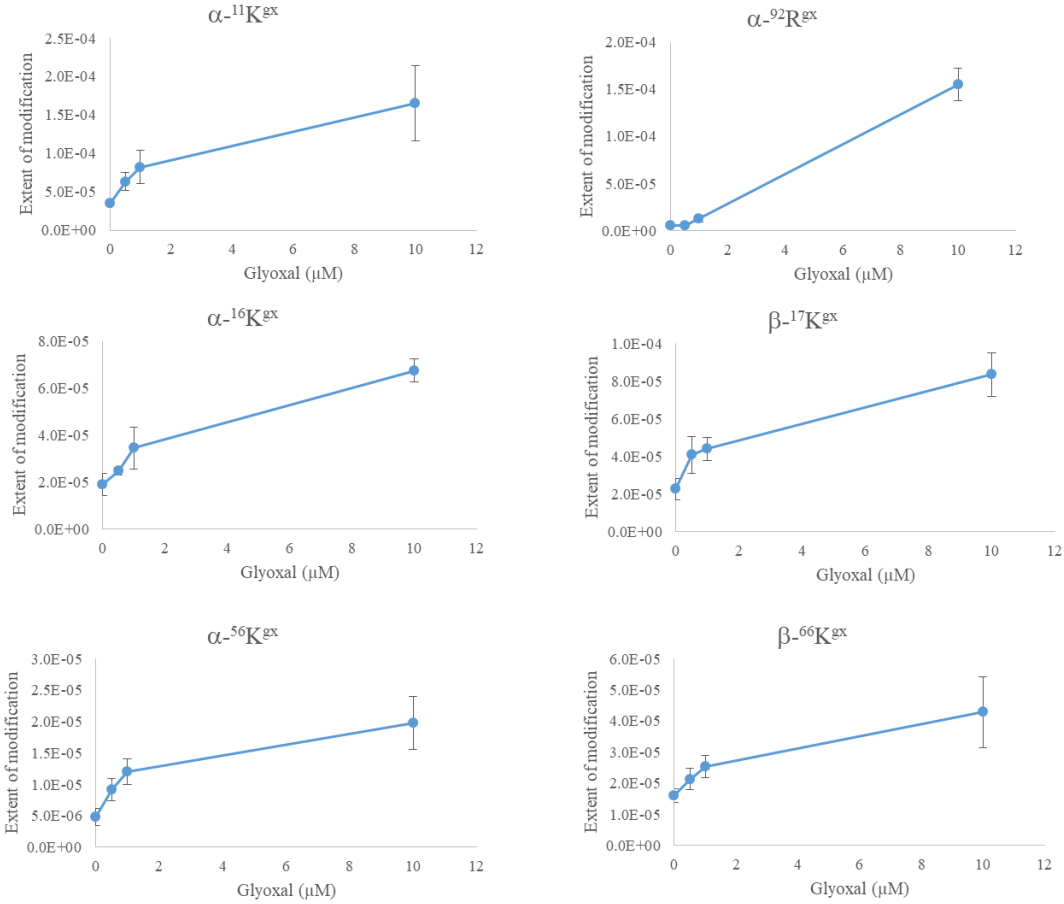

α-11Kgx
Extent of modification
2.5E-04
2.0E-04
1.5E-04
1.0E-04
5.0E-05
0.0E+00
0 2 4 6 8 10 12
Glyoxal (μM)
α-92Rgx
Extent of modification
2.0E-04
1.5E-04
1.0E-04
5.0E-05
0.0E+00
0 2 4 6 8 10 12
Glyoxal (μM)
α-16Kgx
Extent of modification
8.0E-05
6.0E-05
4.0E-05
2.0E-05
0.0E+00
0 2 4 6 8 10 12
Glyoxal (μM)
β-17Kgx
Extent of modification
1.0E-04
8.0E-05
6.0E-05
4.0E-05
2.0E-05
0.0E+00
0 2 4 6 8 10 12
Glyoxal (μM)
α-56Kgx
Extent of modification
3.0E-05
2.5E-05
2.0E-05
1.5E-05
1.0E-05
5.0E-06
0.0E+00
0 2 4 6 8 10 12
Glyoxal (μM)
β-66Kgx
Extent of modification
6.0E-05
5.0E-05
4.0E-05
3.0E-05
2.0E-05
1.0E-05
0.0E+00
0 2 4 6 8 10 12
Glyoxal (μM)

Figure S3.

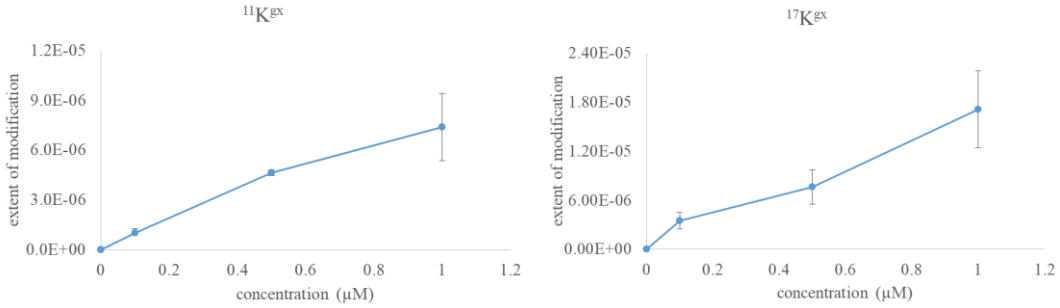
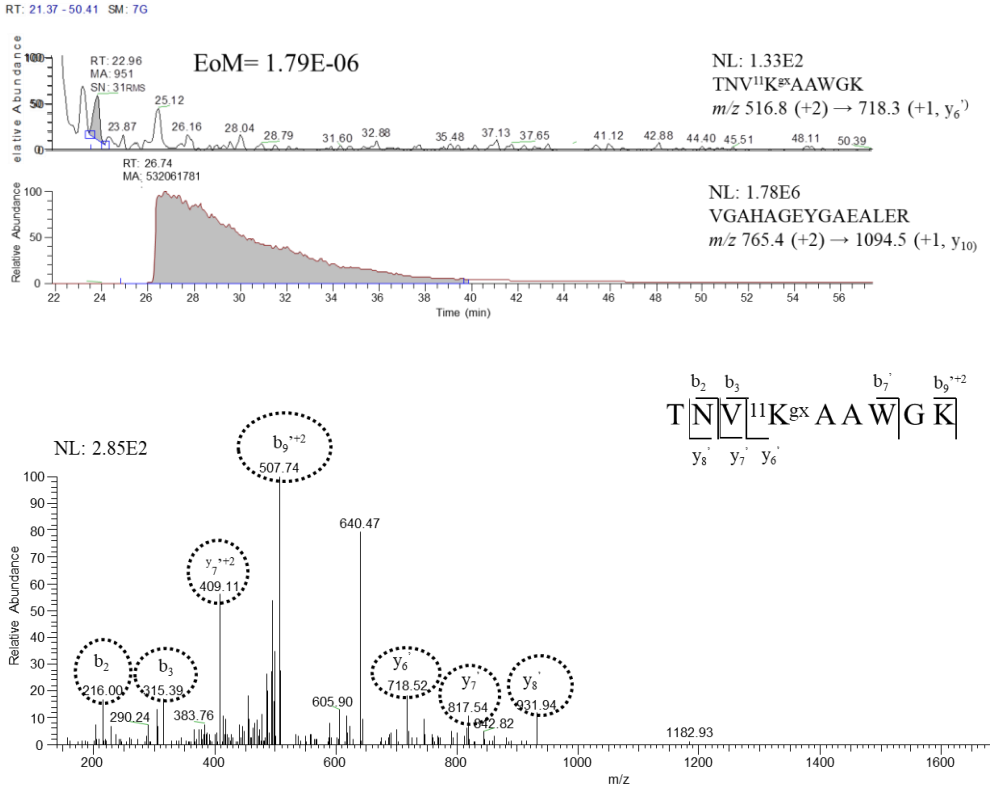
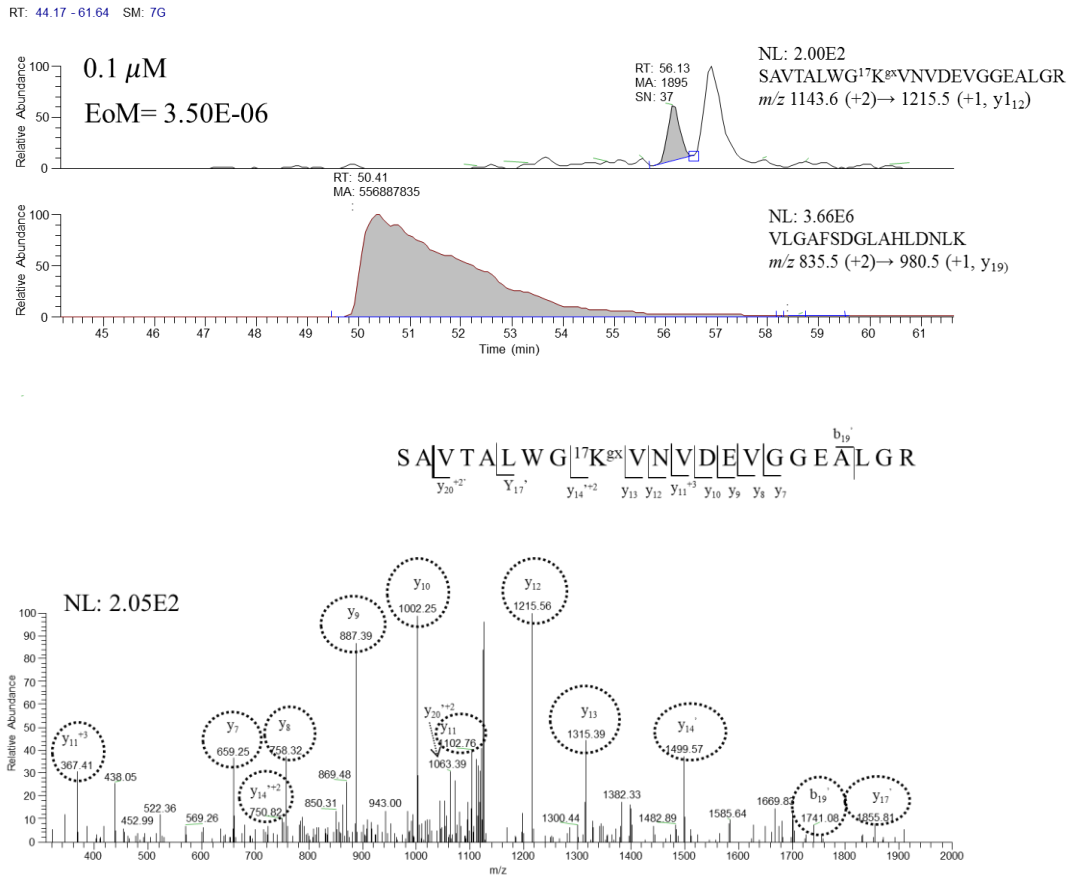

$^{11}K^{ox}$
1.2E-05
9.0E-06
6.0E-06
3.0E-06
0.0E+00
extent of modification
0
0.2
0.4
0.6
0.8
1
1.2
concentration (μM)
$^{17}K^{ox}$
2.40E-05
1.80E-05
1.20E-05
6.00E-06
0.00E+00
extent of modification
0
0.2
0.4
0.6
0.8
1
1.2
concentration (μM)

Figure S4.

(A)


RT: 21.37 - 50.41 SM: 7G
RT: 22.96
MA: 951
SN: 31RMS
EoM= 1.79E-06
NL: 1.33E2
TNV[11]K[gx]AAWGK
m/z 516.8 (+2) → 718.3 (+1, y6')
RT: 26.74
MA: 532061781
NL: 1.78E6
VGAHAGEYGAEALER
m/z 765.4 (+2) → 1094.5 (+1, y10)
Time (min)
T N V [11]K[gx] A A W G K
NL: 2.85E2
b9'+2 507.74
640.47
y7'+2 409.11
b2 216.00
b3 315.39
y6' 718.52
y7' 817.54
y8' 931.94
605.90
290.24
383.76
1182.93
m/z

(B)


RT: 44.17 - 61.64 SM: 7G
0.1 μM
EoM= 3.50E-06
RT: 56.13
MA: 1895
SN: 37
NL: 2.00E2
SAVTALWG[17]K[gx]VNVDEVGGEALGR
m/z 1143.6 (+2)→ 1215.5 (+1, y112)
RT: 50.41
MA: 556887835
NL: 3.66E6
VLGAFSDGLAHLDNLK
m/z 835.5 (+2)→ 980.5 (+1, y19)
Time (min)
S A V T A L W G [17]K[gx] V N V D E V G G E A L G R
NL: 2.05E2
y10 1002.25
y12 1215.56
y9 887.39
y13 1315.39
y14' 1499.57
y7 659.25
y8 758.32
y11+3 367.41
438.05
y14'+2 750.82
869.48
850.31
943.00
1063.39
1382.33
1300.44
1482.89
1585.64
1669.82
b19' 1741.08
y17' 1855.81
m/z