Arginine Modifications by Methylglyoxal: Discovery in a Recombinant Monoclonal Antibody and Contribution to Acidic Species

Chris Chumsae [1,□,*], Kathreen Gifford [1], Wei Lian [2], Hongcheng Liu [1], Czeslaw H. Radziejewski[1] and Zhaohui Sunny Zhou [3]*

1, Protein Analytics, Process Sciences Department, 2, Cell Culture, Manufacturing Sciences Department, AbbVie Bioresearch Center, Worcester, Massachusetts 01605, USA

3, Barnett Institute of Chemical and Biological Analysis, Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115, USA;

SUPPORTING INFORMATION

Table of Contents

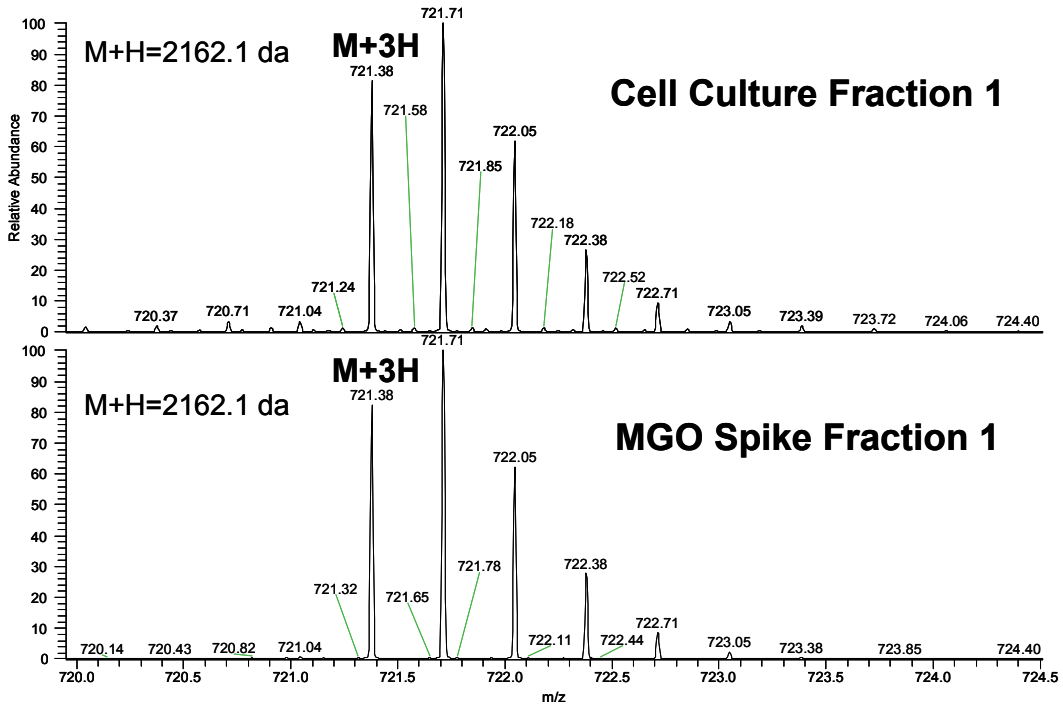


Figure S-1.: Comparison of peptide MS data between acidic peaks from cell culture and acidic fractions from methylglyoxal incubation. The data shows the corresponding mass spectra of a 3+ ion representing a mis-cleaved peptide with a mass increase of 54 daltons.

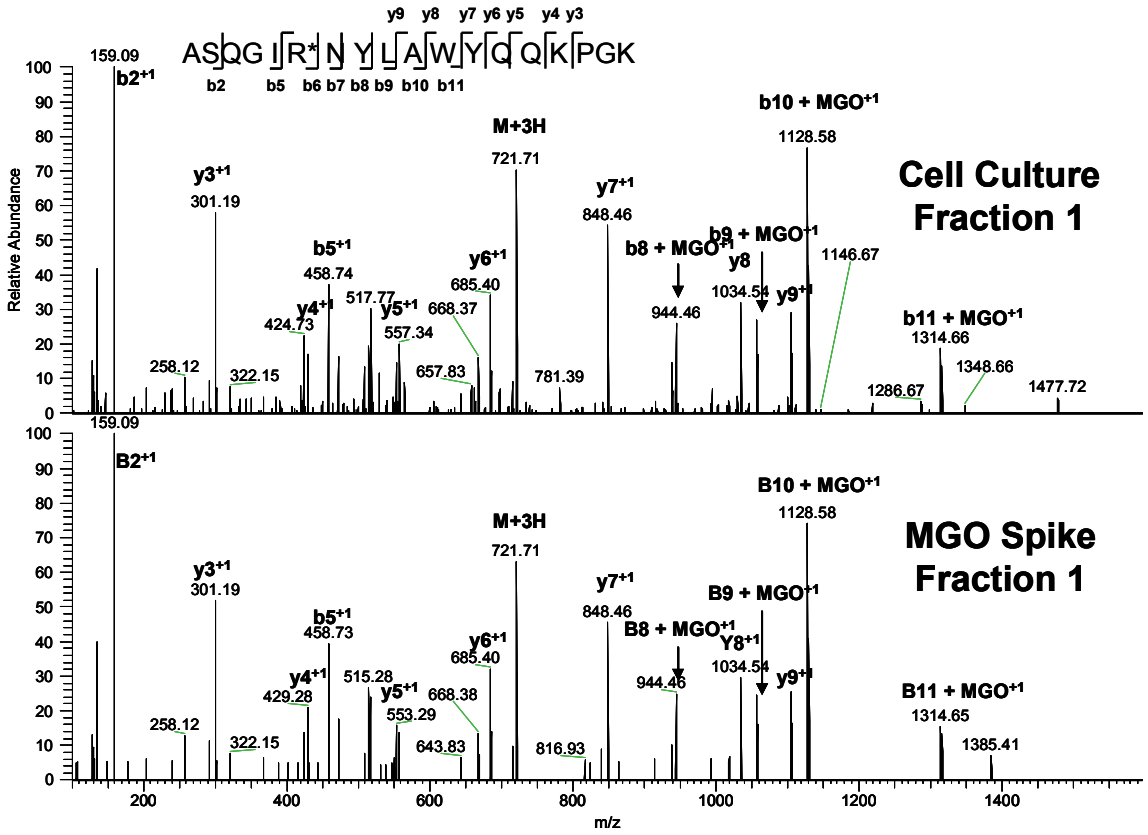


Figure S-2.: Comparison of peptide MS/MS data of the 3+ parent ion between acidic peaks from cell culture and acidic peaks from methylglyoxal incubation. The figure shows the MS/MS spectra of the two peptides from the Cell Culture fraction and from the MGO spike fractions, respectively and confirms that the observed mass shift of +54 Da resides on the mis-cleaved arginine residue.

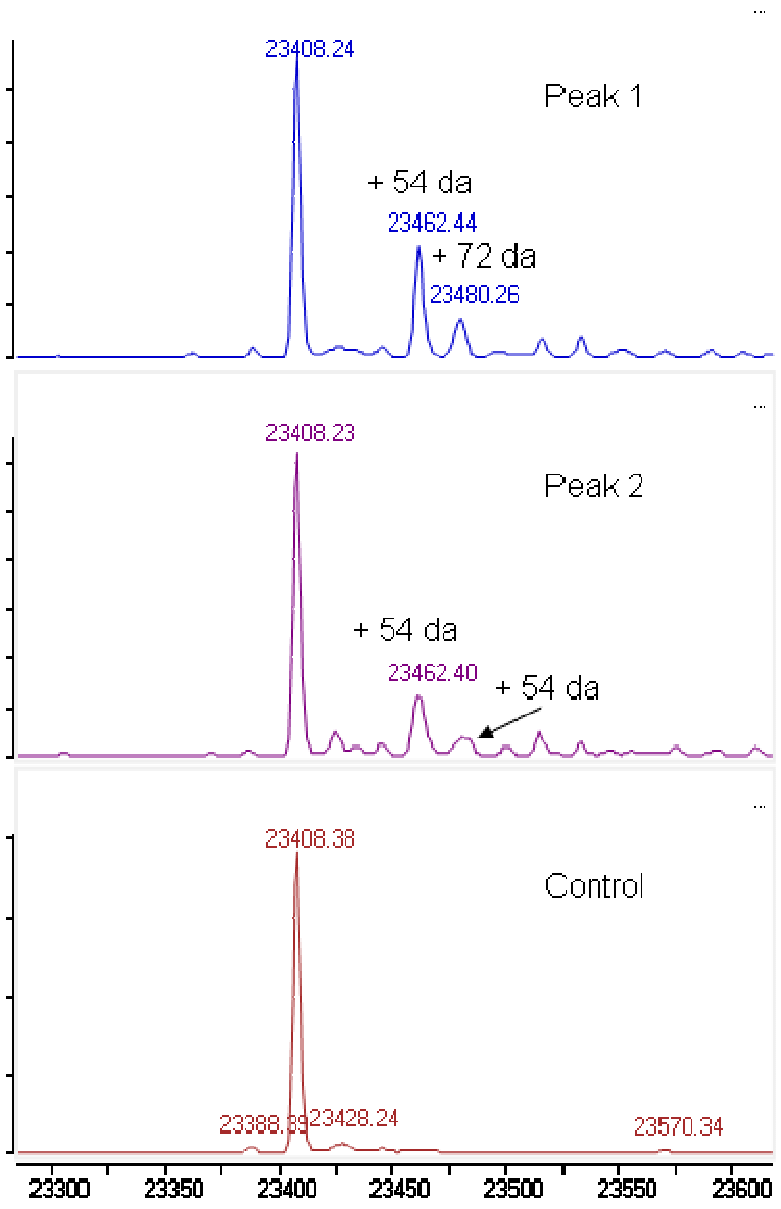


Figure S-3.: Reduced analysis of light chain from peaks 1 and 2 formed during cell culture. In all three panes, the observed mass of 23408.38 da (Control) can be seen which is in good agreement with the theoretical mass of 23408.13 da. In addition to the light chain mass, additional peaks can be seen in the spectra of fraction 1 and fraction 2 which correspond to mass increases of 54 and 72 da greater than the expected mass.

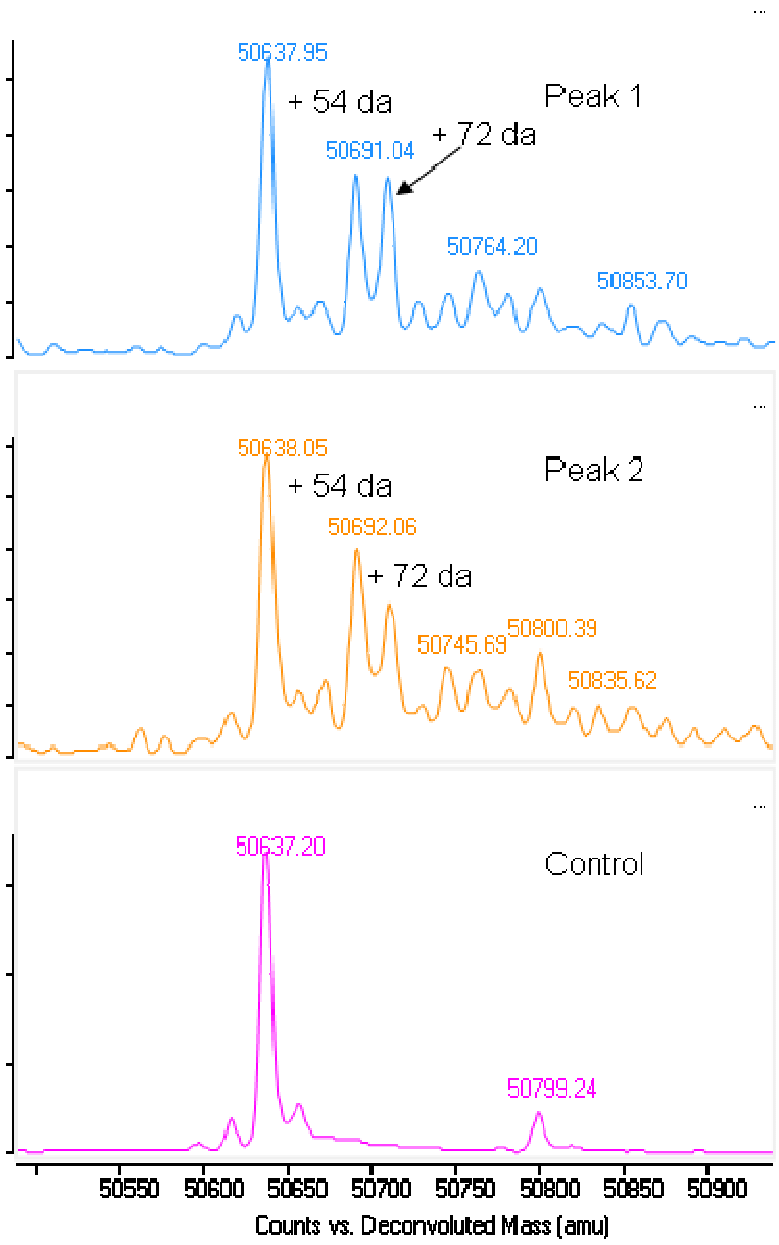


Figure S-4.: Reduced analysis of heavy chain from peaks 1 and 2 formed during cell culture. The heavy chain mass of 50637.20 (Control) can be seen in all three panes of the figure which is in good agreement to the theoretical mass of 50636.78 da (0 gal, 0 Lysine heavy chain). In addition to the heavy chain mass, other peaks are observed in the spectra which correspond to mass increases of +54 da and +72 da over the heavy chain

mass. Multiple lower intensity peaks of greater mass are likely the same MGO modification which has occurred at multiple sites.

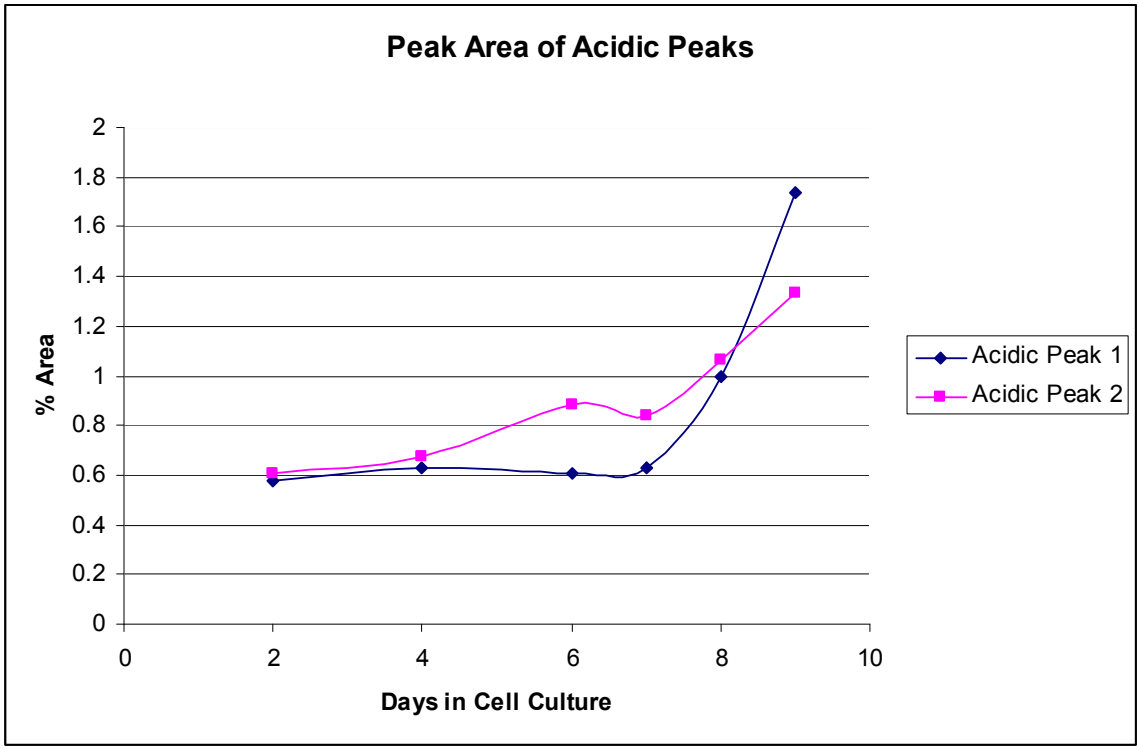


Figure S-5.: The figure shows the relative integrated area of the two acidic peaks analyzed by weak cation exchange chromatography over the duration of the cell culture. The peaks begin to increase at later days in the harvest. Peak 2 increases at day 6 where as peak 1 increases at day 8 of the cell culture. The increase in peak area is presumably due to the antibody becoming modified by methylglyoxal.

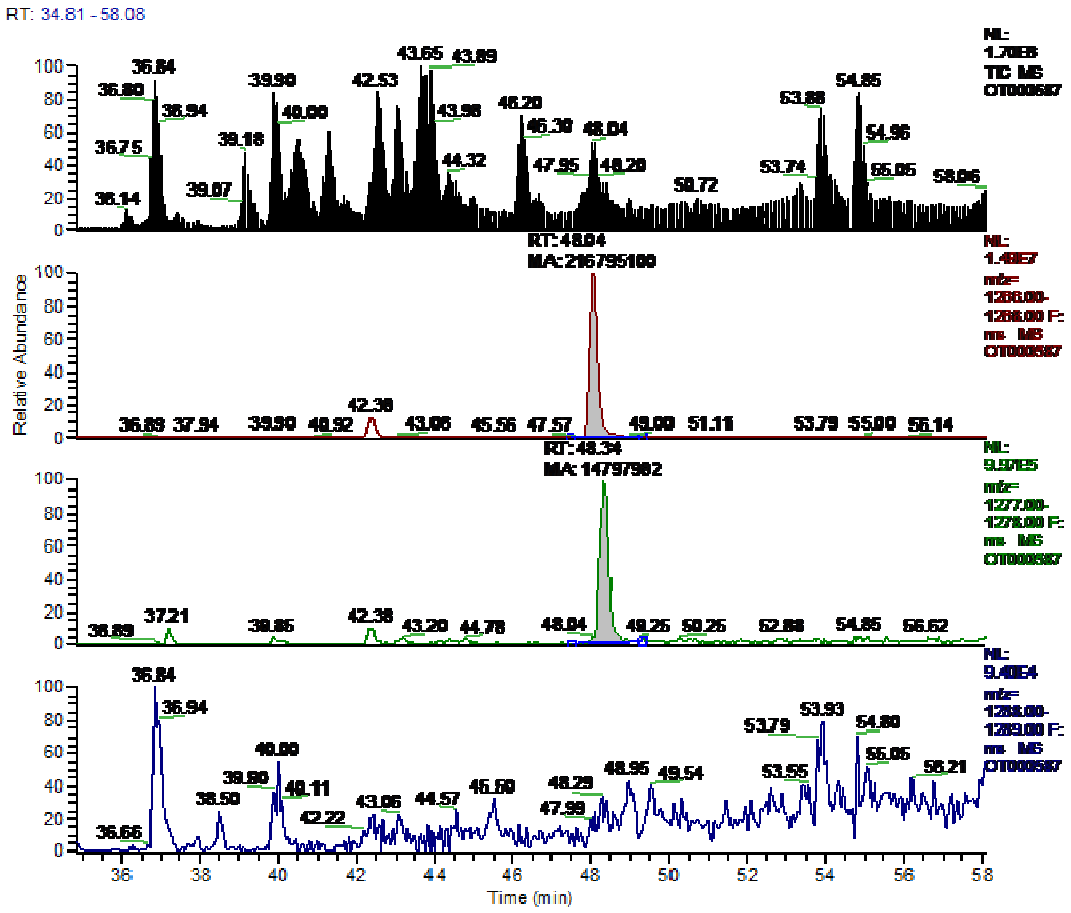


Figure S-6 : The figure shows the total ion current (top), the native peptide (second pane), the peptide modified by one MGO (+54 da) and lastly, a trace showing no evidence of the second arginine within this peptide being modified. The site was identified by previous tryptic digest and the degree of modification was determined by the percent of the respective peaks in the XIC's of the native and MGO modified peptides

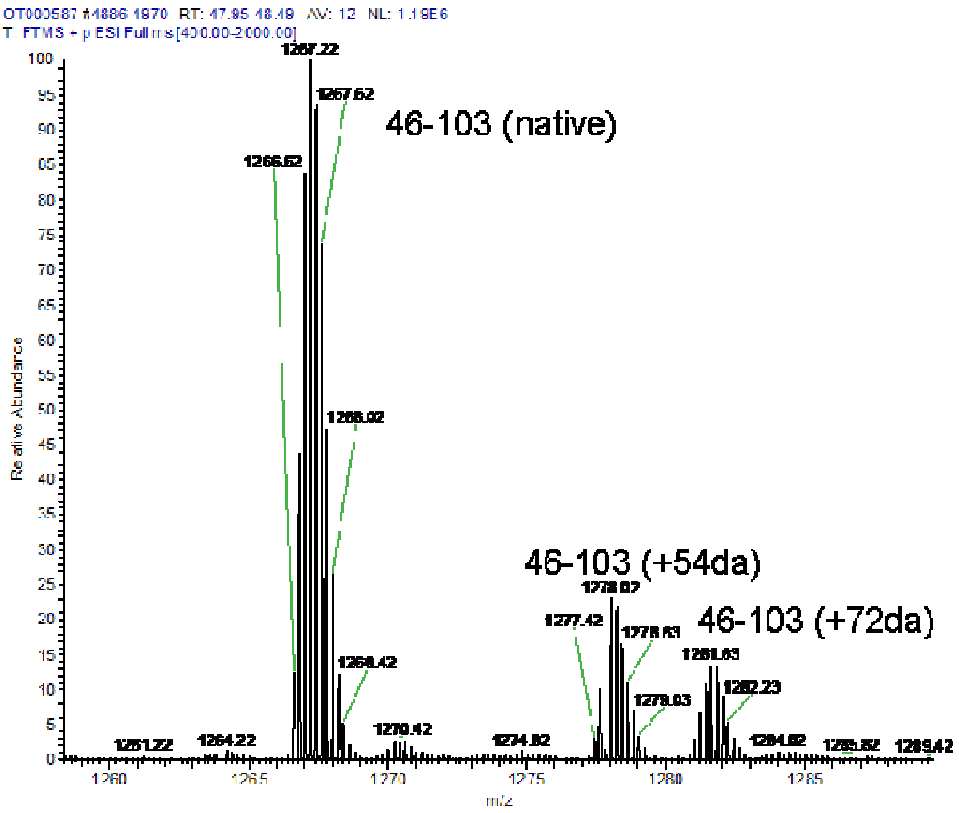


Figure S-7.: The figure displays a mass spectrum of a heavy chain Lys-C peptide showing the isotopic distributions of the +5 charge state (Arg93 internalized). In this manner, the native and the two MGO products of a single modified arginine can be seen within the same spectrum.

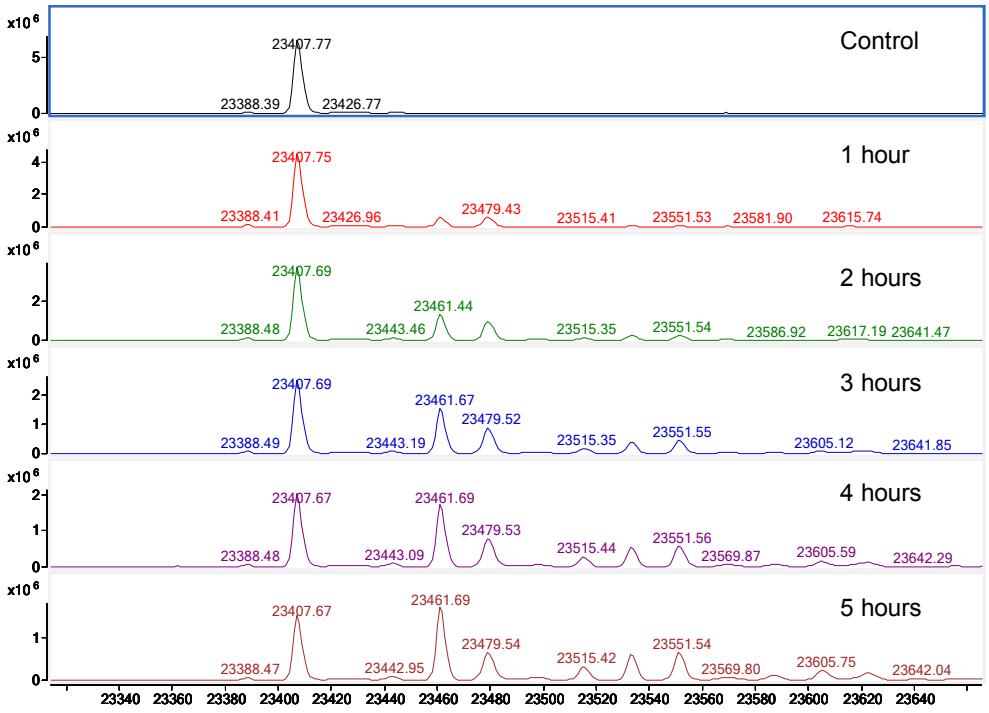


Figure S-8.: Mass spectra of the light chain for a pure 0 Lys are shown over the five hour period. The additional peaks of +54da and +72da have formed and have greatly increased the observed mass heterogeneity. The formation of the additional peaks is in agreement with the observed peaks from the acidic fractions isolated from cell culture. The control was incubated for the entire five hour period but without the addition of methylglyoxal and subsequently showed no added complexity or formation of additional peaks.

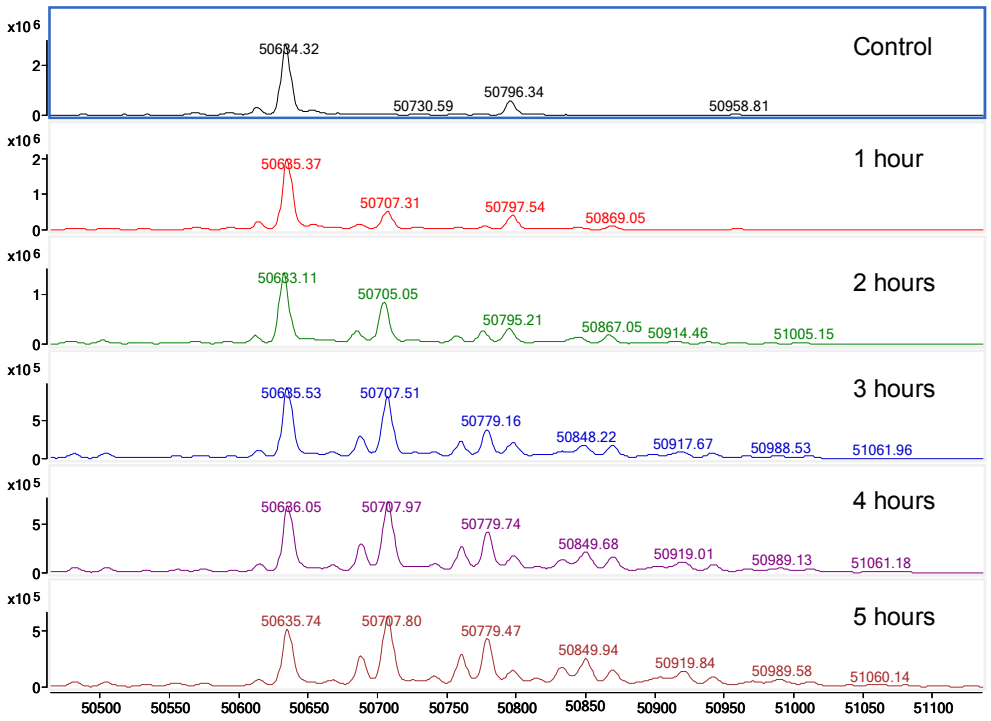


Figure S-9.: Mass spectra of the heavy chain for a pure 0 Lys are shown over the five hour period. The additional peaks of +54da and +72da have formed and have greatly increased the observed mass heterogeneity.

Table S-1.: Results of manual search of methylglyoxal modified peptides found in the recombinant antibody

MGO Modified Tryptic Peptides (mis-cleavages)

ASQGIR*NYLAWYQQKPGK
YNR*APYTFGQGTK
R*TVAAPSVFIFPPSDEQLK
EVQLVESGGGLVQPGR*SLR
DTLMISR*TPEVTCVVVDVSHEDPEVK
EPQVYTLPPSR*DELTK
SR*WQQGNVFSCSVMHEALHNHYTQK

Table S-2.: Results of Sequest algorithm search against the theoretical sequence

Sequence	Activation Type	Modifications	Charge	m/z [Da]	MH+ [Da]	RT [min]	MS Order
EPQVYTLPPSrDELTK	HCD	R11(MGO (R) 72)	2	972.9988	1944.99	27.71	MS2
EPQVYTLPPSrDELTK	CID	R11(MGO (R) 72)	3	649.0014	1944.99	27.72	MS2
EPQVYTLPPSrDELTK	CID	R11(MGO)	3	642.9988	1926.982	27.81	MS2
EPQVYTLPPSrDELTK	HCD	R11(MGO)	3	642.9988	1926.982	27.82	MS2
EPQVYTLPPSrDELTK	CID	R11(MGO)	2	963.9942	1926.981	27.88	MS2
EPQVYTLPPSrDELTK	HCD	R11(MGO)	2	963.9942	1926.981	27.89	MS2
EVQLVESGGGLVQPGrSLR	CID	R16(MGO (R) 72)	2	1027.055	2053.103	32	MS2
EVQLVESGGGLVQPGrSLR	HCD	R16(MGO (R) 72)	2	1027.055	2053.103	32.01	MS2
EVQLVESGGGLVQPGrSLR	CID	R16(MGO)	3	679.0353	2035.091	32.11	MS2
EVQLVESGGGLVQPGrSLR	CID	R16(MGO)	2	1018.05	2035.092	32.13	MS2
EVQLVESGGGLVQPGrSLR	HCD	R16(MGO)	2	1018.05	2035.092	32.15	MS2
YNrAPYTFGQGTK	CID	R3(MGO (R) 72)	2	787.8835	1574.76	17.61	MS2
YNrAPYTFGQGTK	HCD	R3(MGO (R) 72)	2	787.8835	1574.76	17.62	MS2
YNrAPYTFGQGTK	CID	R3(MGO (R) 72)	3	525.5911	1574.759	17.63	MS2
YNrAPYTFGQGTK	HCD	R3(MGO (R) 72)	3	525.5911	1574.759	17.64	MS2
YNrAPYTFGQGTKVEIK	CID	R3(MGO (R) 72)	2	1022.461	2043.916	46.16	MS2
SLrLScAASGFTFDDYAMHWVR	CID	R3(MGO (R) 72), C6(Carboxymethyl)	3	888.4062	2663.204	49.36	MS2
SLrLScAASGFTFDDYAMHWVR	HCD	R3(MGO (R) 72), C6(Carboxymethyl)	3	888.4062	2663.204	49.38	MS2
YNrAPYTFGQGTK	CID	R3(MGO)	2	778.8782	1556.749	17.49	MS2
YNrAPYTFGQGTK	HCD	R3(MGO)	2	778.8782	1556.749	17.5	MS2
YNrAPYTFGQGTK	CID	R3(MGO)	3	519.5878	1556.749	17.56	MS2
YNrAPYTFGQGTK	HCD	R3(MGO)	3	519.5878	1556.749	17.57	MS2
ASQGIrNYLAWYQQKPGK	CID	R6(MGO (R) 72)	3	727.3791	2180.123	32.15	MS2
ASQGIrNYLAWYQQKPGK	HCD	R6(MGO (R) 72)	3	727.3791	2180.123	32.16	MS2
ASQGIrNYLAWYQQKPGK	CID	R6(MGO (R) 72)	2	1090.566	2180.125	32.2	MS2

ASQGIrNYLAWYQQKPGK	HCD	R6(MGO (R) 72)	2	1090.566	2180.125	32.21	MS2
ASQGIrNYLAWYQQKPGK	CID	R6(MGO)	3	721.3756	2162.112	31.52	MS2
ASQGIrNYLAWYQQKPGK	HCD	R6(MGO)	3	721.3756	2162.112	31.53	MS2
ASQGIrNYLAWYQQKPGK	CID	R6(MGO)	2	1081.561	2162.115	31.55	MS2
ASQGIrNYLAWYQQKPGK	HCD	R6(MGO)	2	1081.561	2162.115	31.56	MS2
DTLMISrTPEVTcVVVDVSHEDPE VK	CID	R7(MGO (R) 72), C13(Carboxymethyl)	3	1010.155	3028.451	44.42	MS2
DTLMISrTPEVTcVVVDVSHEDPE VK	HCD	R7(MGO (R) 72), C13(Carboxymethyl)	3	1010.155	3028.451	44.43	MS2
DTLMISrTPEVTcVVVDVSHEDPE VK	CID	R7(MGO), C13(Carboxymethyl)	3	1004.152	3010.442	44.14	MS2
DTLMISrTPEVTcVVVDVSHEDPE VK	HCD	R7(MGO), C13(Carboxymethyl)	3	1004.152	3010.442	44.15	MS2

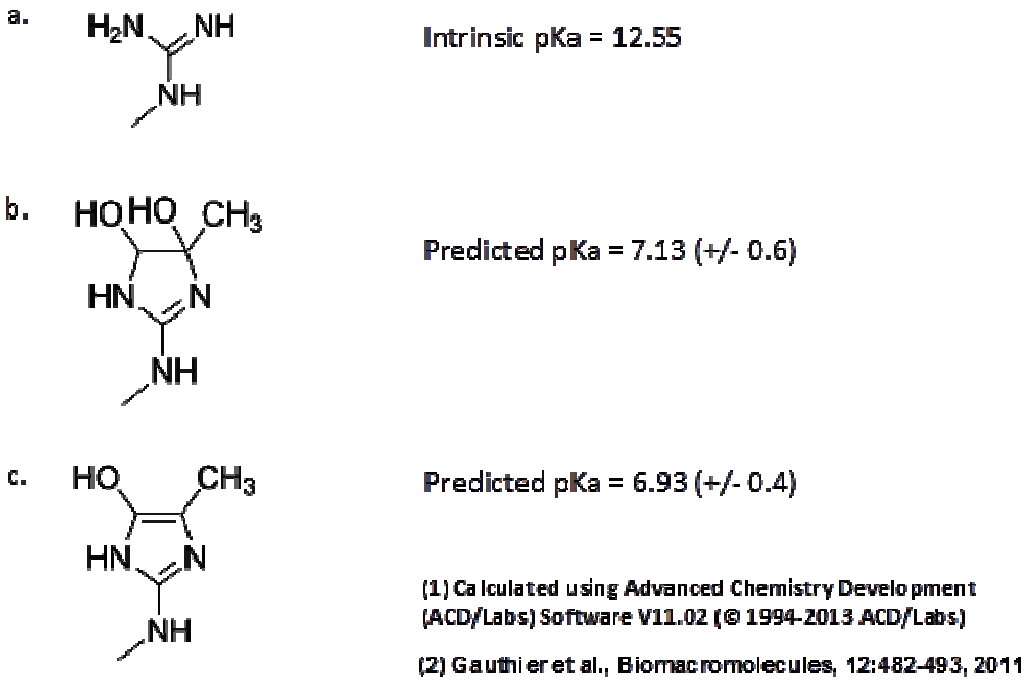


Figure S-10.: Calculated pKa of the core group of arginine and the two products of arginine modification by methylglyoxal. The pKa of the guanidinium core group is significantly depressed to 7.13 and 6.93 for the dihydroxyimidazolidine and hydroimidazolone core groups, respectively

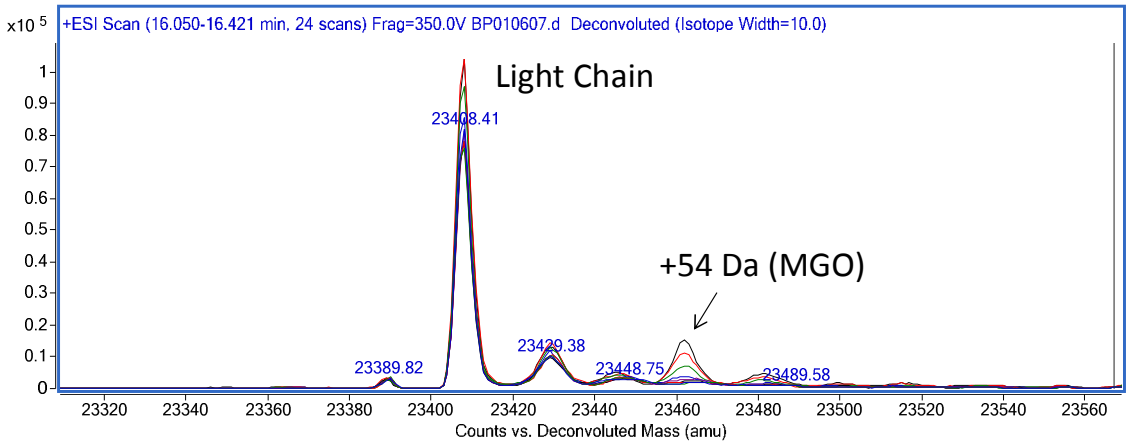
Table S-3: Raw data values of Lys-C peptide peak intensity from Cell Culture WCX fractions 1 and 2

	Fraction 1 Peak Intensities			**Fraction 2 Peak Intensities**		
Site	Unmod.	MGO(+54)	MGO(+72)	Unmod.	MGO(+54)	MGO(+72)
LC30	273429695	138486086	27697217	398154884	86277387.5	19843799
LC93	637430527	54150819	10830164	517745203	52710066.9	12123315
LC108	567693294	2761976.3	552395.27	293643660	980063.272	225414.55
HC16	517056804	73797731	14759546	672482015	90574849.9	20832215
HC259	478982501	47025077	9405015.5	614438486	64274080	14783038
HC359	321068113	41433990	8286798.1	451460954	53981224.7	12415682
HC420	359124190	14302232	2860446.4	416919964	15076683	3467637.1

Table S-4: Relative Intensity of Lys-C peptides from Cell Culture WCX fractions 1 and 2

	Fraction 1 Peak Intensities			**Fraction 2 Peak Intensities**		
Site	Unmod.	MGO (+54)	MGO (+54)	Unmod.	MGO (+72)	MGO (+72)
LC30	1.00	0.506477855	0.216693028	1.00	0.101295571	0.0498394
LC93	1.00	0.08495172	0.101806963	1.00	0.016990344	0.0234156
LC108	1.00	0.004865262	0.003337594	1.00	0.000973052	0.00076765
HC16	1.00	0.142726546	0.134687394	1.00	0.028545309	0.0309781
HC259	1.00	0.098177026	0.104606208	1.00	0.019635405	0.02405943
HC359	1.00	0.129050469	0.119570085	1.00	0.025810094	0.02750112
HC420	1.00	0.039825309	0.036162056	1.00	0.007965062	0.00831727

A. Reduced Light Chain



B. Expanded view of the +54 Da modified light chain

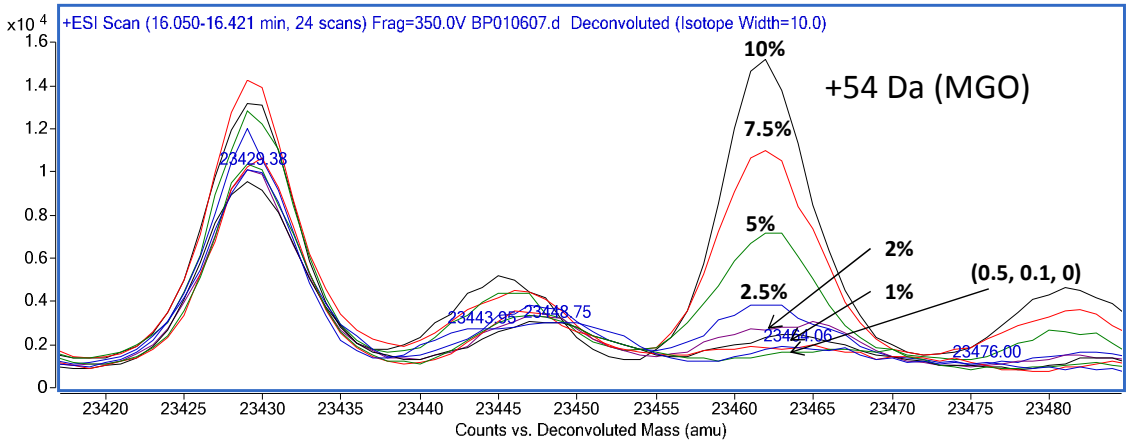


Figure S-11: Detection limit of reduced LC/MS to detect MGO (+54 Da) . The deconvoluted mass spectrum of reduced light chain spiked with MGO modified light chain (top pane is full view and bottom pane focuses on the +54 Da species). Modified light chain was spiked into native light chain to deduce the level at which the +54 Da peak could be detected without an enrichment step. Tha data shows overlaid traces at 10%, 7.5%, 5%, 2.5%, 2%, 1%, 0.5%, 0.1% and 0% modified antibody spikes. The +54 Da peak was detected at 2% modification and was unambiguously identified at 5% modification on our instrument.