Mechanism of irreversible inhibition of Mycobacterium tuberculosis shikimate kinase by ilimaquinone

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Published in:
Biochimica et Biophysica Acta: General Subjects

DOI:
10.1016/j.bbapap.2018.04.007

Publication date:
2018

Document Version
Peer reviewed version

Supplementary material

Figure S1. **Intensities of deconvoluted *MtSK* MS spectra *vs* preincubation time with IQ**. *Mt*SK (1µM) was incubated for 30 hours with 100 µM IQ at 4°C. Intensities of the deconvoluted MS spectra were plotted against preincubation time. With increasing preincubation time, a decrease in the intensities of both forms of free *Mt*SK (19648.68 and 19517.73 Da with and without N-terminal methionine, respectively) was observed suggesting the formation of IQ-adducts (A). Increase of both forms of singly modified (*Mt*SK-IQ) enzyme (19975.18 and 19843.75 Da with and without N-terminal methionine, respectively) was observed reaching a maximum intensity at around 15 hours (B). Increase of doubly modified (*Mt*SK-IQ_2) enzyme (20301.29 and 20170.19 Da with and without N-Terminal methionine) was observed reaching a maximum intensity at around 20 hours (C).

Figure S2. **Deconvoluted ESI-MS spectra for *Mt*SK incubated with 10 µM IQ.** *Mt*SK (1.0 µM) was incubated with 10 µM IQ for 30 hours at 4 °C prior to LC separation and MS analyses of the intact protein. Representative overlapped chromatograms of control *Mt*SK sample (blue line) and *Mt*SK+IQ sample (red line) are shown in panel A. The deconvoluted mass spectrum corresponding to the entire elution envelop (5.16 – 6.62 min) (B) is compared to that of the first (5.16 – 5.44 min) (C), second (5.44 – 5.84 min) (D), and third (5.84 – 6.62 min) (E) elution features of the chromatogram. Masses of 19648.97 and 19517.73 in panel B represent intact *Mt*SK with and without its N-terminal methionine, respectively. Each deconvoluted mass in panels D and E corresponds to addition of an IQ derivative with a mass shift of 326 Da.

Figure S3. **IQ inhibition of LDH, PK, and *Mt*KatG activities according to their respective spectrophotometric assays.** LDH (A), PK (B) and *Mt*KatG (C) were preincubated with 100 µM IQ for 1 hr at 25 °C. Reactions were initiated by the addition of preformed enzyme-inhibitor to the appropriate assay cocktail for each enzyme (see *Materials and Methods*). LDH (A) and PK (B) activities were monitored by decrease in absorbance at 340 nm due to NADH oxidation. *Mt*KatG activity was monitored by decrease in absorbance at 240 nm due to H_2O_2 consumption.

Figure S4. **Deconvoluted ESI-MS spectra for PK incubated with 100 µM IQ.** PK (1.0 µM) was incubated with 100 µM IQ for 30 hours at 4 °C prior to LC separation and MS analyses of the intact protein. The entire deconvoluted mass spectrum (A) of the control (1 hr) and sample (IQ 30 hr) from 6 – 6.8 min shows unmodified PK with molecular weight of 58020 Da. No mass shifts were observed post incubation. Spectra within 7 – 8 min range had high intensities of background noise and were not included.

Figure S5. **Deconvoluted ESI-MS spectra for LDH incubated with 100 µM IQ.** LDH (1.0 µM) was incubated with 100 µM IQ for 30 hours at 4 °C prior to LC separation and MS analyses of the intact protein. Overlapped chromatograms of control LDH sample and LDH+IQ sample are represented in green and black lines respectively. The corresponding chromatograms from 6 – 7.5 min of LDH (A) and LDH+IQ (A) show mainly unmodified protein. Deconvoluted mass spectra corresponding to the peaks eluding at 6.0 – 6.5 min of LDH+IQ shows a preponderance of the unmodified protein (36461 Da) (B). Deconvoluting the LDH+IQ mass spectra from 6.5 –

7.5 min shows the masses of both unmodified (36461 Da) and IQ-modified (36789) LDH (C), with a net mass difference of 326 Da.

Figure S6. Identification of Lys 15, Ser 44, Thr 111, and Ser 77 IQ-adducted peptides by nano-LC-ESI MS/MS analysis. MS/MS spectra of the peptide AVLVGLPGSGKSTIGRR $[M + 3H]^{+3}$ *m/z* 665.43, unmodified y-ion series up to $y_6{*}^{+}$ and shifted by 326.3 from y_7. Likewise, b-ion series are unmodified up to b_{10}^{+} and shifted by 326.3 from b_{11}*, depicting Lys15 as the residued modified by IQ (A). MS/MS spectra of the peptide SIADIFATDGEQEFR $[M + 2H]^{+2}$ *m/z* 1013.05, shows and unmodified y-ion series and a b-ion series shifted by 326.3 from $b_1{*}^{2+}$, identifying Ser 44 as the modification site adducted by ilimaquinone (B). MS/MS spectra of the peptide TGGNTVRPLLAGPDR $[M + 2H]^{+2}$ *m/z* 925.56, shows and unmodified y-ion series and a b-ion series shifted by 326.3 from $b_1{*}^{2+}$, identifying Thr 111 as the modification site adducted by ilimaquinone (C). MS/MS spectra of peptide AALADHDGVLSLGGGAVTSPGVR $[M + 3H]^{+3}$ *m/z* 816.10 shows an unmodified y-ion series up to $y_{12}{*}^{+}$ and shifted by 326.3 from y_{13}. Likewise, b-ion series are unmodified up to b_{10}^{+} and shifted by 326.3 from b_{11}*, depicting Ser77 as the residued modified by IQ (D).

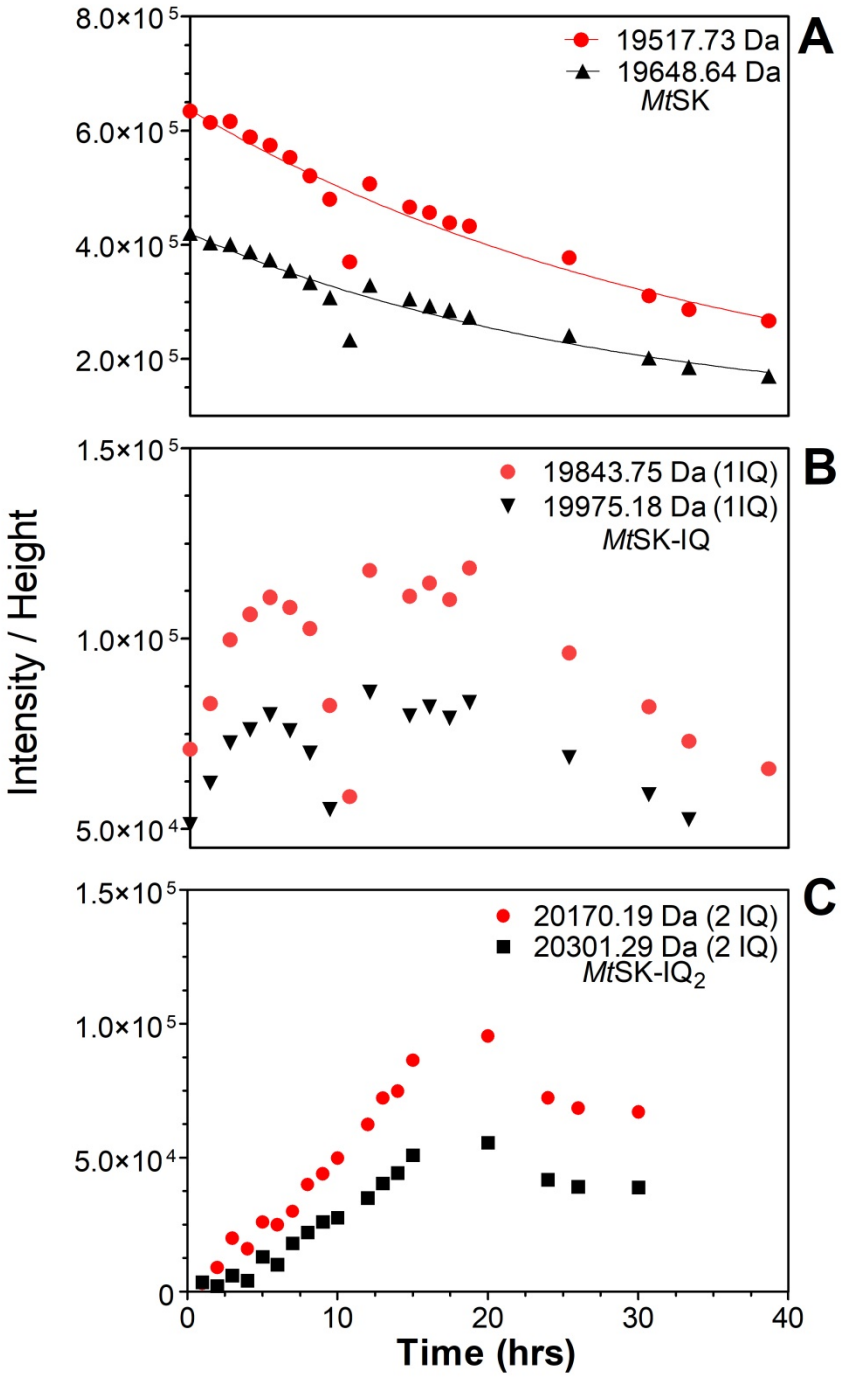
A
19517.73 Da
19648.64 Da
MtSK
B
19843.75 Da (1IQ)
19975.18 Da (1IQ)
MtSK-IQ
C
20170.19 Da (2 IQ)
20301.29 Da (2 IQ)
MtSK-IQ2
Intensity / Height
Time (hrs)

Figure S1

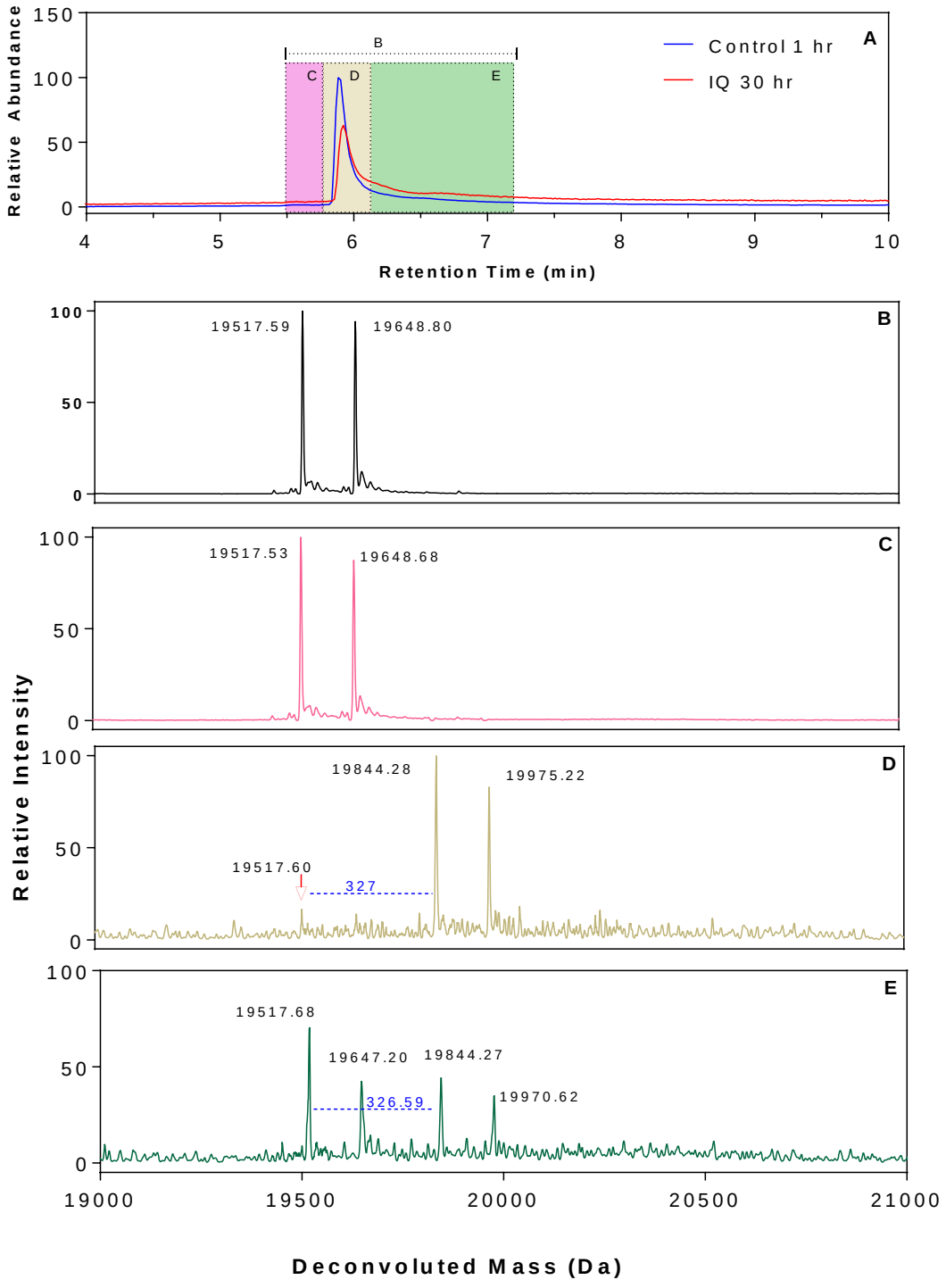
A
Control 1 hr
IQ 30 hr
B
C
D
E
Relative Abundance
Retention Time (min)
B
19517.59
19648.80
C
19517.53
19648.68
D
19844.28
19975.22
19517.60
327
E
19517.68
19647.20
19844.27
19970.62
326.59
Relative Intensity
Deconvoluted Mass (Da)

Figure S2

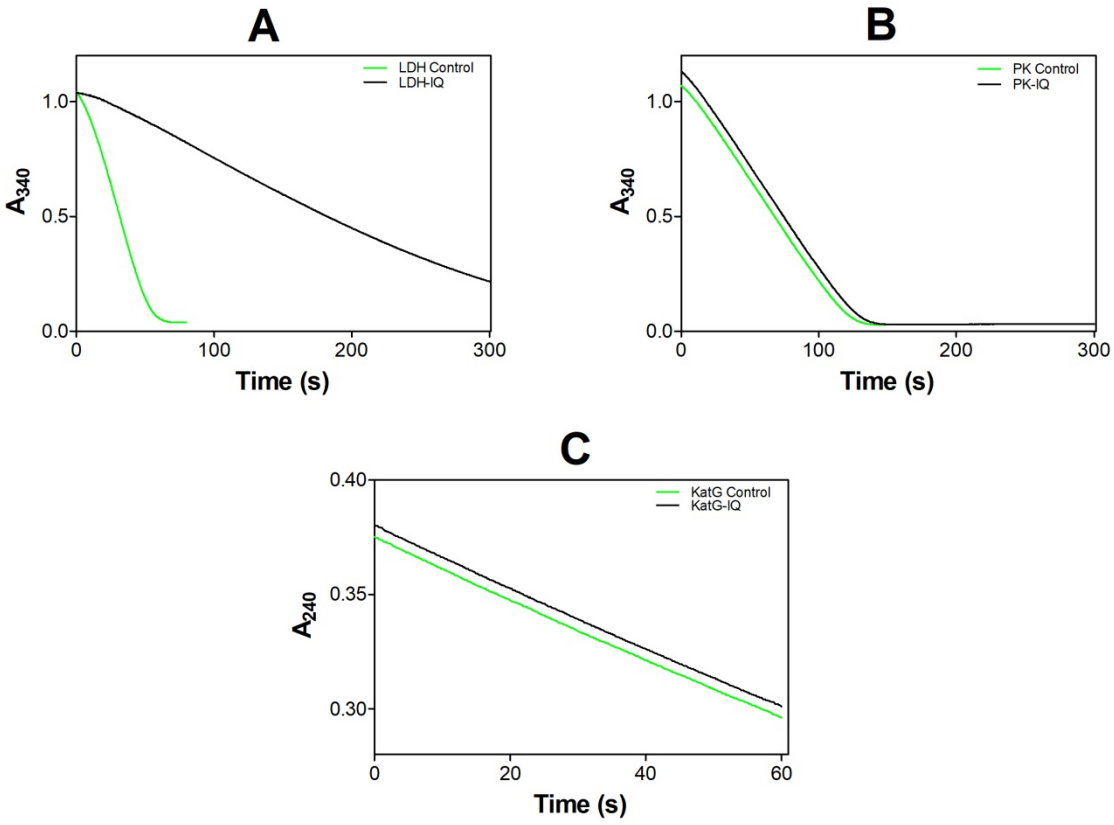
A
LDH Control
LDH-IQ
1.0
0.5
0.0
A340
0
100
200
300
Time (s)
B
PK Control
PK-IQ
1.0
0.5
0.0
A340
0
100
200
300
Time (s)
C
KatG Control
KatG-IQ
0.40
0.35
0.30
A240
0
20
40
60
Time (s)

Figure S3

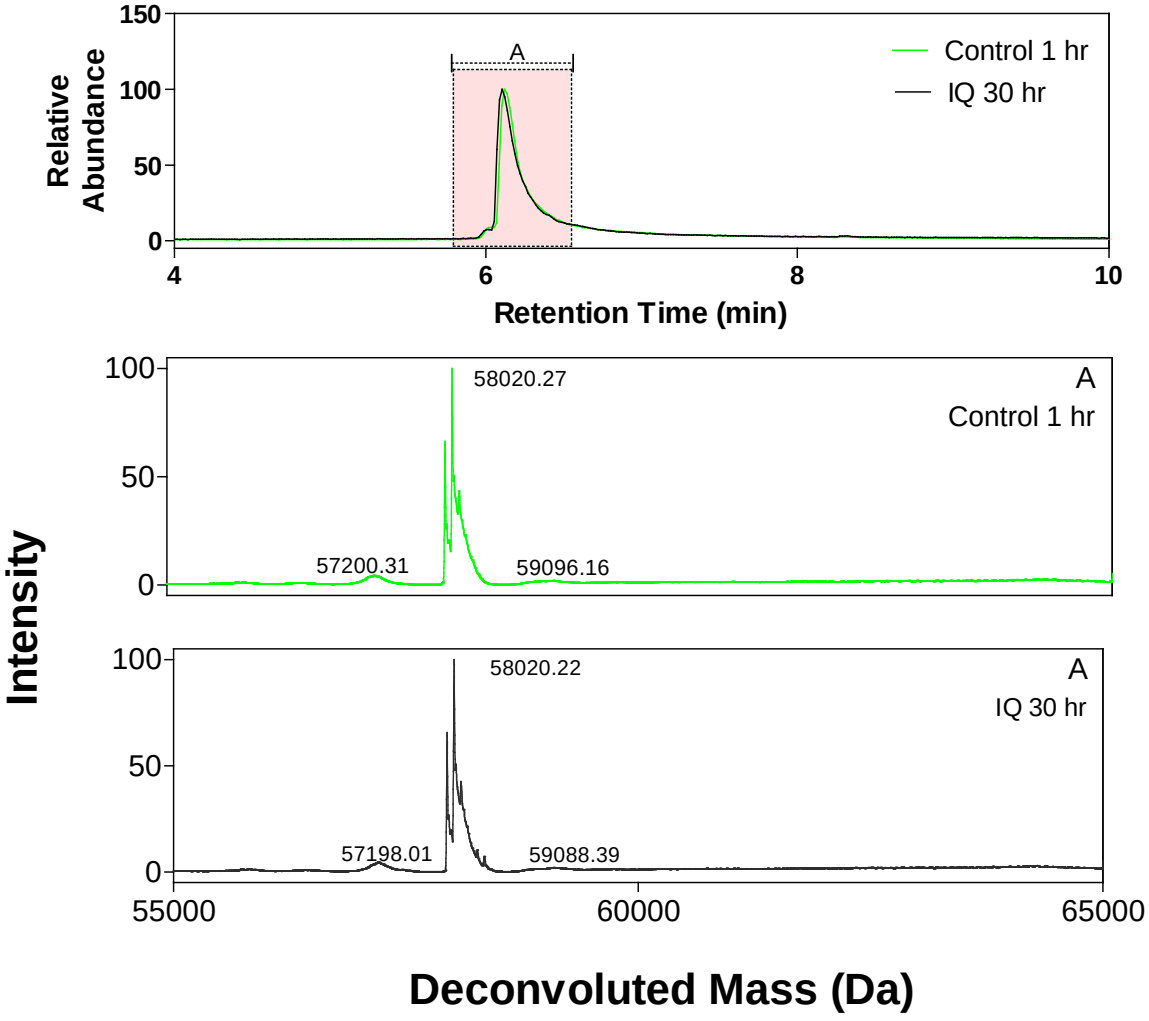
Relative Abundance
150
100
50
0
A
Control 1 hr
IQ 30 hr
4
6
8
10
Retention Time (min)
Intensity
100
50
0
58020.27
A
Control 1 hr
57200.31
59096.16
100
50
0
58020.22
A
IQ 30 hr
57198.01
59088.39
55000
60000
65000
Deconvoluted Mass (Da)

Figure S4

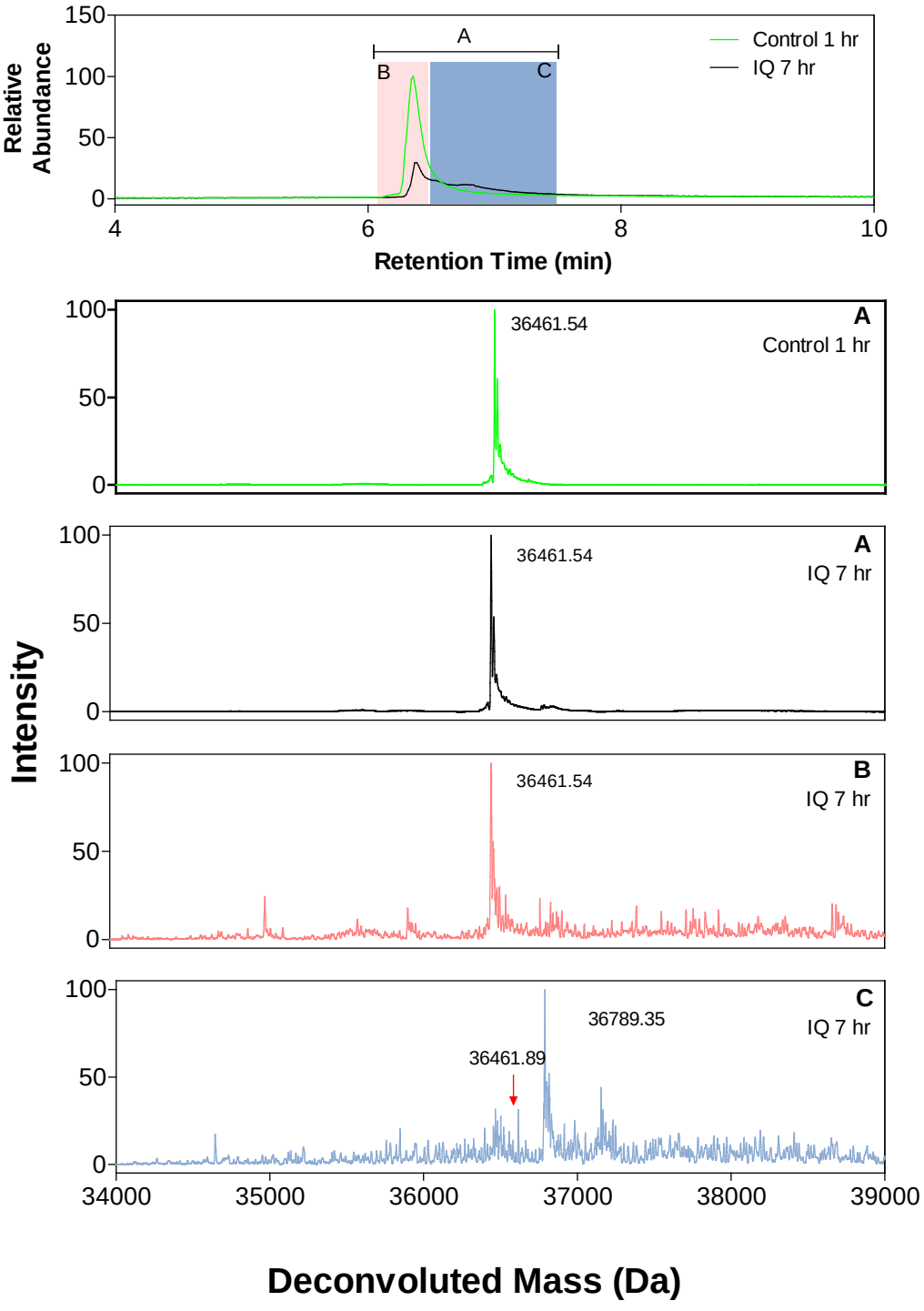
Relative Abundance
150
100
50
0
A
B
C
Control 1 hr
IQ 7 hr
4
6
8
10
Retention Time (min)
Intensity
36461.54
A
Control 1 hr
36461.54
A
IQ 7 hr
36461.54
B
IQ 7 hr
36789.35
36461.89
C
IQ 7 hr
34000
35000
36000
37000
38000
39000
Deconvoluted Mass (Da)

Figure S5

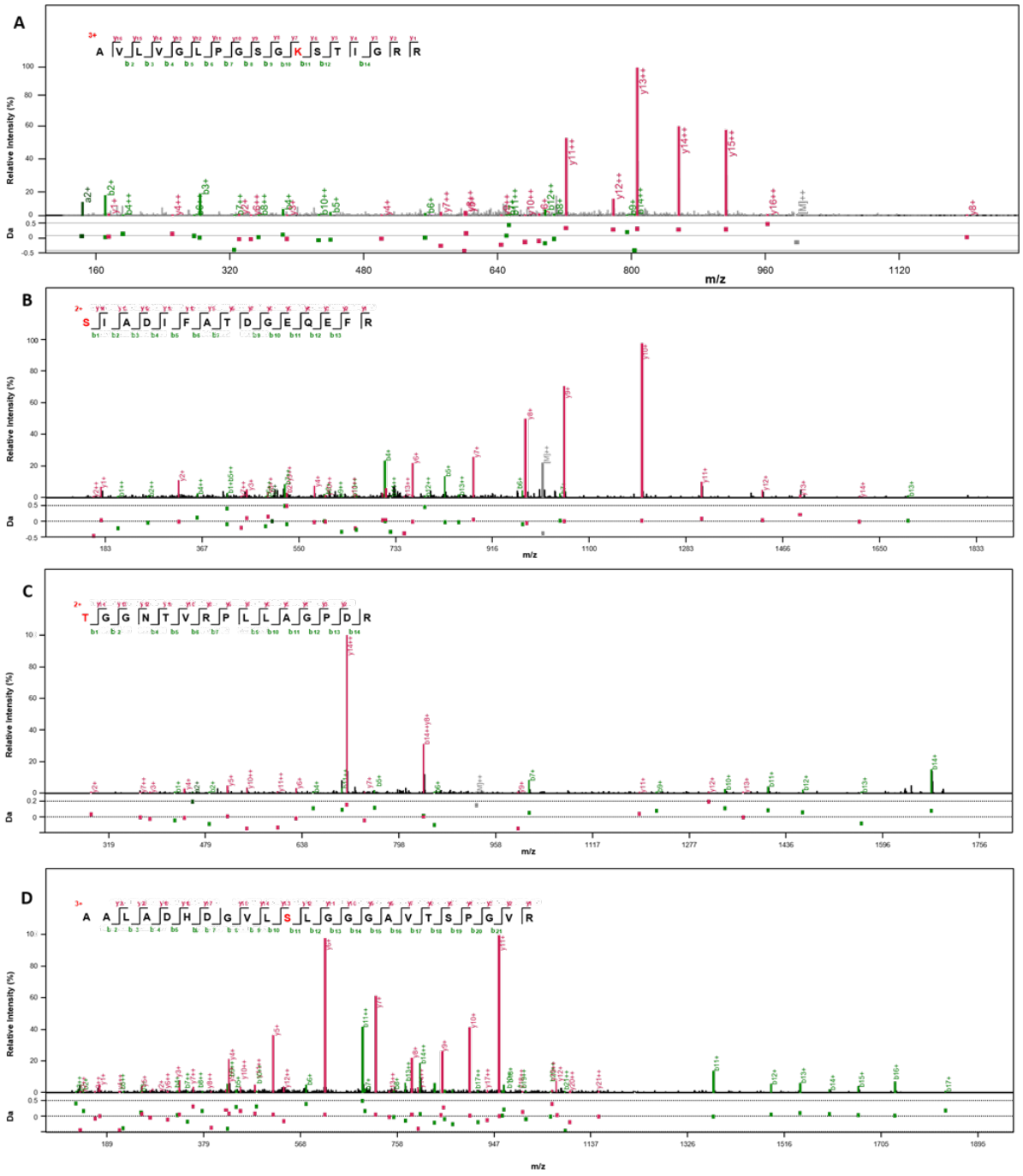

Figure S6