Supporting Information:

IR and UV Photodissociation as Analytical Tools for Characterizing Lipid A Structures

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Experimental Section (Supporting Information)

Materials.

Strain MST10 contains in-frame gene deletions in *lpxT* (*yeiU*) and *eptA*, and harbors two plasmids pAChp0021 and pWScj0256. To generate strain MST10 an in-frame deletion of *eptA* was introduced into strain MST01 (W3110, Δ*lpxT::cat*) by P1 *vir* phage transduction using donor strain CH030 (W3110, Δ*eptA::nptII*).[1] Plasmid pAChp0021 was generated by ligation of the coding sequence of *H. pylori* gene *hp0021* plus 50 bp upstream into the BamH1 and EcoRV restriction sites of plasmid pACYC184 (Novagen). Primer A (5´-GCGCGCGGATCCTCTTATTTAAACAAAAATTTTGTG-3´) and Primer B (5'-GCGCGCGATATCTTAAGGCTTTTTGGGGCT-3´) were used to amplify *hp0021* insert. Plasmid pWScj0256 was generated by ligation of the *C. jejuni cj0256* coding sequence into the SacII and XhoI restriction sites of plasmid pWSK29.[2] Primer C (5´-GCGCGCCCGCGGAAGAAGGAGATATACATGCTTAGATTAACTTGGTTTCAG-3´), containing a Shine-Dalgarno sequence, and Primer D (5´-GCGCGCCTCGAGTCATGGATTTGCCTTAAGTTTAGG-3´) were used to amplify the *cj0256* insert.

Mass spectrometry, Infrared Multiphoton Dissociation, and Ultraviolet Photodissociation.

An online nanoelectrospray setup was used for direct infusion and consisted of a conductive mini microfilter junction from IDEX Health and Science (Oak Harbor, WA) coupled to a New Objectives uncoated PicoTip® nanoESI emitter (Woburn, MA). The spray voltage was applied at 2 kV. Lipid A samples were diluted to 1 μM in 50/50 methanol/chloroform, and were

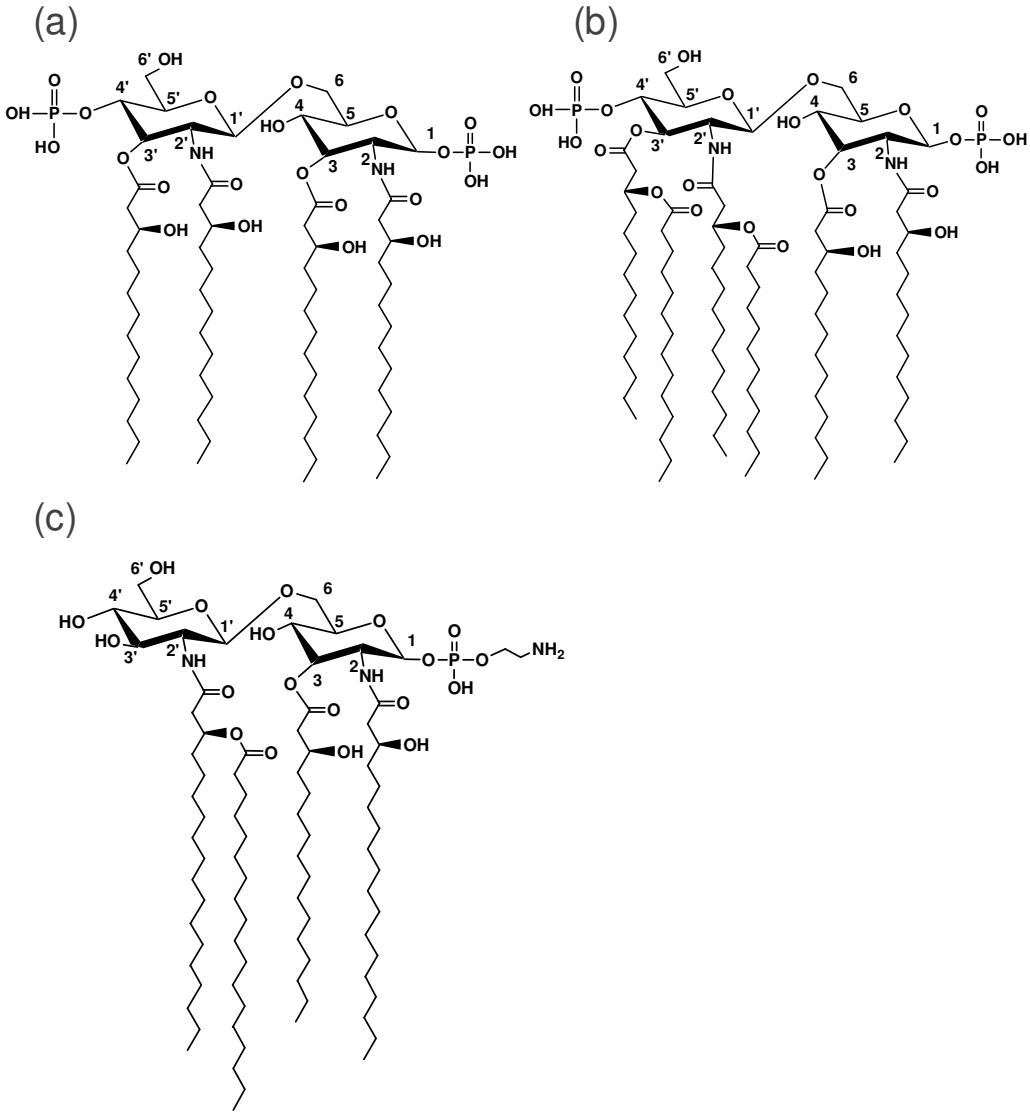
infused at 300 nL/min. Typical parameters such as a *q*-value of 0.25, an activation time of 30 ms, and a normalized collision energy (NCE) of 35% were used for all CID experiments (NCE = (voltage × 30) / (0.000062 × *m/z* × 0.006325) in which 0.000062 is the TickAmpSlope and 0.006325 is the TickAmpInt in the Thermo LTQ software). IRMPD was performed using a *q*-value of 0.1 with the laser operated at 50 watts and an irradiation time of 30 ms. A *q*-value of 0.1 and a laser activation of 5 pulses (8 mJ/pulse) at a 500 Hz rep rate (over a 10 ms period) were used for all UVPD experiments. Activated – electron photodetachment dissociation (a-EPD) was performed using a *q*-value of 0.1 and a laser activation of 5 pulses (8 mJ/pulse) at 500 Hz (over a 10 ms period) to generate the charge reduced, radical species, which was subsequently activated using a normalized collision energy of 35%, *q*-value of 0.25, and an activation period of 30 ms. The maximum injection time for all MS/MS spectra was set to 100 ms.

Experimental Section (Supporting Information) References

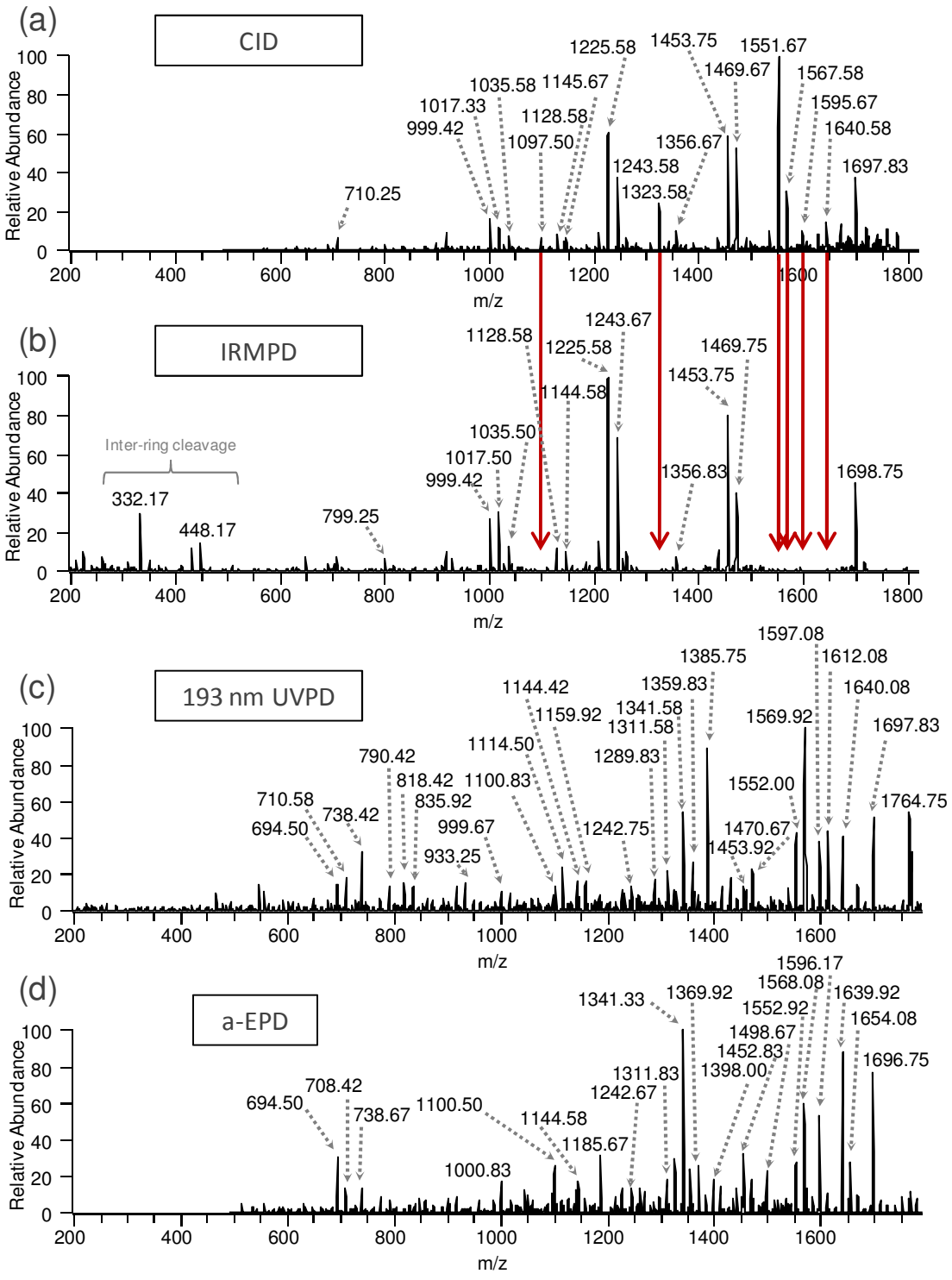
(1) Herrera, C. M.; Hankins, J. V.; Trent, M. S. *Mol Microbiol* **2010**, *76*, 1444-1460.
(2) Wang, R. F.; Kushner, S. R. *Gene* **1991**, *100*, 195-199.

Figures (Supporting Information)

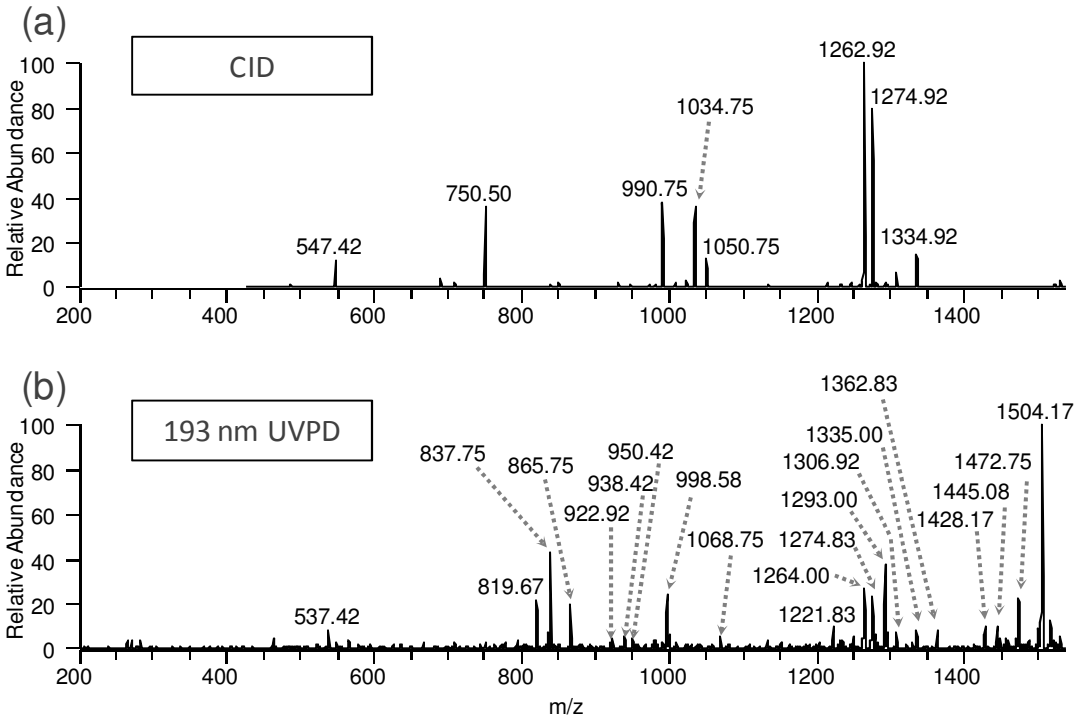
Supplementary Figure 1. Structures of lipid A analytes in this study: (a) lipid IV_A, molecular weight 1404.8, (b) *E. coli* F583 lipid A, molecular weight 1797.2, and (c) *H. pylori* lipid A, molecular weight 1548.2.



Supplementary Figure 2. MS/MS spectra from the dissociation of *E. coli* F583 lipid A by (a) CID, 1-, (b) IRMPD, 1-, (c) 193 nm UVPD, 1-, and (d) a-EPD, 1-•. Red arrows represent the secondary dissociated product ions after IRMPD. The 1- precursor was *m/z* 1796.0 and the 1-• precursor (for a-EPD) was *m/z* 1795.0.


(a)
CID
Relative Abundance
m/z
710.25
999.42
1017.33
1035.58
1097.50
1128.58
1145.67
1225.58
1243.58
1323.58
1356.67
1453.75
1469.67
1551.67
1567.58
1595.67
1640.58
1697.83
(b)
IRMPD
Inter-ring cleavage
332.17
448.17
799.25
999.42
1017.50
1035.50
1128.58
1144.58
1225.58
1243.67
1356.83
1453.75
1469.75
1698.75
(c)
193 nm UVPD
694.50
710.58
738.42
790.42
818.42
835.92
933.25
999.67
1100.83
1114.50
1144.42
1159.92
1242.75
1289.83
1311.58
1341.58
1359.83
1385.75
1453.92
1470.67
1552.00
1569.92
1597.08
1612.08
1640.08
1697.83
1764.75
(d)
a-EPD
694.50
708.42
738.67
1000.83
1100.50
1144.58
1185.67
1242.67
1311.83
1341.33
1369.92
1398.00
1452.83
1498.67
1552.92
1568.08
1596.17
1639.92
1654.08
1696.75

Supplementary Figure 3. MS/MS spectra from the dissociation of *H. pylori* lipid A by (a) CID, 1-, and (b) 193 nm UVPD, 1-. The 1- precursor was *m/z* 1547.2.



Supplementary Figure 4. Structures of phosphorylethanolamine modified lipid A analytes in this study: (a) *H. pylori* lipid A, molecular weight 1548.2, (b) *C. jejuni* lipid A, molecular weight 1922.4, and (c) *E. coli* strain MST10 (W3110 Δ*lpxT*, Δ*eptA*/pAChp0021, pWScj0256), molecular weight 1840.3. The modification is highlighted in pink.