

Supporting Information

Characterization of the Furin Cleavage Motif for HIV-1 Trimeric Envelope Glycoprotein by Intact LC-MS Analysis

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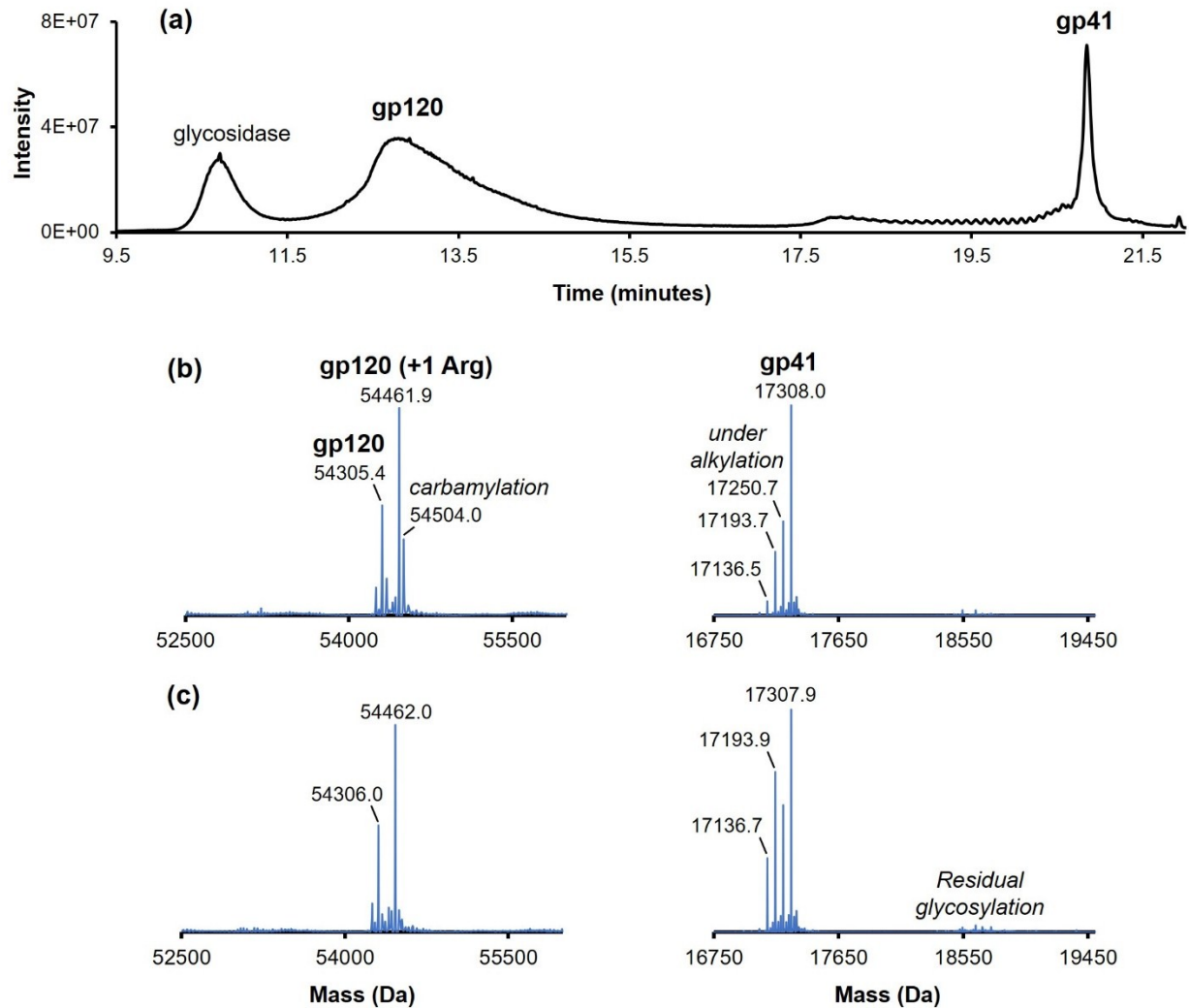


Figure S1. (a) Representative total ion chromatograms of gp120 and gp41 subunits and their deconvoluted MS spectra (gp120 on left and gp41 on right) after using the following sample preparation conditions: 6 M urea and 100 mM DTT at 60°C for 30 min, followed by 150 mM IAM treatment, a buffer-exchange step and overnight deglycosylation with PNGase F at 37°C. (b) with and (c) without the addition of 6 M urea (all other sample preparation conditions held constant). Theoretical masses for gp120 with the addition of 0 and 1 arginine residues were 54,305.6 and 54,461.8 Da, respectively, whereas the theoretical mass for gp41 with 0 arginine residues was 17,307.8 Da.

Table S1. Calculated theoretical average molecular weight (MW) for the reduced and deglycosylated gp120 subunit accounting for percent (%) N-glycan occupancy (based on previously reported LC-MS results for the same HIV Env trimer construct/material).¹

Glycan site #	Reported % N-glycan occupancy	Mass shift (Da)
N88	99%	0.974
N133	100%	0.984
N137	100%	0.984
N156	100%	0.984
N160	100%	0.984
N182	59%	0.581
N185	100%	0.984
N197	79%	0.777
N234	100%	0.984
N262	100%	0.984
N276	100%	0.984
N295	100%	0.984
N301	100%	0.984
N332	100%	0.974
N339	99%	0.984
N355	100%	0.984
N363	100%	0.984
N386	100%	0.984
N392	100%	0.984
N398	100%	0.984
N406	ND*	0.984
N411	ND*	0.984
N448	100%	0.984
N460	100%	0.984
Total expected MW shift upon deglycosylation		23.0 Da
Theoretical MW (Da)		+ 0 Arg + 1 Arg
		53,084.5 53,240.7
Theoretical MW (Da) after deglycosylation		53,107.5 53,263.7

ND = N-glycan occupancy not determined for site; assumed 100% for MW estimation

For the fully alkylated gp120 subunit (+ 57 Da x 21 Cys), the theoretical average MW upon deglycosylation was calculated to be 54,305.6 Da (0 Arg) and 54,461.8 Da (+1 Arg).

Table S2. Calculated theoretical average MW for the reduced and deglycosylated gp41 subunit accounting for percent (%) N-glycan occupancy.¹

Glycan site #	Reported % N-glycan occupancy	Mass shift (Da)	
N611	19%	0.187	
N618	79%	0.777	
N625	8%	0.0787	
N637	100%	0.984	
Total expected MW shift upon deglycosylation		2.03 Da	
Theoretical MW (Da)		+ 0 Arg	+ 1 Arg
		17,134.6	17,290.8
Theoretical MW (Da) after deglycosylation		17,136.6	17,292.8

For the fully alkylated gp41 subunit (+57 Da x 3 Cys), the theoretical average MW upon deglycosylation was calculated to be 17,307.8 Da (0 Arg). No additional arginine residues were observed on the deglycosylated gp41 subunit.

The theoretical average MW for the non-reduced gp140 heterodimer was 70,194.9 Da (0 Arg) and 70,351.1 Da (+ 1 Arg). Upon deglycosylation, the mass shift was 25.0 Da; therefore, the theoretical average mass was adjusted to 70,219.9 Da (0 Arg) and 70,376.1 Da (+1 Arg).

Reference:

1. V.B. Ivleva, J.W. Cooper, F.J. Arnold, Q.P. Lei, *J. Am. Soc. Mass Spectrom.*, 2019, **30**, 1663-1678.