Supplemental material for

Effect of glycosylation and additional domains on thermostability of a family 10 xylanase of Thermopolyspora flexuosa

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Contents:

I. High-resolution mass spectrometry: experimental details

All experiments were performed with a 4.7-T hybrid quadrupole–Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (APEX-Qe™; Bruker Daltonics, Billerica, MA, USA), interfaced to an electrospray ionization (ESI) source (Apollo-II™). Enzyme samples were further desalted by a Sephadex G-25 columns (PD-10; Amersham Pharmacia Bio-tech, Sweden), diluted to a ~10 μM concentration with acetonitrile/water/acetic acid (49.5:49.5:1.0, v/v) solution and directly infused at a flow rate of 1.5 μL/min. ESI-generated ions at *m*/*z* 50-3000 were externally accumulated in the hexapole ion trap (collision cell) for 1.0 s and transferred to an Infinity ICR cell for trapping, excitation and detection. For each spectrum, a total of 256 co-added (1-Mword) time-domain transients were fast Fourier-transformed prior to magnitude calculation and external frequency-to-m/z calibration (ES Tuning Mix; Agilent Technologies, Palo Alto, CA, USA). On-line digestion was performed by following a previously reported protocol (Marie *et al.*, 2000) that was slightly modified (Jänis J. *et al.*, unpublished results). The attempts to reduce disulfides prior to digestion were made by the protocol described earlier (Scigelova, *et al.*, 2001). For collision-induced dissociation (CID) mass spectra, peptide ions of interest were mass-selectively accumulated in the hexapole (quadrupole isolation width 10 *m*/*z*-units) at the static argon pressure of ~4.0×10^{-6} mbar and the hexapole DC-offset was lowered from 0 to –15 V. All data were acquired and processed with the use of Bruker XMASS 7.0.8 software. CID mass spectra were interpreted with the help of GPMAW 8.0 software (Lighthouse Data, Odense, Denmark).

References:

Marie, G., Serani, L., and Laprevote, O. 2000. An on-line protein digestion device for rapid peptide mapping by electrospray mass spectrometry, *Anal. Chem.* 72:5423-5430.

Scigelova, M., Green, P. S., Giannokopulos, A. E., Rodger, A., Crout, D. H. G., and Derrick, P. J. 2001. A practical protocol for the reduction of disulfide bonds in proteins prior to analysis by mass spectrometry, *Eur. J. Mass Spectrom.* 7: 29-34.

II. Accurate mass data for the observed protein forms of *T.reesei* and *E.coli* XYN10A

Table S1. Experimental and calculated masses for the different protein forms detected in the ESI FT-ICR mass spectra of XYN10A with 6xHis-tag expressed in *E. coli* and XYN10A expressed *T. reesei*

T. reesei				
Protein form	mass (exptl), Da[1]	mass (calcd), Da[1]	Δmass, Da	Assignment
1	34120.76 ± 0.05	34120.73	–	Full construct
2	34323.93 ± 0.08	34323.92	+ 203.17	+ GlcNAc
3	34486.03 ± 0.06	34486.06	+ 162.10	+ Man
4	34642.32 ± 0.06		+ 156.29	
5	34722.25 ± 0.13		+ 79.93	
6	34884.26 ± 0.04		+ 162.01	+ Man
E.coli				
Protein form	mass (exptl), Da[1]	mass (calcd), Da[1]	Δmass (Da)	Assignment
1	34943.25 ± 0.15	34942.93	–	Full construct (His6-tagged)

[1]Most abundant isotopic masses averaged over observed charge-state distributions

III. On-line digestion mass spectra

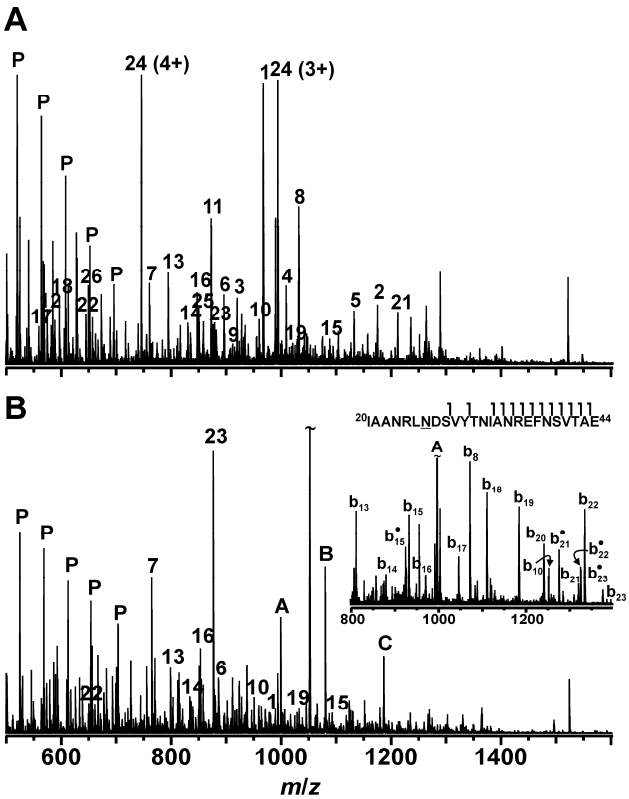

Figure S1: ESI FT-ICR mass spectra of the peptic peptides arising from on-line pepsin digestion of the catalytic domain of XYN10A expressed in *E. coli* (**A**) and *T. reesei* (**B**). The identified peptic peptides are assigned by numbers (1-26) and three observed glycopeptides as A, B and C (for the mass data, see Table S2) The inset in B shows the CID mass spectrum of a triple-charged glycopeptide A (aa 20-44), observed at *m*/*z* ~996. The sequence of A is shown above, with the glycosylated Asn26 underlined. All b-type fragment ions were double-charged, except b_8 and b_{10}. Ions marked with • are b–H_2O fragment ions. Signals from PEG impurity in the samples are assigned as P.

IV. On-line digestion: *E.coli* XYN 10A

Table S2. List of peptic peptides detected in the on-line digestion of *E.coli* XYN 10A

Peptide number	*m/z* (exptl)	*m/z* (calcd)	error (ppm)	Residues & Sequence From-To
1	1932.9519	1932.9442	4.0	1 19 AASTLAEGAAQHNRYFGVA
2	4696.3356	4696.3018	7.2	1 44 AASTLAEGAAQHNRYFGVAIAANRLNDSVYTNIANREFNSVTAE
3	2781.3855	2781.3682	6.2	20 44 IAANRLNDSVYTNIANREFNSVTAE
4	3024.4796	3024.4537	8.6	20 46 IAANRLNDSVYTNIANREFNSVTAENE
5	4526.2151	4526.2104	1.0	20 59 IAANRLNDSVYTNIANREFNSVTAENEMKIDATEPQQGRF
6	1762.8381	1762.8308	4.1	45 59 NEMKIDATEPQQGRF
7	1519.7530	1519.7453	5.1	47 59 MKIDATEPQQGRF
8	3093.5293	3093.5059	7.6	88 114 QQPQWMQNLSGQALRQAMINHIQGVMS
9	1837.9441	1837.9363	4.2	114 128 SYYRGKIPIWDVVNE
10	1908.9868	1908.9734	7.0	114 129 SYYRGKIPIWDVVNEA
11	1750.9145	1750.9043	5.8	115 128 YYRGKIPIWDVVNE
12	1146.6255	1146.6186	6.0	116 124 YRGKIPIWD
13	1587.8496	1587.8409	5.5	116 128 YRGKIPIWDVVNE
14	1658.8852	1658.8780	4.4	116 129 YRGKIPIWDVVNEA
15	3262.6399	3262.6232	5.1	116 143 YRGKIPIWDVVNEAFEDGNSGRRRDSNL
16	1692.8035	1692.7928	6.3	129 143 AFEDGNSGRRRDSNL
17	1117.5198	1117.5152	4.2	144 152 QRTGNDWIE
18	1209.5880	1209.5812	5.6	178 188 NAAKTQAVYNM
19	4112.0248	4111.9996	6.1	247 282 YASVIRDCLAVDRCTGITVWGVRDSDSWRSYQNPLL
20	2720.2917	2720.2799	4.4	251 274 IRDCLAVDRCTGITVWGVRDSDSW
21	3691.8181	3691.7988	5.2	251 282 IRDCLAVDRCTGITVWGVRDSDSWRSYQNPLL
22	1297.6117	1297.6051	5.1	283 293 FDNNGNKKQAY
23	1743.8637	1743.8580	3.3	283 297 FDNNGNKKQAYYAVL
24	2979.4118	2979.4025	3.1	283 307 FDNNGNKKQAYYAVLDALNHHHHHH
25	1699.8126	1699.8080	2.7	294 307 YAVLDALNHHHHHH
26	1253.5590	1253.5551	3.1	298 307 DALNHHHHHH

V. Collision-induced dissociation (CID) measurement of glycopeptide A

Table S3. List of observed fragment ions from the CID measurement of a triply-charged glycopeptide A, observed at *m/z* 995.83 in the on-line digestion of *T.reesei* XYN10A

Fragment ion[1]	*m/z* (exptl)	*m/z* (calcd)	error (ppm)
b_{23}	1384.203	1384.186	12.3
b_{23}-H_2O	1375.190	1375.181	6.7
b_{22}	1333.676	1333.662	10.5
b_{22}-H_2O	1324.669	1324.657	8.9
b_{21}	1284.136	1284.128	6.5
b_{21}-H_2O	1275.131	1275.123	6.7
b_{20}	1240.624	1240.612	9.2
b_{19}	1183.597	1183.591	5.1
b_{18}	1110.063	1110.057	5.7
b_{17}	1045.542	1045.535	6.7
b_{16}	967.4831	967.4846	1.6
b_{14}	874.9462	874.9446	1.8
b_{13}	818.4048	818.4026	2.7
b_{10}	1257.653	1257.644	7.2
b_8	1071.550	1071.544	5.9

[1]All fragment ions are doubly-charged, except b_8 and b_{10} that are singly-charged.