

Comparison of Different Methods for Rapid Identification of Ricin from Castor Bean Extracts by MALDI-MS and ESI-MS

Martin Schär
Marc-André Avondet
Daniel Eglin
Peter Siegenthaler
SPIEZ LABORATORY
Switzerland

Ricin is a large, glycosylated protein with variable N-glycan structures and exists in different varieties. Thus, the *molecular ion* of ricin seems not to be well suited for an unambiguous identification by mass spectrometry [1]. Nevertheless, we assessed the potential to use the (pseudo-) molecular ion signal for rapid ricin identification based on its specific **N-glycan structure** as published in literature [2] and [3]. We analyzed native ricin as well as the glycosylated A- and B-chain using **MALDI linear TOF-MS**, **LC/MS** and **static nanospray**, respectively, and compared the spectra with simulated molecular ion signals.

Mass Spectrum Simulation

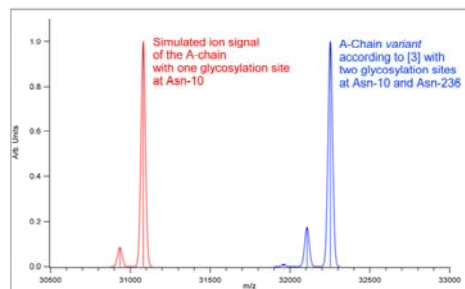


Fig. 1a: Simulated Ricin A-chain.

Computer simulated molecular ion spectrum. Simulation based on N-glycan and protein data from [2], [3] and [4], respectively. Resolution $R=1000$ (FWHM).

LC/MS, Static Nanospray

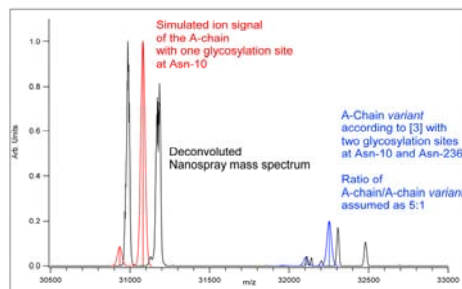


Fig. 1b: Ricin A-chain, glycosylated.

Static nanospray measurement, spray voltage: 700 V. Proxeon NanoES Emitter. ZipTip® C₁₈ clean-up of 0.7 mg/ml glycosylated ricin A-chain (SIGMA). Eluent: 75% MeOH/25% H₂O (0.1% formic acid).

MALDI linear TOF-MS

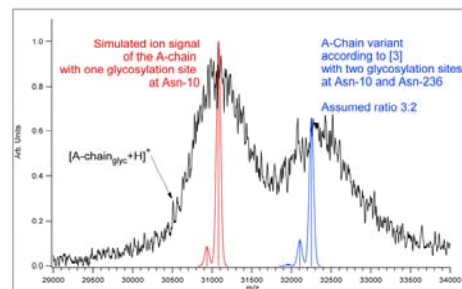


Fig. 1c: Ricin A-chain, glycosylated.

MALDI-MS measurement compared to a simulated mass spectrum with a resolution of $R=500$ (FWHM). Sample: 0.7 mg/ml glycosylated ricin A-chain (SIGMA), clean-up with ZipTip® C₁₈. Matrix: Sinapinic acid.

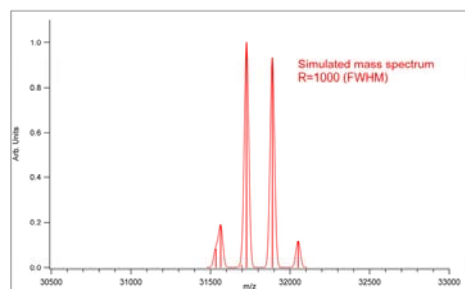


Fig. 2a: Simulated Ricin B-chain.

Computer simulation of the theoretical mass spectrum (glycosylation pattern) of the B-chain based on N-glycan and protein data from [2] and [4], respectively. Resolution $R=1000$ (FWHM).

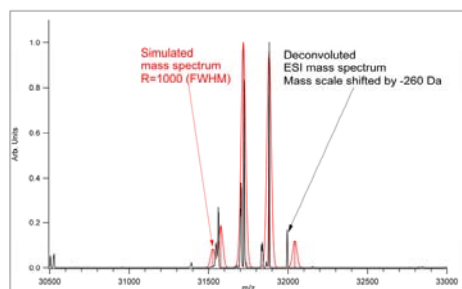


Fig. 2b: Ricin B-chain, glycosylated.

Deconvoluted LC/MS measurement and simulation from Fig. 2a. Note: LC/MS masses shifted by -260 Da. Sample: 1.0 mg/ml glycosylated ricin B-chain (Vector Laboratories). Column: Discovery® BIO Wide Pore C5.

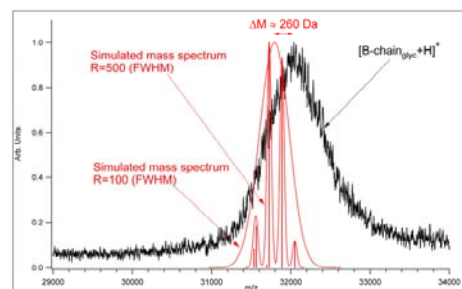


Fig. 2c: Ricin B-chain, glycosylated.

Simulated mass spectra compared to a MALDI-MS measurement of 1.0 mg/ml glycosylated ricin B-chain (Vector Laboratories). Clean-up with ZipTip® C₁₈. Matrix: Sinapinic acid.

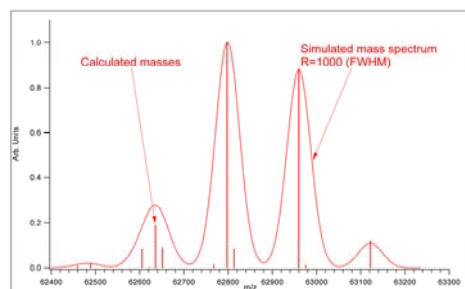


Fig. 3a: Simulated native Ricin.

Simulated molecular ion signal pattern based on N-glycan and protein data from [2] and [4], respectively. A resolution of $R=1000$ (FWHM) was assumed.

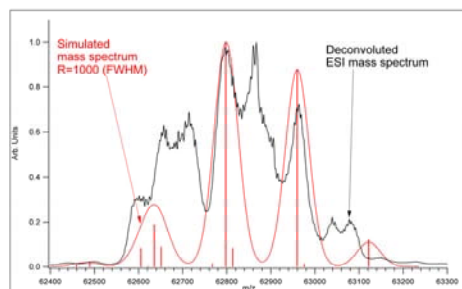


Fig. 3b: Native Ricin.

Deconvoluted LC/MS measurement of 100 ppm ricin (prepared in-house) compared to the computer simulation from Fig. 3a. Column: Discovery® HS C18.

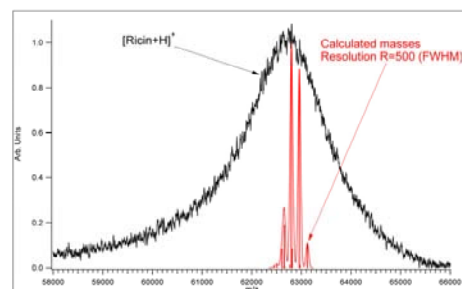


Fig. 3c: Native Ricin.

Simulated mass spectrum vs. MALDI-MS measurement of 1.0 mg/ml ricin (prepared in-house). Clean-up with ZipTip® C₁₈. Matrix: 2,6-DHA/DAC (10:1).

Experimental Conditions

LC/MS, Nanospray: QTrap®, Applied Biosystems/MDS Sciex Turbolonspray®, MDS Sciex Nanospray Ion Source. Agilent Series 1100 HPLC.

Column: Discovery® BIO Wide Pore C5 5μ or HS C18, 5μ, 150x2.1mm. Flow rate: 0.6 ml/min.

Eluent: A: H₂O, B: CH₃CN (0.5% formic acid), 0'-6': 45-70% B in A+B (lin.), 6'-7': 70-95% B in A+B.

MALDI-TOF-MS: Applied Biosystems Voyager DE.

Matrix: I) Saturated sinapinic acid in CH₃CN/H₂O (1:1). II) Saturated 2,6-Dihydroxyacetophenone (2,6-DHA) in H₂O (0.1% TFA) + 0.1 M Diammonium citrate (DAC).

Conclusions

- **N-glycans** of ricin contain no sialic acids: less complex mass spectra can be expected.
- **Glycan patterns** from [1] and [2] do not match well with LC/MS-Data (Fig. 1b, 2b, 3b). Mass difference found between theory and A- as well as B-chain.
- Glycan pattern of native ricin and of A- and B-chain only accessible with **ESI-MS**. "Fingerprint" identification not straightforward due to different ricin varieties and ambiguous deconvolution procedure.
- **MALDI linear TOF-MS:** Spectra questionable for ricin identification. Rapid screening possible.
- **Peptide mass fingerprint/Database** analysis mandatory for unambiguous identification of ricin.

Literature

- [1] D. Despeyroux, N. Walker, M. Pearce, M. Fisher, M. McDonnell, S.C. Bailey, G.D. Griffiths, P. Watts, *Analytical Biochemistry*, **279**, 23 (2000).
- [2] Y. Kimura, S. Hase, Y. Kobayashi, Y. Kyogoku, T. Ikenaka, G. Funatsu, *J. Biochem.*, **103**, 944 (1988).
- [3] Y. Kimura, H. Kusuoku, M. Tada, S. Takagi, G. Funatsu, *Agric. Biol. Chem.*, **54**, 157 (1990).
- [4] <http://www.expasy.org> (P02879, entry name: "RICI_RICCO").

