Significantly Improved in-solution Tryptic Digestion of Proteins

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Overview

- A novel mass spectrometry compatible denaturant, RapiGest™ SF, was applied as a solubilization additive for in-solution endopeptidase digestion of proteins.
- Our study shows that RapiGest™ SF does not inhibit trypsin activity and is degradable under acidic conditions to MS non-interfering products.
- Improved in-solution protein digestion in terms of speed and peptide coverage was observed.

Introduction

Rapid advancements in the mass spectrometry field have enabled more sensitive and accurate identification of proteins. The common strategy is to digest proteins with enzymes and analyze resulting peptides *via* mass spectrometric methods. Enzymatic digestion of complex proteins typically requires overnight in order to obtain optimum digestions. Surfactants are commonly used to disrupt the aggregation of hydrophobic biomolecules prior to enzymatic digestions; however, common denaturants such as SDS and Urea are disruptive to enzyme activity and interfere with mass spectrometry analysis. We employed a novel mass spectrometry compatible denaturant, RapiGest™ SF as an aid for enzymatic digestion of proteins. Unlike SDS and Urea, this denaturant was shown to be compatible with various enzymes. Drastic improvements of protein digestion in terms of speed and peptide recovery are observed. RapiGest™ SF rapidly breaks down to MALDI and LC/MS non-interfering products under low pH conditions, providing additional advantages for MS characterizations of proteins and peptides. Results obtained from the digestion of complex Yeast Ribosomal proteins fractionated from 2-D LC separation showed that more proteins were identified *via* MALDI Peptide Mass Fingerprinting (PMF) when the proteins were reconstituted in RapiGest™ SF.

Experimental

Trypsin Activity Assay

Trypsin activity measurements were carried out by introducing 1 µg/ml of trypsin to 0.25mM N-α-benzoyl-l-arginine ethyl ester (BAEE, pH 7.9) at room temperature [1]. Changes in trypsin activity caused by addition of various of denaturants were measured and compared (Table.1).

Mass Spectrometry Methods

0.1% (w/v) RapiGest™ SF was used to reconstitute proteins before enzymatic digestion. Enzyme to protein ratio used is from 1:100 to 1:50 (w/w). After digestion, RapiGest™ SF was destroyed rapidly by addition of strong acid such as HCl. The degradation products were removed by centrifugation prior to LC-MS analysis. MALDI is tolerant up to 0.25% RapiGest™ SF, hence, it is optional to remove this additive for MALDI-TOF analysis.

Protein digests were mass analyzed either by a Micromass M@LDI™ *R* TOF instrument (Waters) or separated by a RP-HPLC (CapLC®, Waters) then interfaced to an orthogonal acceleration Micromass LCT™ (Waters) *via* an electrospray ionization source. Yeast Ribosomal proteins fractionated from 2-D chromatography separation (cation exchange/reversed phase) were digested with trypsin in the presence or the absence of RapiGest™ SF prior to MALDI-TOF MS analysis (Fig. 3, 4).

Table1. Relative trypsin activity (%) was used to demonstrate the reduction in enzyme activity caused by the following denaturants. RapiGest™ SF (0.1%, 0.5%) causes the least inhibition in trypsin activity among all the denaturants listed. SDS (0.1%, 0.5%) inhibits trypsin activity the most.

	Control	RapiGest™ SF		SDS		Urea		MeOH
		0.1%	0.5%	0.1%	0.5%	2M	4M	50%
Trypsin Activity (%)	100	99	87	20	0.8	85	71	31

Figure 1. LC/MS chromatograms of tryptic digested Myoglobin. (A) Myoglobin solubilized in 0.1% RapiGest™ SF was observed to undergo complete tryptic digestion within 5 minutes. (B) Without the use of 0.1% RapiGest™ SF, Myoglobin was resistant to tryptic digestion. A majority of myoglobin was undigested after 9 hrs.

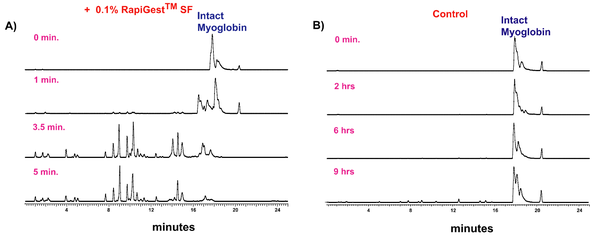


Figure 2. RapiGest™ SF is compatible with non-trypsin endopeptidases. Horse myoglobin (50 pmol/µl) was digested with Asp-N, Lys-C and Glu-C with and without 0.1% (w/v) RapiGest™ SF. (A) After 1 hr incubation at 37 °C with 0.1% RapiGest™ SF, no intact protein was left undigested. (B) Control experiment (no RapiGest™ SF) showed that majority of the myoglobin remained undigested.

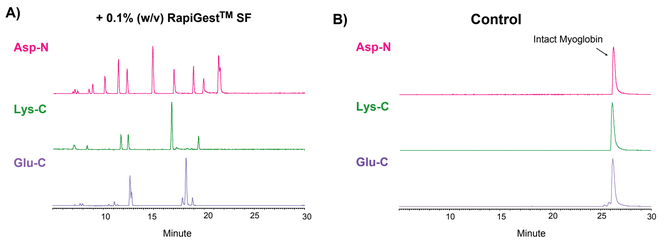


Figure 3. Yeast Ribosomal proteins were separated by multidimensional chromatography (IEX/RP) [2] coupled with online intact mass analysis on a ESI-TOF MS (A). Collected fractions were digested with Promega trypsin for 1 hour in the presence or absence of RapiGest™ SF (control) and analyzed by MALDI-TOF MS (B). Digests were mixed 1:1 with HCCA matrix, and spotted immediately without removing the RapiGest™ SF. MS data were analyzed with Micromass Protein Global Server™ v1.1 software (see Fig. 4 for the summary).

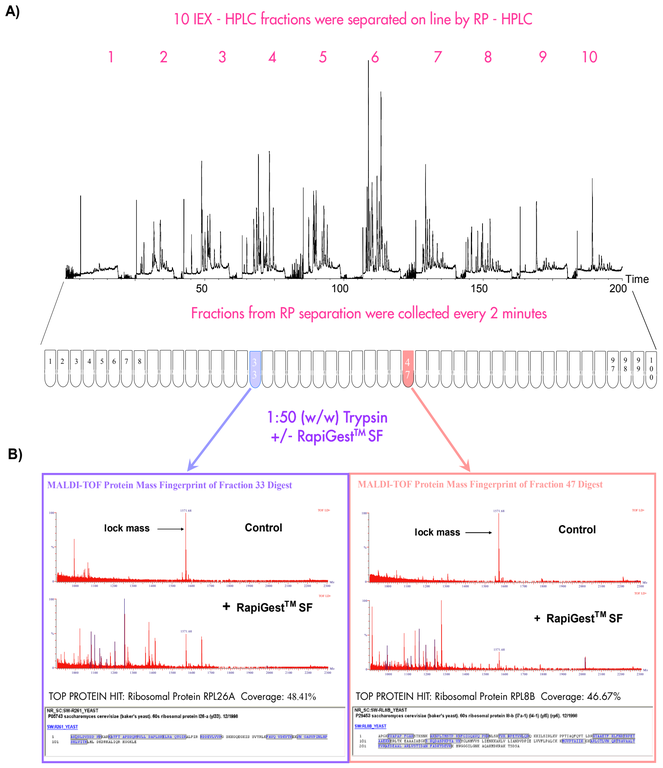
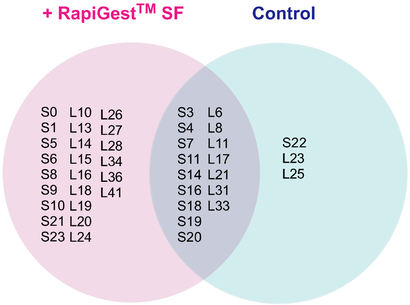


Figure 4. Identification of ribosomal subunits (proteins) was more successful with the use of RapiGest™ SF; 49 ribosomal subunits were identified. Control digestion (no RapiGest™ SF) resulted in identification of only 19 ribosomal subunits.

(S= Small Subunit, L=Large Subunit)



Conclusion

- RapiGest™ SF does not inhibit trypsin activity at moderate concentrations.
- RapiGest™ SF substantially reduces the time required for complete in-solution tryptic digestion of proteins.
- Simplified sample preparation prior to MS analysis.
- Degradation of RapiGest™ SF is optional for MALDI-TOF MS analysis.
- More successful protein identification of complex protein mixtures can be achieved with RapiGest™ SF

References

1. Schwert, G.W. and Takenake, Y. *Biochim. Biophys. Acta* 1955, 16, 570-574.
2. Hongji Liu, Scott J. Berger, Robert S. Plumb, and Steven A. Cohen, *J. Chromatogr., B, in press.*