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Improved Tryptic Digestion of Proteins *via* a Novel Solubilization Factor

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Overview

A novel reagent, RapiGest™ SF, is applied as a denaturant for in-solution tryptic digestion of proteins. RapiGest™ SF does not inhibit trypsin activity and is degradable under acidic conditions to MS non-interfering by-products.

Focus of Study

- Does RapiGest™ SF inhibit trypsin activity
- Time reduction of in-solution tryptic digestion of proteins with RapiGest™ SF
- Is RapiGest™ SF compatible with LC and mass spectrometric methods
- Improvement in the digestion of hydrophobic proteins with RapiGest™ SF

Introduction

Rapid advancements in the mass spectrometry field have enabled more sensitive and accurate identification of proteins. Hydrophobic proteins are troublesome to identify by mass spectrometric methods due to their low solubility. Surfactants are commonly used to disrupt the aggregation of hydrophobic biomolecules prior to enzymatic digestions; however, ionic surfactants such as SDS are disruptive to enzyme activity (*e.g.,* trypsin) and interfere with mass spectrometry analysis (1). We employed a novel reagent (RapiGest™ SF) as an aid for tryptic digestion of proteins. RapiGest™ SF acts as a denaturant like SDS without inhibiting trypsin activity. Results obtained from a trypsin activity assay (2) suggest that RapiGest™ SF does not inhibit trypsin activity even at very high concentrations. 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 by-products under low pH conditions, providing additional advantages for MS characterization of proteins and peptides.

Method

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. Changes in trypsin activity were plotted by measuring the rate of hydrolysis of BAEE to a UV active product, BA, at A_{252nm} (Fig. 1).

Mass Spectrometry Methods

Proteins (2 - 20 µM) were solubilized in 50 mM NH_4HCO_3 or 0.1 - 0.25% (w/w) RapiGest™ SF before tryptic digestion. Trypsin to protein ratio used was 1-2%. Bacteriorhodopsin (~17 µM) was solubilized with 8M urea or 0.25% RapiGest™ SF. After tryptic digestion, RapiGest™ SF was destroyed rapidly by addition of strong acid such as HCl or TFA. The by-products were removed by centrifugation prior to MS analysis.

Protein digests were mass analyzed either by a MALDI-TOF mass spectrometer (M@LDI™ *LR*, Micromass UK Ltd) or separated by RP-HPLC (CapLC®, Waters Corporation) then interfaced to an orthogonal acceleration TOF mass spectrometer (LCT™, Micromass UK Ltd) *via* an electrospray ionization source .

Results

Trypsin Activity

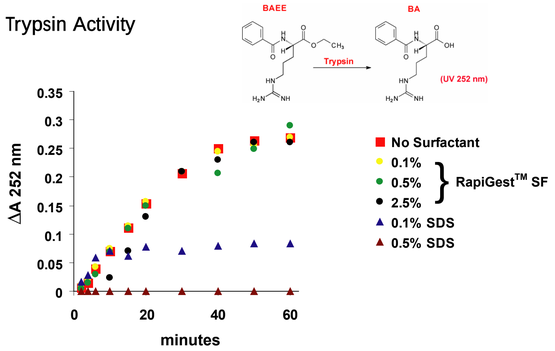

Figure 1. Trypsin cleaves BAEE to BA in the presence or the absence of denaturants. 0.1% to 2.5% RapiGest™ SF shows no inhibition in trypsin activity. On the contrary, trypsin activity was reduced drastically with 0.1% SDS. Further, trypsin activity was completely destroyed with 0.5% SDS.

Compatibility of RapiGest™ SF with MALDI-TOF

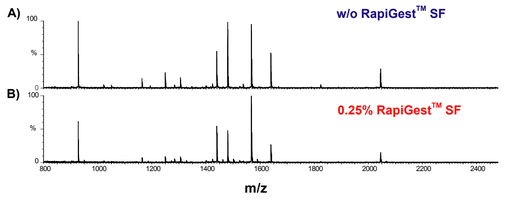

Figure 3. MALDI-TOF mass spectra of 500 fmol of BSA digest. **A)** No additives were introduced to the sample. **B)** 0.25% RapiGest™ SF was initially added then destroyed by the subsequent addition of 50 mM HCl. Signal intensities and the observed peptides are virtually identical.

Time Reduction of Tryptic Digestion

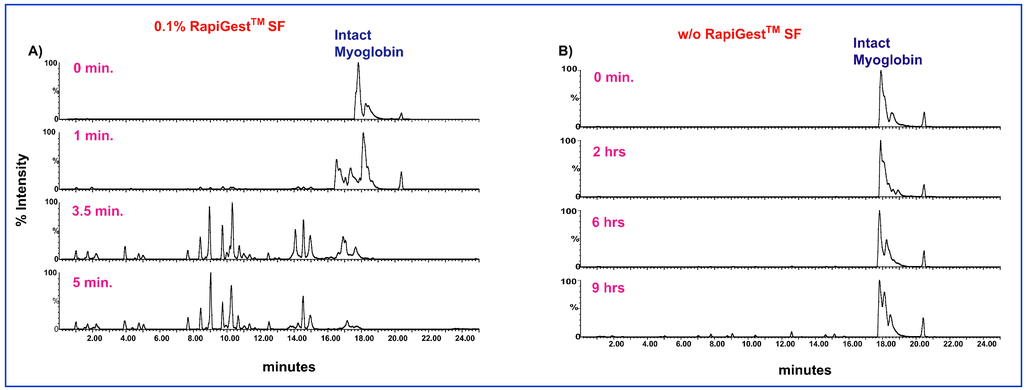

Figure 2. LC/MS TIC of tryptic digested myoglobin solubilized with 0.1% RapiGest™ SF or 50 mM NH_4HCO_3. **A)** Myoglobin treated with 0.1% RapiGest™ SF was observed to undergo complete tryptic digestion within 5 minutes. **B)** Myoglobin in 50 mM NH_4HCO_3 was resistant to tryptic digestion. A majority of myoglobin was undigested after 9 hrs.

Amino Acid Sequence Coverage Enhancement

	Amino Acid Sequence Coverage (%)	
Proteins	50 mM NH_4HCO_3	0.1% RapiGest™ SF
Myoglobin	0%	54%
Bovine Ubiquitin	0%	100%
Ovalbumin	0%	25%
Bovine Serum Albumin	23%	33%

Table 1. Amino acid sequence coverage of the above proteins were compared between two tryptic digestion conditions; digestion in 50 mM NH_4HCO_3 or with 0.1% RapiGest™ SF. The digestion time was 5 minutes. Data was collected by MALDI-TOF MS (mass range: 800 - 3000 amu).

Hydrophobic Proteins

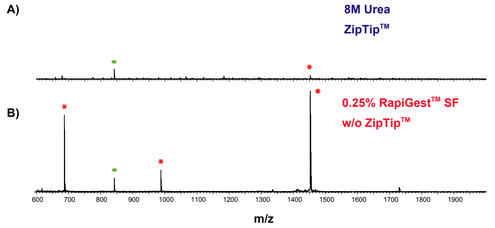

Figure 4. One hour tryptic digestion of Bacteriorhodopsin was mass analyzed by MALDI-TOF MS. Bacteriorhodopsin peptides were labeled as ⋆, trypsin autolysis peaks were labeled as . **A)** Bacteriorhodopsin was solubilized with 8M urea prior to dilution (to 2M) and subsequent tryptic digestion. The digested peptides were desalted with a Millipore ZipTip™. **B)** 0.25% RapiGest™ SF was used to solubilize Bacteriorhodopsin prior to tryptic digestion. The digested sample was spotted directly onto a MALDI plate after the RapiGest™ SF was degraded *via* addition of 50 mM HCl.

Conclusion

- Unlike the commonly used ionic surfactant SDS, RapiGest™ SF does not inhibit trypsin activity
- RapiGest™ SF drastically reduces the time required for an optimum in-solution tryptic digestion of proteins
- RapiGest™ SF decomposes to MS (LC/MS and MALDI-TOF) non-interfering by-products upon acidification
- RapiGest™ SF facilitates the denaturing of hydrophobic proteins and simplifies the sample cleanup

References

1. Rosinke, B.; Strupat, K., Hillenkamp, F.; Rosenbusch, J., Dencher, N.; Kruger, U. and Galla, H. *J. Mass Spectrom.* **1995**, 30, 1462-1468.
2. Schwert, G.W. and Takenake, Y. *Biochim. Biophys. Acta* **1955**, 16, 570-574.