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ACCELERATED ENDOPEPTIDASE DIGESTION OF PROTEINS EMPLOYING AN HPLC AND MS FRIENDLY SURFACTANT

John C. Gebler, Ying-Qing Yu, Martin Gilar, Peter J Lee, Weibin Chen, Amy E Daly
Waters Corporation, Milford MA 01757

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Overview

Endopeptidase digestion of proteins into peptides followed by MALDI-MS or LC/MS/MS analysis is critical for protein identification. The use of proteolytic enzymes such as trypsin is well defined and considered a standard protocol. Nevertheless, a number of challenges remain such as sluggish protein digestion, incomplete digestion (low peptide yield), miscleavages, or proteolytic resistance. Several works have been published describing methods to overcome these deficiencies by addition of denaturants (urea, organic solvents), application of heat, and so forth. Generally, it is the tertiary structure of the proteins that makes them proteolytically resistant. The addition of strong denaturants such as SDS does disrupt protein folding, however, this often results in rapid loss of enzymatic activity of the endopeptidase. Also, most denaturants are difficult to remove and interfere with mass analysis and RP HPLC separations. In this work we report on an acid labile surfactant that does not suppress endopeptidase activity and is easily removed prior to mass analysis or RP HPLC. We have observed up to a 2-orders of magnitude improvement in digestion speed (example 20 hours to 20 mins).

Introduction

- Modern mass spectrometric technologies enable more sensitive and accurate identification of proteins.
- The common strategy is digesting proteins with enzymes to peptides followed by mass analysis.
- Obtaining complete enzymatic digestion of proteins or complex protein mixtures is very challenging.
- Surfactants are commonly used to disrupt the aggregation of hydrophobic biomolecules, but are often disruptive to enzyme activity and suppress ion-signals during mass spectrometry analysis.
- We employed a novel HPLC and mass spectrometry compatible surfactant, RapiGest™ SF, as an aid for enzymatic digestion of proteins.
- Unlike SDS and Urea, this surfactant was shown to be compatible with trypsin and other enzymes.
- Significant improvements of protein digestion in terms of speed and peptide recovery were observed.
- Upon acid treatment the surfactant rapidly breaks down to MALDI and LC/MS non-interfering products.
- At low concentrations (< 0.05%, w/v), RapiGest SF does not pose significant interferences.

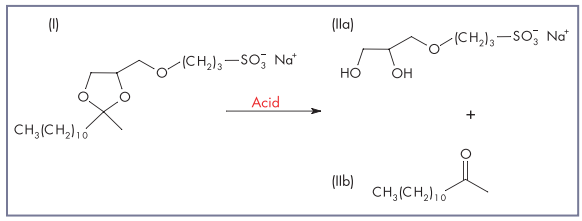

Figure 1. RapiGest (I) and acid degradation products (IIa) and (IIb).

Methods

Trypsin Activity Assay

Trypsin activity measurements were carried out with a colormetric assay which followed the hydrolysis of N-α-benzoyl-L-arginine ethyl ester (BAEE, pH 7.9) at room temperature (Table.1).

Trypsin Digestion of Proteins

0.05% to 0.1% (w/v) RapiGest SF was used to reconstitute proteins before enzymatic digestion. Trypsin to protein ratio was 1:100 to 1:50 (w/w). Digestion with trypsin was done under standard conditions (ammonium bicarbonate buffer and incubation at 37 °C). After digestion, the surfactant was destroyed by the addition of strong acid such as HCl, TFA, or formic (half life, 7 min at pH 2.0). The degradation products were removed by centrifugation prior to mass analysis or

chromatography. Resulting peptides from digestion were analyzed with a MALDI-TOF MS instrument (Waters® Micromass® MALDI R or L/R) or LC/MS with a capillary RP-HPLC (Waters CapLC®) interfaced to an orthogonal acceleration ESI-TOF MS instrument (Waters Micromass LCT™).

Results

Additive	None	RapiGest		SDS		Urea		MeOH
Amount	—	0.1%	0.5%	0.1%	0.5%	2M	4M	50%
Trypsin Activity	100%	99%	87%	20%	1%	85%	71%	31%

Table 1. Relative trypsin activity (%) in the presence of different denaturants.

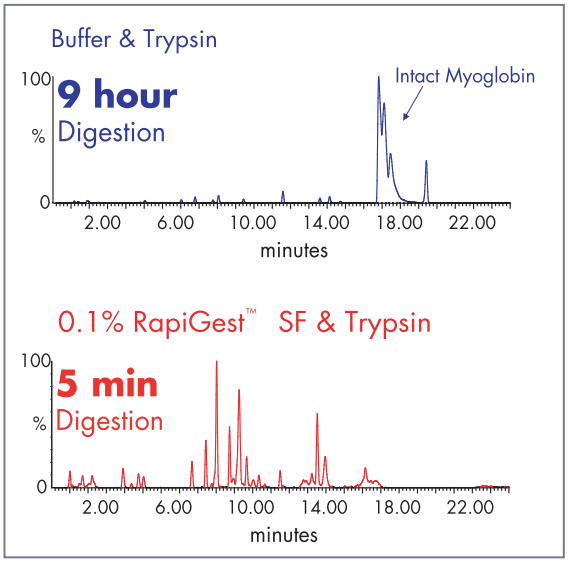

Figure 2. Accelerated digestion in the presence of the denaturant RapiGest. Control in buffer/trypsin (Blue) and RapiGest/trypsin (Red). Data presented at TIC chromatograms (LC/ESI-TOF).

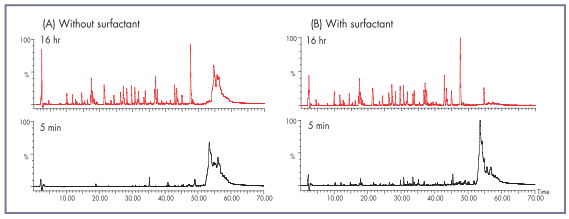

Figure 3. Spectra of peptides derived from trypsin digestion of Enolase with and without RapiGest. (A) Without the surfactant, after 16 hours some undigested Enolase remains. (B) With RapiGest, after 16 hours the intact Enolase has been consumed. Results were similar for the digestion of BSA, Bovine Hemoglobin, ADH, and Phosphorlase B.

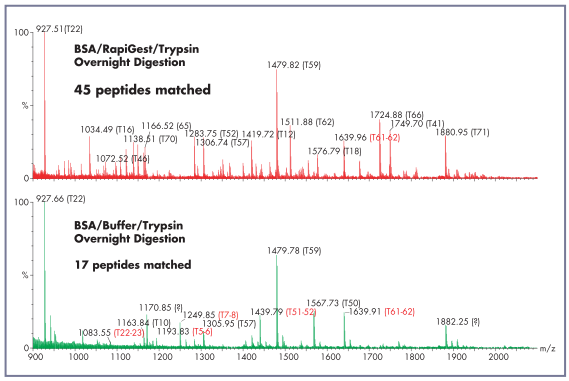

Figure 4. MALDI analysis of trypsin digested BSA. Red, results of digestion with RapiGest. Green, results of digestion in buffer (no RapiGest). Note difference in number of peptides matched from database searching. The number of miscleavages (peptides masses labeled red) is reduced when the surfactant is employed.

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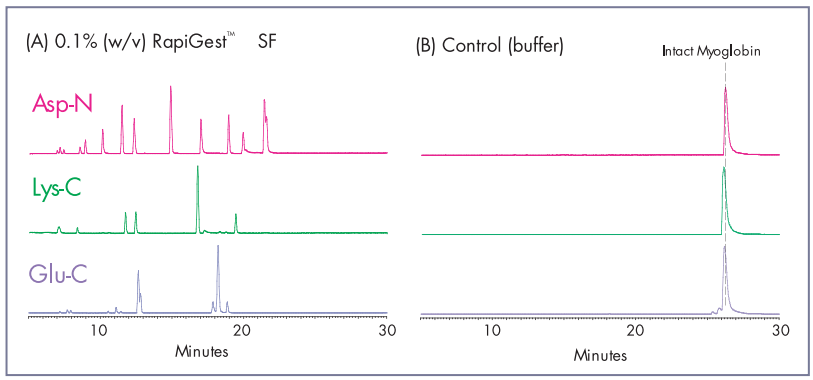

Figure 5. RapiGest SF is compatible with non-trypsin endopeptidases. Horse Myoglobin (50 pmol/µl) was digested with Asp-N, Lys-C and Glu-C with and without 0.1% (w/v) RapiGest SF. (A) After 1 hr incubation at 37 °C with 0.1% surfactant, no intact protein was left undigested. (B) Control experiment (buffer only) showed that majority of the Myoglobin remained undigested.

Conclusions

- RapiGest SF does not inhibit endopeptidase 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 LC and MS analysis. The surfactant does not interfere with LC/MS analysis in low quantities
- Improves the enzymatic digestion of membrane proteins.
- RapiGest simplifies digestion and sample handling

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

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