Selective Derivatization of Cysteines for the Enhancement of UV Photodissociation of Peptides

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EXPERIMENTAL
Derivatization
Derivatization by MCB was performed by incubating a 1:1 mole ratio of MCB peptide in 10μM HFIP/HEPES buffer for 5 minutes at room temperature.

Derivatization by ABD-F was carried out with a 1:1 mole ratio of ABD-F peptide in HFIP at room temperature. Following the derivatization, a small aliquot of the sample was removed from excess reagent by using C18 spin columns and dried for ESI mass analysis.

Mass Spectrometry
Samples were spotted on a 100 μl concentration via ESI on a ThermoFinnigan LCQ Deca instrument equipped with a CapNANO source. Photolyzed at both 355 and 308 nm were performed using an 8 ns pulse at 20 Hz.

RESULTS AND DISCUSSION
Reduction of Disulfide Bonds
MCB and ABD-F will selectively and efficiently react with the free sulfydryl groups of cysteines. Peptides, if denatured earlier in order to inhibit thiol oxidation, are reacted with either reagent (Figure 2A). However following a derivatization (DTT) selection of the peptides, all peptides were modified by the MCB or ABD-F reagents resulting in mass shifts of 191 Da on 150 Da, respectively. The number of adducts parallel the number of cysteines in each peptide.

UVPD versus CID of ABD-F Modified Peptides
The ABD-F derivatization proved extremely efficient, yielding peptides with all cysteines modified. The resulting modified peptides were characterized by CID and UVPD in order to determine whether the modified ion could be easily detected and the impact of the derivatization on the overall peptide fragmentation pattern. By comparing UVPD and CID of unmodified and modified peptides, it can be observed that some peptides are not detected by UVPD whereas they are detected by CID, suggesting that the UVPD reaction is sensitive to the presence of the derivatization reagent (Figure 3B).

UVPD versus CID of MCB-Modified Peptides
The MCB reaction was also extremely efficient, yielding modified peptides with large abundances in the UV mass spectra. These peptides were also analyzed by CID and UVPD and the results are shown in Figure 4. Comparing UVPD and CID of the unmodified and modified peptides, it can be observed that the UVPD reaction was not sensitive to the presence of the derivatization reagent. This suggests that the derivatization reagent is not detected by UVPD and that the UVPD reaction is insensitive to the presence of the derivatization reagent.

CONCLUSIONS
By selecting the proper MCB and ABD-F derivatization reactions with UVPD and CID, all the disulfide peptides can be photolyzed. In general, the UV photolysis reaction is most effective in the unmodified peptides. UVPD of modified peptides is somewhat more sensitive to the presence of the derivatization reaction, which can be observed in some cases.

REFERENCES

Figure 1. Sequence Coverage Comparisons for CID and UVPD of ABD-F: Sematostatin.

Figure 2. Sequence Coverage Comparisons for CID and UVPD of ABD-F: Sematostatin.

Figure 3. Sequence Coverage Comparisons for CID and UVPD of ABD-F: Sematostatin.

Figure 4. Sequence Coverage Comparisons for CID and UVPD of ABD-F: Sematostatin.

Figure 5. Sequence Coverage Comparisons for CID and UVPD of ABD-F: Sematostatin.