DEST: A Novel Amidinating Protein Cross-linking Reagent

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Introduction

Advancing technologies in mass spectrometry, including high resolution and high mass accuracy, have led to a resurgent interest in chemical cross-linking as a method of studying protein structures and interactions, because it is now possible to identify peptide cross-links formed from proteolysis of derivatized protein samples with reasonably high confidence.1, 2

Nevertheless, analytical challenges hinder what can currently be accomplished. For example, the detection of cross-linked peptides in the proteolytic digests of derivatized proteins is often impaired by the combination of their low stoichiometric yield and the presence of other peptide species. Development of a simple and efficient technique for enriching cross-linked peptides from all components of proteolytic digests, including dead-end modified peptides, is therefore worth pursuing. Another challenge in cross-linking is ensuring that the reaction of a particular covalent probe does not have a deleterious effect on the stability of native protein structure. Amine-reactive succinimidyl esters are frequently used even though their labeling eliminates the native basicity of amines and thereby potentially disturbs native protein structure. Although amine-reactive imidates introduce basic amine modifications, these reagents are effective only at alkaline pH, so their use may perturb native protein structure.

Each of these shortcomings is addressed by the use of a new protein cross-linking reagent, known as DEST (diethylsuberthioimidate).

Method

Diethyl suberthioimidate (DEST) was prepared from ethanethiol and Diethyl suberonitrile (9 mmol) in anhydrous dichloromethane (1:3 v/v) was added to Suberonitrile (9 mmol) in anhydrous dichloromethane (1:3 v/v) was added to

SCX Enrichment of DEST Cross-links

Interpeptide DEST cross-links were separated from other components of a trypic digest using SCX chromatography. Since the basicity of amines is maintained upon amidation, interpeptide DEST cross-links contain six strongly basic residues. They therefore adsorb more strongly to SCX material than unmodified and dead-end modified peptides, which contain only 2 and 3 strongly basic residues, respectively.

Conclusion

We have introduced a novel bifunctional thioimidate cross-linking reagent (diethyl suberthioimidate, DEST) that modifies amines without sacrificing their basicity. Study of a model system by LC-MS demonstrated that DEST is effective under physiological conditions. This reagent is therefore a compelling alternative to imidate and succinimidyl ester reagents for structural studies, because it does not require alkaline pH for reactivity, does not perturb the electrostatic properties of a protein, and is thus less likely to lead to artificial conclusions about native protein structure.

The reagent also has a particularly attractive analytical characteristic in that the interpeptide cross-links it forms can be readily detected by LC-MS/MS and easily separated from other components of tryptic digests using strong cation exchange chromatography. The use of this novel amidinating protein cross-linking reagent holds great promise for large-scale structural analysis of complex systems.

References