Unique Fragmentation of DEST Cross-Linked Peptides Facilitates Their Identification

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OVERVIEW

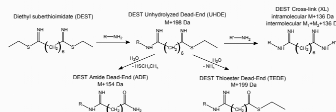
- Unique fragmentation of DEST cross-linked peptides
- New algorithm for identifying DEST cross-linked species

INTRODUCTION

Chemical cross-linking combined with mass spectrometry has been used as an alternative approach to elucidate protein structures and interactions.[1-3] However, mass spectrometric identification of the cross-linked peptides is often hampered by both the complexity of the created peptide mixture and the complicated fragmentation of cross-linked species. Previously, we developed a novel cross-linking reagent, diethyl suberthioimidate (DEST), that yields amidinated residues after reaction and preserves the charges on primary amines.[4] In some proton deficient cases, cross-linked peptides were selectively dissociated at the amidinated sites. This feature can be beneficial for identifying cross-linked species. In this work, we studied the fragmentation of DEST cross-linked peptide ions produced by MALDI and observed that there was preferential cleavage at the cross-linked sites upon CID. A new algorithm was designed to identify cross-linked peptides based on this feature of their MALDI MS/MS spectra.

METHODS

Horse heart cytochrome c was prepared at 5μM. The reaction was carried out in a physiologically relevant buffer with DEST present at a 100:1 reagent to protein ratio for 12 hours at room temperature. The reaction was quenched by the addition of Tris. The cross-linked proteins were digested with trypsin in a solution containing Rapigest. After enrichment via SCX chromatography, cross-linked peptides were separated by nanoscale reverse-phase liquid chromatography and then directly spotted onto a MALDI plate using a robot. Peptide ions were created, isolated and fragmented by CID on an MALDI-LTQ XL mass spectrometer. Cross-linked species were identified from their spectra by manual interpretation and reference to previous work.[4] A new algorithm was developed to facilitate the identification of DEST cross-linked species based their unique fragmentation pattern.



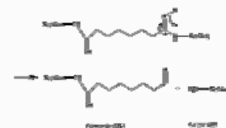
Scheme 1. Major reaction products of DEST cross-Linking

RESULTS

1. Unique Fragmentation of DEST Cross-linked Peptides

Amidine bonds are known to be subject to reversal when deprotonated by extremely basic solutions (i.e. pH >12)[5]. We believed that fragmentation of the amidine bonds of a cross-link would become more significant in MS/MS spectra of proton-deficient species, such as those produced by MALDI.

The proposed mechanism for this fragmentation process is demonstrated in Scheme 2. In theory, both of the amidine bonds of the linkage in cross-linked peptides can be cleaved and four different fragments can be formed: α peptide, α+XL, β peptide, and β + XL.



Scheme 2. Mechanism for the preferential cleavage at the amidine cross-link bond

2. Mass Spectrometric Analysis of Cross-Linked Peptides

Figure 1 is an MS/MS spectrum of [73]K*YIPGTK [79] cross-linked to [54]NK*GITWK [60] in different charge states. Preferential cleavages at the amidine bonds of a cross-link are observed when the peptide is singly charged. α+XL and β + XL are the most intense fragments in the MS2 spectrum

α+XL and β + XL can be further isolated in the trap for MS3 experiments. Cross-linked sites can be confirmed from their MS3 spectra (displayed in Figure 2).

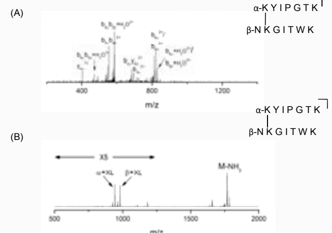


Figure 1. MS/MS spectra of the same cross-linked peptide (A) in ESI and (B) in MALDI

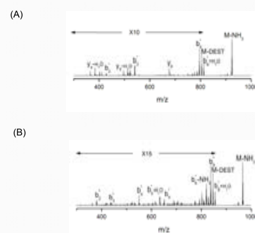


Figure 2. MALDI-LTQ MS/MS/MS spectrum of (A) α+XL (K*YIPGTK) and (B) β+XL (NK*GITWK)

3. Fragment Ion Intensities

Four kinds of fragments can be formed by cleavage of the cross-link bonds: α peptide, α +XL, β peptide, and β + XL. The appearance of these fragment ions and their intensities in MS2 are mainly determined by the fragment basicities.

- α+XL and β+XL are always observed in MS/MS spectra due to their amidinated residues at cross-linking sites.
- α and β are only seen occasionally when arginine is in the peptide sequence (shown in Figure 3)
- Occasionally only one peptide chain is observed when this chain contains many basic residues (shown in Figure 4)

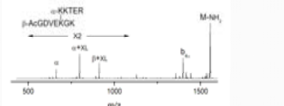


Figure 3. MALDI-LTQ MS/MS/MS spectrum of [87] K*KTER [91] cross-linked to [1] AcGDVEK*GK [7]

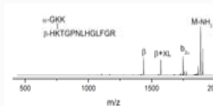


Figure 4. MALDI-LTQ MS/MS/MS spectrum of [6]GK*K[8] cross-linked to [26]HK*TGPNLHGLFGR[38]

4. New Algorithm

We developed a simple algorithm for identifying cross-linked peptides based on the unique fragmentation of DEST cross-linked peptides that searches through all the precursor masses and corresponding MS/MS peak lists. Any precursor associated with fragment peaks that match Equations 1 and 2 will appear in the search result.

$$m(\alpha+XL) + m(\beta+XL) - m(\text{precursor}) = 137.10733 \quad (1)$$

$$m(\alpha+XL) - m(\alpha) = 136.10005 \quad (2)$$

The cross-linked peptides that were manually identified are listed in Table 1. Eight of the cross-linked peptides (highlighted) were quickly uncovered using our algorithm.

Table 1 Identified Cross-linked Peptides

m/z	α+XL	β+XL	cross-link
1184.818	KK (411.283)	AcGDVEKGK (910.454)	K87-K5
1312.700	KK (411.283)	AcGDVEKGKK (1038.490)	K87-K5
1474.727	KTER (669.455)	KYIPGTK (942.636)	K88-K73
1570.800	KKTER (797.545)	AcGDVEKGK (910.545)	K87/88-K5
1575.888	KTER (669.437)	MIFAGIKK (1043.544)	K88-K86
1698.991	KKTER (797.460)	AcGDVEKGKK (1038.512)	K87/88-K5
1788.037	KYIPGTK (942.498)	NKGITWK (982.583)	K73-K55
1760.958	KTER (669.314)	EDLIAYLKK (1228.564)	K88-K99
1849.065	KYIPGTK (942.475)	MIFAGIKK (1043.562)	K73-K86
1888.987	KKTER (797.443)	EDLIAYLKK (1228.63)	K87/88-K99
1901.010	GKK (468.230)	HKTGPNLHGLFGR(1569.780)	K7-K27
2666.272	MIFAGIKK (1043.607)	EETLMEYLENPKK (1759.830)	K86-K72
3264.708	KYIPGTK (942.480)	NKGITWKEETLMEYLENPK (2459.182)	K73-K55

- The use of Equation 1 worked most efficiently for the identification of cross-linked peptides
- Most of the cross-linked peptides identified in MALDI-LTQ are in the mass range of 1000-2000 Da.

CONCLUSIONS

- Singly-charged, collisionally activated DEST cross-linked peptides were found to undergo preferential cleavage at cross-linking sites.
- Intense peaks for α+XL and β+XL product ions were observed in most cases. The intensities of α+XL and β+XL are mainly dependent on the basicities of the individual peptide chains.
- A new algorithm was developed for the facile identification of cross-links based on this unique fragmentation propensity.

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