Probing Protein-Protein Interactions with Chemical Cross-linking and Differential Nano-LC-MS

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Most cellular processes are mediated by the interaction networks of macromolecules[1]. An understanding of the recognition between macromolecules is the first step in deciphering cell machinery and protein interaction networks. Strategies based on chemical cross-linking have the potential to detect components and analyze the spatial organization of the interacting protein assemblies[2]. In addition, the rigorous-identification of the cross-linked peptides by mass spectrometry can pinpoint the regions involved in the protein interaction interface. However, to date the unambiguous identification of cross-linked peptides has been hard to achieve, due to cross-linker reactivity at multiple sites, low stoichiometry of cross-linking reactions, and the interference of surface modifications and intramolecular cross-links. Such studies will be even more difficult for homo-molecular complexes, since the intermolecular and intramolecular cross-links can not be distinguished by the sequence of cross-linked peptides.

To facilitate mining the cross-linked peptides from normal tryptic peptides, nonspecifically cleaved peptides, cross-linker modified peptides and other contaminants, MS-Bridge, a new program in our ProteinProspector package was developed as an aid to find the mass values that may indicate the putatively cross-linked peptides in the protealytic digestion mixture of cross-linked protein complex. Taking advantage of the power of on-line capillary HPLC with nanoelectrospray ionization using a QqTOF mass spectrometer, a generic methodological strategy using chemical cross-linking and differential nano-LC-MS has been established to probe protein-protein interface with high sensitivity and accurate structural details.

GGH-ecotin D137Y was used as a model protein and cross-linked by primary amine reactive cross-linker, disuccinimidyl suberate (DSS). Covalently cross-linked dimer was separated from monomer by reducing 1D SDS-PAGE. Both dimer and monomer bands were cut out and in-gel digested with trypsin. The tryptic digestion mixtures were analyzed by nano-LC-MS. The LC-MS runs of both the dimer and monomer tryptic digestion mixtures were thoroughly compared in searching for cross-linked peptides. In this way, the normal tryptic peptides, surface modified peptides and intramolecular cross-linked peptides were excluded, and the additional cross-linked peptides can be correctly assigned as the intermolecular cross-links on the protein interface. After pre-selection for cross-linked peptides by MS-Bridge, the putatively cross-linked peptides were subjected to collision-induced-dissociation (CID), and the resulting sequence information not only identified the peptides involved in the cross-links but unambiguously established the location for each member of the cross-links in the protein dimer. As shown in Fig.1, the peptide at 2680.44 (670.85^{4+} and 894.46^{3+}) was the dominant cross-linked species in the dimer, and the cross-linking sites G-3 and K135 could be established based on the sequence information provided by low-energy CID spectrum (Fig.2). This is consistent with our GGH-Ni (II) oxo-complex cross-linking studies on the protein[3]. In addition, three other intermolecular cross-linked peptides have been identified corresponding to two more pairs of cross-links, namely K131-K135 and K126-K135. A retrospective analysis of the wild-type ecotin X-ray crystal structure has shown that the cross-linked residues are all on the dimeric interface. The sequence overlapping in the peptide at 2318.23, corresponding to cross-linking pair K131 and K135, elucidated that the cross-link is indeed formed on the dimer interface, thus further validating our strategy in searching for intermolecular cross-linking. Differential MALDI-MS of dimer and monomer digestion mixtures was examined on the same samples for differential nano-LC-MS, and four out of seven peptides

were proven to be false positives. Having established those optimal conditions, we applied our method to GRP94, which is the endoplasmic reticulum analog of hsp90 and exists as homodimer under physiological conditions. One cross-linked peptide has been identified corresponding to the cross-link between K547 and K663. The cross-linked residues are within the C-terminal domain of the protein and very close to the GRP94 dimerization-domain, representing amino acids 676-719[4].

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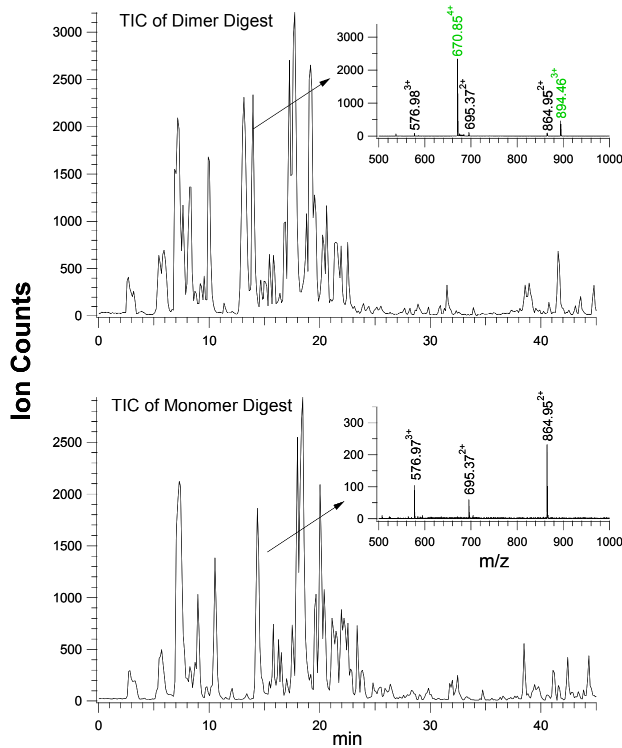

Fig.1. Differential nano-LC-MS-MS in searching for cross-linked peptides

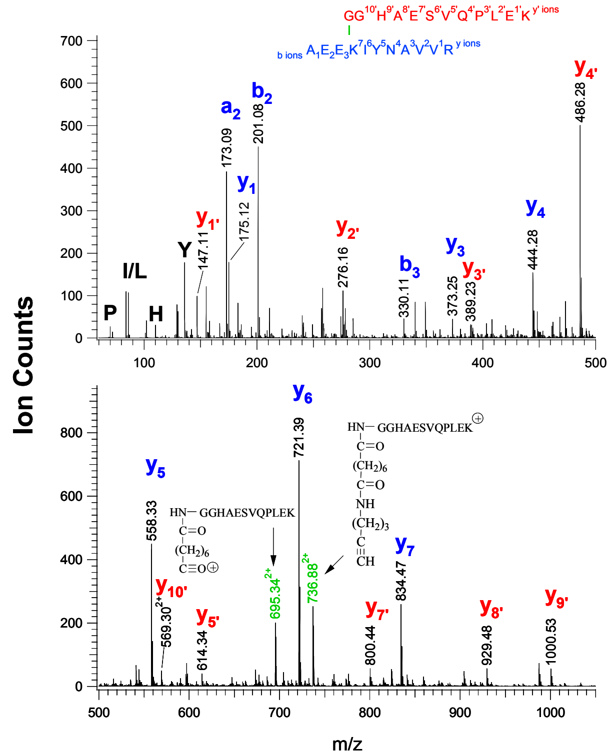

Fig. 2. CID spectrum of cross-linked peptide at 2680.37