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Elucidating protein inter- and intramolecular interacting domains using chemical cross-linking and matrix-assisted laser desorption ionization–time of flight/time of flight mass spectrometry

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ABSTRACT

Among many methods used to investigate protein/protein interactions, chemical cross-linking combined with mass spectrometry remains a vital experimental approach. Mapping peptides modified by cross-linker provides clues about proteins' interacting domains. One complication is that such modification may result from intra- but not intermolecular interactions. Therefore, for overall data interpretation, a combination of results from various platforms is necessary. It is postulated that the secretory isoform of gelsolin regulates several biological processes through interactions with proteins such as actin, fibronectin, vitamin D-binding protein, and unidentified receptors on the surface of eukaryotes; it also has been shown to self-assemble eventually leading to the formation of homo-multimers. As such, it is an excellent model for this study. We used four cross-linkers with arm length ranging from 7.7 to 21.7 Å and MALDI-TOF/TOF mass spectrometry as the analytical platform. Results of this study show that MALDI-based mass spectrometry generates high quality data to show lysine residues modified by cross-linkers and combined with existing data based on crystallography (Protein Data Bank, PDB) can be used to discriminate between inter- and intramolecular linking.

A variety of methods are used to study three-dimensional structures of proteins, their self-assembly into homo-polymeric complexes, and interactions with other proteins and nonproteinaceous molecules. Methods used for such studies include X-ray crystallography, fluorescence resonance energy transfer (FRET),[1] chemical cross-linking, mass spectrometry [1] also with ion mobility techniques [2], circular dichroism (CD), and atomic force microscopy [3], as well as a combination of these methods [4], each being complementary with specific strengths and limitations [5–12]. For example, FRET can be used if the proximity of two fluorescent groups is between 10 and 100 Å; however, it requires labeling of proteins [13]. X-ray crystallography requires growing of crystals and can be affected by disorder in unit cell or distortion in crystallization.

Chemical cross-linking has been utilized since the 1940s as a tool to study protein complexes, structural features, and stability as well as for practical applications such as formation of toxoids [14,15]. Recently, chemical cross-linkers regained interest. Cross-linking reactions combined with mass spectrometry can be used in rapid detection of interacting domains of proteins [16]. Further development of cleavable and noncleavable cross-linkers of various lengths that target specific chemical groups, advancement in high-throughput crystallography [17], and mass spectrometry of proteins and peptides allowed a much broader application of cross-linking to address questions related to protein–protein interactions, changes in three-dimensional structures, etc. [18].

Matrix-assisted laser desorption ionization (MALDI) is a soft ionization technique widely used in proteomic studies [19]. Moreover, MALDI-TOF (time of flight) mass spectrometry is broadly applicable for investigating many posttranslational modifications and protein/peptide quantitation, including isobaric tags for relative and absolute quantitation (iTRAQ), formylation of serine (Ser) and threonine (Thr) residues [20], protein modifications induced by polyacrylamide gel [21], and various aspects of signal transduction [22]. Although development of electrospray ionization-based mass spectrometry unquestionably advanced the application of this type of ionization to study posttranslational modification, MALDI-TOF/TOF with collision-induced dissociation (CID) remains one of the major platforms for studying protein and protein/peptide drug modification in biopharmaceutical research [23]. Furthermore, new cross-linkers such as the α-cyano-4-hydroxycinnamic acid (CHCA)-tagged cross-linker JMV 3378 have been developed to selectively enhance MALDI-TOF signal and compare spectra using

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[1] *Abbreviations used:* ACN, acetonitrile; cGSN, cytoplasmic GSN; CHCA, α-cyano-4-hydroxycinnamic acid; DTT, dithiothreitol; FRET, fluorescence resonance energy transfer; GSN, gelsolin; MALDI, matrix-assisted laser desorption ionization; pGSN, plasma GSN; TFA, trifluoroacetic acid; TOF, time of flight.

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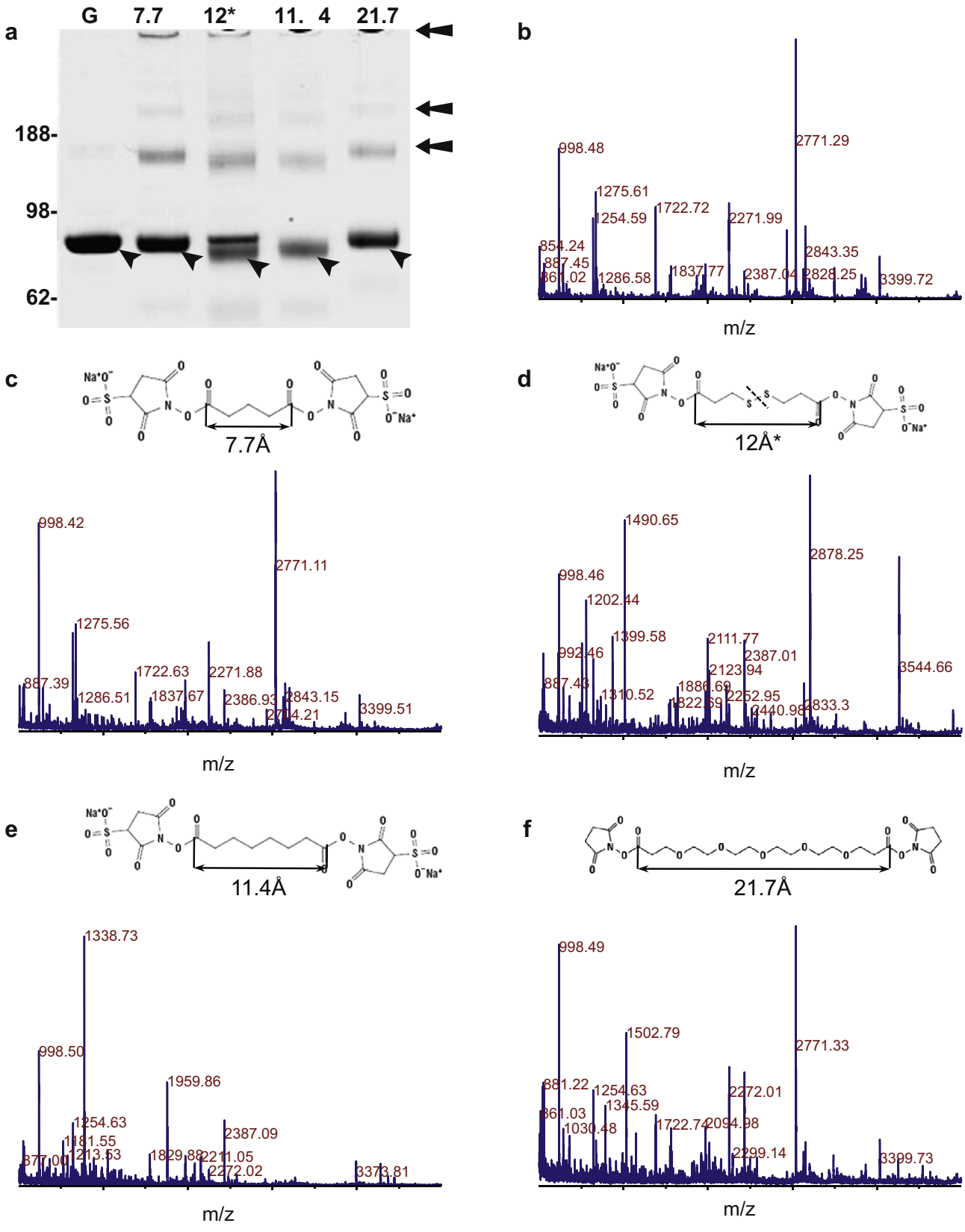

Fig.1. Chemical cross-linking of human pGSN. (a) 1-DE analysis of native human pGSN purified from human plasma (G). Lanes represent 1-DE analysis of the same preparation of pGSN after chemical cross-linking with four linkers. Chemical structures and space lengths of respective linkers are presented in panels c–f. (b) Representative MALDI-TOF spectrum of pGSN tryptic digests. Panels c–f represent MALDI-TOF spectra of tryptic digests of pGSN after cross-linking with respective linkers showing significant changes in MALDI-TOF spectra resulting from cross-linking.