Design and Synthesis of Protein Chemical Crosslinkers: A Modular Approach

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Contents

Abstract

The study of protein structure and interactions is pivotal in understanding the function and malfunction of complex biological systems. The structures of some proteins are unable to be determined using traditional high resolution biophysical techniques, requiring the development of amenable low resolution alternatives. Chemical Crosslinking Mass Spectrometry (CXMS) is one technique which can be used to probe protein structure through the formation of covalent linkages between protein residues. The formation of these links is facilitated by chemical crosslinking reagents.

Widespread use of the CXMS technique has been hampered primarily by analytical challenges pertaining to the detection and identification of crosslinked species using Mass Spectrometry (MS). Attempts to mitigate the challenges have been made by modifying the structure of chemical crosslinkers through the addition of functional groups such as affinity tags, isotope labels and cleavable bonds. Crosslinkers combining more than one type of functional group (combination crosslinkers) present the most promising targets for CXMS applications, combining the benefits of each functional group. However, combination crosslinkers are not commercially available, thus necessitating in-house synthesis. Incorporating more than one functionality also results in more complex molecular structures and synthetic processes, making the crosslinkers difficult to adapt to suit a particular experiment. Consequently, the use of combination crosslinkers has been limited to date to a small number of studies.

The research presented in this thesis describes the development of a modular chemical crosslinker design and corresponding synthetic protocol for the synthesis of combination crosslinkers. The modular crosslinker structure can be readily modified to include a range of functional groups using a small number of different reactions, including amide coupling and O-alkylation, and commercially available starting materials such as Boc-serine, from a minimum of five synthetic steps. The utility of the synthetic process was validated through the synthesis of a crosslinker containing an alkyne functional group, which can be used to attach a biotin affinity tag through alkyne-azide Huisgen Cyclisation.

Synthesis of two custom designed combination crosslinkers utilising alkyne tags and cleavable bonds is also described. The function of the cleavable bonds was established using collision induced dissociation processes within the mass spectrometer. Ensuring that a crosslinker is effective in probing quaternary structure and protein-protein interactions is essential as the investigation of these structures is a major goal of CXMS. Therefore, a crosslinking assay using *Staphylococcus aureus* biotin protein ligase, which forms homodimers when substrate bound, was also developed using the commercially available crosslinkers Disuccinimidyl Suberate (DSS) and Dithiobis(succinimidyl) Propionate (DSP), to enable the efficacy of crosslinkers synthesised using the modular synthetic protocol to be determined.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Abbreviations

Arom	Aromatic Chemical Shift
ATP	Adenosine Triphosphate
Boc	tert-Butyloxycarbonyl
BPL	Biotin Protein Ligase
CID	Collision Induced Dissociation
Cryo-EM	Cryo-electron Microscopy
CXMS	Cross Linking Mass Spectrometry
CXL	Chemical Cross Linking
Da	Daltons
kDa	kilo-Daltons
DC	Direct Current
DDA	Dithiodiglycolic Acid
DEA	Diethylamine
DCM	Dichloromethane
DIC	N'N-diisopropylcarbodiimide
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
DSP	Dithiobis(succinimidyl) Propionate
DSS	Disuccinimidyl Suberate
EDC-HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride
ECD	Electron Capture Dissociation
EI	Electron Ionisation
eq	Equivalents
ESI	Electrospray Ionisation
FAB	Fast Atom Bombardment
Fmoc	Fluorenylmethyloxycarbonyl
h	hour(s)
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HDX	Hydrogen/Deuterium Exchange

HDX-MS	Hydrogen/Deuterium Exchange Coupled to Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
HTH	Helix-Turn-Helix
HOBt	1-Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
IMMS	Ion Mobility Mass Spectrometry
IR	Infrared
MALDI	Matrix Assisted Laser Desorption Ionisation
MCP	Microchannel Plate
MeO	Methyl Ester Protecting Group
MeOH	Methanol
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MS/MS/MS	3-Stage Tandem Mass Spectrometry
m/z	Mass to Charge Ratio
nESI	Nano-Electrospray Ionisation
NHS	N-hydroxy Succinimide
NMR	Nuclear Magnetic Resonance Spectroscopy
PBS	Phosphate-Buffered Saline
Q-ToF	Quadupole-Time of Flight
R_f	Retention Factor
RF	Radio Frequency
Rxn	Reaction
SaBPL	*Staphylococcus aureus* Biotin Protein Ligase
SAXS	Small Angle X-ray Scattering
$SOCl_2$	Thionyl Chloride
TFA-NHS	N-trifluoroacetoxy Succinimide
TFAA	Trifluoroacetic Anhydride
THF	Tetrahydrofuran
ToF	Time of Flight
TLC	Thin Layer Chromatography
WT	Wild Type

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