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Summary of doctoral dissertation

„Intermolecular disulfide bridges application in the study of peptide splicing mechanism catalyzed by proteases and in formation of peptide conjugates”

RNA splicing takes place during transcription of RNA which is a common process to generate mRNA. Autocatalytic reaction of certain proteins that excise their internal fragments named inteins out of their precursors and ligate the flanking regions (exteins) to form a mature protein, discovered in the early 90’s of the last century, was named *protein splicing*. More recently, ***peptide splicing*** has been defined as formation of a peptide whereby two noncontiguous fragments of the peptide are joined together in a proteasome [1,2]. The authors proposed a mechanism of proteasome catalyzed peptide splicing, where α-amino group of a *C*-terminal fragment liberated at one of the proteasome catalytic sites performs a nucleophilic attack on the acyl-enzyme intermediate containing the *N*-terminal fragment at a second catalytic site to form a new peptide bond. More recently, this mechanism was confirmed experimentally by Mishto *et al.* [3] using high resolution mass spectrometry in conjunction with bioinformatic methods. They named it the direct transpeptidation. Another mechanism considered by the authors named hydrolysis and transpeptidation, required the hydrolysis and formation of the new acyl-enzyme intermediate that precedes the transpeptidation step. Colgrave *et al*. [4] and my group [5] in parallel demonstrated that analogues of SFTI-1 while incubated with trypsin or chymotrypsin, yielded spliced peptides. They shown, that upon incubation in such process two reactive sites of these analogues were cleaved, the middle fragment was removed, and a monocyclic SFTI-1 or an analogue [Phe^{5}] SFTI-1 was formed. In order to study mechanism of splicing catalyzed by serine proteinases, I designed and synthesized a series of monocyclic (with disulfide bridge only), two-peptide-chain SFTI-1 analogues. These peptides are composed of *N*- and *C*-terminal fragments of SFTI-1 or its analogues, connected by a disulfide bridge [6,7]. The incubation of such peptides with proteinase resulted in the synthesis of the peptide bond that gave monocyclic SFTI-1 inhibitors. By applying HPLC/MS and X-ray analyses I proved that regardless of the extension at the *C*-terminus by Ser of the first chain or by Lys at the *N*-terminus of the second chain, spliced monocyclic SFTI-1 was formed. Using $H_2{}^{18}O$ I confirmed that similar to the mechanism of peptide splicing catalyzed by proteasome, also the splicing of SFTI-1 analogues follows a direct transpeptidation mechanism [7].

The next aim of my work was to obtain a new class of compounds with antimicrobial activity. These compound are bioconjugates: peptidic hybrids composed of two peptides with antimicrobial activity and peptide-based conjugates. The combination of the appropriate drug molecule with a peptide in the bioconjugate might lead to the product with improved pharmacological profile as compared with the parent compounds. Designed peptide-based conjugates composed of the antibiotic-nystatin (Nys), and selected fragments of cathelicidins (a guinea pig and ring-necked pheasant). Both components were connected by disulfide bond, which is readily reduced in the intracellular environment, so that the transported molecule with therapeutic effect is rapidly released. Disulfide bridges have been extensively used in targeted drug delivery [8], owing to their reducible nature. In order to form a disulfide bond between nystatin with selected fragments cathelicidins, I used two compounds: 4-(pyridin-2-yldisulfanyl)butyric acid and dithiobis(succinimidyl propionate) (DSP). I was able to conjugate peptide to nystatin in solution using 4-(pyridin-2-yldisulfanyl) butyric acid and Lomant's reagent, although due to low yield, the conditions need to be optimized. Designed peptidic hybrids composed of two peptides with antimicrobial activity. First peptide is a modified 15-29 fragment of human neutrophil peptide 1 (HNP-1), a member of α-defensins. This peptide displays antimicrobial activity against a broad spectrum of pathogens, the closest to that of full length HNP-1. Second peptide is human lactoferrampin (LFampH) which is the fragments 269-285 of human lactoferrin – a monomeric 80-kDa metal-binding glycoprotein belonging to the transferrin family. These peptide also exert a broad-spectrum activity against several fungi, bacteria, and viruses. I synthesized two series of compounds consisting of two peptides (analogue of LFampH and analogue of HNP-1) conjugated by isopeptide bond and disulfide bridge. In order to form an intermolecular disulfide bridge I introduced appropriate modifications into the peptide chains. Their antimicrobial, especially antifungal activity will be determined in the laboratory of Prof. Tzi Bun Ng from the School of Biomedical Sciences and Department of Microbiology, Chinese University of Hong Kong.

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