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„Analogues of SFTI-1 which undergo peptide splicing as vectors for introducing cytotoxic peptides or fluorescent probes into the cells”

Trypsin inhibitor isolated from sunflower seeds (SFTI-1) is one of the most widely studied inhibitors of serine proteases. SFTI-1 belongs to the Bowman-Birk family of inhibitors, among which it is one of the smallest and most active compounds. It is a circular peptide, consisting of 14 amino acid residues, and its structure is stabilized by a disulfide bridge. The reactive site P_1-P_1' is located between residues Lys^5 and Ser^6, and therefore SFTI-1 forms a complex of 1:1 stoichiometry with cognate enzymes displaying trypsin-like specificity [1]. Due to its compact structure and high inhibitory activity, SFTI-1 is a good starting point for the design of compounds with new therapeutic properties.

In the last decade it was proven that in the catalytic chamber of proteasome immunogenic peptides are formed as a result of joining together two non-continuous fragments of peptides [2]. The observed process was named peptide splicing. Few years ago Łęgowska *et al*. [3] discovered that dimeric analogues of SFTI-1 containing two reactive sites can also undergo peptide splicing. In the reaction with cognate serine proteinase (trypsin or chymotrypsin) the reactive sites of double sequence analogues of SFTI-1 were cleaved and the middle fragment (insert peptide) was removed. Moreover, in this reaction the formation of monocyclic SFTI-1 or its analog [Phe^5]SFTI-1, which contained only one reactive site, was observed.

Because of the fact that analogues of SFTI-1 possess significant proteolytic resistance and are able to cross the cell membrane [4], I decided to explore the possibilities of introduction into the cells, as part of insert peptides, synthetic moieties which could be utilized as therapeutic or diagnostic agents. Understanding the mechanism of this process, and the setting of minimum requirements (optimum chain length, primary structure) of the middle fragment undergoing peptide splicing would allow for the design of peptides (peptidomimetics) with potential

application properties. The desired analogues of SFTI-1 after the crossing the cell membrane of tumor cells or microorganisms- would undergo peptide splicing inside the cells and release the middle fragment with particular function, forming at the same time a proteinase inhibitor to reinforce its destructive functions.

The main aim of my work was the design and synthesis of SFTI-1 analogues with different lengths and sequences, which contained in the middle fragment a compound with specific profile of biological activity or a fluorescence marker, allowing its use in therapy or medical diagnosis.

Within the doctoral dissertation I have designed and synthesized 20 new SFTI-1 analogues of different lengths and sequences that comprised one of the cytotoxic peptides: RGD or GRGDNP, or fluorophore molecule(s). My intention was to obtain peptides, which upon penetration of the cells underwent proteolysis with the release of the active peptide or fluorescence, and the simultaneous formation of monocyclic SFTI-1.

Among the new SFTI-1 analogues undergoing peptide splicing, I selected the one with optimal structure. Moreover, I proved that SFTI-1 is an excellent base structure allowing the introduction of peptides having the desired biological activity into the cells while improving its stability, as well as increasing their activity. In addition, the obtained results show that peptides containing molecules displaying FRET are a promising tool to track proteolysis within cells, and may be of potential use in diagnostics.

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