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Synthesis and assessment of amyloidogenic activity of the C-peptide fragments

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Introduction

In the recent years, the research on amyloidogenic peptides have become a key area of scientific research in the field of biotechnology and medicine. The amyloidogenic peptides have significantly contribution in understanding the pathological mechanisms associated with neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Moreover, it has been found that undesirable aggregation of metabolic hormones (insulin and amylin) could be also a source of the progress of type I and II diabetes. It is known that the diabetes mellitus is a group of physiological dysfunctions characterized by hyperglycaemia resulting directly from insulin resistance (in the case of type 2 diabetes mellitus-T2DM), inadequate insulin secretion/production, or excessive glucagon secretion (in type 1 diabetes mellitus-T1DM) [1,2]. Although the aetiology of diabetes may differ from T1DM to T2DM, common features, namely formation of insoluble amyloid deposits within different tissue, may occur during the progression of the both diseases. It has been proven that amyloid deposits consist largely of aggregated insulin molecules (especially during long-term subcutaneous application of the hormone during T1DM) and both insulin and amylin chains in the case of T2DM. Amylin is co-secreted with insulin from pancreatic β cells, and due to its tendency to aggregate within pancreatic islet cells in type 2 diabetes mellitus (T2DM) is so-called as human amyloid polypeptide (hIAPP) [3,4]. The amyloidogenic properties of peptides could be also favoured by factors like acidic pH, drastic changes in temperature, contact with hydrophobic surfaces or variations in ionic strength of solution. Although there are reports which put hypothesis that amyloid deposits may also include other structures. Currently, researchers are increasingly focusing on the tendency to aggregate C-peptide and its input on another metabolic disorders [5,6].

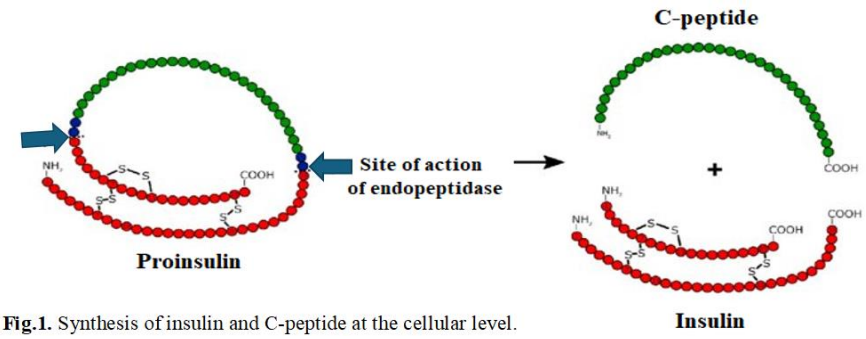

Fig.1. Synthesis of insulin and C-peptide at the cellular level.

Initially, peptide C, produced in the cell during the proteolytic transformation of proinsulin into insulin and co-secreted in equimolar amounts into the circulatory system together with insulin, was considered as a by-product whose action did not significantly affect the functioning of the body. However, its activity has now been confirmed at the stage: a) promoting proper folding of proinsulin; b) initiating the process of creating disulphide bridges within the hormone; c) supporting interactions enabling controlled secretion of insulin from the pancreas. On the other hand, there is some controversy regarding reported effects of the C-peptide. Its beneficial effects have been demonstrated in long-term treatment in type 1 diabetes. In contrast to this, C-peptide in type 2 diabetes could induce proinflammatory and proatherogenic effects.

Results and Discussion

Based on these inconsistencies, one of the directions of our research includes studies on the tendency to aggregation of C-peptide fragments under *in vitro* conditions.

To verify this hypothesis, C-peptide was divided into five shorter fragments (Table 1). As it was identified for insulin [7], we also would like to detect the shortest potential sequences within C-peptide responsible for the growth of amyloid fibril (so-called hot-spot fragments).

Table 1. The synthesized C-peptide fragments.

(1)	H_2N-^{57}EAEDLQV^{63}-OH
(2)	H_2N-^{64}GQVELG^{69}-OH
(3)	H_2N-^{70}GGPGAG^{75}-OH
(4)	H_2N-^{76}SLQPLA^{81}-OH
(5)	H_2N-^{82}LEGSLQ^{87}-OH

The proposed peptide chains were synthesized according to the Fmoc/tBu methodology on a 2-chlorotrityl chloride resin using a triazine coupling reagent [8]. In the second stage of the research, a spectrophotometric and microscopic tests were carried out to assess their tendency to aggregation.

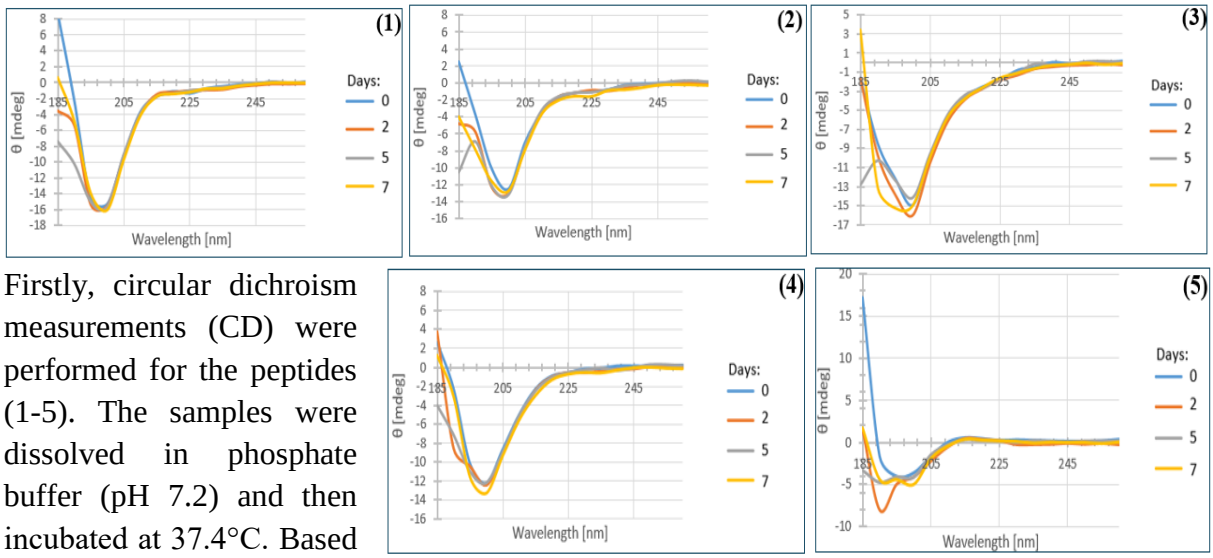

Figure 1. CD measurements registered for the analyzed C-peptide frgaments.

Firstly, circular dichroism measurements (CD) were performed for the peptides (1-5). The samples were dissolved in phosphate buffer (pH 7.2) and then incubated at 37.4°C. Based on the registered CD spectra we conclude that first four peptides (1-4) showed stable secondary structure during 7 days. In turn, the fragment (5) was the most susceptible to conformational changes. For the

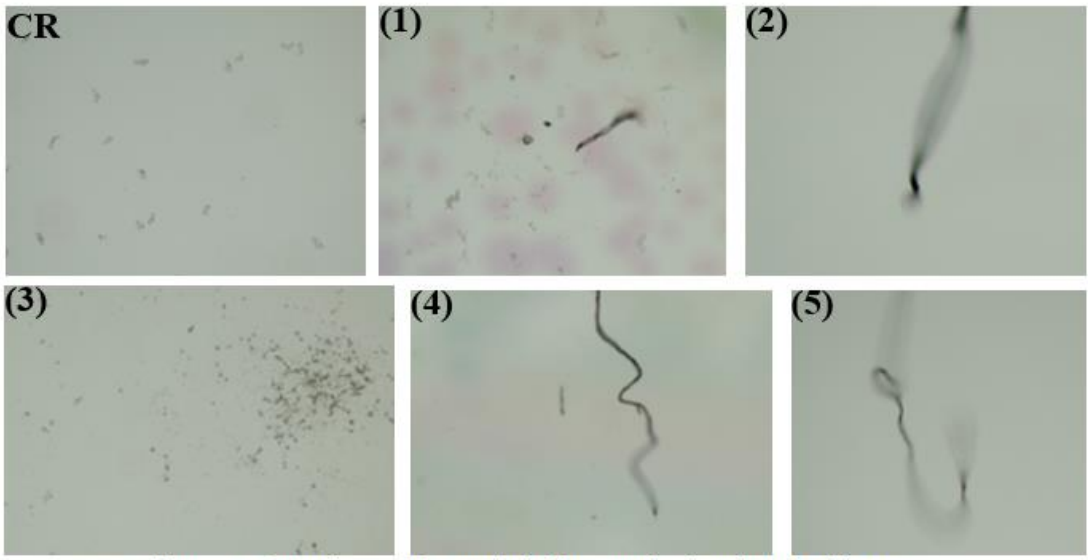

Figure 2. The microscopic analysis for samples incubated with CR.

microscopic studies carried out for the incubated samples, additionally stained with a dye that selectively interacts with amyloid fibers (namely Congo red, CR), the highest density of fibrillar structures was also identified in the fifth, C-terminal sequence, marked in locants 82-87. This is most likely related to the presence of the active site in this area, information about which can be found in the literature [9].

Moreover, the unmature amyloid fibers were observed for the samples (1), (2) and (4) but there were unstable. On the other hand, in the case of the third peptide (70-75), the predisposition to form aggregates was the weakest compared to the rest of the synthesized amino acid chains.

Conclusions

The research carried out so far point out the tendency to aggregate some of the analyzed sequences, while opening a new research approach to searching for new functions of C-peptide as a biomarker in the diagnosis of diseases related to metabolic disorders. As part of further experiments, it is planned to repeat the aggregation tests for structures composed of at least seven amino acids and at a reduced pH.

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