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Research Article

The EW Peptide Regulates Gene Expression and HIF1α Protein Synthesis in Patients with Diabetic Foot

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Abstract

The immunomodulatory drug Thymogen (EW peptide) regulates the reactions of cellular, humoral immunity and nonspecific resistance of the body. The EW peptide was previously shown to be effective in the repair of lower extremity ulcers in patients with diabetes mellitus. This correlated with the normalization of the HIF1α protein concentration in blood plasma. The aim of this work is to elucidate the molecular mechanism of the EW peptide's effect on the synthesis of HIF1α. Molecular modeling and ligand docking methods were used to demonstrate the possibility of binding the EW peptide to the double-stranded DNA sequences GGAG, AGGA, CCGG, GGTG, AGTC in various HIF1α gene promoters. These data suggest that the clinical efficacy of the EW peptide in diabetic foot patients is due to its ability to regulate gene expression and HIF1α protein synthesis.

Keywords: *EW Peptide; HIF1α; Hypoxia; Diabetes Mellitus; Diabetic Foot*

Abbreviations

HIF1α: Hypoxia-Inducible Factor 1α; dsDNA: Double-Stranded Deoxyribonucleic Acid; DM: Diabetes Mellitus; mRNA: Matrix Ribonucleic Acid; Thymogen: EW Dipeptide (α-Glutamyl-α-Tryptophan)

Introduction

According to the International Diabetes Federation, during the period from 2009 to 2019, the number of people with diabetes mellitus (DM) increased almost 2-fold. It has been established that genetic predisposition can be one of the factors causing complications and leading to an increased mortality risk in DM. Impaired expression of the *HIF1α* gene is one of the main risk factors and reasons for the unfavorable course of DM [1].

HIF1α (Hypoxia-inducible factor 1α) is a regulator of oxygen homeostasis in hypoxia. In DM, tissues are in a state of hypoxia, and adaptive responses are impaired due to insufficient activation of HIF1α signaling. This effect is the result of impaired HIF-1α function induced by hyperglycemia. In addition, in diabetic foot syndrome, there is a violation of the HIF1α synthesis, which correlates with the severity of the disease. In this regard, HIF1α is considered as a therapeutic target in the development of drugs for the treatment and prevention of DM [2].

In the pharmaceutical market of the Russian Federation, the immunomodulatory drug Thymogen (peptide EW) is known as a dipeptide that regulates the reactions of cellular, humoral immunity and nonspecific resistance of the organism. Thymogen stimulates regeneration processes in case of their inhibition by normalizing cellular metabolism. Thymogen stimulates the differentiation of lymphocytes, normalizes the amount of T-helpers, cytotoxic T-lymphocytes and their ratio in patients with various immunodeficiency states. The EW peptide regulates the growth of blood vessels, skin fibroblasts and has an antitumor effect [3-6].

Administration of the EW peptide promoted the activation of reparative processes in ischemic tissues of patients with diabetic foot syndrome. After a 3-week course of application of the EW peptide in patients with diabetic foot, the level of HIF1α in blood plasma decreased by 70% compared to this indicator before treatment, thus practically reaching the values characteristic of healthy people. This was accompanied by epithelization of ulcers on the feet of patients with DM, while in the control group (standard therapy) no such effect was observed. Thus, the reparative effect of the EW peptide on lower limb ulcers in DM was associated with its antihypoxic effect induced by the regulation of HIF1α synthesis [7]. These clinical data were consistent with the results of an earlier pilot study. In the model of streptozotocin diabetes mellitus in rats, the EW peptide contributed to the normalization of tissue oxygenation and decrease in HIF1α synthesis [8].

It is known that the mechanism of action of many ultrashort (2-7 amino acid residues) peptides is due to their ability to regulate gene expression and protein synthesis in animals and humans. Ultrashort peptides can penetrate the nucleus and nucleolus of cells and interact with the nucleosome, histone proteins, single- and double-stranded DNA (dsDNA). DNA-peptide interactions play an important role in template synthesis reactions, including sequence recognition in gene promoters, replication, transcription, and repair. A study using the DNA microarray technology revealed the ability of the EW peptide to regulate the expression of a wide range of genes: *MT-ATP6 (ATP6), MT-ND1, MT-ND4, MT-CO1, AK2, HBA1,2, COP1, PDLIM5 (Enh2), HSP90AB1, HSPBAP1 (Pass1), HLA* [9]. These genes encode proteins that perform an antioxidant, stress-protective function and regulate cellular metabolism and immunogenesis. Violation of the expression of these genes leads to the development of various diseases. For example, changes in the expression of HLA family genes are the cause of the development of DM and immunopathology [10].

Objective of the Study

The objective of this work is to elucidate the molecular mechanism of the effect of the EW peptide on the synthesis of HIF1α.

Materials and Methods

The possibility of interaction of the EW peptide with dsDNA regions in the classical linear B-form was analyzed by methods of molecular modeling and ligand docking using the ICM-Pro software (Molsoft LLC, USA) and supercomputer technologies of the St. Petersburg Nuclear Physics Institute Named after B.P. Konstantinov, National Research Center "Kurchatov Institute". Based on the properties of complementarity and symmetry of the spatial structure of dsDNA in the classical B-form, 136 unique dsDNA sequences consisting of 4 base pairs were obtained. With the help of specifically developed software scripts in the ICM language, spatial structures of DNA receptors in the classical linear B-form were generated, consisting of two repeating pairs of nucleotides (the first and the last 4 pairs were AT-rich regions) and alternating sequences of the studied site [11]. The binding site of the peptide in the dsDNA molecule was defined as the central alternating sequences. Virtual screening of the studied ligands was performed in the internal force field of the ICM-Pro software package (ICMFF) [12] and the DockScan algorithm (Molsoft LLC). When calculating, global optimization of the entire flexible ligand in the receptor field was performed. Using the Monte Carlo method, stochastic conformations of the peptide were obtained with further local minimization of the energy gradient [13]. The generation of new peptide conformations was carried out according to the maximum number of searches of the ligand angular parameters (Thorough = 30), which was selected based on the reproducibility of the docking results [11]. After the virtual screening procedure, the obtained DNA-peptide complexes were sorted in ascending order of their Score function. For further analysis of the resulting complexes, the most energetically favorable position of each ligand was retained. Information about the nucleotide sequences of the promoters of various variants of the *HIF1A* gene was obtained from the PubMed database.

Results and Discussion

Table 1 lists the most energetically favorable complexes of the EW peptide with dsDNA. The EW peptide can interact with the GGAG dsDNA sequence with an ICM-Score function value of -32.6 (Figure 1) or with the AGAC dsDNA sequence with an ICM-Score function value of -32.1.

When interacting with the GGAG dsDNA region, the EW peptide forms 4 intermolecular hydrogen bonds at the side of the minor groove with nitrogen atoms in guanine G5, G6 and adenine A7 positions. When interacting with the AGAC dsDNA region, the EW peptide forms 4 intermolecular hydrogen bonds at the side of the minor groove: with nitrogen atoms in guanine G6, adenine A7 positions, and with a phosphate backbone in thymine T20, T21 positions.

The positions of the EW peptide in complexes with the GGAG and AGAC dsDNA sequences coincide. In the central part of dsDNA, guanine G6 forms a hydrogen bond with the carbonyl group of the main chain of the peptide, and adenine A7 forms a hydrogen bond with the nitrogen atom of the C-terminal tryptophan. The replacement of guanine G5 with adenine A5 results in the loss of the hydrogen bond donor for the N-terminal glutamic acid, and hence the loss of the energetic contribution of the hydrogen bond. However, in the case of interaction with the AGAC dsDNA region, the value of the van der Waals interaction energy decreased, and the polarization of chemical bonds increased, which made a positive contribution to the interaction energy (Table 1 and figure 1).

In the promoters of the second and third variants of the gene encoding the human HIF1A protein, dsDNA sequences were found, which, according to the calculation data, were complementary to the EW peptide (Table 2 and 3). The most probable dsDNA sequence in terms of the minimum interaction energy, which is complementary to the EW peptide, GGAG, occurred once in the direct sequence of the promoter of the second variant of the *HIF1A* gene and twice - in the reverse sequence. Also, in the direct sequence of the promoter of the second variant of the *HIF1A* gene, the AGGA dsDNA sequence was found, which is complementary to the EW peptide and ranks 9th in terms of the probability of DNA-peptide interactions in table 2. In the reverse sequence of the promoter of the second variant of the HIF1A gene, the CCGG dsDNA sequence was found, which is complementary to the EW peptide and ranks 5th in terms of the probability of formation of

№	DNA sequence	ICM-Score	Hbond	Hphob	VwInt	Dsolv	SolEl	Eintl
1	GGAG	-32.6	-11.0	-4.0	-25	16.7	7.04	0.0
2	AGAC	-32.1	-7.5	-4.1	-30	17.2	2.31	0.7
3	CGCG	-31.7	-10.7	-4.0	-26	18.3	9.05	0.0
4	CGGG	-31.2	-9.7	-3.7	-26	16.3	6.65	0.0
5	CCGG	-29.1	-10.0	-3.7	-25	18.0	10.50	0.0
6	AGTC	-29.1	-5.5	-4.1	-31	15.8	2.55	0.0
7	CAGG	-28.9	-10.0	-3.7	-25	16.9	9.31	0.0
8	GGTG	-28.9	-9.2	-3.8	-26	17.6	7.78	0.0
9	AGGA	-28.7	-9.5	-3.7	-25	18.2	7.26	0.0
10	AGGT	-28.3	-9.1	-3.7	-26	18.3	6.95	0.0

Table 1: *The most energetically favorable complexes of the EW peptide with dsDNA.*

Description of abbreviations: DNA: The central area of dsDNA under investigation; Score: Assessment of the probability of ligand binding to the receptor; Hbond: Energy contribution of the hydrogen bond; Hphob: Energy contribution of hydrophobic action; VwInt: Van der Waals interaction energy; Dsolv: Hydrogen bond desolvation energy of donor and acceptor; SolEl: Electrostatic solvation energy upon ligand binding; Eint: The value of the internal energy of the ligand conformation.

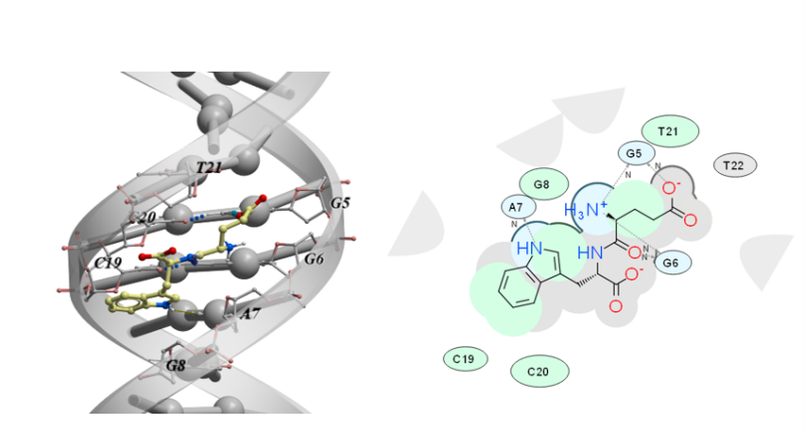

Figure 1: *Binding of the EW peptide to the GGAG dsDNA region. The green color shows the hydrophobic region, the blue color shows the hydrogen bond acceptors, and the gray color shows the surface accessible to the solvent.*

the dsDNA-EW peptide complex in table 2. In the promoter of the third variant of the gene encoding the human HIF1A protein, the GGTG and AGTC dsDNA sequences complementary to the EW peptide were found. In the first variant of the gene encoding the HIF1A protein in humans, no dsDNA sequences complementary to the EW peptide were found.

The human HIF1A protein isoform encoded by the second gene variant has a shorter amino acid sequence than isoform 1. Human HIF1A isoform 3 contains an alternative 5'terminal exon and is transcribed. HIF1A isoform 3 is an activator of T-lymphocytes in humans [14].

Homo sapiens hypoxia inducible factor 1 subunit alpha (HIF1α), transcript variant 2, mRNA https://www.ncbi.nlm.nih.gov/nuccore/NM_181054
Localization of the GGAG dsDNA sequence
Direct gene sequence AGTGCACAGT GCTGCCTCGT CTGAGGGGAC A**GGAG**GATCA CCCTCTTCGT CGCTTCGGCC
Reverse gene sequence TCACGTGTCA CGAC**GGAG**CA GACTCCCCTG TCCTCCTAGT G**GGAG**AAGCA GCGAAGCCGG
Localization of the AGGA dsDNA sequence
Direct gene sequence AGTGCACAGT GCTGCCTCGT CTGAGGGGAC **AGGAGGA**TCA CCCTCTTCGT CGCTTCGGCC
Localization of the CCGG dsDNA sequence
Reverse gene sequence TCACGTGTCA CGACGGAGCA GACTCCCCTG TCCTCCTAGT GGGAGAAGCA GCGAAG**CCGG**

Table 2: *Localization of dsDNA sequences, complementary to the EW peptide, in the promoter of the second variant of the gene encoding the human HIF1α protein.*

Homo sapiens hypoxia inducible factor 1 subunit alpha (HIF1α), transcript variant 3, mRNA https://www.ncbi.nlm.nih.gov/nuccore/NM_001243084
Localization of the GGTG dsDNA sequence
Direct gene sequence AATAAGT**GGT G**GTTACTCAG CACTTTTAGA TGCTGTTTAT AATAGATGAC CTTTTCTAAC
Localization of the AGTC dsDNA sequence
Reverse gene sequence TTATTCACCA CCAATG**AGTC** GTGAAAATCT ACGACAAATA TTATCTACTG GAAAAGATTG

Table 3: *Localization of dsDNA sequences, complementary to the EW peptide, in the promoter of the third variant of the gene encoding the human HIF1α protein.*

Conclusion

Experimental and clinical studies revealed the ability of the EW peptide to promote the repair of trophic foot ulcers in DF. The anti-hypoxic and reparative effect of the EW peptide is associated with its ability to regulate the synthesis of the HIF1α protein in tissues and blood plasma [7,8]. Since many biologically active ultrashort peptides regulate gene expression by binding to their promoters in dsDNA, it has been suggested that the EW peptide can regulate the expression of the HIF1α immunopathology gene [10].

Using the methods of molecular modeling and ligand docking, it was found that the EW peptide could bind with a high probability to the dsDNA sequences GGAG, AGAC, CGCG, CGGG, CCGG, AGTC, CAGG, GGTG, AGGA, AGGT. Of all the listed dsDNA sequences, GGAG has the highest complementarity to the EW peptide (Figure 1). In the promoters of different variants of the genes encoding the HIF1α protein in humans, the GGAG dsDNA sequence appeared 3 times. dsDNA sequences AGGA, CCGG, GGTG, and AGTC, complementary to the EW peptide, were also found in these promoters.

Thus, the clinical efficacy of the EW peptide in patients with diabetic foot and DM is based on its ability to regulate gene expression and HIF1α protein synthesis.

Conflict of Interest

Authors declare no any conflict of interest.

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