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Original paper

In silico investigation of the potential interaction between two entomological peptides and human androgen receptor

SORIN DRAGA[1], EMILIA BUSE[1], DIANA ENE[1], SABINA SERBU[1]*, BOGDAN PURCAREANU[1,3], LAURA OLARIU[1,2]

[1]Biotehnos S.A., Otopeni, Ilfov, Romania
[2]Academy of Romanian Scientists, Bucharest, Romania
[3]University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Polizu Street No. 1-7, 011061, Bucharest, Romania

Abstract

Objective: We aim to evaluate the potential interaction of two insect hemolymph peptides, MDF3 and MDF4, with the human androgen receptor, on the premise that the proliferative effects of the two peptides are (at least in part) a consequence of AR binding.

Methods: We employed a bioinformatic approach for the prediction of protein-peptide interaction and peptide aggregation, using various *in silico* on-line tools such as docking servers, aggregation prediction servers and visualization and analysis software in order to evaluate our results.

Results: Our evaluation indicates that MDF3 and MDF4 interact with the androgen human androgen receptor by binding to a helix shown to be involved the receptor dimerization. Out of the two peptides, MDF3 appears to form a more extensive bond network with the receptor.

Conclusion: Our analysis indicates that MDF 3 and 4 may be able to activate the human androgen receptor and warrant further investigation of the potential effect on receptor function. MDF3 appears to be the most promising out of the two peptides and its interaction should be further evaluated by both computational and experimental methods.

Keywords Human androgen receptor, peptides, docking, molecular modeling.

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*Corresponding author: SABINA SERBU, Biotehnos S.A., Gorunului 3-5 street, Otopeni, Ilfov, Romania, Tel./Fax +4031-710.23.83
E-mail: sabina.serbu@biotehnos.com

Introduction

Peptides represent short chains, with a variable length between 2 and 50 aminoacids. Peptide therapeutics have played a notable role in medical practice since the 1920s (VECCHIO, TORNALI, BRAGAZZI, MARTINI, 2018). A growing number of peptide drugs are approved in the US and other major markets, and peptides continue to enter clinical development. Peptide drug discovery has diversified beyond its traditional focus on endogenous human peptides to include a broader range of structures identified from other natural sources or through medicinal chemistry efforts (LAU, 2018). Entomological peptides from the hemolymph represent an area of increased interest, showing numerous therapeutic benefits and has resulted in the field of pharma-entomology.

Based on the number of species, insects offer twice the biodiversity of plants and microorganisms put together. If we consider that almost half the drugs currently on the market are derived from plants or micro-organisms, insects can represent a significant untapped source of novel therapeutics (DIMARCQ, 2003).

Lymanthria Dispar peptides represent a potential source for therapeutic peptides: extensive work on insects was done by Loeb's group, to identify a number of peptides present in the hemolymph, brain and testis. Among these are ecdysiotropin, a peptide indentified for the first time in pupae brains (MEOLA, LOEB, KOCHANSKY, WAGNER et al, 1997; LOEB, KOCHANSKY, WAGNER, WOODS et al, 1998), midgut differentiation factors (MDFs) 1 and 2 (LOEB, 1999) and midgut differentiation factors 3 and 4. Among various peptides, *Lymantria dispar* pupae hemolymph contains MDF 3 and 4 which have been shown to promote stem cell differentiation, with a peak activity detected at 10^6 M (MDF3, with the corresponding aminoacid sequence: EEVVKNAIA) and 10^8 M (MDF4, with the corresponding aminoacid sequence: ITPTSSLAT), respectively, falling within the physiological range for bioactivity (LOEB, 2002).

The human androgen receptor is a type of nuclear receptor, well known for its involvement in cell proliferation and differentiation (LUCAS, NASCIMENTO, PISOLATO, PIMENTA et al, 2014). Other insect growth factors, such as ecdysone and 20-ecdysone have been shown to have anabolic and proliferative effects, possibly mediated by interaction with the androgen receptor, in the absence of the ecdysteroid receptor, and ecdysone has been shown activate the mineralocorticoid receptor (LU, WANG, GE, DWORKIN et al, 2018). Wound-healing effects of ecdysteroids have also been described, with 20-ecdysone in liposomes shown to shorten the duration of skin repair after superficial wounding and stimulation of keratinocyte differentiation *in vitro* (LAFONT, 2003).

Anabolic effects on rats were also reported (SYROV, 2000) along with growth-promoting effects in pigs (KRATKY, 1997) and Japanese quails (KOUDELA, TENORA, BAJER, MATHOVA et al, 1995; SLÁMA, KOUDELA, TENORA, MATHOVÁ, 1996).

Considering that Lymanthria is a rich source for bioactive substances, of which ecdysones are biologically active in humans, we aim to evaluate the potential interaction of MDF3 and MDF4 peptides with the human androgen receptor, on the premise that the proliferative effects are (at least in part) a consequence of AR binding. To this end, we employed *in silico* docking methods to asses if the two peptides bind in a region relevant to AR activation.

Materials and Methods

The crystal structure of the human androgen receptor binding domain was obtained from the PDB database (BERMAN, WESTBROOK, FENG, GILLILAND et al, 2000) (PDB code: 2AMA). The structure was chosen because of the good sequence coverage, good resolution and lack of mutations.

The primary sequences of the two peptides were obtained from the literature (Loeb et al, 2002) and were submitted to the PEP-FOLD server (THÉVENET, SHEN, MAUPETIT, GUYON et al, 2012), in order to generate corresponding tri-dimensional structures. The best models were further submitted to Z-DOCK 3.0.2 web server (PIERCE, 2011), in order to obtain structures of the complexes.

The structures with the best Z-scores were refined using the FlexPEP Dock webserver (London, Raveh, Cohen, Fathi et al, 2011), in order to account for inherent peptide flexibility. The results were further evaluated for the presence of hydrogen bonds, hydrophobic, ionic and aromatic interactions, using PIC (Protein interaction calculator), (TINA, 2007).

Further, we evaluated the aggregation propensity for both our peptides, using the PASTA webserver (WALSH, SENO, TOSATTO, TROVATO, 2014). Visualization was performed with Molsoft ICM Browser (FERNANDEZ-RECIO, 2002) and Pymol (The PyMOL Molecular Graphics System, Version 0.99rc6, Schrödinger, LLC.).

Results

Our results show that the two peptides tend to adopt a helical conformation and bind a helix located on the ligand binding domain of the AR. The interactions between the two peptides and corresponding helix are mediated by a series of hydrogen bonds and other interactions (hydrophobic and ionic in the case of MDF3 and hydrophobic in the case of MDF4). Neither peptide is predicted to form aggregates. Structurally, both peptides are predicted to be intrinsically disordered, based on primary sequence analysis. However, MDF3 is predicted to have some residual secondary structure (see Discussion section below and Table 3).

Discussion

The protein–protein interface formed during AR dimerization is stabilized by numerous aminoacid interactions, some of which are located in the alpha-helix

interacting with our peptides (NADAL, PREKOVIC, GALLASTEGUI, HELSEn et al, 2017), that could lead to the potential inhibition of receptor dimerization. Targeting the AR dimerization has been indicated as a potential therapy for prostate cancer (DALAL, BAN, LI, MORIN et al, 2018).

MDF3 interacts with the AR receptor by forming a series of hydrogen bonds, hydrophobic interactions and an ionic interaction (see Tables 1, 2 and 3 and Figure 2) while MDF4 interacts by forming a hydrogen bond and a series of hydrophobic contacts with the AR (see Table 3). This could be partially explained by the fact that MDF4 is predicted to be 100% random coil, while MDF3 is predicted to be 66% alpha-helix and 33% random coil, thus facilitating ligand-receptor interaction in a more stable manner, as opposed to a more transient interaction in the case of MDF4 (see Table 5).

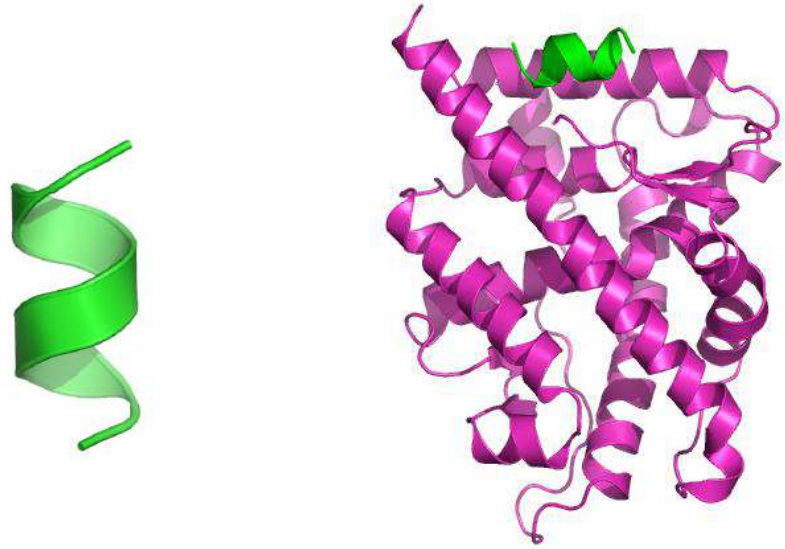

Figure 1. Figure representing the tri-dimensional structures of the MDF3 peptide (left) and the complex formed between MDF3 and the androgen receptor (right).

Table 1. Hydrogen bonds formed between donor-acceptor aminoacid pairs of the MDF3 peptide and the androgen receptor

Protein-Protein Side Chain-Side Chain Hydrogen Bonds												
DONOR			ACCEPTOR				PARAMETERS					
POS	CHAIN	RES	ATOM	POS	CHAIN	RES	ATOM	MO	Dd-a	Dh-a	A(d-H-N)	A(a-O=C)
836	A	LYS	NZ	6	-	ASN	ND2	-	3.06	9.99	999.99	999.99
2	-	GLU	OE1	832	A	MET	SD	1	3.71	3.2	110.41	999.99
2	-	GLU	OE1	832	A	MET	SD	2	3.71	4.71	16.48	999.99
2	-	GLU	OE2	832	A	MET	SD	1	2.55	1.64	139.02	999.99
2	-	GLU	OE2	832	A	MET	SD	2	2.55	2.94	58.03	999.99
6	-	ASN	ND2	832	A	MET	SD	1	3.89	3.36	112.18	999.99
6	-	ASN	ND2	832	A	MET	SD	2	3.89	3.59	98.88	999.99

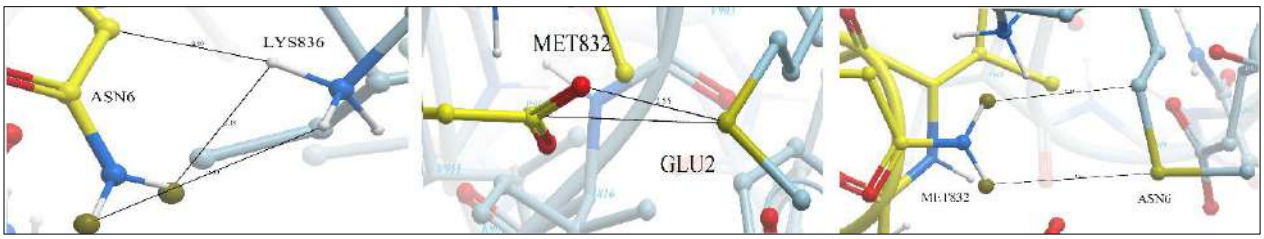

Figure 2. Detailed view of a few representative hydrogen bonds formed between MDF3 and the androgen receptors.

Table 2. Hydrophobic interactions formed between the androgen receptor and MDF3

Hydrophobic Interactions within 5 Angstroms				
Position	Residue	Chain	Position	Residue
832	MET	A	3	VAL
835	ILE	A	3	VAL
835	ILE	A	7	ALA
856	PHE	A	7	ALA
856	PHE	A	8	ILE
856	PHE	A	9	ALA
914	ILE	A	4	VAL
916	PHE	A	4	VAL
916	PHE	A	7	ALA
916	PHE	A	8	ILE

Table 3. Ionic interactions formed between the androgen receptor and MDF3

Ionic Interactions within 6 Angstroms				
Position	Residue	Chain	Position	Residue
836	LYS	A	2	GLU

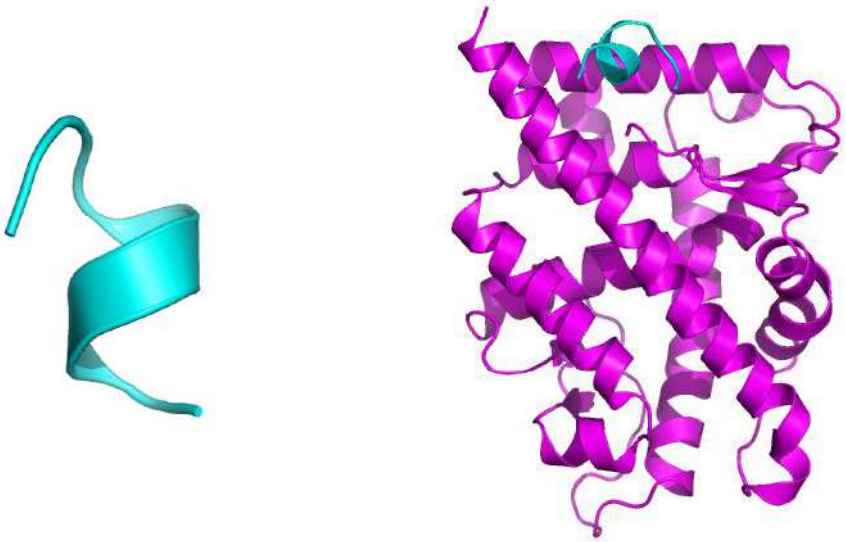

Figure 3. Figure representing the tri-dimensional structures of the MDF3 peptide (left) and the complex formed between MDF4 and the androgen receptor (right).

Table 4. Hydrogen bonds formed between donor-acceptor aminoacid pairs of the MDF4 peptide and the androgen receptor

Protein-Protein Mine Chain-Side Chain Hydrogen Bonds												
DONOR			ACCEPTOR				PARAMETERS					
POS	CHAIN	RES	ATOM	POS	CHAIN	RES	ATOM	MO	Dd-a	Dh-a	A(d-H-N)	A(a-O=C)
860	A	THR	OG1	9	-	THR	OXT	-	3.41	9.99	999.99	114.84

Table 5. Hydrophobic interactions formed between the androgen receptor and MDF4

Hydrophobic Interactions within 5 Angstroms				
Position	Residue	Chain	Position	Residue
832	MET	A	3	PRO
835	ILE	A	3	PRO
914	ILE	A	1	ILE
916	PHE	A	1	ILE

Aggregation prediction

Considering that the peptides analysed here may hold therapeutic benefit, we evaluated their aggregation potential. This is an important factor when considering heterologous expression, but also in the context of preparative chromatographic purification.

Table 6. Aggregation propensity of MDF3 and MDF4, along with disorder and secondary structure prediction

Peptide name	length	# amyloids	best energy	% disorder	% α-helix	% β-strand	% coil
MDF3	9	0	-4.119255	100	66.67	0	33.33
MDF4	9	0	-0.237392	100	0	0	100

Limitations

We acknowledge that our *in silico* evaluation has certain limitations, mainly as a consequence of the inherent dynamical nature of proteins and protein-protein interactions. Further simulation of the AR receptor dimer, both in complex with the peptides and alone, is warranted in order to provide a better understanding of the consequences of peptide binding. This, most likely, would involve extensive molecular dynamics simulations. Also, we must consider the fact that, regardless of how refined the simulation protocols would be, any such results would have to be experimentally validated.

Conclusions

Our analysis indicates that MDF 3 and 4 may be able to activate the human androgen receptor and warrant further investigation of the potential effect on receptor function. MDF3 appears to be the most promising out of the two peptides and its interaction should be further evaluated by both computational and experimental methods.

Conflict of Interest

The authors have no conflict of interest to declare.

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