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Molecular Recognition Theory of the complementary (antisense) peptide interactions

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Abstract

Molecular Recognition Theory is based on the finding of Blalock et al. (Biochem. Biophys. Res. Commun. 121 (1984) 203–207; Nature Med. 1 (1995) 876–878; Biochem. J. 234 (1986) 679–683) that peptides specified by the complementary RNAs bind to each other with higher specificity and efficacy. This theory is investigated considering the interaction of the sense peptides coded by means of messenger RNA (read in 5′→3′ direction) and antisense peptides coded in 3′→5′ direction. We analysed the hydropathy of the complementary amino acid pairs and their frequencies in 10 peptide–receptor systems with verified ligand–receptor interaction. An optimization procedure aimed to reduce the number of possible antisense peptides derived from the sense peptide has been proposed. Molecular Recognition Theory was also validated by an "in vivo" experiment. It was shown that 3′→5′ peptide antisense of α-MSH abolished its cytoprotective effects on the gastric mucosa in rats. Molecular Recognition Theory could be useful method to simplify experimental procedures, reduce the costs of the peptide synthesis, and improve peptide structure modelling.

Keywords: Molecular; Recognition; Antisense; Peptide; RNA

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Introduction

Advances in the understanding of the protein structure, and the development of new techniques for the peptide synthesis have resulted in a wide application of peptides in biomedicine (Hurby and Matsunaga, 2002). The genome project has resulted in a large number of gene sequences that code for known proteins, and in nucleotide sequences of currently unknown proteins/peptides that may be synthesized and investigated to provide new structures or drugs (Heal et al., 2002a; Hurby and Matsunaga, 2002).

In natural systems a protein is synthesized in such a way that the genetic information, stored within DNA, is firstly transcribed into the messenger RNA string and then translated by means of the ribosome, messenger RNA template and related transfer RNA anticodons into the protein (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Doolittle, 1985; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998; Štambuk and Konjevoda, 2003; Tamarin, 2002). Molecular Recognition Theory (MRT) is based on an observation that peptides specified by the complementary RNA codons bind to each other with higher specificity and efficacy then peptides not specified by the complementary codons (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998). It has been shown that, according to the Kyte and Doolittle scale (Kyte and Doolittle, 1982), the codons for hydrophilic amino acids are complemented by the codons for hydrophobic amino acids and vice versa (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998; Štambuk and Konjevoda, 2003). The neutral amino acids specified by the same scale pair with neutral comlementary amino acids (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Kyte and Doolittle, 1982; Štambuk, 1998; Štambuk and Konjevoda, 2003). This theoretical concept defined by Blalock, Bost and Smith has been successfully applied to more than 40 complementary peptide–receptor systems, and it is thought to be a valuable tool for deriving new biologically active peptides and antibodies and performing selective peptide–receptor modulation (Baranyi et al., 1995; Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998; Štambuk and Konjevoda, 2003).

Due to the degeneracy of the genetic code each amino acid is usually coded by several codons, which in turn results in more than one complementary/antisense pair (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998; Tamarin, 2002). The latter leads to the large number of possible complementary peptides, even for relatively short peptide motifs.

Methods

Complementary ligand–receptor pairs

Complementary pairs defined by means of the MRT are presented in Table 1. Differences in Kyte and Doolittle (1982) hydropathic scores of the complementary

Table 1. Number of possible complementary (antisense) pairs of amino acids within peptides transcribed in 3′→5′, 5′→3′ and in both directions

Amino acid (a.a.)[a]	3′→5′ antisense	5′→3′ antisense	3′→5′ & 5′→3′
I (Isoleucine)	Y	Y, N, D	N, D, Y
V (Valine)	H, Q	H, N, D, Y	H, Q, N, D, Y
L (Leucine)	N, E, D	E, K, Q	N, E, D, K, Q
F (Phenylalanine)	K	K, E	K, E
C (Cysteine)	T	T, A	T, A
M (Methionine)	Y	H	Y, H
A (Alanine)	R	R, G, S, C	R, G, S, C
G (Glycine)	P	P, S, T, A	P, S, T, A
T (Threonine)	C, W	G, S, C, R	C, W, G, S, R
W (Tryptophan)	T	P	T, P
S (Serine)	S, R	R, G, T, A	S, R, G, T, A
Y (Tyrosine)	M, I	I, V	M, I, V
P (Proline)	G	G, W, R	G, W, R
H (Histidine)	V	V, M	V, M
D (Aspartic acid)	L	I, V	L, I, V
E (Glutamic acid)	L	L, F	L, F
N (Asparagine)	L	I, V	L, I, V
Q (Glutamine)	V	L	V, L
K (Lysine)	F	F, L	F, L
R (Arginine)	A, S	A, S, P, T	A, S, P, T
Pairs per 20 a.a.	27	52	64

[a]Codons are given in Table 2.

pairs in 3′→5′ direction were analysed by the *k*-means clustering procedure (Table 2) (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998). Pearson correlation was used to describe hydropathy relationship of the sense and antisense amino acid pairs (Fig. 1).

The frequencies of sense-antisense pairs within randomly selected 10 peptide–receptor systems were analysed to evaluate the MRT (Table 2) (Bajpai. et al., 1991; Blalock and Bost, 1986; Bost et al., 1985; Gartner et al., 1991; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Konjevoda et al., 2001; Soffer et al., 1987; Štambuk, 1998):

1. $ACTH_{1-24}$/α-melanotropin (α-MSH)
2. β-amyloid$_{1-15}$,
3. angiotensin II,
4. epidermal growth factor,
5. interleukin 2,
6. met-enkephalin,
7. prolactin,

Table 2. Complementary (antisense) codons and related amino acids that interact as ligand–receptor pairs in 3′→5′ messenger RNA reading frame direction

Ligand (5′→3′)	Receptor (3′→5′)	KDL	KDR	ΔKD	Z	No.
I (AUA)*	Y (UAU)*	4.5	−1.3	5.8	1.02	0
V (GUU, GUC)	Q (CAA, CAG)	4.2	−3.5	7.7	1.35	3
V (GUG, GUA)	H (CAC, CAU)	4.2	−3.2	7.4	1.30	5
L (UUG, UUA)	N (AAC, AAU)*	3.7	−3.5	7.2	1.26	2
L (CUC, CUU)	E (GAG, GAA)	3.7	−3.5	7.2	1.26	6
L (CUG, CUA)	D (GAC, GAU)	3.7	−3.5	7.2	1.26	5
F (UUU, UUC)	K (AAA, AAG)	2.7	−3.9	6.6	1.16	4
C (UGU, UGC)*	T (ACA, ACG)	2.5	−0.7	3.2	0.56	1
M (AUG)	Y (UAC)	1.9	−1.3	3.2	0.56	7
A (GCG, GCU, GCC, GCA)	R (CGC, CGA, CGG, CGU)	1.8	−4.5	6.3	1.11	8
G (GGG, GGU, GGA, GGC)	P (CCC, CCA, CCU, CCG)	−0.4	−1.6	1.2	0.21	10
T (ACA, ACG)*	C (UGU, UGC)	−0.7	2.5	−3.2	−0.56	1
T (ACC)*	W (UGG)	−0.7	−0.9	0.2	0.04	0
W (UGG)*	T (ACC)	−0.9	−0.7	−0.2	−0.04	1
S (UCG, UCA, AGC, AGU)	S (AGC, AGU, UCG, UCA)	−0.9	−0.9	0	0.00	7
S (UCU, UCC)*	R (AGA, AGG)	−0.9	−4.5	3.6	0.63	3
Y (UAC)*	M (AUG)*	−1.3	1.9	−3.2	−0.56	2
Y (UAU)	I (AUA)	−1.3	4.5	−5.8	−1.02	6
P (CCC, CCA, CCU, CCG)	G (GGG, GGU, GGA, GGC)	−1.6	−0.4	−1.2	−0.21	6
H (CAC, CAU)	V (GUG, GUA)	−3.2	4.2	−7.4	−1.30	6
D (GAC, GAU)	L (CUG, CUA)	−3.5	3.7	−7.2	−1.26	8
E (GAG, GAA)	L (CUC, CUU)	−3.5	3.7	−7.2	−1.26	7
N (AAC, AAU)	L (UUG, UUA)	−3.5	3.7	−7.2	−1.26	5
Q (CAA, CAG)	V (GUU, GUC)	−3.5	4.2	−7.7	−1.35	2
K (AAA, AAG)	F (UUU, UUC)	−3.9	2.7	−6.6	−1.16	7
R (CGC, CGA, CGG, CGU)	A (GCG, GCU, GCC, GCA)	−4.5	1.8	−6.3	−1.11	3
R (AGA, AGG)*	S (UCU, UCC)	−4.5	−0.9	−3.6	−0.63	1

KDL = Kyte & Dollittle hydropathy of ligand peptide.
KDR = Kyte & Dollittle hydropathy of receptor peptide.
ΔKD = difference in amino acid pair hydropathy.
Z = Z value of ΔKD (with respect to the mean).
No. = number of matching pairs in 10 verified ligand–receptor peptide complexes (* = less frequent pairings).

8. transferrin,
9. vitronectin,
10. von Willebrand factor.

Peptides

Substances, i.e. investigated peptides, were:

1. α-MSH peptide: a.a. sequence SYSMEHFRWGKPV (>97% purity, SIGMA®, USA);

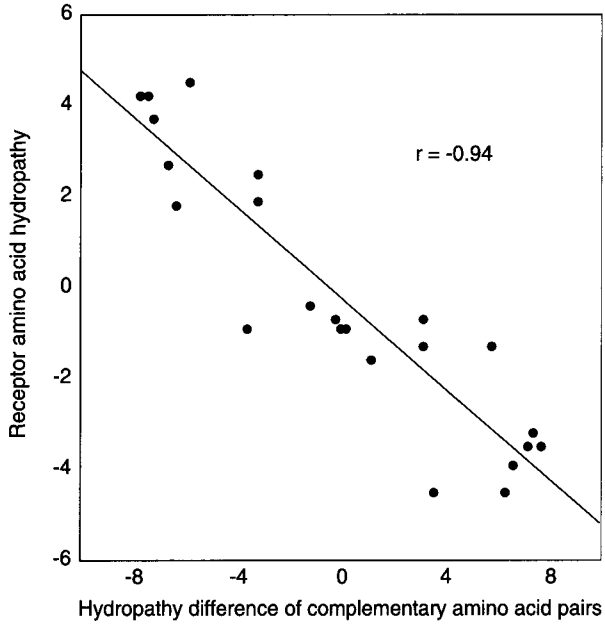

Fig. 1. Pearson correlation between hydropathy difference of complementary amino acid pairs and receptor amino acid hydropathy according to Kyte and Doolittle ($r = -0.94$).

2. antisense peptide: a.a. sequence RMRYLVKATPFGH (>97% purity, CBI, USA).

"In vivo" testing of Molecular Recognition Theory

The ability of the 13 a.a. peptide with an amino acid sequence antisense (in $3' \rightarrow 5'$ direction) to α-MSH was investigated with respect to its ability to block the biological effects of α-MSH. The influence of the antisense peptide on protective effects of α-MSH on the gastric mucosa was studied on classic model of the ethanol induced gastric lesions (Konjevoda et al., 2000; Konjevoda et al., 2001; Oates and Hakkinen, 1988). The lesions were induced with intragastric application of 1 ml 70% ethanol per rat according to the standard protocol (Konjevoda et al., 2000; Konjevoda et al., 2001; Oates and Hakkinen, 1988). Animals were randomized in four groups of six rats each. Tested peptides were dissolved in 0.9% NaCl up to the volume of 1 ml and administered intraperitoneally, 1 h prior to inducing gastritis. We observed 4 groups of animals treated with: physiological saline, α-MSH (2 mg/kg), equimolar concentration of antisense peptide (2 mg/kg), and 1:1 mixture of α-MSH and antisense peptide (2 mg/kg of each substance). Following the sacrifice 1 h after the 70% ethanol administration, hemorrhagic gastric area was assessed in mm^2 by means of the digital camera (JVC) and an image analysis software (SFORM, VAMS,

Zagreb, Croatia) (Konjevoda et al., 2000; Konjevoda et al., 2001). The protocol of the investigation complied with the European Community guidelines for the use of experimental animals, and was approved by the ethics committee. Statistical analysis and data plotting was performed with GraphPad Prism Software (version 4.0). The results were compared by means of Kruskal-Wallis test and Dunn's multiple comparison test (p-values below 0.05 were considered statistically significant).

Results

Evaluation of Molecular Recognition Theory

A total number of 27 possible amino acid complementary pairs arises when amino acids of the sense peptide coded in $5' \rightarrow 3'$ messenger RNA direction are complemented to antisense amino acids of the peptides read in $3' \rightarrow 5'$ direction of the string (Tables 1 and 2). $3' \rightarrow 5'$ complements to codons AUC and AAU for isoleucine, and ACU for threonine are stop codons (UAG, UUA and UGA) and consequently they are omitted from Table 2.

Kyte and Doolitttle hydropathic amino acid scores of the complementary (antisense) peptide pairs exhibit strong negative correlation to the receptor amino acid values ($r = -0.94$, Fig. 1). Differences in the score hydropathy analysed by the k-means clustering procedure (Table 3) show that the codons coding for hydrophilic amino acids are mostly complemented by the antisense codons for hydrophobic ones (7/8), and vice versa (7/11). Otherwise, matching amino acid belongs to the neutral group (Table 3). Neutral amino acids are in most cases (5/8) complemented by means of the neutral ones, and in the few cases of exception either by polar (1/8) or nonpolar amino acids (2/8) (Table 3).

Thirty-eight peptide motifs with experimentally confirmed interactions of the ligand–receptor type were analysed for the complementarity of particular amino acid pairs and codons read in $3' \rightarrow 5'$ direction (Table 2). Out of these 38 motifs 19 were receptor sequences while the others represented complementary ligands of the receptors. Frequencies of particular sense-antisense pairs presented in Table 2 show that different antisense receptor amino acids bind with different frequencies to the same sense amino acid of the peptide ligand (e.g. pair VH is more frequent than VQ, pairs LD and LE are more frequent than LN).

In contrast to the small number of 27 possible antisense amino acids read in $3' \rightarrow 5'$ direction, 52 possible antisense amino acid combinations for 20 possible amino acids of the sense peptide were obtained in $5' \rightarrow 3'$ direction (Table 1). Consequently, for a peptide of the amino acid chain length $\mathbf{n}$ the average number of all possible antisense peptide substitutions $\mathbf{N}$ read in $3' \rightarrow 5'$ direction is relatively small, i.e., $\mathbf{N} = \mathbf{n}^{1.35}$ (Table 1). In $5' \rightarrow 3'$ direction there exists much more possible substitutions ($\mathbf{N} = \mathbf{n}^{2.6}$), while the number of all possible interacting antisense motifs rises dramatically when both directions are observed ($\mathbf{N} = \mathbf{n}^{3.2}$, Table 1).

Table 3. Clustering of Kyte & Doolittle hydropathy differences in complementary messenger RNA pairs read in 3′→5′ direction extracts 3 basic groups of antisense pairs (polar, nonpolar and neutral)

Cluster 1 of 3 contains 11 cases (nonpolar ligand→polar receptor)						
Members		Statistics				
Case	Distance	Variable	Minimum	Mean	Maximum	St. Dev.
Pair V-Q	1.75	KD DIFFERENCE	3.20	5.95	7.70	1.76
Pair V-H	1.45					
Pair L-N	1.25					
Pair L-E	1.25					
Pair L-D	1.25					
Pair F-K	0.65					
Pair A-R	0.35	Nonpolar (hydrophobic) a.a.: I, V, L, F, M, C, A				
Pair I-Y*	0.15					
Pair M-Y*	2.75	* = combined to neutral a.a.				
Pair C-T*	2.75					
Pair S-R*	2.35					
Cluster 2 of 3 contains 8 cases (polar ligand→nonpolar receptor)						
Members		Statistics				
Case	Distance	Variable	Minimum	Mean	Maximum	St. Dev.
Pair Y-I*	1.12	KD DIFFERENCE	−7.70	−6.93	−5.80	0.63
Pair R-A	0.62					
Pair K-F	0.33					
Pair D-L	0.27	Polar (hydrophilic) a.a.: H, D, E, N, Q, K, R				
Pair E-L	0.27					
Pair N-L	0.27	* = combined to neutral a.a.				
Pair H-V	0.47					
Pair Q-V	0.77					
Cluster 3 of 3 contains 8 cases (neutral ligand→neutral receptor)						
Members				Statistics		
Case	Distance	Variable	Minimum	Mean	Maximum	St. Dev.
Pair G-P	2.45	KD DIFFERENCE	−3.60	−1.25	1.20	1.85
Pair S-S	1.25					
Pair W-T	1.05					
Pair P-G	0.05	Neutral a.a.: G, T, W, S, Y, P				
Pair T-C*	1.95					
Pair T-W	1.45	* = combined to nonpolar or polar a.a.				
Pair Y-M*	1.95					
Pair R-S*	2.35					
Distance metric is Euclidean distance (*k*-means splitting)						
Variable	Between SS	df	Within SS	df	*F*-ratio	
KD DIFFERENCE	784.978	2	57.782	24	163.021	
TOTAL	784.978	2	57.782	24		

It is interesting to note that 14 out of 20 amino acids pairs with a single amino acid pair in both 3′→5′ and 5′→3′ directions (i.e., IY, VH, LE, FK, CT, AR, GP, TC, SR, YI, PG, HV, EL, KF), only arginine posseses two pairs RA & RS (Table 2). Five

amino acids—methionine, tryptophan, glutamine, aspartic acid and asparagine, pair with different antisense amino acids in $3' \rightarrow 5'$ and $5' \rightarrow 3'$ directions (i.e., MY, MH, WT, WP, QV, QL, DL, DI, DV, NL, NI and NV; Table 2). According to this arrangement, 20 amino acids have a relatively small number of 28 possible antisense pairs.

"In vivo" effects of complementary peptides

The effects of α-MSH and its antisense peptide on the cytoprotection of rat gastric mucosa are presented in Fig. 2. Significantly less ethanol induced lesions of the gastric mucosa was observed when α-MSH was administered intraperitoneally at the concentration of 2 mg/kg (Kruskal-Wallis test $p = 0.0085$, Dunn's multiple comparison test $p < 0.05$). Antisense peptide obtained by α-MSH sequence transcription in $3' \rightarrow 5'$ direction, did not exhibit statistically significant difference of the gastric lesion area when the result was compared to the control untreated animals (Dunn's multiple comparison test $p > 0.05$; Fig. 2). Same was valid when the combination of both peptides was applied. This proved that antisense peptide neutralizes cytoprotective effects of α-MSH on the gastric mucosa (Fig. 2).

Discussion

Molecular Recognition Theory is based on the result of Blalock, Smith and Bost (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984) that hydropathic character of an amino acid residue is related to the middle letter of the messenger RNA codon. Within antisense peptides transcribed in $3' \rightarrow 5'$ or $5' \rightarrow 3'$ messenger RNA direction hydrophilic and hydrophobic patterns of amino acid polarity are changed into the opposite ones. The patterns of neutral amino acids remain neutral (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Heal et al.,

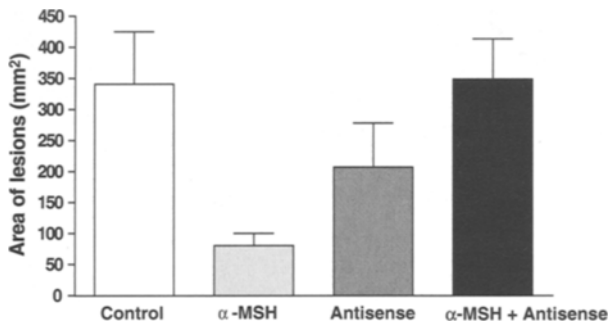

Fig. 2. The effects of α-MSH and corresponding antisense peptide on the cytoprotection of rat gastric mucosa.

2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998). According to Blalock this remarkable characteristic of the genetic code is responsible that sense and antisense peptide pairs have mutually complementary shapes (hypothetic secondary and tertiary structures) which results in their interaction (Blalock and Smith, 1984; Heal et al., 2002a; Hurby and Matsunaga, 2002; Štambuk, 1998).

β-strand peptide conformation and structural similarity of the interacting peptides is thought to provide better binding affinity of the sense-antisense pairs (Heal et al., 2002a; Hurby and Matsunaga, 2002). However, some authors point out that hydropathic complementaity per se is not responsible for the interaction between sense and antisense peptides (Heal et al., 2002a).

We analysed all possible complementary amino acids pairs that could arise from the sense $5' \rightarrow 3'$ and antisense $3' \rightarrow 5'$ messenger RNA interaction. Such complementary $3' \rightarrow 5'$ readings of messenger RNA residues correspond to transfer RNA anticodons used for the sense peptide synthesis on the ribosome (Štambuk, 1998; Tamarin, 2002). *k*-means clustering of hydropathic score differences between all possible pairs of sense-antisense peptide complexes in Table 3 confirmed the assumption of Blalock et al. (Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984) that in opposite RNA strands hydrophilic and hydrophobic patterns of amino acids are interchanged, while the neutral remain unchanged. It is worth mentioning that for almost 30% (8/27) of the sense-antisense pairs deviations from this rule are observed (Table 2). Deviations from the hydropathy relationships assumed by the Molecular Recognition Theory might be one of the reasons that in some cases the patterns of interacting sense-antisense peptides do not follow theoretical assumptions (Heal et al., 2002a; Hurby and Matsunaga, 2002). It is known that relatively small change of the amino acid molecular polarity might influence the secondary protein structure (Štambuk and Konjevoda, 2003), thought to be relevant for the interaction of sense and antisense peptides (Blalock and Smith, 1984; Heal et al., 2002a; Hurby and Matsunaga, 2002).

Another interesting observation is that some antisense amino acids are more frequently observed in complementary pairs rather than others (Table 2). The results, obtained by analysis of 10 peptide–receptor complexes with ligand–receptor interaction, suggested that further studies on a larger data-sets are needed in order to prove that observed frequency patterns have a biological relevance. If so, frequently observed antisense amino acid patterns in the ligand–receptor systems might facilitate binding and/or positive biological effects of such peptide–receptor interactions, while antisense amino acid patterns rarely found in those systems might be the one that lead to the negative results.

We tested the possibility that antisense peptide modulates biological response to a well known and pharmacologically relevant peptide hormone (Blalock and Bost, 1986). α-MSH was selected since it represents the first 13 amino acids of ACTH molecule (Catania et al., 2000; Lipton and Catania, 1997). Binding of peptides that were specified by complementary RNAs of the first 24 amino acids of the ACTH served as an experimental evidence to support classic results of Blalock and Bost (1986). Our "in vivo" results presented in Fig. 2 are in line

with the results of "in vitro" binding done by Blalock and Bost (1986). It is clearly shown that antisense peptide successfully blocks protective effects of α-MSH on the gastric mucosa. α-MSH is known to be highly efficient in the standard cytoprotective model of the ethanol induced gastric lesions, that was used in this study (Konjevoda et al., 2000; Konjevoda et al., 2001; Oates and Hakkinen, 1988). It is important to note that this result indicates that antisense peptides could be used to modulate systemic "in vivo" biological responses of the potent peptide hormone that exerts strong stress-protection (Catania et al., 2000; Lipton and Catania, 1997).

The biological modulation (neutralization) of sense peptide effects by means of antisense peptides may arise from:

1. binding of sense and antisense peptide into molecular complexes, leaving low levels of sense peptide to elicit its expected biological effects;
2. partial antagonization of the sense peptide receptor by means of sense-antisense peptide complex, resulting in the disturbance of the receptor function;
3. combination of 1 and 2; and
4. other biological effects of antisense peptide that may not be explained by the involvement of sense peptide and its receptors.

In the case that the sequence of antisense peptide is unknown because of the missing nucleotide sequence, or the structure of antisense peptide read in $3' \rightarrow 5'$ direction does not posses expected biological activity, alternative amino acid residues that pair with sense peptide amino acids may be used for molecular design purposes (Table 1). Those alternative residues represent peptide amino acids arising from antisense read in $5' \rightarrow 3'$ direction or combined $3' \rightarrow 5'$ and $5' \rightarrow 3'$ directions (Table 1). It is worth mentioning that such transcriptions give rise to a dramatically increased number of possible antisense peptide amino acid combinations, a fact not favourable for a peptide or drug design purposes (Results, Table 1). Molecular Recognition Theory deals with relatively short motifs ranging from $\geqslant 4$ to < 30 amino acids (Baranyi et al., 1995; Bajpai. et al., 1991; Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Bost et al., 1985; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998) and consequently antisense peptide readings in $3' \rightarrow 5'$ direction could be more suitable for the rational peptide design of the interacting ligand–receptor systems.

Although various experimental protocols verified the Molecular Recognition Theory (Baranyi et al., 1995; Bajpai. et al., 1991; Blalock, 1995; Blalock and Bost, 1986; Blalock and Smith, 1984; Bost et al., 1985; Heal et al., 2002a, b; Hurby and Matsunaga, 2002; Štambuk, 1998) a single, conclusive, and reproducible algorithm for efficient antisense peptide design that had been verified by several independent research groups is missing.

Possible applications of the Molecular Recognition Theory in biomedicine are related to peptide vaccine development, and antibody or drug design. The method could also simplify experimental procedures, improve peptide structure modelling and reduce the costs of the peptide synthesis.

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