Antisense Peptide Technology

Subjects: Engineering, Biomedical

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Antisense peptide technology (APT) is based on a useful heuristic algorithm for rational peptide design. It was deduced from empirical observations that peptides consisting of complementary (sense and antisense) amino acids interact with higher probability and affinity than the randomly selected ones. This phenomenon is closely related to the structure of the standard genetic code table, and at the same time, is unrelated to the direction of its codon sequence translation.

Keywords: antisense ; complementary ; peptide ; binding ; genetic code ; bioengineering

1. Introduction

The concept of sense and antisense (i.e., complementary) peptide interaction was developed in the early 1980s by Root-Bernstein, Biro, Blalock, Mekler, Siemion, and others [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20]. First, it was theoretically assumed and later empirically observed that peptides consisting of amino acids specified by sense and antisense sequences interact with higher probability and affinity than randomly selected peptides (**Table 1** and **Table 2** , **Figure 1**). This approach was successfully applied to the investigations of more than 50 ligand–acceptor (receptor) systems, including the immune response to viral subunits and related manipulations with an epitope and paratope design [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22].

Table 1. Standard genetic code table.

First Letter (5′)	Second Letter				Third Letter (3′)
	U	C	A	G	
U	F	S	Y	C	U
	F	S	Y	C	C
	L	S	stop	stop	A
	L	S	stop	W	G
C	L	P	H	R	U
	L	P	H	R	C
	L	P	Q	R	A
	L	P	Q	R	G
A	I	T	N	S	U
	I	T	N	S	C
	I	T	K	R	A
	M	T	K	R	G
G	V	A	D	G	U
	V	A	D	G	C
	V	A	E	G	A
	V	A	E	G	G

Table 2. Direction of translation specifies amino acid pairing.

Amino Acid	Antisense 3′ → 5′	Antisense 5′ → 3′	Consensus
F	K	K, E	K
L	D, E, N	E, Q, K	E
I	Y	N, D, Y	Y
M	Y	H	
V	H, Q	H, D, N, Y	H
S	S, R	G, R, T, A	R
P	G	G, W, R	G
T	W, C	G, S, C, R	C
A	R	R, G, S, C	R
Y	M, I	I, V	I
H	V	V, M	V
Q	V	L	
N	L	I, V	
K	F	F, L	F
D	L	I, V	L
E	L	L, F	L
C	T	T, A	T
W	T	P	
R	A, S	A, S, P, T	A, S
G	P	P, S, T, A	P

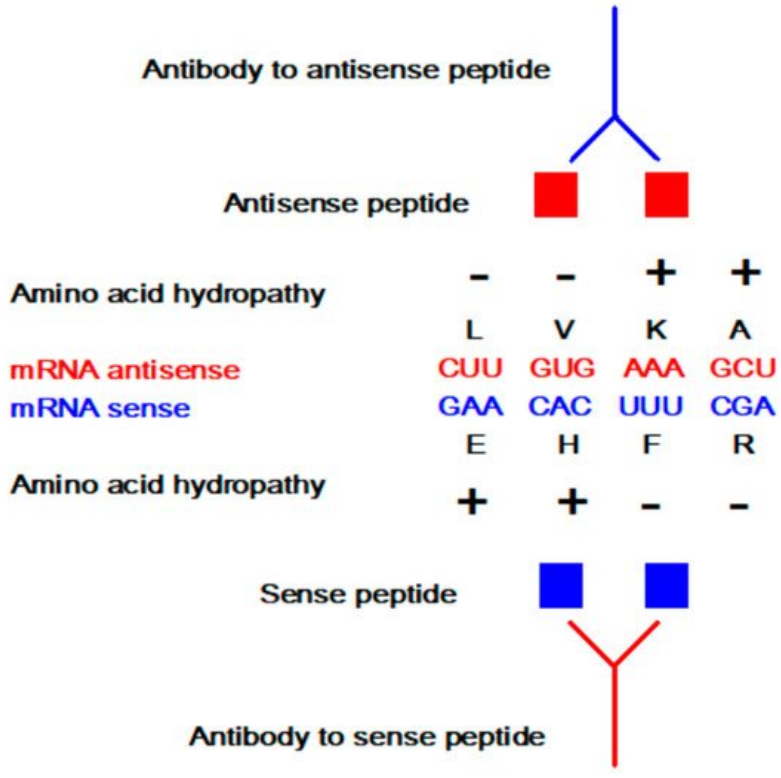

Figure 1. Modeling of sense-antisense peptide binding and related epitope-paratope interactions.

Sense peptides are essential and specific parts of viral and other proteins that elicit normal and pathologic immune responses [6][7][8][9][14][19][20][21]. Using antisense peptide technology, they could be utilized to derive targeted tests for different antibody (Ab), hormone, growth factor, or cell subpopulations [4][5][6][7][8][9][13][14][17][18][19][20][21][22][23]. The potential of antisense peptides is twofold: 1. as future diagnostic tests targeting protein epitopes or paratopes of interest, or 2. as future therapeutic agents that target specific parts of antigens to selectively modify host immune response (e.g., an antisense peptide may disrupt or modify different factors like virulence, replication or host defense) [6][7][8][9][13][14][18][19][20][21]. Consequently, sense-antisense peptide interactions may serve as a useful starting point for: 1. the development of biochemical assays for the evaluation of the immune response, and 2. modeling and design of new peptide binders for specific proteins and their receptors.

2. Antisense Peptide Technology (APT)

The antisense peptide binding preference for the complementary sense sequence provides the opportunity to build a technology platform for the development and implementation of new immunochemical procedures and assays which use antisense peptides instead of the primary and/or secondary antibodies. APT is based on a heuristic algorithm for rational peptide design of the interacting ligand-receptor (acceptor) sequences specified by the complementary codons (**Table 1** and **Table 2**, **Figure 1**) [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24]. Heuristic methods reduce solution space by focusing on results based on the reduced set of criteria—in this case, complementarity rules defined by the standard genetic code (SGC) table [19][24].

Four main problems of sense and antisense peptide applications in immunochemistry are comparable to the reasons for the lack of success of the synthetic peptide vaccines [25], while the fifth topic addressed is related to the application of antisense/complementary sequences in bioengineering [18][19][20][24]:

- reliance on continuous epitopes,
- overconfidence in ligand specificity,
- amino acid bias in characterizing ligand-acceptor (receptor) interactions,
- difficulties in the estimation of structure-function relationships between specific ligand–acceptor (receptor) pairs,
- amino acid coding, complementarity, and frameshifts.

Each of these specific problems is worth addressing.

2.1. Reliance on Continuous Epitopes

Epitopes and paratopes are not structural features of molecules [25][26]. They are entities characterized by a recognizable identity and defined by mutual complementarity [25][26]. Edmundson et al. [25][26][27] proposed the contact model of “flexible keys and adjustable locks” for epitope and paratope interaction. An epitope may be characterized as continuous and discrete [25][26]. Modeling of continuous epitopes by means of APT is often used for the sequences between 5 and 15 amino acids. The application of APT to discontinuous epitopes, between 10 and ≈20 amino acids, is more complicated, and similarly to Abs, often requires complex procedures that involve precise definition in structural terms, i.e., X-ray crystallography [7][20][25]. Discontinuous epitopes consist of amino acid side chains of two to five separate protein fragments that are brought together by the folding of the peptide chains—which act as a scaffold [25].

Recent computational docking methods for protein and peptide interactions, and progress in peptide library-use concerning synthetic and/or structurally modified peptides, enable comparative studies and engineering of both continuous and discontinuous peptides and selection of potential motifs/lead compounds [20][28][29][30][31]. Novel protein-peptide docking procedures are based on different aspects of interaction studies, including inhibitor screening, model prediction, experimental data interpretation, specificity of prediction, and design of interfering peptides [28].

2.2. Overconfidence in the Ligand Specificity

Selective targeting of peptide motifs (epitopes) could be achieved via APT (**Figure 2**a), with certain advantages, disadvantages, and differences with respect to the antibodies. When an antisense peptide is used, its small size—in comparison with the antibody—enables depth of tissue penetration [20]. The binding affinity measurements for antisense peptides are often in the micromolar K_d range, while the values for the antibodies are in the nano- to micromolar range, and maximum care must be applied to the selection of peptides with optimal affinity, i.e., the lowest possible K_d [19][20].

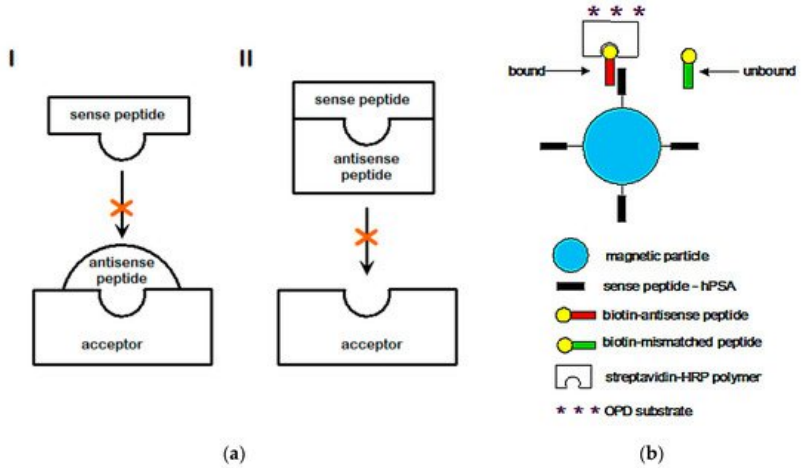

Figure 2. (**a**) Possible interactions of the complementary peptides: (I) acceptor-antisense binding, and (II) sense-antisense binding. (**b**) Schematic representation of peptide-based magnetic particle enzyme immunoassay (MPEIA) according to Štambuk et al. [20].

Different methods have been used to evaluate sense-antisense peptide interactions, ranging from microtiter plate assay methods (immunoassays) to high-performance affinity chromatography, and other related techniques [2][3][4][5][6][12][13][14][15][18][19][20].

Standard biochemical methods for this type of analysis include enzyme-linked immunosorbent assay (ELISA), magnetic particle enzyme immunoassay (MPEIA, **Figure 2**b), and microscale thermophoresis. Meanwhile, the use of other methods, usually depending on the experimental design, includes tryptophan fluorescence spectroscopy, biosensor-based surface plasmon resonance, resonant mirror (RM) biosensor assays, electrospray ionization mass spectrometry, and NMR spectroscopy [2][3][4][5][6][12][13][14][15][18][19][20][32]. Although the results of binding affinity measurements may vary from method to method, recent comparative measurements involving tryptophan fluorescence spectroscopy, microscale thermophoresis (MST), and MPEIA showed consistent results for these simple, quick, and inexpensive methods that could be used for high-throughput screening [18][19][20]. However, binding affinity and biological activity are not synonymous because high affinity is not necessarily accompanied by high activity [32][33].

2.3. Amino Acid Bias in Characterizing Ligand-Acceptor (Receptor) Interactions

The huge difference in the number of possible antisense peptides available for sequence selection and possible database screening depends on the direction of the mRNA translation. The standard genetic code table specifies the translation of antisense (or complementary) peptides in two directions. **Table 2** shows that 27 antisense amino acid pairs are derived by the 3′ → 5′ translation direction and significantly more (52 pairs) are obtained using the 5′ → 3′ direction algorithm [16][17][18][19][20]. The latter result is due to the fact that 5′ → 3′ antisense translation of the genetic code is based on 16 groups of codons, while 3′ → 5′ antisense translation depends on only four codon groups [19]. According to Siemion et al. [13][19], there are three main hypotheses concerning the interaction of sense-antisense peptides based on complementary coding principles.

The Mekler-Blalock antisense hypothesis is based on the hydropathic complementarity principle of sense and antisense peptide interactions, named Molecular Recognition Theory (MRT), which is independent of the direction of triplet reading, since the central (second) base of the coding triplet specifies the hydropathy of the amino acid [7][8][9][13][19].

According to Root-Bernstein, the antisense approach in the 3′ → 5′ direction applies to peptides of <20 amino acids that may lack specific secondary and tertiary structure [3][4][5][6][18][19][20]. Such design leads to significantly fewer antisense peptides and represents a plausible solution for the screening of bioactive ligands [3][4][5][6][18][19][20].

The Siemion hypothesis of sense-antisense peptide interaction is based on the periodicity of the genetic code, i.e., the Siemion one-step mutation ring of the code, and the resulting sense-antisense amino acid pairs are in most cases similar to the 3′ → 5′ translation direction [13][19].

The clustering of amino acid pairs, according to interaction preference, is defined by the complementary U ↔ A and C ↔ G bases of the second codon base. The second codon base, according to Woese, specifies the physicochemical properties of the amino acids [24][34]. Therefore, it is not surprising that diverse amino acid properties—like hydrophobicity, hydrophilicity, lipophilicity, and molecular descriptors of contact potential (Miyazawa-Jernigan), hydrophobic moment, and intrinsic disorder—follow the identical sense and antisense complementarity clustering scheme that is associated with

molecular interaction at the peptide level (≥4 aa) [7][8][9][10][11][12][13][14][15][16][17][18][19][20][21][22][23][24]. In a recent article, Štambuk et al. [19] emphasized that “the natural genetic coding algorithm for sense and antisense peptide interactions combines elements of amino acid physico-chemical properties, stereochemical interactions, and bidirectional transcription”. The relationship of the genetic code and amino acid polarity with respect to protein structure and temperature conditions are discussed in reference [24] and the related Data in Brief article.

2.4. Difficulties in the Estimation of Structure-Function Relationships between Specific Ligand-Acceptor (Receptor) Pairs

The effects of an antisense ligand on its sense receptor (acceptor) may arise from the biological modulation and/or neutralization of the sense peptide effects by means of [6][7][17][35]:

- peptides binding into molecular complexes (leaving none or low levels of sense peptide to elicit its own biological effects),
- total or partial antagonization of the sense peptide receptor by means of its complexation with an antisense ligand,
- combination of the first two factors,
- other biological or biochemical effects of an antisense peptide that cannot be explained by the involvement of a sense peptide and its receptors (e.g., generation of bioactive antibodies to peptides and/or their complexes, cellular receptor, and growth factor modulation).

Each of those points should be carefully analyzed in the context of receptor binding and the biological effects observed under specific experimental designs. For example, in proliferative and biochemical studies involving cellular receptors and serum/plasma proteins, experimental results may be modified by the anticoagulant used—heparin, citrate, or EDTA [36][37].

Amino acid isomerization may also be an important factor in modifying protein or peptide structure, interaction, and receptor binding properties [16][19][33][38][39][40].

2.5. Amino Acid Coding, Complementarity, and Frameshifts

Amino acid coding with respect to complementary protein constructs, mutation, and frameshifts have been studied by many authors, including Arques and Michel [41][42], Bartonek et al. [43], McGuire and Holmes [21], Štambuk [44][45], Wichmann et al. [46][47], and Youvan et al. [48][49][50].

A recent article by Bartonek et al. [43] showed that a frameshifting mechanism could be an effective evolutionary strategy for generating novel proteins with mostly unchanged physicochemical properties. Nevertheless, an important aspect of frameshift coding related to antisense/complementary sequences needs to be addressed. In 1996, Arques and Michel [41] [42] identified a complementary circular code of trinucleotides (X) which on average has the highest occurrence in the reading frame (X_0) compared to the two shifted frames (X_1 and X_2).

This code was found in the protein coding genes of bacteria, archaea, eukaryotes, plasmids, and viruses [42][51]. It enables the reading frames to be retrieved in genes without start codons and with a window length of ≥13 nucleotides [41][42]. The frame X_0 consists of 12 amino acids (A, N, D, Q, E, G, I, L, F, T, Y, V), while frames X_1 (A, R, C, I, L, K, M, P, S, T, V) and X_2 (A, R, C, Q, G, H, L, P, S, W, Y) have 11 amino acids each [41][42][51]. With respect to the antisense codon and amino acid translation in the 5′ → 3′ direction, the X_0 frame of the circular code is self-complementary, and X_1 and X_2 frames are mutually complementary [41][42]. In 1999, Štambuk showed that the combinatorial necklace model enables the use of coding theory arithmetic in the analyses of the genetic code and circular code antisense translations [24][44][45][52].

Two seemingly opposite biological coding rules are characteristic for the interpretation of the SGC frameshifts and related mathematics—including complementary transformations within frames. They both deal with the mechanisms of translation error-control and flexibility and could have an important impact on SGC repertoire manipulations.

The first coding rule is that X_0, X_1, and X_2 frames of the circular code distinguish three possible reading frames of the protein-coding sequence since hidden stop codons in X_1 and X_2 prevent off-X_0-frame protein translation—this procedure is often named ambush hypothesis [53][54], and it is thought to ensure accurate translation.

Paradoxically, the second coding rule—related to SGC flexibility—is that stop codon readthrough may be promoted by the nucleotide environment, with glutamine (Q), tyrosine (Y), and lysine (K) inserted at UAA and UAG stop codons, whereas

tryptophan (W), cysteine (C), and arginine (R) could be inserted at a UGA stop codon [55][56].

Considering bioengineering modeling, a reduced number of amino acids in frames X_0, X_1, and X_2 match the criteria for the use of simplified amino acid alphabets for engineering purposes and related sample space reductions [57]. Consequently, we measured the relationships of the main amino acid (aa) properties addressed by Bartonek et al. [43] in the frames X_0, X_1, and X_2 of the complementary circular code [41][42]. The factors of amino acid polarity, secondary structure, molecular volume, diversity, and electrostatic charge by Atchley et al. [58] were correlated to scales of nucleobase/amino acid interaction preferences for guanine (GUA), purines (PUR), and pyrimidines (PYR) [43][59].

A significant rise in the correlation of amino acid polarity to preference scales for guanine GUA, PUR, and PYR was observed in frame X_0 (**Table 3**). In frame X_1 (shifts +1 and −2), we found a strong correlation between codon and amino acid diversity factor and GUA, PUR, and PYR scales (**Table 3**). This observation is not surprising, since Atchley et al. [58] reported that diversity factor exhibits a highly significant correlation to amino acid physiochemical attributes and substitution matrices, and the X_1 frame is specified by the second codon base, which is associated with the majority of such information [24][34][60].

Table 3. Correlations between amino acid factors and preference scales in frames 0 and +1 (−2).

Parameter	Polarity (20 aa)	Polarity (X_0, 12 aa)	Diversity (X_1, 11 aa)
GUA—nucleobase preference	**−0.54 ***	**−0.63 ***	**0.71 ***
PUR—nucleobase preference	**−0.07**	**−0.49 ***	**0.82 ***
PYR—nucleobase preference	**0.06**	**0.49 ***	**–0.85 ***

* $p < 0.05$ (Pearson's R); aa = amino acid.

However, in frame X_2 (shifts +2 and −1), correlations between physiochemical factors and nucleobase preference scales were not significant. This observation is in agreement with recent findings that, contrary to X_1, the frame X_2 of the complementary circular code is less optimized than the SGC to reduce the effects of +2 and −1 frameshifts, in particular with respect to the physicochemical properties of amino acids [51].

A rise in correlation among amino acid factors and nucleobase preference scales in frames X_0 and X_1 of the circular codes may reflect the importance of the first two bases for the variables encoding scheme [24][34][60], and points to a possible application of GUA, PUR, and PYR scales [43][59] to different genetic code analyses. In our opinion, comparative investigations of complementary circular code and SGC—concerning frameshifts, error-correction, evolution, and biological engineering—seem to be justified.

As emphasized by Choi et al. [61], "ribosome is intrinsically susceptible to frameshift before its translocation and this transient state is prolonged by the presence of a precisely positioned downstream mRNA structure". Additionally, according to Rozov et al. [62], ribosome also "prohibits the G-U wobble geometry at the first position of the codon–anticodon helix". Therefore, it is not surprising that programmed ribosomal frameshifting enables reverse-genetics approaches and the construction of modified viruses with engineered deletions and/or foreign inserts [63].

Such engineering procedures could be used: 1. for artificial control of gene expression at the translation level, and 2. to generate differentiable marker vaccines and modified live virus vaccines [61][63]. More details on the challenges and perspectives of reverse vaccinology (RV) approaches may be found in Van Regenmortel [64] and Moxon, Reche, and Rappuoli [65].

3. Perspective

The applicability of APT was confirmed recently for the magnetic particle enzyme immunoassay (MPEIA, **Figure 2**b) and immunohistochemical procedures [19][20]. This opens a perspective for the development of a new class of efficient immunochemical assays based on short peptide technology [18][19][20]. Additionally, it was also shown that modern computational methods enable a new approach to the studies of sense and antisense peptide interactions [20]. Several free web-based services for protein structure prediction and modeling (e.g., I-TASSER, Phyre2, PEP-FOLD 3, CABS-dock) enable accurate protein-peptide docking, i.e., in silico search for the peptide binding sites [20][28].

Small molecules and peptides may be also used for blocking protein-protein and protein-peptide interactions. In addition to NMR and X-ray crystallographic methods and mutational data, computational and virtual spectroscopy methods—such as the informational spectrum method (ISM)—could be also used to define hot spots in proteins [18][20]. An APT-based approach is also useful for peptide interaction and pharmacophore modeling [32][35]. The application of artificial proteins in the context of APT is also a plausible method to derive new antisense modulators of the protein interactions [19][24][66][67].

APT could be easily adapted to magnetic and polystyrene bead assays, conventional ELISAs, and multiplex assays, so it is possible to achieve two major lines of quick and sensitive assay development: 1. MPEIAs read with appropriate absorbance readers, and 2. Multiplex ELISAs read with appropriate imagers (e.g., with a high-resolution chemiluminescence readers for printed microtiter plates) [19][20].

Developing new immunoassays is important for situations such as the infection outbreaks due to the possibility to design—in a relatively short time—quick, inexpensive, and simple assays that could be automated to obtain medium/high throughput screenings of particular binders, peptide motifs, and antibodies, etc. If carefully selected, such laboratory techniques enable the experimental application of different laboratory procedures which, depending on the experimental design, may be used for:

- selection of different targets and evaluation of complementary (sense–antisense) peptide binding;
- quantification of specific antibodies, peptides, and proteins;
- design of MPEIAs and Multiplex ELISAs tailored for a specific purpose.

The benefits of APT outweigh the costs of medium/high throughput screening and random peptide libraries and could lead to considerable savings in time and money. Practical applications and benefits of APT application are:

1. Quick design and validation of the complementary ligands and acceptors;
2. Computational validation and virtual screening of different protein and peptide structures;
3. Rationalization of peptide library screening;
4. The tests can be produced in a short period of time;
5. The tests will be made composite (according to the LEGO principle) and will consist of less expensive and commercially available components;
6. The time required to obtain results is shorter (since no antibody production is needed);
7. The test enables large quantity sample testing using standard laboratory equipment (since it does not require special reagents or complicated sampling processing);
8. The tests are likely to prove important for the investigation of the immune response, disease pathogenesis, and clinical outcome of different infections;
9. Designed antisense peptides (and anti-antisenses [21]) may also provide a basis for further development of vaccines and lead compounds for different diseases;
10. Detection of mutant strains is quicker since new antisense peptide motifs could be synthesized, evaluated for binding, and easily linked to magnetic particles in a short period of time, which avoids the antibody production process;
11. A green chemistry approach significantly reduces or avoids the loss of animal life.

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