CROATICA CHEMICA ACTA
CCACAA **79** (3) 379–383 (2006)

ISSN-0011-1643
CCA-3103
Original Scientific Paper

Alpha-melanotropin Peptide: Structure and Ligand–Receptor Recognition

Karlo Houra,[a] Nikola Štambuk,[b,*] Paško Konjevoda,[b] Alenka Boban-Blagaić,[c] Tomislav Bruketa,[d] and Biserka Pokrić[b]

[a]*Department of Neurosurgery, University Hospital »Sestre Milosrdnice«, Vinogradska 29, HR-10000 Zagreb, Croatia*

[b]*Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia*

[c]*Department of Pharmacology, School of Medicine, University of Zagreb, Šalata 4, HR-10000 Zagreb, Croatia*

[d]*Clinical Hospital Center Zagreb, Kišpatićeva 12, HR-10000 Zagreb, Croatia*

RECEIVED NOVEMBER 28, 2005; REVISED FEBRUARY 27, 2006; ACCEPTED MARCH 16, 2006

The hydropathic profile, secondary structure, epitope and binding site of the α-melanotropin molecule were investigated. It was shown that the standard algorithm according to Kyte and Doolittle may be combined with complex methods of the secondary structure prediction to extract the information relevant for the epitope location and modeling. The binding ligand-receptor motifs of the hormone were investigated by means of the SSpro8 method. The Molecular Recognition Theory combined with an NCBInr protein database search was applied to find the possible paratope (receptor) structures for the predicted α-melanotropin epitope (ligand). The described concept constitutes a useful and simple set of procedures for deriving new biologically active peptides and antibodies and also for performing modulation of peptide-receptor interaction.

Keywords
alpha-melanotropin
protein structure
ligand
receptor
recognition

INTRODUCTION

Pro-opiomelanocortin (POMC) hormone is synthetized in the gland.[1] Several important and biologically active peptides are derived from the large POMC precursor molecule.[1,2] Among them are melanotropins (α-, β- and γ-MSH), potent endogenous inhibitors of inflammation.[1,2] Melanotropins share a common amino acid motif HFRW and exert their effects by activating seven-transmembrane domain G-protein-coupled melanocortin receptors (MCR).[2–4] During the last decade, five members of this receptor family, MC1R to MC5R, have been characterized and cloned.[1–4]

Proteolytic cleavage of POMC is done by pro-hormone convertases PC1 and PC2.[2] First, PC1 leads to the adrenocorticotrophic hormone (ACTH) and subsequently PC2 leads to the formation of alpha-melanotropin, *i.e.,* α-MSH.[2] Alpha-melanotropin is an ancient, evolutionally conserved, tri-decapeptide that corresponds to the first 13 amino acids of ACTH.[2,5,6] It is the most widely studied peptide in the context of inflammation, involved in the host reaction to infectious and inflammatory stimuli in humans.[2,5,6] Some of its protective mechanisms include a compensatory increase in the presence of inflammation,[5–7] reduction of proinflamatory cyto-

* Author to whom correspondence should be addressed. (E-mail: stambuk@irb.hr)

kine synthesis, and antipyretic effects.[2,5–7] Anti-inflammatory effects of the peptide are thought to be mediated through MCR1, MCR3, MC4 and MCR5.

In this study, we investigated and modelled hydrophobicity, secondary structure, receptor binding site, antigenicity and molecular recognition of the α-melanotropin peptide and its possible receptor targets.

METHODS

Hydrophobicity Analyses

The hydrophobicity profile of α-melanotropin was analyzed by means of the algorithm by Kyte & Doolittle.[8,9] The values of amino acid hydrophobicity presented in Table I were averaged over a segment of 7 residues and plotted (Figure 1). This procedure enables the prediction of exposed loops, including epitopes.[9]

Secondary Structure Prediction

Analysis and prediction of the α-melanotropin structure (Table II) was done by means of the SSpro8 structure prediction method.[10–11] SSpro is a server for protein secondary structure prediction based on an ensemble of 11 BRNNs (bidirectional recurrent neural networks).[10–11] This secondary structure prediction procedure is an extension to SSpro and adopts the full DSSP 8-class output classification as follows:

1. H: α-helix
2. G: 3_{10}-helix
3. I: π-helix
4. E: β-strand
5. B: β-bridge
6. T: β-turn
7. S: bend
8. C: the rest

The system is available online (based on PSI-BLAST profiles) and may be found at the address: http://www.igb.uci.edu/tools/scratch/.

Molecular Recognition of the Receptor

According to the Molecular Recognition Theory (MRT), sense and antisense peptides have mutually complementary shapes, which results in their interaction.[12–18] This is due to the fact that in antisense (complementary) peptides, due to the genetic code, hydrophilic and hydrophobic patterns of amino acid polarity are changed into the opposite ones.[12–18] Table I presents complementary (antisense) peptide pairs arising from the genetic code table.[14–16]

MRT was used to extract an antisense peptide complementary to α-melanotropin and its paratope motif located at the amino acid positions 5–8. This quadripeptide (LVKA) is an MRT antisense transcript of the α-melanotropin epitope predicted by means of the Kyte & Doolittle algorithm and SSpro8 structure prediction method (Figure 1, Tables I-II). A ProteinInfo sequence search of the NCBInr database was used to locate molecular receptors for the LVKA natural ligand (EHFR motif of α-melanotropin).

TABLE I. Antisense (complementary) pairs of amino acids for peptides and proteins

Amino acid (aa)	Kyte & Doolittle hydropathy	Natural antisense (transcribed 3'→5')	Antisense (transcribed 5'→3')
I (Isoleucine)	4.5	Y	Y, N, D
V (Valine)	4.2	H, Q	H, N, D, Y
L (Leucine)	3.7	N, E, D	E, K, Q
F (Phenylalanine)	2.7	K	K, E
C (Cysteine)	2.5	T	T, A
M (Methionine)	1.9	Y	H
A (Alanine)	1.8	R	R, G, S, C
G (Glycine)	–0.4	P	P, S, T, A
T (Threonine)	–0.7	C, W	G, S, C, R
W (Tryptophan)	–0.9	T	P
S (Serine)	–0.9	S, R	R, G, T, A
Y (Tyrosine)	–1.3	M, I	I, V
P (Proline)	–1.6	G	G, W, R
H (Histidine)	–3.2	V	V, M
D (Aspartic acid)	–3.5	L	I, V
E (Glutamic acid)	–3.5	L	L, F
N (Asparagine)	–3.5	L	I, V
Q (Glutamine)	–3.5	V	L
K (Lysine)	–3.9	F	F, L
R (Arginine)	–4.5	A, S	A, S, P, T

Kyte & Doolitttle hydropathic amino acid scores of the antisense peptide pairs in Table I exhibit strong negative correlation to the receptor amino acid values (r = –0.94).[16] Codons coding for hydrophilic amino acids are mostly complemented by the antisense codons for hydrophobic ones (87.5 %), and *vice versa* (63.6 %).[16] Otherwise, matching amino acids belong to the neutral group.[16] Neutral amino acids are in most cases (62.5 %) complemented by the neutral ones, and in the few cases of exception either by polar (12.5 %) or nonpolar amino acids (25 %).[16]

RESULTS AND DISCUSSION

Sequence Analyses

The simplest analysis of a protein sequence is a hydrophobicity plot of the amino acid hydropathy along the chain.[8,9,19] It is used to predict the positions of exposed and buried residues, antigenic sites, membrane-spanning regions and turns between elements of the secondary

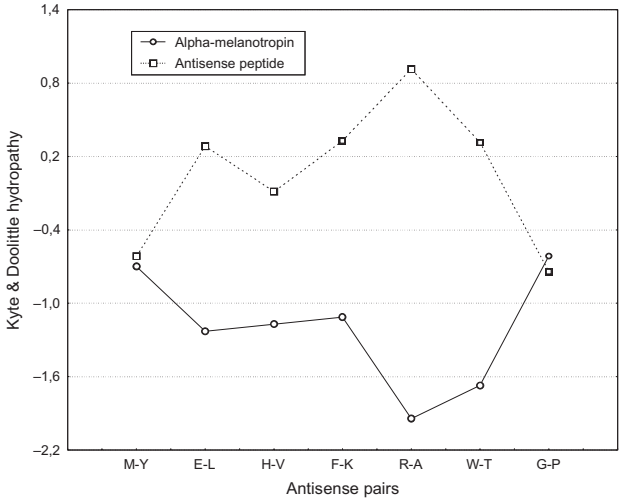

Figure 1. Hydropathy patterns of α-melanotropin and its 3'→5' antisense peptide over a 7-residue-segment (amino acids 4–10).

structure.[8,9,19] The standard Kyte & Doolittle algorithm uses amino acid hydropathy values averaged over a short segment, 7 residues, to predict exposed loops/epitopes. The transmembrane regions are analyzed by averaging a segment of 15 to 19 residues.[8,9] Positive values, usually >1.6, point to the transmembrane segment, while negative ones point to the exposed loops/antigenic site.[8,9]

The analysis of the α-melanotropin sequence, with Kyte & Doolittle hydropathy values averaged over a segment of 7 residues, is shown in Figure 1. It can be clearly seen that hydrophilicity is prominent for the α-melanotropin amino acids 5–9 with the peak at amino acids 8 (R) and 9 (W). The hydrophilic amino acid motif 6–9 (HFRW) is known as the part of the melanotropin molecule important for its receptor binding.[2–4] This motif is shared by all melanotropin peptides (α-, β- and γ-MSH).[2] The peptide structure was additionally investigated by means of the SSpro8 method. Table II confirmed that the first four amino acids of the α-melanotropin binding site EHFRW (aa 5–9) constitute an extended strand, *i.e.,* β-strand, whereas the last amino acid W along with the next one G belong to a part which was defined as β-turn.

Molecular Recognition

The Molecular Recognition Theory is based on the observation that peptides consisting of amino acids specified by the complementary (antisense) RNA codons bind to each other with higher specificity and efficacy than peptides not specified by the complementary codons.[12–18] According to this theory, sense-antisense peptide pairs behave as ligand-receptor systems.[12–16]

Interacting antisense pairs are often defined from the genetic code table read in natural 3'→5' direction

TABLE II. Secondary structures of α-melanotropin and its 3'→5' antisense peptide predicted by means of the SSpro8 method

α-melanotropin
S Y S M E H F R W G K P V
* * * E E E E E T T * * *
Antisense peptide
R M R Y L V K A T P F G H
* * E E H E E * * * T * *

(Table I).[16] Reverse reading in 5'→3' direction may be also applied for the antisense peptide design.[16] The latter is due to the fact that the hydropathic character of an amino acid residue is related to the middle letter of the messenger RNA codon triplets, which remains the same regardless of the direction of transcription.[13,16] This concept has been successfully applied to more than 40 complementary peptide-receptor systems.[13–18]

Table I shows that in contrast to the small number of 27 possible antisense amino acids read in 3'→5' direction, 52 possible antisense amino acid combinations for 20 possible amino acids of the sense peptide were obtained in 5'→3' direction. Consequently, for a peptide of the amino acid chain length *n*, the average number of all possible antisense peptide substitutions *N* read in 3→5' direction is relatively small, *i.e.,* $N = n^{1.35}$, whereas in 5'→3' direction many more possible substitutions exist ($N = n^{2.6}$) (Table I, Figure 2). Therefore, antisense peptide readings in 3→5 direction are more suitable for rational peptide design of the interacting ligand-receptor systems, or antigen-antibody complexes.[16]

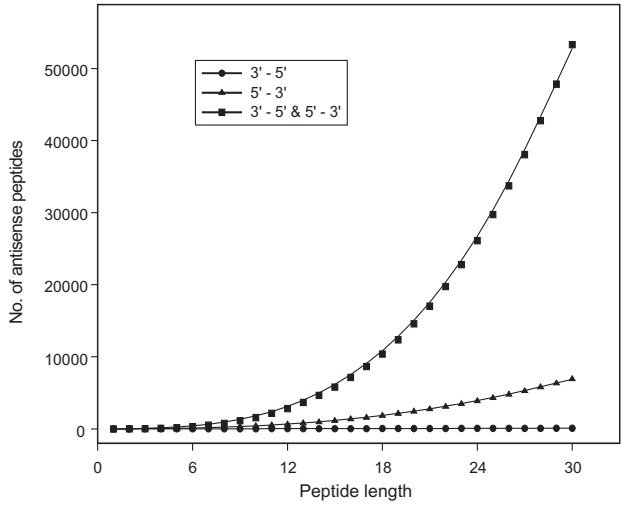

Figure 2. Average number of possible antisense peptide amino acid substitutions arising from the sense peptide readings in 3'→5', 5'→3' and in both directions.

Bioactive Sites

The peaks of hydrophilicity are found at the position of extended peptide motifs/epitopes, which are often successful in raising antibodies.[9,19] We applied the Molecular Recognition Theory to define the motif LVKA as a possible receptor site for the α-melanotropin receptor binding motif EHFR (3'→5' and 5'→3'). Quadripeptide EHFR at α-melanotropin positions 5–8 is predicted to be of β-strand structure (Table II). It has been reported that β-strand peptide conformation and structural similarity of the interacting peptides provide better binding affinity of the sense-antisense pairs.[15–16] Two α-melanotropin amino acids at positions 9–10 were excluded since they belong to β-turn (Table II). An NCBInr protein database search of the motif LVKA confirmed that it is present, as a possible binding site for α-melanotropin, in a number of G-protein-coupled receptors, T cell receptor and antibody/immunoglobulin chains (Table III). The analysis is consistent with the known data on possible receptor sites for the α-melanotropin and its immunomodulatory functions,[1–7] and Blalock's observation that antibodies interact as a result of complementary shape and reflect the form of the peptides.[13]

Binding sites predicted for the α-melanotropin in Table IV indicate that some of the known immunomodulatory functions of this hormone may be explained by means of the Molecular Recognition Theory, *i.e.,* ligand-receptor interactions of the complementary peptides. Immune system molecules sharing fragments of antisense peptide could account for the effects of α-melanotropin on lymphocytes, antibodies and neutrophils.[2,5,6] Štambuk *et al.*[16] showed recently that cytoprotective effects exerted by α-melanotropin were also abolished by means of its antisense peptide. This confirmed the *in vitro* binding results of Blalock and Bost.[12,16]

Empirical observations of specific interactions between complementary (antisense) peptides suggest that the new concept of the proteomic code could prove valuable in bridging the gap between gene and protein coding systems.[15] Štambuk *et al.*[20–22] reported that the secondary protein structure could be accurately predicted from the mRNA strings. However, a comparative analysis of the interacting 2D and 3D peptide structures within sense-antisense MRT pairs is missing at the moment. An extended, *i.e.*, β-strand, peptide conformation of the interacting sense-antisense pairs is thought to provide better binding,[15,16] though some authors point out that hydropathic complementarity *per se* is not responsible for the interaction between sense and antisense peptides.[15,16] A single, conclusive, and reproducible algo-

TABLE III. Receptor and antibody binding sites for the α-melanotropin motif EHFR according to the Molecular Recognition Theory

α-melanotropin antisense motif LVKA	Version [Homo sapiens]	Location *
Putative G-protein coupled receptor	gi|20152314|dbj|BAB89338.1|	165–168
G-protein-coupled receptor	gi|2668608|gb|AAC51905.1|	8–11
Sphingolipid GPCR-1 (EDG1)	gi|49457532|emb|CAG47065.1|	8–11
Seven transmembrane helix receptor	gi|21928418|dbj|BAC05802.1|	144–147
T-cell receptor beta-chain	gi|338983|gb|AAA61017.1|	30–33
Immunoglobulin heavy chain	gi|47600815|emb|CAG29711.1|	11–14
Immunoglobulin alpha-1 chain	gi|553351|gb|AAA52733.1|	3–6
Immunoglobulin gamma-chain	gi|474960|gb|AAA58691.1|	30–33
Ig kappa chain variable region	gi|16076485|emb|CAC94463.1|	4–7
Ig heavy chain variable region	gi|10636616|emb|CAC10680.1|	11–14
IgE variable region	gi|1197507|emb|CAA64959.1|	29–32

TABLE IV. Immune system molecules sharing the fragments of α-melanotropin 3'→5' antisense motif RHRYLVKATPFGH

RHRYLVKATPFGH	Molecule [Homo sapiens]	Location*
RYLV******	Ig hc variable/VHDJ region	102–105/103–106
RYLVK****	T-cell receptor beta-chain	7–11, 16–20, 28–32, 36–40
RYLVK****	Interleukin 8 receptor type 2/B	154–158, 159–163
****LVKATP***	Ig hc variable region	5–10
*******ATPFG*	Ig hc VHDJ region	102–106

rithm for efficient antisense peptide design, verified by several independent research groups, is missing.[15,16]

CONCLUSION

Protein sequence analysis based on simple hydropathy plots may be combined with complex methods of the secondary structure prediction in order to extract the information relevant for the epitope location and modelling. Following this first step, the Molecular Recognition Theory with a subsequent database search may be applied to find possible receptor/paratope structures for particular epitope ligands. The described concept could constitute a useful and simple set of procedures for deriving new biologically active peptides, antibodies and for performing peptide-receptor modulation.

Acknowledgement. – This work was supported by the Croatian Ministry of Science, Technology and Sports.

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SAŽETAK

Alpha-melanotropni peptid: Struktura i prepoznavanje liganda i receptora

Karlo Houra, Nikola Štambuk, Paško Konjevoda, Alenka Boban-Blagaić, Tomislav Bruketa i Biserka Pokrić

Istraživan je hidropatski profil, sekundarna struktura, epitop i vezno mjesto molekule α-melanotropina. Pokazano je kako se standardni algoritam prema Kyte i Doolittleu može kombinirati s kompleksnijim metodama predikcije sekundarne strukture kako bi se dobila relevantna informacija o položaju i modeliranju epitopa. Vezanje ligand-receptor motiva navedenog hormona istraživano je pomoću SSpro8 postupka. kako bi se pronašli mogući paratopi (receptori) za predviđene α-melanotropinske epitope (ligande). Primijenjeno je i pretraživanje NCBInr proteinske baze podataka pomoću teorije molekularnog prepoznavanja. Opisani koncept predstavlja koristan i jednostavan skup postupaka za izvođenje novih biološki aktivnih peptida i protutijela, kao i za provođenje modulacije interakcije sustava peptid-receptor.