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ANTISENSE SEQUENCES OF ANTIGENIC PEPTIDES ARE FOUND IN MHC CLASS II MOLECULES

William Campbell and Hidechika Okada

Department of Molecular Biology, Nagoya City University School of Medicine, Nagoya 467, Japan

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SUMMARY The sequence of the well known antigenic peptide OVA 323-339 as well as other peptides containing reiterative motifs of the core region essential for binding to Class II MHC molecules was compared for homology with the DNA derived antisense peptide sequences corresponding to the alpha chain of the mouse IA^d molecule. Homology was obtained within the sequence found in the transmembrane region of the molecule. Increased binding of the peptides containing the reiterative motifs based on the OVA 323-339 sequence may be due to the increased number of contact sites on IA^d molecules which were identified as being complementary in a sense-antisense manner.

It has been recently demonstrated that synthetic peptides derived from the non coding strand of DNA can bind with high affinity and specificity to peptides derived from the coding DNA strand (1,2,3). The interaction has been related to the tendency in the genetic code for codons of hydrophilic amino acids to be complemented by codons on the complementary strand for hydrophobic amino acids (4,5). A possible consequence of the above interactions is that many peptides such as hormones can assume amphiphilic secondary structures in the presence of another amphiphilic one such as a receptor site or membrane site (6).In fact, in the presence of their respective receptors, peptide hormones have been predicted to form amphiphilic conformations (7,8). The above information has been used to generate antisense peptides specified by RNA sequences complementary to the mRNA for ACTH (9), γ-endorphin (10), ribonuclease S peptide (1), luteinizing hormone releasing hormone (LHRH) (11), angiotensin II (12), fibronectin (13) and

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interleukin 2 (14). Because the binding of sense and antisense peptides may result from one peptide being an "internal image" of the other, then it follows that an antibody to the antisense peptide should bind to the receptor for the same peptide. This has indeed proven to be true for ACTH (9), γ-endorphin (10), LHRH (11), angiotensin II (12) and fibronectin (13). Such antibodies have been used to affinity purify the receptors for the above ligands and have also been shown to mimic the action of a hormone on its receptor (9,12). Another practical application of sense-antisense peptide interactions was recently demonstrated when the complementary sequence coding for a segment in cystatin C (an inhibitor of cysteine proteases) suggested to be the active site of inhibitory activity was used to search for complementary sequences in a protein data bank (15). Homology was found in the beta chain of human C4. The authors were then able to demonstrate specificity and saturability of the interaction between cystatin C and the complementary deduced peptide as well as inhibition of the interaction between C4 and cystatin C by the antisense peptide. The concept that complementary peptides are capable of binding hormones in receptor like fashion was used to demonstrate that ligands and receptors such as EGF, IL-2 and transferrin contain complementary regions of nucleic acid (16). This was done by comparing the ligand DNA sequences to complementary antisense sequences of the receptors. Significant homology was detected, that is four out of six nucleotides in succession were homologous and because of the redundancy in the genetic code, five out of six amino acids were homologous in each case. Furthermore the homologous segments corresponded to complementary regions in the ligand binding portions of the receptors. Based on the fact that sense and antisense peptides have affinity for each other, we investigated whether or not any interaction of this kind could be

involved in the binding and presentation of an antigenic peptide in the context of Class II MHC molecules. We chose the well characterized immunogenic peptide fragment OVA 323-339 as well as reiterative motifs based on this sequence. In this article we show that these peptides contain sequences which are complementary in sense-antisense fashion to a sequence found in the transmembrane region of the alpha chain of IA^d molecules and we offer an explanation for the increased binding of these reiterative motif sequences.

MATERIALS AND METHODS

Homology searches were conducted by generating an amino acid sequence corresponding to the RNA sequences of the target protein for example, MHC Class II IA^d, as well as the complementary non-coding strand (antisense) RNA sequences read in antiparallel fashion (Fig.1). These antisense peptide sequences were compared to the sense peptide eg. OVA 323-339 for homology using the GENETYX homology search program (Software Development Corporation, Tokyo, Japan). The program identifies all possible amino acid matches for a given overall percentage homology. This generates a large data file which is then searched with a "search and find" command using Wordstar (Microsoft Corp./NEC, Tokyo, Japan). The search and file command was for two or more consecutive amino acid matches. From the data obtained, we could then identify regions with potential binding affinity for each other based on sense-antisense interaction. Hydropathic profiles were generated using the Kyte and Doolittle method with an averaging of 7 amino acids (17). These profiles were overlapped in antiparallel fashion to identify regions of significant hydropathic complementarity. Amino acid sequences were compared to protein sequences in a protein data base, PC GENE, Databank Release 2, 1989 (Intellegenetics Inc., Mountain View, CA 94040, USA) containing 10,856 protein sequences.

RESULTS AND DISCUSSION

Based on the above considerations, we reasoned that some region in MHC molecules may contain hydropathic complementarity for immunogenic peptides and that this may be in part responsible for the binding of peptide fragments for presentation. We therefore compared the antiparallel DNA-derived consensus peptide sequences of IA^d molecules to that of the sequence OVA 323-339 as well as to other synthetic peptides whose sequences are based on the OVA sequence (Table 1). OVA 323-339 is known to be a good binder of

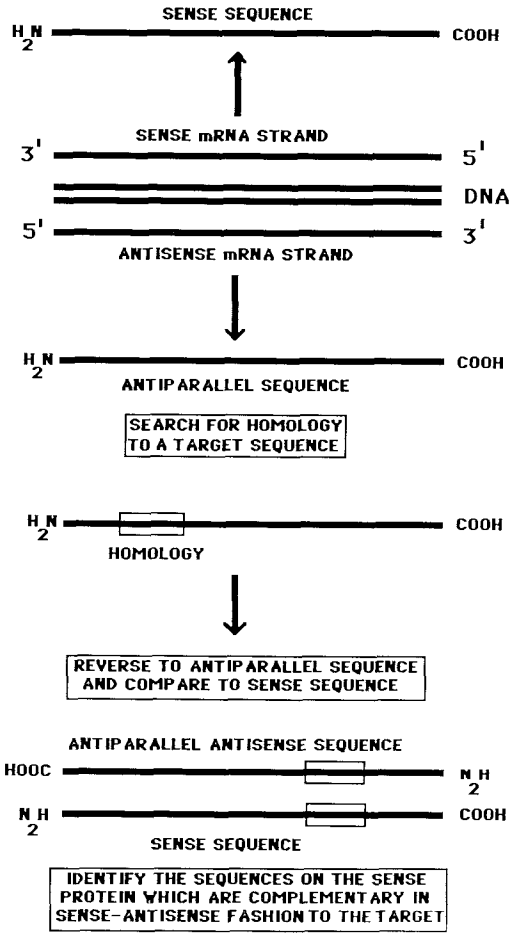

Fig 1. Schematic outline of the strategy used to search for sense-antisense complementarity.

IA^d molecules and is also known to be effective in stimulation of MHCII restricted T cell proliferation (18). Surprisingly, our results showed homology to be present in the

TABLE 1

CLASS II BINDING OF REITERATIVE ANALOGS TO IA^d MOLECULES

PEPTIDE	SEQUENCE	RELATIVE BINDING CAPACITY[a]
OVA 323-339	ISQAVHAAHAEINEAGR	1.00
ROII	VHAAHAEINVHAAHA	1.62
ROIII	VHAAHAVHAAHAEIN	24.80
ROIV	AHAAHAAHAAHAAHAA	34.00

a. Data were obtained from A. Sette et al. (19).

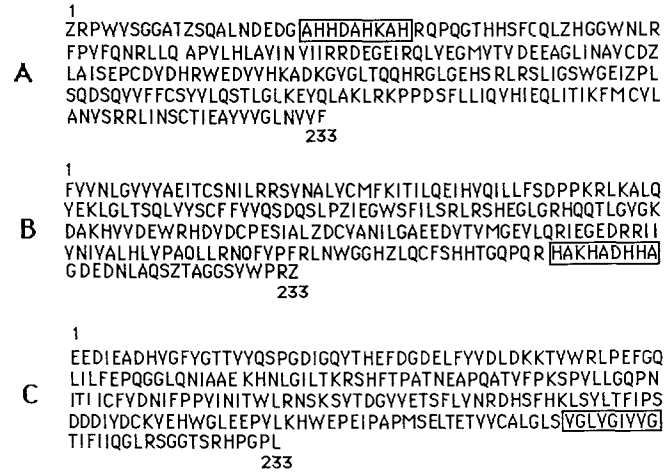

Fig 2. Amino acid sequences used to identify regions of antisense complementarity. A) Antiparallel antisense IA^d sequence; B) Reversed antiparallel antisense IA^d; C) IA^d sequence. Boxed in areas identify the region in IA^d which is complementary in antisense manner to OVA peptide and the reiterative motifs shown in Table 1. Numbers 1 and 233 are amino acid residue numbers from the amino to carboxy terminus.

transmembrane region of the alpha chain of IA^d (Fig 2). When the sequences in this region were compared for homology to a protein data base, none was found. When sequences of synthetic peptides were used that have been reported to exhibit higher binding to IA^d (19), then a much better fit occured, that is, more amino acids lined up (Fig 3). This was true for the reiterative sequence VHAAHAVHAAHAEIN (ROIII) but not for VHAAHAEINVHAAHA (ROII) in which the reiterative sequences were separated by a spacer. These results corresponded with the experimental values

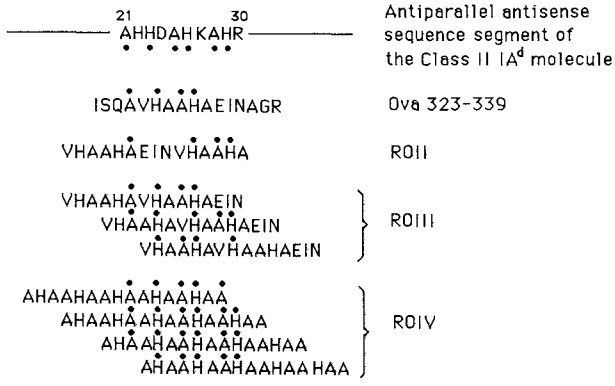

Fig 3. Alignment of the antiparallel antisense sequence of IA^d molecules with Ova 323-339, ROII, ROIII and ROIV.

obtained for binding (19). When the reiterative motif containing only alanine and histidine AHAAHAAHAAHAAHAA (ROIV), which showed the highest binding to IA^d was used to search for homology on the complementary antiparallel derived peptide sequence of IA^d alpha chain, several contact points were found all in close proximity, which may account for the higher binding of reiterative motifs. These peptides containing reiterative sequences may be able to bind not only to the peptide binding pocket in IA^d molecules but also elsewhere as in this case in the transmembrane region. We therefore conclude that more than one binding site exists in IA^d for the motif VHAAH found in OVA 323-339 and that these multiple sites may somehow act synergistically to enhance binding. The fact that the site exists in the transmembrane region raises the possibility that presentation of peptide may be facilitated in some way during or after assembly of IA^d molecules and that binding in this region may generate a signal for some change, perhaps conformational which renders such peptides as OVA 323-339 immunodominant. Another possibility may be that peptide bound in the transmembrane region may be exposed to the bottom of the antigen binding pocket.

Evidence is accumulating that the hydropathic profile rather than specific amino acids is responsible for playing the major role in sense-antisense peptide recognition. Indeed, it has been shown that it is possible to alter the strength of interaction between a target peptide and a computer derived antisense peptide whose sequence is obtained by maximizing hydropathic complementarity (20). When the hydropathic profiles of these peptides are overlapped, a high degree of complementarity can be seen. In view of this fact, we compared the hydropathic profiles of sense and antisense IA^d alpha chain sequences overlapped in antiparallel fashion. We found that the transmembrane region in

the antiparallel antisense sequence where OVA 323-339, ROIV and the reiterative sequence containing only alanine and histidine showed homology is also the most hydropathically complementary (Fig 4). In addition, we believe that by searching for regions of high hydropathic complementarity between two protein sequences, we may be able to predict domains that would have affinity for each other. If the number of amino acids averaged for plotting hydropathic profiles is optimized, then the number of regions of high complementarity are relatively few (21), however, these may represent regions in different molecules potentially capable of interacting with each other. Hydropathic recognition may play a role in several biological processes such as receptor-ligand recognition, contributing ("chaperoning") the assembly of macromolecular structures and self recognition of sequence stretches within the same molecule. Much work needs to be done on understanding sense-antisense peptide recognition, but one thing that remains clear and is demonstrated in this paper is the

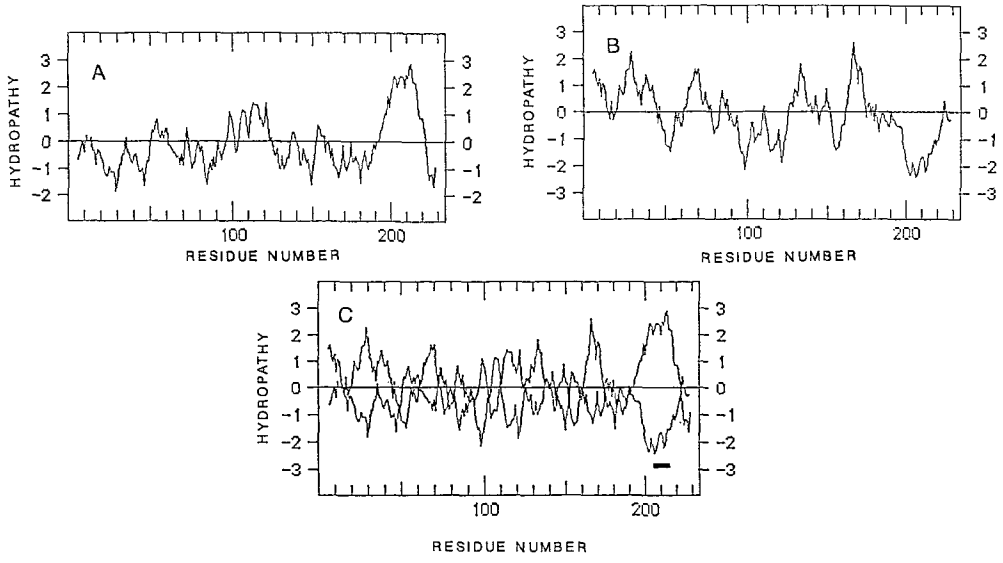

Fig 4. Hydropathic profiles using the Kyte-Doolittle method averaging seven amino acids of the IA^d sense and IA^d antisense sequences. **A**) IA^d; **B**) Reversed antiparallel IA^d; **C**) Overlapped IA^d and reversed antiparallel IA^d. Region of antisense complementarity is indicated by the solid horizontal bar.

usefulness of this phenomenon as a tool for predicting the potential interaction between proteins.

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