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Assessing the preferred solution conformation of an interacting sense–antisense (complementary) peptide pair

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ABSTRACT

Sense peptides and corresponding antisense peptides, are capable of making specific interactions. Such interactions may result from inter-peptide side-chain/side-chain contacts or because peptides adopt mutually complementary three-dimensional shapes. Using a combined ^{1}H NMR spectroscopy/molecular modeling approach to study the interactions between one sense peptide and its corresponding antisense peptide, data are produced that provide clear support for the former hypothesis.

By definition, a sense peptide is one whose amino acid residue sequence is coded for by the codon sequence from a coding region of the sense (positive) strand of DNA (read 5′ → 3′). Conversely, an antisense (complementary) peptide is coded for by the antisense (complementary) codon sequence (read 5′ → 3′) of the corresponding antisense (negative) strand of DNA. Specific interactions have been observed and characterized between corresponding sense–antisense peptide pairs in numerous systems, although the field remains controversial owing to difficulties experienced in understanding the bases of such specific interactions.[1] Applications of these interactions have been demonstrated in over 40 widely differing systems,[1] for instance in the identification of an activator of $\alpha_{IIb}\beta_3$ integrin,[2] and a specific inhibitor of tumor necrosis factor-α (TNF-α).[3] Such results are gradually leading to the possibility of sense–antisense peptide interaction-based oligopeptidic drug design.[1] In our own case, our primary interest in sense–antisense peptide interactions derived from an interest to modulate interleukin-1β (IL-1β) mediated effects. IL-1β belongs to the interleukin-1 family (IL-1F) of cytokines, and is a key, early stage effector in a number of immune and inflammatory response pathways, including the autoimmune response triggered by the debilitating disease rheumatoid arthritis.[4] Given this interest, a family of antisense peptides (principally, VITFFSL-NH_2, C-terminal amide) was devised that was shown to bind specifically to a 7-residue surface loop or β-bulge known as the Boraschi loop (QGEESND; residues 48–54 of the mature protein) of IL-1β leading to measurable inhibition of IL-1β mediated biological responses in vitro[5–7] and even in vivo.[8]

One main theory of interaction is the molecular recognition theory (MRT), according to which sense and corresponding antisense peptides are influenced by opposite internal forces, and therefore adopt mutually complementary three-dimensional shapes with respect to each other in solution.[1] The other main theory of interaction has been called the Mekler–Idlis (M–I) pair theory.[1] According to this theory, sense–antisense peptide interactions are made possible by inter-peptide contacts between the side-chains of sense peptide codon-directed amino acid residues and corresponding

Abbreviations: TNFα, tumour necrosis factor-α; IL-1β, interleukin-1 β; IL-1F, interleukin-1 family; M–I, Mekler–Idlis; MRT, molecular recognition theory; DMSO, dimethylsulphoxide; COSY, correlation spectroscopy; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotational nuclear Overhauser effect spectroscopy; MD, molecular dynamics; vdW, van der Waals; RMSD, root mean square deviation; AP-ITD (rest), anti-parallel main chain alignment of sense–antisense peptide pair with side-chains interdigitated, NMR restraints; AP-ITD (unrest), anti-parallel main chain alignment of sense–antisense peptide pair with side-chains interdigitated, no NMR restraints; AP-PLA (rest), anti-parallel main chain alignment of sense–antisense peptide pair with side-chains parallel planar (stacking), NMR restraints; AP-PLA (unrest), anti-parallel main chain alignment of sense–antisense peptide pair with side-chains parallel planar (stacking), no NMR restraints; P-ITD (rest), parallel main chain alignment of sense–antisense peptide pair with side-chains interdigitated, NMR restraints; P-ITD (unrest), parallel main chain alignment of sense–antisense peptide pair with side-chains interdigitated, no NMR restraints; P-PLA (rest), parallel main chain alignment of sense–antisense peptide pair with side-chains parallel planar (stacking), NMR restraints; P-PLA (unrest), parallel main chain alignment of sense–antisense peptide pair with side-chains parallel planar (stacking), no NMR restraints; MM–PBSA, molecular mechanics–Poisson-Boltzmann surface area.

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antisense (complementary) codon-directed amino acid residues in the antisense peptide. Such specific, through space inter-peptide contacts have been proposed to be limited in the first instance to 26 non-overlapping pairs of amino acid residues that are derived by analysis of the genetic code and its complement.[9] Schematic illustrations of interactions between VITFFSL-NH_2 and QGEESND-NH_2 are shown according to the M–I pair theory. Particular reference is made to two opposing modes of interaction where amino acid residue side chains align plane parallel (**PLA**; M–I Miller Hypothesis) or are interdigitated (**ITD**; M–I Chaiken Hypothesis) (Fig. 1).

Previously, interactions between antisense peptide VITFFSL-NH_2 and IL-1β were demonstrated by the resonant mirror biosensor. K_d, values were measured in the low μM region upwards.[5–7] In a similar way, specific interactions involving other sense–antisense peptide pairs have been characterized by other solid–liquid phase techniques such as high-performance affinity chromatography. In the absence of X-ray crystallographic information, NMR spectroscopy studies have been attempted to characterize sense–antisense peptide interactions.[10] However, these have proved difficult to implement given the weakness of sense–antisense peptide interactions in the first place and also the tendency of sense and corresponding antisense peptide pairs to be hydropathic opposites of each other and therefore mutually insoluble in aqueous buffer or polar organic solvents. In our case we opted for a simple staged process to:

1. use ^{1}H NMR and ^{13}C NMR spectroscopy to diagnose specific interactions between antisense peptide VITFFSL-NH_2 and its corresponding sense peptide (QGEESND-NH_2), in dimethyl sulfoxide (DMSO), a mutually acceptable solvent.
2. deliver on sufficient experimental NMR data restraints to enable molecular dynamic (MD) structure simulations of the QGEESND-NH_2/VITFFSL-NH_2 mixed peptide pair with restraints.

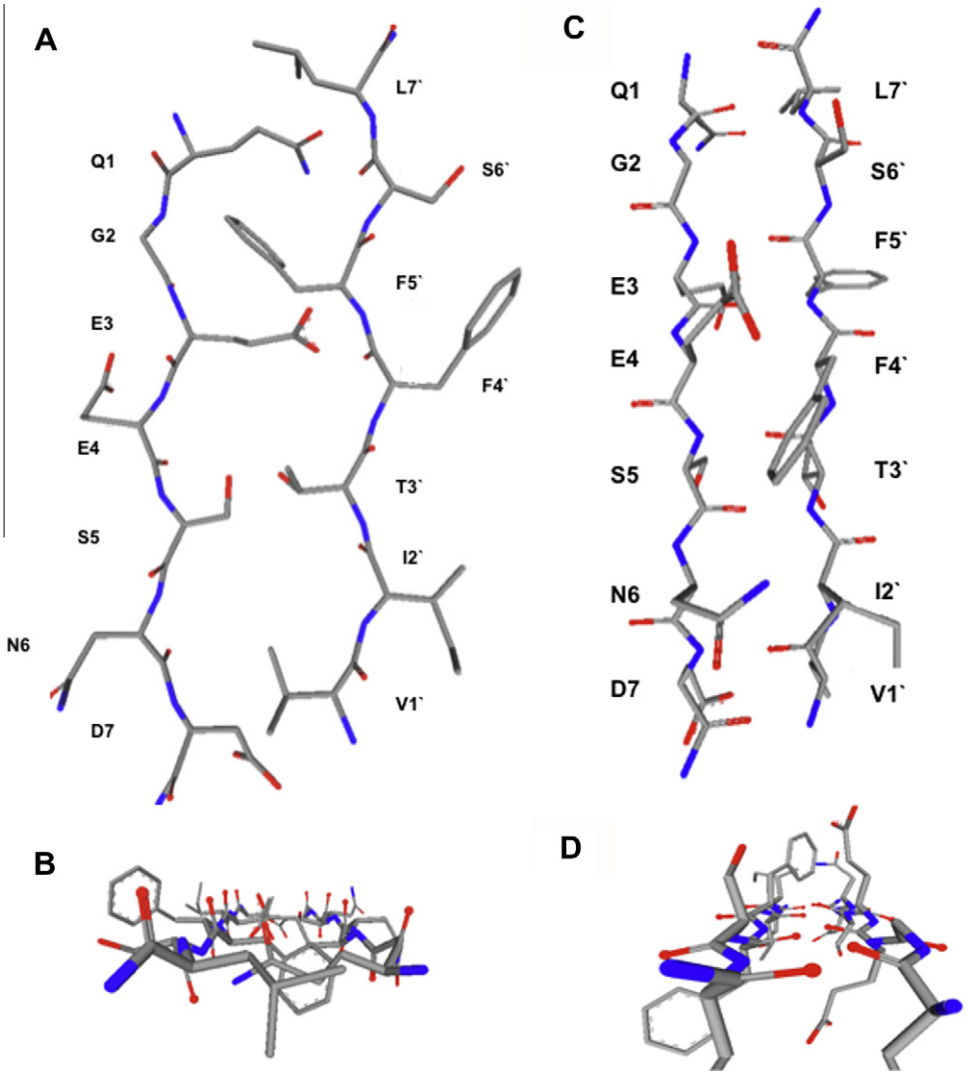

Figure 1. Approximate 3D representations of a sense heptapeptide QGEESND-NH_2 with a corresponding antisense heptapeptide, VITFFSL-NH_2. According to M–I pair theory, each sense codon-directed amino acid residue of the sense peptide should be in a position to interact with the corresponding antisense codon-directed amino acid residue of the antisense peptide. For this specific interaction requirement to be met, both peptides must be aligned mutually antiparallel and in extending conformations as shown. Specific inter-strand side-chain interactions between sense and corresponding antisense amino acid residues may then be enabled in one of two distinct ways. According to the M–I Chaiken hypothesis, side-chains are interdigitated (**ITD**) holding strands together principally via van der Waals interactions. See (A) top and (B) end view (where Leu7′ [L7′] of the antisense and Gln1 [Q1] of the sense peptide are in the left and right foreground, respectively). According to the M–I Miller hypothesis, interactions between sense and corresponding antisense amino acid residues are made possible with side chains aligned plane parallel (**PLA**) (above and below the plane of the strands) so enabling mixed mode binding interactions (van der Waals, electrostatic, hydrogen bonding etc.) to take place. See (C) top and (D) end view (where Leu7′ [L7′] of the antisense and Gln1 [Q1] of the sense peptide are in the left and right foreground, respectively).