Solution structure of the human chemokine HCC-2: A monomeric representative of the CC-chemokine subtype

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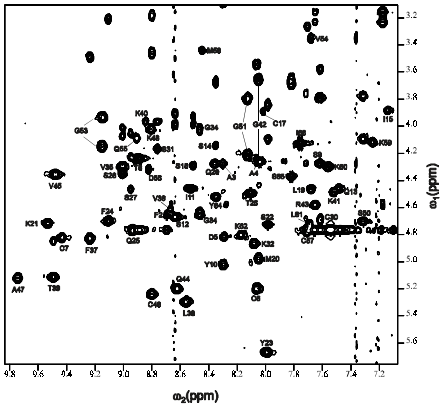
Introduction

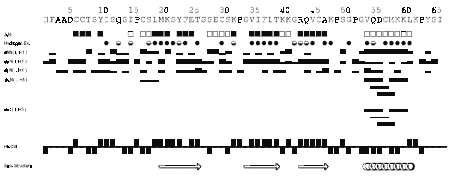
HCC-2 is a member of the chemokine protein family, a group of small proinflammatory cytokines (8-10 kDa) that are involved in the selective recruitement of a large variety of leukocytes as well as their activation [1,2]. Two main subfamilies, CXC and CC chemokines, are distinguished by the relative position of the first two of four conserved cysteines. Those are separated by one amino acid or directly adjacent to each other [3]. CC chemokines stimulate monocytes, T-lymphocytes, basophiles and eosinphiles, whereas CXC chemokines, like interleukin-8, act predominantly on neutrophiles [4,5]. All chemokines signal through G-protein-coupled seven-transmembrane domain receptors [6].

Recently, the gene of HCC-2 was identified and localized on chromosome 17 [7]. Its translated sequence consists of 113 amino acids containing a putative signal peptide of 21 residues and the mature peptide HCC-2 of 92 amino acids. Unexpectedly, recombinant expression of HCC-2 resulted in different N-terminally truncated forms of HCC-2 depending on the expressing system. In P. pastoris, HCC-2 containing 66 amino acids corresponding to sequence positions 48-113 was obtained [7], whereas expression in E. coli resulted in HCC-2 of 68 residues (positions 46-113), and the complete propeptide HCC-2(22-113) [8]. These derivatives were shown to induce chemotaxis on human monocytes, T-Lymphocytes, human dendritic cells and, to a lesser degree, in eosinophiles [7,8,9]. It was demonstrated, that the effects of HCC-2 are mediated through the CCR-1 and CCR-3 receptor. In contrast to most other CC chemokines, HCC-2 contains two additional cysteine residues forming a third disulfide bond. These six cysteines were also found for human Ckb8 as well as some murine homologues [10].

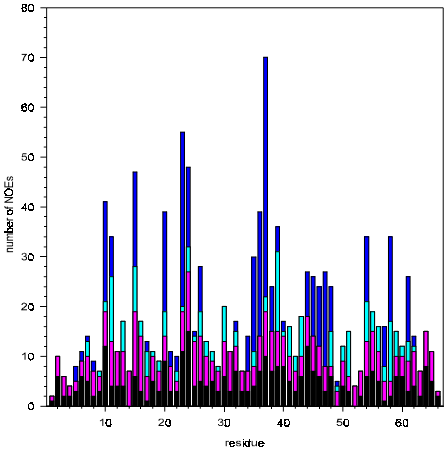
The structure of the 66-residue HCC-2 was determined by two-dimensional NMR-spectroscopy, using 770 NOE distance restraints and 58 dihedral angle restraints for the structure calculations. After exclusion of the four N- and four C-terminal residues which are disordered in solution, the structure is well-defined showing a backbone RMSD of approximately 0.6 Å.



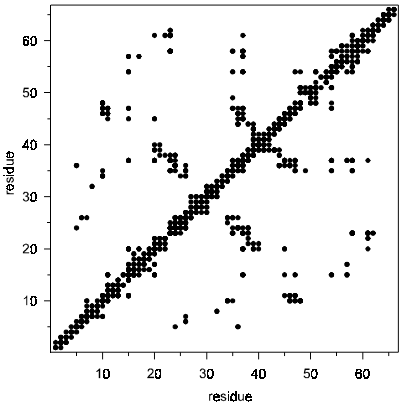
Fingerprint region of a 2D Clean-TOCSY (τ_m=80 ms) spectrum of HCC-2. The spectrum was recorded at 298 K at pH 3.0 (10 % D_2O added as a spin lock signal). The C_αH-NH cross peaks are labelled.



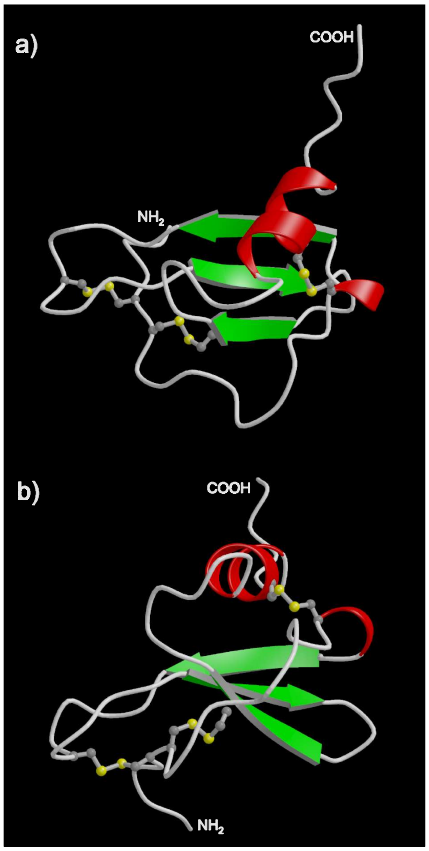
Secondary structure of HCC-2: Summary of sequential and medium-range NOEs, slowly-exchanging amide protons, vicinal backbone NH-C_αH coupling constants ($J_{\alpha N}$) and chemical shift indices (HA-CSI) [11]. The relative strengths of the NOEs, categorized as strong, medium or weak, by cross-peak intensity, are indicated by the width of the horizontal bars. Values of $^3J_{HNa}$ > 8Hz or < 6 Hz are denoted by black or white squares, respectively. Backbone amide protons that were observable 4h or 20h after transfer of the protein sample from H_2O to D_2O are marked by half-filled circles and black circles, respectively. Positive or negative chemical shift indices are denoted by black rectangles above or below the axis, respectively. Regions of β-sheet are typified by positive chemical shift indices, and α-helix by negative [11]. In the bottom line the secondary structure assignments are depicted.



Distribution of intraresidual (black), sequential (red), medium range (cyan) and long range (blue) NOEs over the sequence. The height of the bars indicates the number of NOEs.

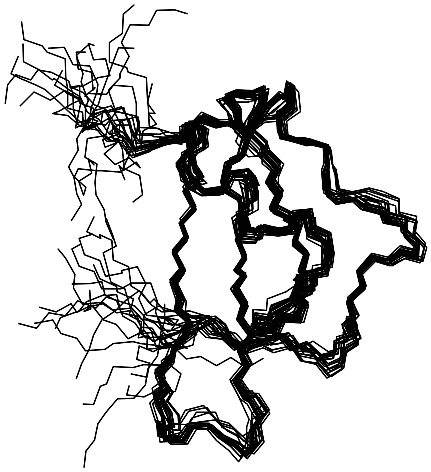


Summary of the spatial interactions between the amino acids of HCC-2. Dots in the matrix plot represent NOEs that were observed between the corresponding residues. The length and position of the helices and β-sheets can be deduced from this presentation.

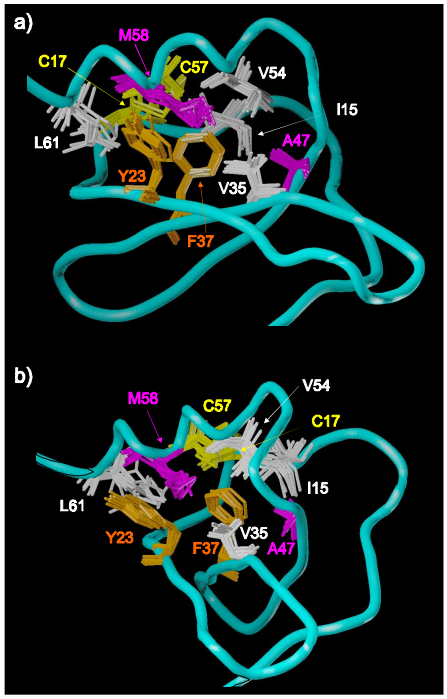


Schematic ribbon drawing indicating the elements of regular secondary structure. Figure drawn with MOLSCRIPT [12] and Raster3D [13]. The disulfide bonds (Cys6-Cys30, Cys7-Cys46, Cys17-Cys57) are shown in stick representation. The views in a) and b) are rotated 90° about the horizontal axis.

Backbone overlay of HCC-2 (orange), RANTES (blue) and MIP-1β (green) CC-chemokines in tube representation. (Figure drawn with Sybyl 6.4).



Backbone overlay of a family of 20 HCC-2 structures. The structures were accepted by the criterion of the best energy function after the restrained molecular dynamics calculation.



Detailed view on the region of hydrophobic interactions defining the relative orientation of the C-terminal α-helix with respect to the β-sheet. Residues involved in hydrophobic contacts are shown as a best-fit superposition of the 10 lowest-energy structures (Figure drawn with Sybyl 6.4). The views in a) and b) are rotated by approximately 70°.

Conclusions:

HCC-2 is a monomer even at millimolar concentrations, contrasting earlier studies of other chemokines such as RANTES or MIP-1β that form multimers at this concentration. Since chemokines are generally believed to act as monomers at physiological concentrations, HCC-2 provides a model system in which the structure is not influenced by quaternary interactions.

The presence of the additional disulfide bond does not perturb the typical chemokine fold consisting of a triple stranded antiparallel β-sheet and a C-terminal α-helix. There are, however, numerous subtle differences between HCC-2 and related CC-chemokines, including the distribution of hydrophobic and aromatic residues as well as the orientation of the loops connecting the β-strands.

References

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