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Sequence specific ^{1}H, ^{13}C and ^{15}N resonance assignment of rat CD2 domain 1

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Biological context

A detailed analysis of the determinants of protein-protein interactions is the key to an understanding of the recognition processes involved in the immune response. Among the large number of proteins that are important to these processes, the cell surface glycoprotein CD2 is probably one of the best characterised, although many aspects of its role still remain to be clarified. Three-dimensional structures of human and rat CD2 have been determined in solution (Driscoll et al., 1991; Withka et al., 1993) and in crystal form (Jones et al., 1992) and its interaction with its binding partner CD58 (in rat CD48) has been characterised extensively by biochemical and spectroscopic methods. The current model for the CD2–CD48 interaction (van der Merwe et al., 1995), derived from the conserved CD2 homodimer-contact found in the crystal, predicts a finely tuned, specific matching of charged residues in the binding interface. This pattern is in contrast to most protein–protein interfaces, where charged residues play a less significant role. The mode of interaction raises questions about the specificity of binding, the stability of the complex, the importance of individual charged residues and the domain and side chain flexibility involved in the adhesion process.

For detailed NMR studies aimed at analysing details of protein-protein interactions (see e.g. McAlister et al., 1996) at high resolution, specific residue properties, such as side chain pK_a values of titrating residues and backbone and side chain dynamics, heteronuclear experiments are indispensable. It was therefore necessary as a first step to extend the original, limited assignment of CD2 domain 1 (Driscoll et al., 1991) to a heteronuclear resonance assignment incorporating as many ^{13}C and ^{15}N resonances as possible.

Methods and results

In order to improve the yield of protein expression (as compared to previous work of Driscoll et al., 1991) the nucleotide sequence encoding residues 23–121 (domain 1) of rat CD2 (SwissProt accession code: CD2_RAT) was recloned into a pET-21b expression vector (Novagen) by PCR. A *Nde*1 restriction site immediately preceding the codon for residue 23 and a stop codon immediately followed by a *Bam*HI restriction site after the codon for residue 121 were introduced to facilitate the cloning. The resulting construct was transformed into *E. coli* bacterial strain BL21(DE3) ($F^{-}ompT\ \ hsdS_B$ (r_B^- m_B^-) *gal dcm*(DE3)) for expression of the gene product. The cells were grown overnight in 100 mL of modified M9 medium (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.5 g/L NaCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 10 μM $FeSO_4$, 2 g/L glucose, 1 mL/L vitamins solution (1000× concentration: 0.5 g/L choline chloride, 0.5/L g folic acid, 0.5 g/L panthotenic acid, 0.5 g/L nicotinamide, 1 g/L myo-inositol, 0.5 g/L pyridoxal/HCl, 0.5 g/L thiamine/HCl, 0.05 g/L riboflavin, 1 g/L biotin), 1 mL/L of micronutrients (1000× concentration: 30 μM $(NH_4)_6MO_7$, 0.4 mM H_3BO_3, 30 μM $CoCl_2$, 10 μM $CuSO_4$, 80 μM $MnCl_2$, 10 μM $CnSO_4$) and 50 μg/L carbenicilin. The overnight culture was spun down and the cells transferred to 1 L of fresh media containing 1 g/L ^{15}N $(NH_4)_2SO_4$ and 2 g/L of ^{13}C D-glucose. The cells were induced at an OD_{600} of 0.4 with 0.025 mM IPTG, doubling the concentration of IPTG every hour for the next 3 h. The cells were harvested 6 h after induction, lysed

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Supplementary material: One table with acquisition parameters of the 3D triple resonance experiments used in the assignment of CD2.

in 50 mM malonate pH 5.2 and the cell debris pelleted by centrifugation at 20 000 g for 30 min. The supernatant was loaded onto an S-sepharose column and the protein eluted using a salt gradient followed by Superose-12 (Pharmacia) gel-filtration in 20 mM phosphate pH 5.3. The final sample was obtained after a further ion exchange step using a S-10 (Bio-Rad) column with stepwise pH elution (6.5, 7.5 and 9.0 in 20 mM phosphate buffer) with CD2 eluting at pH 7.5. All buffers used in the purification contained 0.5 mM EDTA and 0.1 mM PMSF. The protein was concentrated using centripep3 concentrators (Amicon). Protein concentration was determined by UV absorption at $\lambda = 280$ nm using an extinction coefficient of $\epsilon_{280} = 13940\ M^{-1}cm^{-1}$. The overall yield of purified CD2 domain 1 was ≈35 mg per L of culture medium as compared to 5 mg/L (Driscoll et al., 1991; McAlister et al., 1996).

NMR experiments for the initial assignment were performed on a 1.5 mM uniformly $^{15}N/^{13}C$ enriched sample of CD2 at 25 °C in 90% H_2O/10%D_2O in the presence of 20 mM phosphate buffer, pH 5.0. Measurements were performed at a proton frequency of 600 MHz on a Varian Unity Plus spectrometer equipped with a 5 mm triple resonance/shielded Z-gradient probe and three RF channels.

Spectra were processed with nmrPipe and analysed with XEASY. Chemical shifts for ^{1}H, ^{15}N and ^{13}C were referenced to TSP = 0 ppm, as proposed by Wishart et al. (1995).

Extent of assignments and data deposition

The resonance assignment was made from scratch, independent of the assignment originally obtained for the structure determination of rat CD2 domain 1 (Driscoll et al., 1991). Backbone ^{1}H, ^{15}N and ^{13}C resonances were assigned by the basic set of triple resonance experiments, i.e. HNCO, HNCACB, HN(CO)CACB and CBCACOCAHA. The backbone assignments were then extended into the side chains using 3D HCCH-TOCSY and HCCH-COSY experiments. The triple resonance experiments were used essentially as proposed originally by Kay et al. (1990) and most recently reviewed by Clore and Gronenborn (1998). Aromatic side chains were assigned from adapted ^{13}C CT-HSQC experiments (Vuister and Bax, 1992) and connected to the backbone resonances via (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE experiments (Yamazaki et al., 1993). Sidechain carbonyl carbons were assigned using 2D and 3D versions of H(C)CO and HC(C)CO-TOCSY spectra (modified from Kay et al., 1990).

Essentially complete assignments were obtained for ^{1}H, ^{15}N and ^{13}C resonances. Not assigned are the side chain ^{15}N resonances in lysine and histidine residues. Assignments are deposited with the BioMagResBank (http://www.bmrb.wisc.edu/), accession number 4109. They serve as basis for a variety of biophysical studies including the examination of backbone/side chain dynamics, pKa determination, analysis of protein-protein interactions and protein folding pathways. The assignments were originally determined for a pH value of 5.0. It was discovered, however, that a significant proportion of chemical shifts shows a substantial dependence from pH between 3.0 and 8.0 so that additional backbone and partial side chain assignments are provided at pH values of 3.0 and 7.5. These were obtained mainly from extensive pH titration experiments supported by triple resonance experiments in cases of overlap during the titration.

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