Journal of Biomolecular NMR, **24:** 365–366, 2002.
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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the Coxsackievirus and Adenovirus receptor domain 1

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Received 11 September 2002; Accepted 7 October 2002

Key words: Adenovirus, CAR, Coxsackievirus, NMR assignments

Biological context

Coxsackievirus, which is a major cause of viral cardiac infections (Abelmann, 1973), and adenovirus, which is responsible for respiratory, gastroenteric and ocular infections as well as a popular vector for gene therapy (Horwitz, 1996), enter their cellular targets by first binding to CAR (coxsackievirus and adenovirus receptor), which is expressed in a wide variety of tissue types (Bergelson et al., 1997; Tomko et al., 1997). The normal cellular function of CAR is thought to be involved in homophilic cell adhesion in the developing brain (Honda and Kuwano, 2000). The gene for CAR codes for a 365 amino acid protein, which consists of an extracellular domain followed by transmembrane and intracellular domains. The extracellular domain of CAR consists of 2 Ig domains termed CAR-D1 and CAR-D2, which possess all of the necessary characteristics for viral attachment and entry (Wang and Bergelson, 1999). CAR-D1 is sufficient for adenovirus binding; the coxsackievirus binding site is presently unknown. Recently, the crystal structures of CAR-D1 in isolation and CAR-D1 bound to adenovirus fiber head have been obtained (van Raaij et al., 2000; Bewley et al., 1999). As a first step toward obtaining the CAR-D1 solution structure, near complete assignment of the CAR-D1 ^{1}H, ^{13}C and ^{15}N is presented.

Methods and experiments

Human CAR-D1 (residues 21-144) was subcloned into the *BamHI/EcoRI* restriction sites of the pGEV2 expression vector (Huth et al., 1997). The resulting construct consists of the IgG-binding domain of streptococcus (termed PG) followed by a thrombin cleavage site and CAR-D1. Expression was obtained in *Escherichia coli* strain BL21(DE3) by the addition of IPTG (final concentration = 0.8 mM) at OD_{600} = 0.8 and growing the cultures at 37 °C for 4 hours. ^{13}C/^{15}N- and ^{15}N-labeled CAR-D1 were prepared by growing the appropriate *E. coli* strain in M9 minimal media supplemented with 2 g/l ^{13}C-glucose (Isotec, Miamisburg, OH) and 1 g/l ^{15}N-ammonium chloride (Martek Biosciences, Columbia, MD). The majority of PG-CAR-D1 was found in the inclusion bodies. PG-CAR-D1 was solubilized in 6 M Gdn-HCl, 100 mM Tris-HCl/pH 7.3 and partially purified on a S200 size exclusion column. PG-CAR-D1 was purified to near homogeneity by reverse phase chromatography. PG-CAR-D1 was renatured in 100 mM Tris-HCl/pH 7.3, 100 mM NaCl, 3 mM glutathione, 0.3 mM glutathione disulphide at 4 °C. PG was cleaved from CAR-D1 by the addition of thrombin at a concentration of 38 μg/ml for 1 h at room temperature. Thrombin was removed by passage through a benzamidine column and PG was separated from CAR-D1 by reverse phase chromatography. CAR-D1 was then renatured in 100 mM Tris-HCl/pH 7.3, 100 mM NaCl at 4 °C and concentrated by ultrafiltration using a 10 kDa cutoff. The identity of CAR-D1 was confirmed by mass spectrometry (Research Resources Center, University of Illinois at Chicago). Initial NMR experiments were obtained on CAR-D1 prepared in 50 mM PO_4/pH 6.0. Subsequently, it was observed that CAR-D1 samples prepared in 50 mM d-formate/pH 3.0 exhibited superior spectra (as judged by average H_N T2 and spectral dispersion) and thus all subsequent NMR experiments were performed in the low pH buffer. All NMR experiments were performed on a Bruker 600 MHz DRX spectrometer equipped with a triple-axis pulsed field

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Figure 1. ^{15}N-edited HSQC of CAR-D1 acquired at 600 MHz. Numbering corresponds to that of human CAR (van Raaij et al., 2000). G19 and S20 are cloning artifacts. Minor peaks are unmarked. Sidechain asparagine and glutamine resonances are marked by horizontal lines. Note that the crosspeak for W57ε is missing, presumably due to exchange line-broadening. Experimental conditions were 1 mM ^{13}C/^{15}N-labeled CAR-D1, 50 mM formate/pH 3.0, 10% D_2O at 293 K.

gradient probe. Spectra were processed by nmrPipe and visualized with nmrDraw (Delaglio et al., 1995). Backbone resonances were assigned by a standard set of 3D heteronuclear NMR experiments including: HNCA, HNCACB, HN(CO)CA, CBCA(CO)NH, and HNCO. The sidechain resonances were assigned by 2D TOCSY, 2D ^{13}C-edited HSQC, 3D ^{15}N-edited TOCSY-HSQC, 3D H(C)CH-TOCSY, 3D (H)CCH-TOCSY, and 3D CC(CO)NH experiments. Assignments were confirmed with 2D NOESY, 3D ^{13}C-edited NOESY-HSQC (mixing time = 100 ms), 3D ^{15}N-edited NOESY-HSQC (mixing time = 120 ms).

Extent of assignments and data deposition

Near complete assignment of the CAR-D1 backbone ^{1}H, ^{13}C and ^{15}N has been achieved by using a standard set of heteronuclear NMR experiments listed above. Unassigned backbone resonances include: (i) ^{15}N of the proline residues (P25, P40, P47, P52, P61, P85, P126 and P141), (ii) ^{13}CO of the residues preceding prolines (T24, L39, S46, G51, S60, Y84, A125 and K140), (iii) the ^{1}H and ^{15}N of the N-terminal residue (G19), (iv) the ^{13}CO of the C-terminal residue (A144). The ^{15}N-edited HSQC of CAR-D1 is shown in Figure 1. Interestingly, numerous residues exhibited 'minor form' peaks, ranging in intensity from 10–25% of the 'major form' peak. The majority of the minor peaks could be assigned from their proximity to a major peak in the ^{15}N-edited HSQC, identification of the amino acid type from the HNCACB, and their placement from the CBCA(CO)NH. Residues exhibiting minor peaks include: S20, S21, I22, M28, I29, K31, K33, G34, Y38, L45, G51, I55, I59, S60, A62, D63, N64, V67, Q69, I72, L73, G76, D77, K78, I79, Y83, Y84, D86, K88, H92, F93, D97, L98, G101, D102, A103, V107, L110, Q111, L112, D114, G116, T117, G127, I133, H134, L138, K140, S142, G143, A144. Potential sources for the minor peaks include proline isomerization states, cysteine redox state, and side chain conformations of aromatic groups. The source of these minor peaks will require determination of the CAR-D1 solution structure, which is presently underway. In addition, >95 % of the ^{1}H, ^{13}C and ^{15}N sidechain resonances of the major form of CAR-D1 were assigned. The chemical shifts of the CAR-D1 major conformation have been deposited in the BioMagResBank under the accession number BMRB-5516.

Acknowledgement

This work was supported by the American Heart Association Scientist Development Grant 0030374Z.

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