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ARTICLE

^{1}H, ^{13}C and ^{15}N assignments of a camelid nanobody directed against human α-synuclein

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Abstract Nanobodies are single chain antibodies that are uniquely produced in Camelidae, e.g. camels and llamas. They have the desirable features of small sizes (Mw < 14 kDa) and high affinities against antigens (Kd ~ nM), making them ideal as structural probes for biomedically relevant motifs both in vitro and in vivo. We have previously shown that nanobody binding to amyloidogenic human lysozyme variants can effectively inhibit their aggregation, the process that is at the origin of systemic amyloid disease. Here we report the NMR assignments of a new nanobody, termed NbSyn2, which recognises the C-terminus of the intrinsically disordered protein, human α-synuclein (aS), whose aberrant self-association is implicated in Parkinson's disease.

Keywords Camelid antibody · Nanobody · Alpha-synuclein · Intrinsically disordered protein · Parkinson's disease

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Biological context

Heavy-chain antibodies (HCAb) are uniquely produced in Camelidae with a single chain to achieve antigen recognition as compared to four chains—two heavy and two light chains—in conventional antibodies (Hamers-Casterman et al. 1993). As a result of the single-chain domain architecture of the variable domain of HCAbs (VHH), there are only three hypervariable loops (H1-3) available to achieve antigen recognition instead of six loops (H1-3 and L1-3) in conventional antibodies. The reduced number of hypervariable loops for antigen binding in VHHs is in part compensated by a much longer H3 and increased sequence variability in H1 that serve to expand the conformational space accessible to the hypervariable loops. These loops can generate a large repertoire of sequences for antigen recognition by forming a large interaction surface, which can be either flat or convex, enabling VHHs to bind in the clefts of target molecules, such as enzyme active sites, locations that are usually inaccessible to conventional antibodies (De Genst et al. 2006).

VHHs isolated by phage-display are commonly referred to as Nanobodies (Nbs). Given the versatility, the high expression yield and the typically high thermal and chemical stabilities of Nbs, they have become an attractive tool for studying molecular recognition phenomena in protein aggregation. We have previously shown that the

binding of an Nb variant, cAb-HuL6, to the amyloidogenic D67H and I56T variants of human lysozyme can effectively inhibit the formation of amyloid fibrils in vitro (Dumoulin et al. 2003). This has led to further developments of other Nbs that target different regions of the human lysozyme variants with similar inhibitory effects against protein aggregation (Chan et al. 2008). Nbs have, for example, the potential to be elicited towards, and to be able to detect, transiently populated oligomeric forms of aggregation-prone proteins whose structural epitopes may be absent or different from their monomeric counterparts (Chiti and Dobson 2008).

We have recently identified an Nb, termed NbSyn2, which has been elicited against human α-synuclein (aS), an intrinsically disordered protein with a molecular weight of 14 kDa. Fibrillar aggregates of aS are the main constituent of Lewy bodies—the cellular hallmarks of Parkinson's disease (Spillantini et al. 1997). Despite the absence of a defined three-dimensional structure of aS, NbSyn2 is able to recognise the C-terminus of the monomeric protein with high affinity (Kd ∼ 10 nM). Importantly, we have recently found that NbSyn2 is able to bind to oligomeric and fibrillar forms of aS (unpublished data). This Nb has, therefore, great potential to be used as a molecular probe of the structures and dynamics of aS both in vitro and in vivo. In order to obtain further insights into the mechanism by which NbSyn2 recognises different forms of aS, we aim to carry out detailed structural elucidation and interaction studies on the aS-NbSyn2 system using solution state NMR spectroscopy. Here, we report the ^{1}H, ^{15}N and ^{13}C assignments of NbSyn2 in its free form, making it the third set of NMR assignments of VHHs to be discussed in the literature to date (Vranken et al. 2002; Renisio et al. 2002).

Methods and experiments

The DNA sequence of NbSyn2 was cloned into an expression vector with a hexahistidine (His_6) tag at its C-terminus. $^{13}C/^{15}N$ labelled protein was expressed in the *E. coli* strain WK6 grown in minimal (M9) media containing 1 g/L $^{15}NH_4Cl$ and 3 g/L ^{13}C D-glucose. The protein was subsequently purified a Ni-column (Ni–NTA Superflow; Qiagen) followed by gelfiltration chromatography (Superdex 75 16/60; Pharmacia) to >95% homogeneity as judged by SDS–PAGE. The purified protein was then concentrated to ca. 300 μM and buffer-exchanged into 20 mM sodium acetate at pH 4.8.

The resonance assignments were achieved following a computer-aided procedure as described previously (Hsu et al. 2009). All experiments were carried out at 25°C using a Bruker Avance 700 MHz spectrometer equipped with a cryogenic triple resonance probe (Bruker BioSpin). Briefly, HNCA, CBCA(CO)NH, HNCACB, HNCO and ^{15}N-TOCSY-HSQC spectra were recorded for backbone assignments, and 2D constant-time [^{13}C–^{1}H] HSQC, 3D [^{13}C,^{15}N]-simultaneously edited NOESY-HSQC, 3D HcCH-COSY and 3D HCCh-TOCSY spectra were recorded for side-chain assignments. All NMR data were processed and analysed by TopSpin (Bruker BioSpin), NMRPipe (Delaglio et al. 1995) and Sparky (http://www.cgl.ucsf.edu/home/sparky/) software packages.

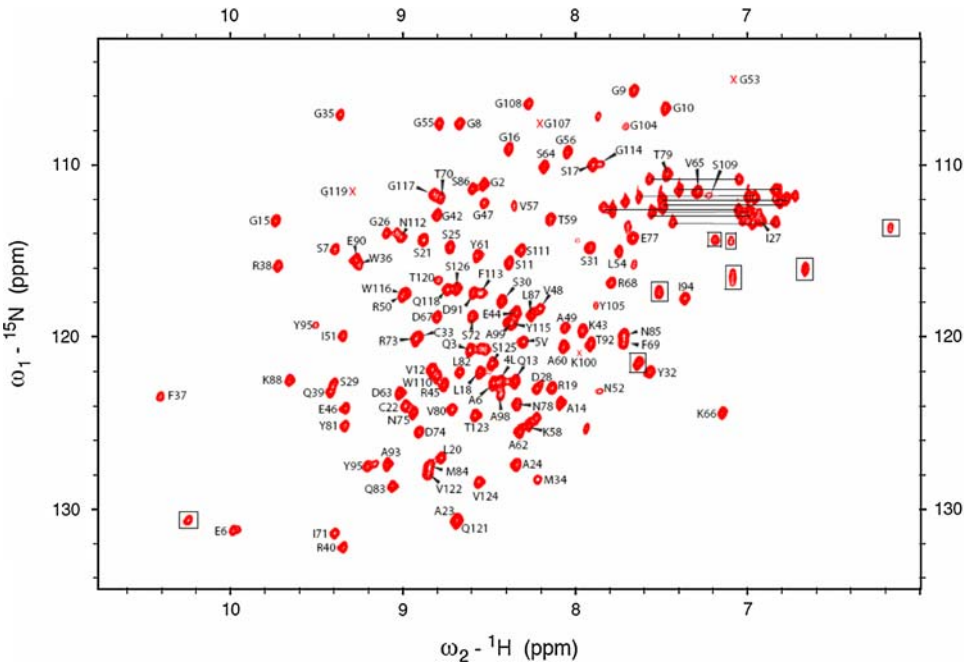

Fig. 1 Assignments of the 2D [^{15}N–^{1}H] HSQC spectrum of NbSyn2 recorded at 25°C and at a ^{1}H frequency of 700 MHz. Pairs of side-chain NH_2 resonances are connected by *horizontal lines* and aliased side-chain resonances are *boxed*. *Red crosses* indicate the positions of severely broadened correlations

Extent of assignment and data deposition

We have assigned 97% of the expected backbone ^{1}H–^{15}N correlations (117 out of 121). The missing ones correspond to C97, F101, S102 and C106. The latter three residues may be partially solvent exposed in the loop regions based on homology modelling (not shown). In addition, 98% of ^{1}Hα (123 out of 126), and 94% of all ^{13}CO, ^{13}Cα and ^{13}Cβ (338 out of 359) resonances of NbSyn2 have been assigned. The overall completeness of the assignments (excluding aromatic side-chains) is 95% (1,269 out of 1,330 atoms). Most missing assignments correspond to Cδ of glutamates and Cγ of arginines and cysteines. Note that G26 exhibits a clear minor conformation in the HNCACB and CBCA(CO)NH spectra, showing distinct chemical shifts along the ^{1}H^{N} and ^{15}N dimensions, while those of the corresponding Cα and Cβ are very similar. Based on homology modelling, G26 is located in a β-turn region next to H1. Additionally, G53, K100 and G119 exhibit very broad lines in the fingerprint region of the [^{15}N–^{1}H] HSQC spectrum of free NbSyn2, indicative of conformational fluctuations and/or solvent exchange of the amide protons of these residues (Fig. 1). Solvent exchange-related line broadening is particularly pronounced for the loop residues; a large number of [^{15}N–^{1}H] correlations of these residues become broadened beyond detection when the pH value is increased from 4.8 to 7.4 (data not shown). The assignments have been deposited in the BMRB under the accession number 16305. The secondary structure propensities derived from secondary chemical shifts are included as electronic supplementary material.

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