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ARTICLE

Backbone and side-chain ^{1}H, ^{13}C and ^{15}N resonance assignments of LEN, a human immunoglobulin κIV light-chain variable domain

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Abstract ^{1}H, ^{13}C and ^{15}N resonance assignments are presented for a recombinant 114 amino acid human immunoglobulin (Ig) κIV light-chain variable domain (V_L) LEN, which displays a high degree of sequence identity with another human Ig κIV V_L, SMA. While SMA is highly amyloidogenic in vivo and in vitro and has been linked to the pathogenesis of light-chain amyloidosis, LEN is non-amyloidogenic in vivo and can be converted to the amyloid state only in vitro under destabilizing conditions. Measurements of longitudinal and transverse amide ^{15}N relaxation rates confirm that, as expected, LEN is a dimer at physiological pH and typical concentrations used for NMR studies, and the analysis of secondary chemical shifts indicates that the protein has a high β-sheet content. These findings are consistent with previously published biophysical data and the high-resolution X-ray structure of LEN.

Keywords Immunoglobulin light-chain · Bence Jones protein · Light-chain amyloidosis · Protein misfolding · NMR spectroscopy

Biological context

The conversion of soluble peptides and proteins into non-crystalline, high-molecular-weight aggregates underlies some 40 pathological conditions in humans, broadly referred to as protein conformational diseases or amyloid diseases (Chiti and Dobson 2006). Light-chain amyloidosis (AL) is one of the most prevalent systemic diseases of this kind, and is characterized by the extracellular deposition of amyloid fibrils in organs and tissues, most commonly in the kidneys and heart, which leads to rapidly progressive organ failure (Solomon and Weiss 1995; Falk et al. 1997). The amyloidogenic precursor proteins in AL are usually immunoglobulin (Ig) light-chain variable domains (V_L) of the λ or κ type, produced by clonal plasma cells in the bone marrow; AL patients typically have elevated plasma cell burdens, secrete in urine homodimers composed of free monoclonal Ig light-chains, commonly referred to as Bence Jones proteins, and in ~10–15% of the cases also suffer from multiple myeloma (Solomon and Weiss 1995; Falk et al. 1997; Kyle and Gertz 1995).

Interestingly, only a relatively small fraction of Ig V_Ls are amyloidogenic in vivo (Solomon and Weiss 1995; Falk et al. 1997), and multiple studies have established the links between mutations in Ig V_L amino acid sequences, reduced protein stability and the capacity of these proteins to misfold and assemble into amyloid fibrils under physiological conditions (Hurle et al. 1994; Raffen et al. 1999; Kim et al. 2000). High-resolution X-ray structures of several amyloidogenic and non-amyloidogenic Ig V_L domains have also been reported (Epp et al. 1975; Huang et al. 1994; Schormann et al. 1995; Huang et al. 1997; Bourne et al. 2002; Wall et al. 2004; Baden et al. 2008), revealing the existence of both large and more subtle structural perturbations—typically involving residues near the dimer interface—that appear to be associated with the reduced thermodynamic stability and increased amyloidogenicity of certain Ig V_Ls relative to others. However, relatively little remains known at atomic resolution about the partially

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folded intermediates involved in Ig V_L aggregation and protein conformation in amyloid fibrils.

In order to provide such insights, we have recently commenced solution and solid-state NMR studies focused on two highly homologous (~93% sequence identity) 114-residue human Ig κIV V_L domains, LEN and SMA, which can be readily prepared in recombinant form and exhibit drastically different amyloidogenic propensities in vivo (Wilkins Stevens et al. 1995). Specifically, SMA was the main component of amyloid deposits isolated from an AL patient, whereas LEN, which differs from the germline sequence κIV by a single somatic mutation (S29N) and from SMA at eight amino acid positions (S29N, K30R, P40L, Q89H, T94H, Y96Q, S97T, I106L) was extracted as a Bence Jones protein from a multiple myeloma patient with no symptoms of AL (Wilkins Stevens et al. 1995). These amyloidogenic propensities have been correlated with different thermodynamic stabilities of the two proteins (Raffen et al. 1999) and are also mirrored in vitro; at physiological pH, SMA assembles into amyloid fibrils with relative ease, while LEN is a stable dimer under these conditions and can be converted to the amyloid state only under destabilizing conditions (e.g., in the presence of denaturant, ~1–2 M guanidinium hydrochloride) (Wilkins Stevens et al. 1995; Raffen et al. 1999). Extensive subsequent studies of SMA and LEN aggregation carried out using a variety of biophysical techniques by Fink and coworkers (Khurana et al. 2001; Souillac et al. 2002; Souillac et al. 2003) revealed that the dimer dissociation is a critical step in initiating protein aggregation and that multiple partially folded intermediates appear to play a role in the conversion of these Ig V_L domains to amyloid fibrils. As an initial step in our NMR studies aimed at elucidating the detailed atomic level mechanisms of SMA and LEN aggregation, we present the solution-state backbone and side-chain assignments of LEN at pH 6.5. We note here that ^{1}H, ^{13}C and ^{15}N resonance assignments of a related fibrillogenic Ig λVI V_L were also recently reported (Gutierrez-Gonzalez et al. 2007).

Methods and experiments

Protein expression and purification

The plasmid encoding for LEN (Wilkins Stevens et al. 1995) was kindly provided by Dr. Fred J. Stevens (Argonne National Laboratory). Uniformly ^{15}N- and ^{13}C, ^{15}N-labeled LEN samples were prepared according to published procedures (Wilkins Stevens et al. 1995) with minor modifications. Briefly, for ^{13}C, ^{15}N-LEN, electro-competent *Escherichia coli* C41(DE3) cells transformed with the LEN plasmid were grown at 30°C and 110 rpm in a modified M9 minimal medium (Cai et al. 1998) containing 100 μg/mL carbenicillin, 1 g/L $^{15}NH_4Cl$, 3 g/L ^{13}C-D-glucose (Cambridge Isotope Laboratories) and 0.5 g ^{13}C, ^{15}N-enriched Isogro growth medium (Isotec/Sigma-Aldrich); for ^{15}N-LEN the minimal medium contained 3 g/L natural abundance D-glucose and 0.5 g ^{15}N-Isogro. Protein expression was induced at OD_{600} ~0.8 by the addition of isopropyl-β-D-thiogalactoside to a final concentration of 1 mM. Cells were grown for an additional 16 h and harvested by centrifugation at 4,000*g* and 4°C for 10 min.

The periplasmic extract which contained LEN was isolated as described previously (Wilkins Stevens et al. 1995) by incubating the cells for 1 h at 4°C in Tris EDTA–sucrose pH 8.0 buffer containing lysozyme. Following centrifugation at 27,000*g* and 4°C for 15 min, the supernatant that included the periplasmic fraction was dialyzed against 10 mM Tris, pH 8.0 and applied at a flow rate of 0.5 mL/min to two 1 mL HiTrap Q XL cartridges (GE Healthcare) connected in series and equilibrated with 10 mM Tris, pH 8.0. The flow, through fraction containing LEN was subsequently dialyzed against 10 mM sodium acetate, pH 5.0 and applied at a flow rate of 0.5 mL/min to two 1 mL HiTrap SP XL cartridges (GE Healthcare) connected in series and equilibrated with 10 mM sodium acetate, pH 5.0; LEN was eluted from the cartridges with a 0–100 mM NaCl gradient. The protein was exchanged into 20 mM Tris, 100 mM NaCl, and pH 7.2 buffer by using an Amicon Ultra-15 3,000 molecular-weight-cut-off centrifugal device (Millipore) and applied to a HiLoad 16/60 Superdex 75 gel filtration column (GE Healthcare) equilibrated with the same Tris buffer. Fractions containing LEN were collected and pooled together, and the protein identity and purity confirmed by SDS–PAGE and MALDI–TOF mass spectrometry. Typical yields of isotopically enriched LEN (determined from absorbance at 280 nm with an extinction coefficient of 24,535 M^{-1} cm^{-1}) were ~12–14 mg per 1 L of cell culture.

NMR spectroscopy

Uniformly ^{15}N- and ^{13}C, ^{15}N-labeled samples used for NMR studies consisted of LEN at a concentration of 1.7 mM and 1.5 mM, respectively, in aqueous solution containing 20 mM sodium phosphate, 100 mM NaCl, 7% (v/v) D_2O and 0.02% (w/v) NaN_3 at pH 6.5 in a total volume of 300 μL in Shigemi microcells. NMR spectra were recorded at 25°C on Bruker DMX-600 and DRX-800 MHz spectrometers equipped, respectively, with a TXI room-temperature probe with triple-axis gradients and a QXI cryogenic probe with *z*-axis gradients. Sequential backbone ^{1}HN, ^{15}N, $^{13}C'$, $^{13}C\alpha$ and $^{13}C\beta$ assignments were obtained using a suite of triple-resonance 3D HNCO, HNCA, HN (CO) CA and HN (CA) CB experiments based

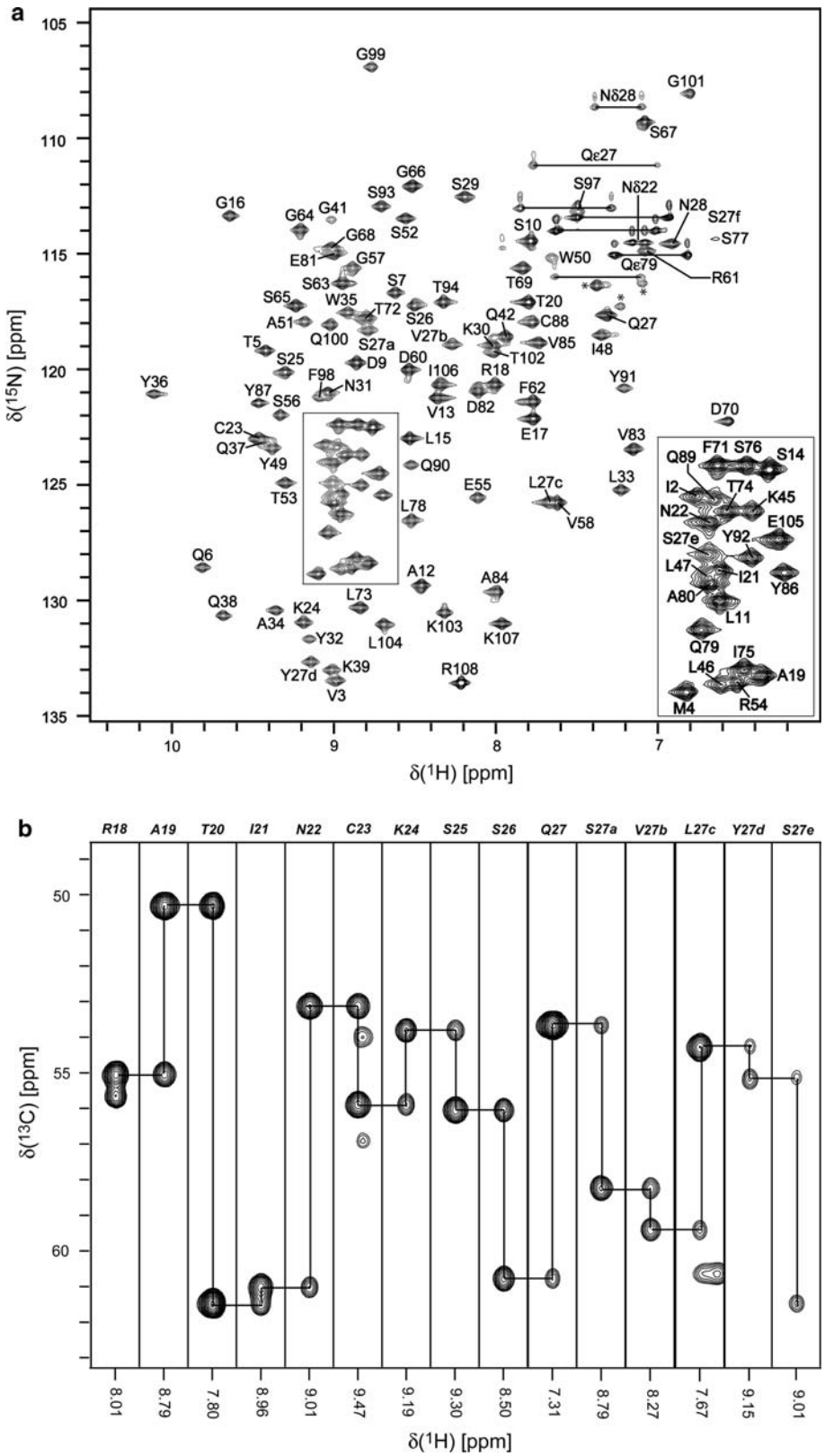

Fig. 1 **a** 800 MHz ^{1}H–^{15}N HSQC spectrum of ^{15}N-labeled LEN at pH 6.5 and 25°C. The Asn and Gln amide side-chain resonances are indicated by *horizontal lines*. Aliased Arg $^{15}N\varepsilon$ signals are marked by *asterisks*. **b** Representative strips from a 800 MHz 3D HNCA spectrum of ^{13}C, ^{15}N-labeled LEN

on the pulse schemes of Kay and co-workers (Yamazaki et al. 1994). The $^{1}H\alpha$ and side-chain ^{1}H, ^{13}C and ^{15}N resonances were assigned using 3D H(C)CH-TOCSY, (H)CCH-TOCSY, ^{15}N-TOCSY-HSQC and ^{15}N-NOESY-HSQC spectra (Cavanagh et al. 1996). NMR spectra were processed using NMRPipe (Delaglio et al. 1995) and

analyzed using NMRDraw (Delaglio et al. 1995) and Sparky (Goddard and Kneller 1993). ^{1}H chemical shifts were referenced to external TSP, and ^{13}C and ^{15}N shifts were referenced indirectly (Cavanagh et al. 1996).

Residue-specific longitudinal (R_1) and transverse (R_2) relaxation rates and steady-state heteronuclear {^{1}H}–^{15}N NOEs for backbone amide ^{15}N nuclei were obtained at 600 MHz ^{1}H frequency using the pulse schemes of Farrow et al. 1994. Spectra were processed and analyzed using the NMRPipe/NMRDraw software package (Delaglio et al. 1995).

Assignments and data deposition

Figure 1 shows a 2D ^{1}H–^{15}N HSQC spectrum of LEN recorded at 800 MHz ^{1}H frequency, as well as representative strips from a 3D HNCA spectrum illustrating sequential backbone assignments. In summary, backbone amide ^{1}H and ^{15}N chemical shifts were obtained for 106 of 108 non-proline residues, with the exceptions being D1 and Y96. Several residues in the HSQC spectrum, including S27e, S27f, Y32, G41, W50, S77, Q90, Y91, S97, F98 and G99, exhibit notably attenuated intensities most likely due to conformational exchange. According to the X-ray structure of LEN (Huang et al. 1997) all of these residues are found in the loop regions with the majority present in the vicinity of the dimer interface. Nearly complete assignments (97.2%) of the ^{1}Hα, ^{13}Cα, ^{13}Cβ and ^{13}C′ chemical shifts were obtained, with P43 and P95 being the only residues that could not be assigned. In addition, chemical shifts were obtained for 94.1% of the remaining aliphatic ^{1}H and ^{13}C side-chain resonances, as well as for ^{15}Nδ–^{1}Hδ of N22 and N28 and ^{15}Nε–^{1}Hε of Q27 and Q79. The chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 16463.

The ^{13}Cα and ^{13}Cβ secondary shifts, $\Delta\delta$(Cα) and $\Delta\delta$(Cβ), respectively, were used to evaluate the secondary structure of LEN. The plot of $\Delta\delta$(Cα)–$\Delta\delta$(Cβ), shown in Fig. 2a, indicates that the majority of residues adopt a β-strand conformation and that the locations of different secondary structure elements predicted on the basis of chemical shifts are in good agreement with the X-ray structure of LEN. Finally, in order to probe the oligomeric state of LEN, the ^{15}N R_2/R_1 ratios for 96 residues with {^{1}H}–^{15}N NOE values greater than 0.75 (Fig. 2b) were used to estimate the overall protein rotational correlation time within the ModelFree software package (Palmer et al. 1991). These measurements provided average amide ^{15}N relaxation time constants of $T_1 = R_1^{-1} = 1,056 \pm 8$ ms and $T_2 = R_2^{-1} = 54.0 \pm 0.4$ ms, yielding an estimated rotational correlation time of $\tau_c = 13.9 \pm 0.1$ ns for LEN. This τ_c value indicates that at physiological pH LEN (M $\sim$ 13 kDa) is a dimer at concentrations required for NMR studies. This observation is consistent with the dimerization constant of approximately 4×10^5 M^{-1} determined for LEN (Wilkins Stevens et al. 1995), both

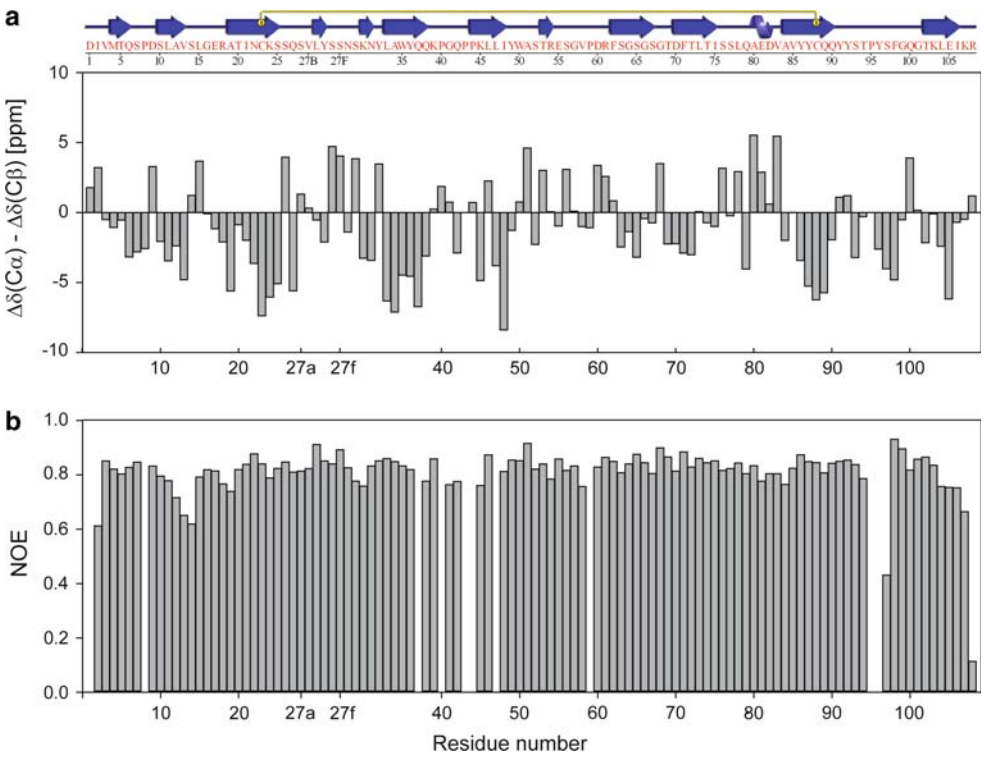

Fig. 2 **a** Plot of $\Delta\delta$(Cα)–$\Delta\delta$(Cβ) as a function of residue number. Above the plot, the schematic secondary structure diagram of LEN indicates the disulfide bond in *yellow* and the location of β-strands and α-helix found in the X-ray structure (PDB entry 1LVE) (Huang et al. 1997). Residue numbering is according to Kabat et al. 1991. **b** Heteronuclear {^{1}H}–^{15}N NOE values for LEN determined at 600 MHz ^{1}H frequency and 25°C as a function of residue number. For residues where the NOE could not be determined, the NOE value was set to zero

published (Wilkins Stevens et al. 1995) and our own gel filtration chromatography data (not shown) which indicate that LEN elutes as a dimer at concentrations $> \sim 0.5$ mM, as well as the LEN crystal structure.

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