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NMR backbone resonance assignments of the prodomain variants of BDNF in the urea denatured state.

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Abstract

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family of proteins which plays a central role in neuronal survival, growth, plasticity and memory. A single Val66Met variant has been identified in the prodomain of human BDNF that is associated with anxiety, depression and memory disorders. The structural differences within the full-length prodomain Val66 and Met66 isoform could shed light on the mechanism of action of the Met66 and its impact on the development of neuropsychiatric-associated disorders. In the present study, we report the backbone ^{1}H, ^{13}C, and ^{15}N NMR assignments of both full-length Val66 and Met66 prodomains in the presence of 2 M Urea. These conditions were utilized to suppress residual structure and aid subsequent native state structural investigations aimed at mapping and identifying variant-dependent conformational differences under native-state conditions.

Keywords

BDNF; Val66Met; prodomain; neurotrophin; urea; intrinsically disordered proteins

Biological Context

Human brain derived neurotrophic factor (BDNF) possesses a single nucleotide polymorphism (rs6265), that results in a Val66Met substitution, that is carried by ~25% of the overall population. The Val66Met substitution is located within the prodomain region of BDNF and has been demonstrated to significantly predispose its carriers to depression, anxiety and memory problems (Chen et al. 2006; Egan et al. 2003; Martinowich et al. 2007). Full length BDNF protein is expressed as a pre-pro-protein that is processed in the ER generating proBDNF, which is subsequently trafficked to the Golgi where proBDNF may be cleaved by proteases to generate an isolated prodomain and mature BDNF (mBDNF). The mBDNF protein strengthens synaptic contacts and initiates trophic neuronal responses (Teng et al. 2010). However, during early development or injury, the unprocessed proBDNF can be secreted by neurons inducing neurite retraction through the interaction with the p75 neurotrophin receptor (p75NTR) in combination with Sortilin or SorCS2 receptors (Teng et al. 2005).

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We have recently demonstrated that the isolated Met66 prodomain produces a degenerative gain-of-function inducing neuronal growth cone retraction, whereas the Val66 prodomain is inert. Our initial studies utilized a truncated version of the human prodomain (BDNF residues 23–113) optimized for solubility (Anastasia et al. 2013). To gain further insight into Val66Met polymorphism activity we initially examined by NMR the full-length prodomain isoforms (residues 19–128). Our preliminary characterization of the full-length Val66 and Met66 prodomains indicated a significant number of missing resonances, using non-denaturing native-like conditions. Here we have assigned the backbone resonances using denaturing conditions (2 M Urea) in order to identify and assign these missing peak intensities. These assignments will enable subsequent NMR titration experiments to native conditions for detailed structural characterization of the conformational differences between the Val66 and Met66 BDNF prodomains variants.

Methods and experiments

Protein cloning, expression and purification

We utilized pET28 plasmid (Novagen, Madison, WI, USA) constructs containing the human BDNF Val66 or Met66 prodomain sequence (amino acids 19–128) preceded by an N-terminal His-6-SUMO tag (Mossessova and Lima 2000). This construct was transformed in BL21(DE3) pLysS cells (Invitrogen, Grand Island, NY, USA). For isotopically labelling (^{13}C,^{15}N) the protein was expressed using the media swap protocol as previously described (Marley et al. 2001). Briefly, cells were grown in 3 liters of LB broth, then centrifuged and washed with M9 salts, then concentrated and resuspended into 750 ml of minimal media containing 4 g/L of ^{13}C glucose as the sole carbon source and 1 g/L of $^{15}NH_4Cl$ as the sole nitrogen source. The cells were given a growth recovery period, followed by induction of protein express with 0.5 mM IPTG for 3 hours at 37 °C. Cells were harvest by centrifugation, lysed by sonication in 6 M guanidine hydrochloride, 50 mM sodium phosphate pH 7.0 buffer, then pelleted by centrifugation for 5 min at 6000 g. The supernatant was loaded on a Qiagen Ni-NTA column for capture of the His-tagged construct, and then washed using 8 M Urea, 50 mM sodium phosphate pH 6.0 buffer. Elution was achieved using 8 M Urea, 600 mM imidazole, 50 mM sodium phosphate pH 6.0 buffer. Samples were dialyzed overnight into 20 mM Tris, 100 mM NaCl pH 8.0, and then cleaved to remove the Sumo tag by incubation with Ulp1 protease for 1 hour at room temperature. Subsequently, the sample was negatively selected in a second Ni-NTA column, leaving cleaved prodomain in the flow (His_6-tagged Sumo and His_6-tagged Ulp1 enzyme were retained in the Ni-NTA resin). Prodomain was collected and further purified by HPLC using C18 Vydac preparative column using a 0.1% formic acid-H_2O/ acetonitrile gradient. Prodomain fractions were collected and lyophilized for subsequent NMR studies. The protein purity was checked by SDS-PAGE and MALDI-TOF-MS. Protein concentrations were quantified by UV absorbance and Bradford analysis.

NMR Spectroscopy

Uniformly ^{15}N- and $^{13}C/^{15}N$- labeled samples of the full-length Val66 and Met66 prodomain were prepared in 2 M Urea, 100 mM NaCl, 10 mM Tris, 10 mM glycyl-glycine, 10 mM piperazine, 0.5 mM EDTA, and 0.1 mM DSS pH 7.2 at 300 μM concentration. D_2O

was added to the NMR sample prior to measurement to a 7% final concentration. All NMR experiments were carried out at 278 K, on Bruker Avance III 600MHz (Weill Cornell Medical College) and Bruker Avance 900MHz (NYSBC) spectrometer equipped with triple resonance cryogenic probes. The ^{1}H chemical shifts were referenced with respect to DSS, the ^{15}N and ^{13}C chemical shifts were referenced indirectly using IUPAC referencing ratios (Markley et al. 1998). Sequential backbone assignments were obtained using combinations of triple resonance HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH and (H)N(COCA)NH 3D spectra. Spectra were processed using NMRPipe (Delaglio et al. 1995) and TopSpin 3.2 (Bruker, Rheinstetten, Germany) followed by the analysis with the program NMRFAM-Sparky (Lee et al. 2015) and Pine (Bahrami et al. 2009)

Assignments and data deposition

The overlay 2D ^{15}N-HSQC spectrum of the Val66 (blue) and Met66 (red) spectra is shown with the labeled peak assignments (Fig. 1a). In 2M Urea, the HSQC displays uniform peak intensities and chemical shift dispersion consistent with an intrinsically disordered protein. Collectively, 101 residues out of 104 possible non-proline resonances could be identified in both Val66 and Met66. All the assigned backbone chemical shifts have been deposited in the BioMagResBank under the access number BMRB 27102 for the Val66 prodomain and BMRB 27103 for the Met66 prodomain. The amide peak assignments are 97% complete with the exception of S17, M18 and E111. Residues S17 and M18, were introduced to the N-terminus of prodomain during the SUMO cleavage purification tag site. E111 within the C-terminal region could not be specifically assigned to sequence, due to either overlap or exchange. The secondary structure populations were quantified based on the chemical shifts of the $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ resonances using δ2D (Camilloni et al. 2012) (http://www-vendruscolo.ch.cam.ac.uk/d2D) which indicate that both prodomains in 2 M Urea are predominantly unstructured (Fig. 1b,c), with only subtle differences under these conditions.

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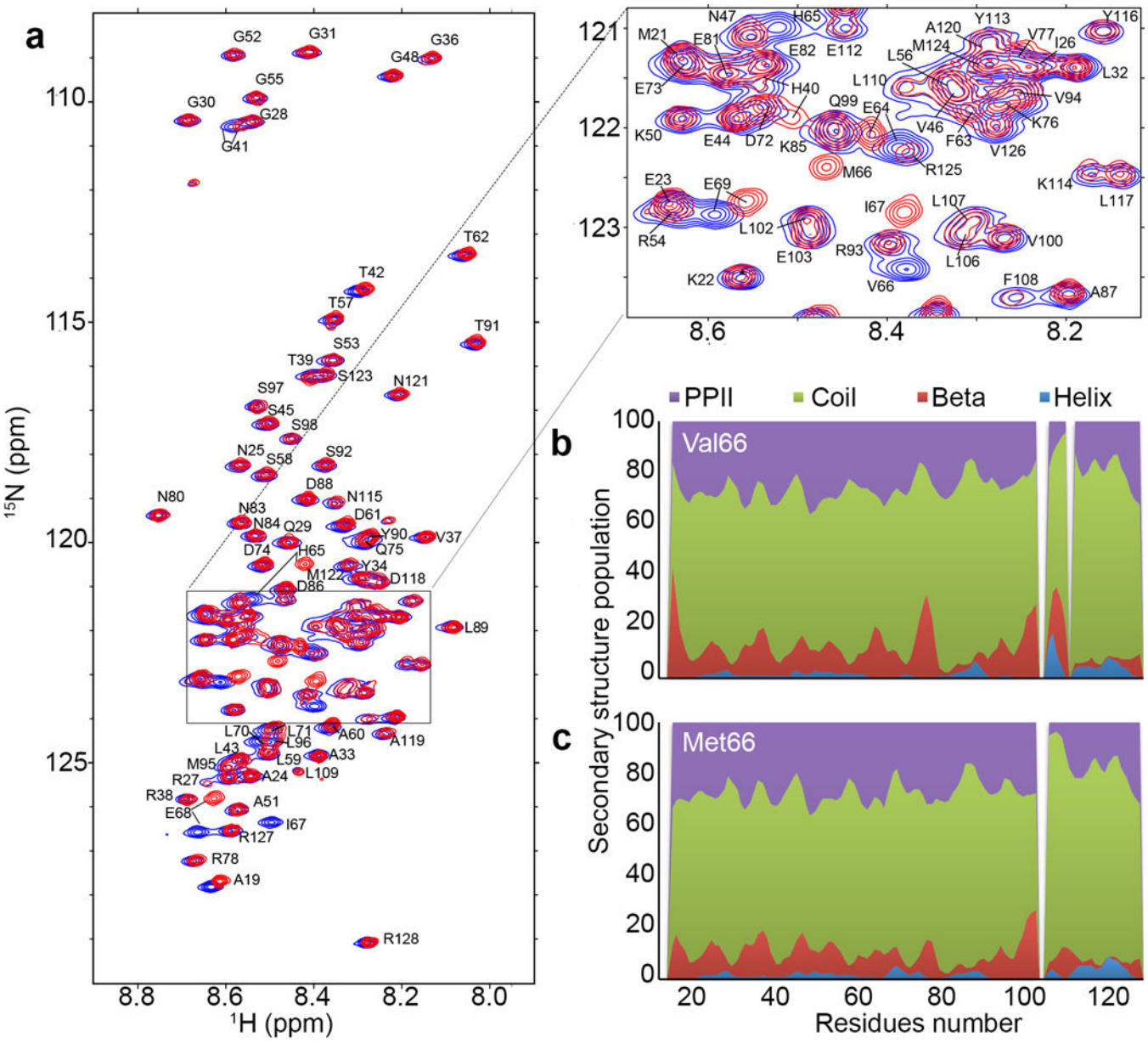

Figure 1).

Amide resonance assignments for Val66 and Met66 prodomains of BDNF. **a.** Overlay 2D ^{1}H-^{15}N HSQC spectrums for the BDNF Pro-domain isoforms Met66 (red) and Val66 (blue). The resonance assignments for the backbone signals are provided in the spectra. These spectra are similar, with the exception of several peaks shifted near the N-terminal (A19), His40 regions (H40-T42), the isoform substitution site V/M-66 (H65-E69). The inset at the top right is an enlarged view of the most crowded spectral region. **b, c** Secondary structures populations predicted using δ2D program for **b.** Val66 prodomain and **c.** Met66 prodomain.