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Biochemical and Biophysical Research Communications 360 (2007) 128–134
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Nogo-B receptor possesses an intrinsically unstructured ectodomain and a partially folded cytoplasmic domain

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Received 26 May 2007
Available online 13 June 2007

Abstract

RTN4/Nogo proteins containing three isoforms have been implicated in a large and diverse spectrum of biological functions. By contrast, only two functional receptors were known for them, namely NgR binding the 66-residue ectodomain shared by all three Nogos and NgBR specifically binding Nogo-B. The 297-residue NgBR was recently identified to be essential for stimulating chemotaxis and morphogenesis of endothelial cells but its structural property still remains completely unknown. In the present study, we expressed and subsequently conducted bioinformatics, CD and NMR characterization of NgBR and its two dissected domains. Very surprisingly, our results indicate that the NgBR ectodomain is intrinsically unstructured without both secondary and tertiary structures while the cytoplasmic domain is only partially folded with secondary structures but without a tight tertiary packing. Therefore, NgBR is a very rare example showing that the entire ectodomain of a transmembrane receptor could be predominantly disordered and the results presented here may bear important implications in understanding NgBR functions in the future.

Keywords: Nogo proteins; Nogo-B receptor; Intrinsically unstructured; Partially folded; Bioinformatics; CD spectroscopy; NMR spectroscopy

Recently RTN4/Nogo proteins have received intense studies because they have been identified to be implicated in a variety of critical cellular processes including CNS neuronal regeneration, vascular cell spreading and chemotaxis, apoptosis, interaction with β-amyloid protein converting enzyme (BACE), formation/maintenance of the tubular network of the endoplasmic reticulum (ER) [1–5]. Nogo proteins are composed of three alternative splice forms, namely 1192-residue Nogo-A, 373-residue Nogo-B, and 199-residue Nogo-C. All three isoforms contain a conserved reticulon homology domain (RHD) in which a 66-residue extracellular domain termed Nogo-66 is capable of inhibiting neurite growth and inducing growth cone collapse [1,2]. This 66-residue inhibitory domain has been shown to be anchored on the oligodendrocyte surface and to bind its receptor NgR at a very high affinity [2]. In particular, intervention in the Nogo-66-NgR binding has been extensively validated to be a promising strategy for developing therapeutic agents to prompt adult CNS axonal regeneration.

Due to their highly diverse spectrum of biological functions, it has been extensively proposed that in addition to NgR, Nogo proteins should have many other unidentified receptors and binding partners. Indeed, we have previously demonstrated that Nogo-A and Nogo-B might be associated with the third SH3 domain of Nck2 adaptor protein via a short proline-rich motif over its N-terminus [6]. Strikingly, a novel transmembrane protein has been recently identified to be a specific receptor for the amino terminus of Nogo-B (AmNogo-B) and thus termed NgBR [7]. The 297-residue NgBR is a hypothetical protein with its ectodo-

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0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.031

main showing no homology with any known protein but with its cytoplasmic domain bearing a high similarity to the cis-prenyltransferase family of lipid-modifying enzymes. However, despite this sequence similarity, no lipid transferase activity was detected for the NgBR cytoplasmic domain [7]. The NgBR protein is involved in stimulating chemotaxis and morphogenesis of endothelial cells and was found to be highly expressed in mouse heart, liver, kidney, and pancreas. NgBR is not only colocalized with Nogo-B during angiogenesis *in vivo*, but also specifically interacted with AmNogo-B and consequently mediates chemotaxis and 3D tube formation of endothelial cells *in vitro* [7].

Structural characterization of NgBR represents an essential step to insight the molecular mechanism underlying the interaction between AmNogo-B and NgBR as well as to gain rationales for further development of agents of therapeutic interest. Therefore in the present study we initiated cloning and expression as well as further CD and NMR characterization of NgBR and its two dissected domains. Very surprisingly, the obtained results indicate that the NgBR ectodomain is intrinsically unstructured while its cytoplasmic domain is only partially folded. Our present findings may provide important clues for further understanding of functional roles of NgBR.

Materials and methods

Bioinformatics characterization. To facilitate the experimental studies here, we have utilized a variety of bioinformatics tools to pinpoint the sequence and structural properties of NgBR. ELM [8] was used to spot out the signal peptide sequence and other short functional motifs. PREDATOR [9] was employed to predict the secondary structures while IUPRED [10,11] and VSL2B [12,13] for identification of the disorder tendency of NgBR.

Cloning, expression and purification of NgBR and its dissected domains. Total RNA was extracted from NIH 3T3 mouse cell pellet using Trizol Reagent. Full-length NgBR RNA was reversely transcribed into DNA by RT-PCR using One-step RT-PCR kit. Bioinformatics analysis revealed that as shown in Fig. 1A, the 297-residue NgBR contained a putative signal sequence (1–46), an ectodomain (47–120), a type 1A transmembrane domain (121–139) and a cytoplasmic domain (140–297) [7,8]. Therefore,

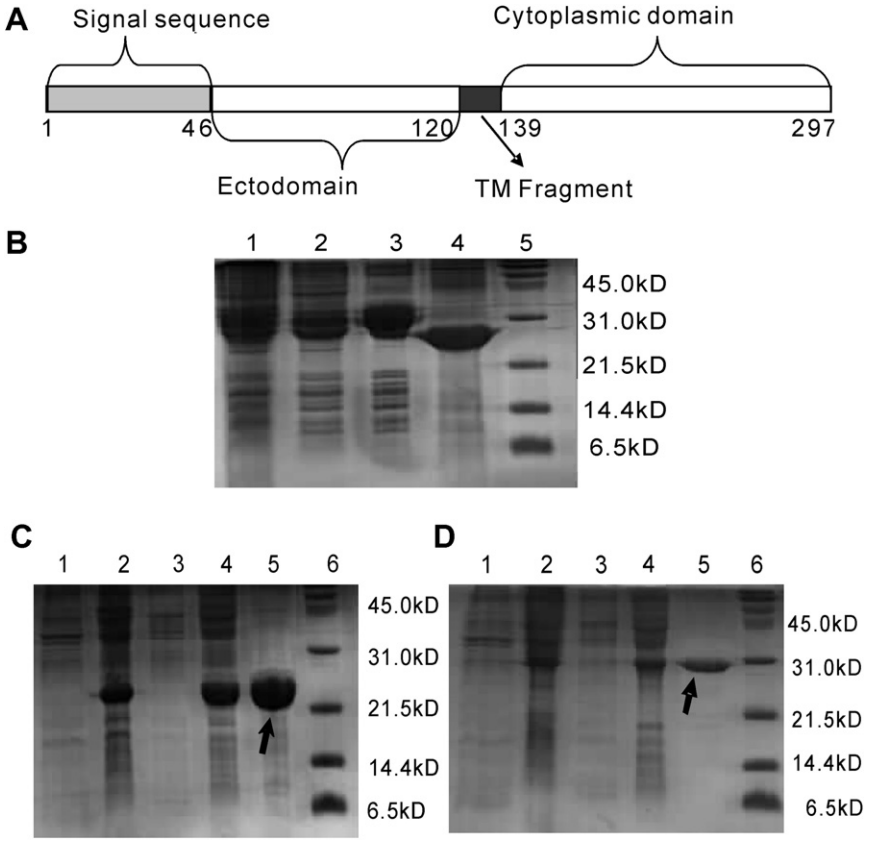

Fig. 1. Domain organization of NgBR and its expression and purification. (A) Bioinformatics characterization reveals that NgBR contains a putative signal sequence (1–46), an ectodomain (47–120), a type 1A transmembrane domain (121–139) and a cytoplasmic domain (140–297). (B) Coomasie brilliant blue stained SDS–PAGE gel showing the expression and affinity-purification of the NgBR ectodomain. Line 1: total cell extract after IPTG induction; line 2: supernatant of the cell lysate; line 3: GST beads with bound GST-fused ectodomain; line 4: GST beads after in-gel thrombin cleavage; line 5: protein molecular weight markers. (C) Coomasie brilliant blue stained SDS–PAGE gel showing the expression and affinity-purification of the NgBR cytoplasmic domain. Line 1: total cell extract before IPTG induction; line 2: total cell extract after IPTG induction; line 3: supernatant of the cell lysate; line 4: pellet of the cell lysate; line 5: Ni-agrose beads with bound cytoplasmic domain in the presence of 8 M urea; line 6: protein molecular weight markers. (D) Coomasie brilliant blue stained SDS–PAGE gel showing the expression and affinity-purification of the full-length of NgBR. Line 1: total cell extract before IPTG induction; line 2: total cell extract after IPTG induction; line 3: supernatant of the cell lysate; line 4: pellet of the cell lysate; line 5: Ni-agrose beads with bound NgBR in the presence of 8 M urea; line 6: protein molecular weight markers.

DNA fragments encoding the full-length NgBR with the signal peptide removed as well as its ectodomain and cytoplasmic domain were amplified by PCR and subsequently cloned into His-tagged vector pET32a or GST-tag vector pGEX 4T-1. DNA sequences were confirmed by automated DNA sequencing, and the recombinant proteins were expressed in *Escherichia coli* BL21 cells. Briefly, the cells were cultured at 37 °C to an OD_{600} of 0.6 and then IPTG was added to a final concentration of 1.0 mM to induce expression of the recombinant proteins for 4 h at 37 °C. The GST-fused ectodomain was purified by affinity binding to the glutathione-Sepharose 4B resin and then the pure ectodomain was obtained by in-gel thrombin cleavage followed by HPLC purification on a semi-preparative RP-18 column (Vydac). The His-tagged cytoplasmic domain and full-length NgBR were expressed in *E. coli* BL21 cells but they were found only in the inclusion body. As a consequence, they were purified by Ni^{2+}-affinity chromatography under denaturing condition in the presence of 8 M urea followed by further HPLC purification on a reverse-phase C4 column (Vydac). For heteronuclear NMR experiments, ^{15}N-labeled proteins were prepared following the similar expression and purification procedure except for growing *E. coli* cells in M9 medium instead of LB medium. The $(^{15}NH_4)_2SO_4$ salt was used for ^{15}N-isotope labeling as previously described [14]. The identities of all recombinant proteins described here were verified by MALDI-TOF mass spectrometry.

Circular dichroism (CD) and NMR experiments. All CD experiments were performed on a Jasco J-810 spectropolarimeter equipped with a thermal controller using 1 mm path length cuvettes. The NgBR ectodomain was dissolved in 20 mM phosphate buffer (pH 6.0). However, the cytoplasmic domain and full-length proteins of NgBR were found to be totally insoluble in salty buffer and thus were solubilized in salt-free water (pH 4.5) as we recently described [15,16]. The far-UV CD spectra were recorded between 190 and 260 nm with protein concentrations of ~20 μM while the near-UV CD spectra were recorded between 250 and 360 nm with protein concentrations of ~150 μM at 25 °C. Final CD spectra were obtained by adding and averaging data from three independent scans.

NMR samples were prepared by dissolving the lyophilized proteins in 450 μL of 20 mM phosphate buffer (pH 6.0) for the NgBR ectodomain or Milli-Q water for the cytoplasmic domain and full-length proteins of NgBR (pH 4.5), with an addition of 50 μL of D_2O for NMR spin-lock. HSQC experiments were acquired on an 800 MHz Bruker Advanced spectrometer equipped with pulse field gradient units at 298 K as previously described [14–16]. Spectral processing and analysis were carried out using NMRpipe [17] and NMRview [18].

Results

Cloning and expression of NgBR and its dissected domains

The full-length NgBR, as well as its cytoplasmic domain and ectodomain were successfully cloned into pGEX 4T-1 or pET32a vector with BamHI/XhoI restriction sites and expressed as GST-fused or His-tagged proteins. The NgBR ectodomain was expressed as a GST fusion protein which was purified under native condition (Fig. 1B). After removing the GST by in-gel thrombin cleavage, the NgBR ectodomain was further purified by HPLC. On the other hand, the His-tagged cytoplasmic domain and full-length

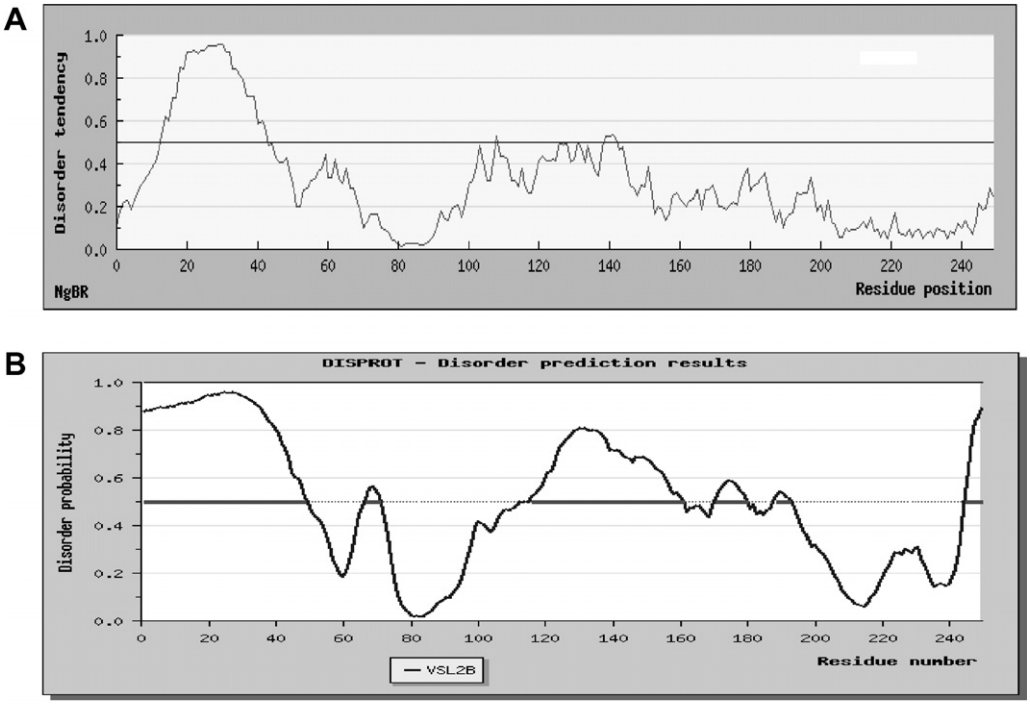

Fig. 2. Bioinformatics analysis of NgBR. (A) The globularity and disordered regions of NgBR assessed by IUPRED; and (B) by VSL2B. (C) Amino acid sequence of the NgBR ectodomain with prolines residues highlighted.

NgBR were found to exist in the inclusion body. As such the two recombinant proteins were first purified by Ni^{2+}-affinity column under denaturing condition (Fig. 1C and D) followed by HPLC purification. The molecular weights of all three recombinant proteins determined by MALDI-TOF mass spectrometry matched those predicted from their amino acid sequences.

The lyophilized proteins of the full-length NgBR and its cytoplasmic domain were found to be highly insoluble in salty buffer, but strikingly could be solubilized in salt-free water (pH 4.5) to high concentrations, as we previously discovered [10,11]. However, we failed to adjust their solution pH to a high value because addition of a tiny amount of NaOH would result in a rapid aggregation. On the other hand, the solubility of the NgBR ectodomain was very high in salty buffer and thus all studies on it were conducted in 20 mM phosphate buffer (pH 6.0).

Bioinformatics characterization

As seen in Fig. 2A and B, analysis of NgBR by IUPred and VSL2B indicated that the N-terminal 50 residues out of the 74-residue NgBR ectodomain had a very high tendency to be disordered while the cytoplasmic domain was much more structured despite also owning a relatively disordered fragment (Fig. 2B). Consistent with this, secondary structure prediction by PREDATOR indicated that the majority of the ectodomain was random coil while the large portion of the cytoplasmic domain formed helix and β-sheet secondary structures (results not shown). Interestingly, examination of the amino acid sequence revealed an interesting fact that up to 10 proline residues existed in the 74-residue ectodomain (Fig. 2C). Interestingly, high proline content has been previously proposed to be associated with the lack of a defined tertiary structure [19,20].

Structural characterization of the NgBR ectodomain

Fig. 3A present the far-UV CD spectrum of the NgBR ectodomain which owned the largest negative signal at ~198 nm and had no positive signal at ~190 nm, indicating that it was predominantly unstructured. Furthermore, no significant difference was detected for its near-UV CD spectra in the absence and presence of 8 M urea (Fig. 3B), suggesting that the no tight tertiary packing existed in the NgBR ectodomain.

More specifically, the ^{1}H–^{15}N NMR HSQC spectrum of the NgBR ectodomain displayed very narrow spectral dispersions over both dimensions (~0.75 ppm for ^{1}H and ~20 ppm for ^{15}N) as well as NMR resonance peaks could be observed for almost all residues (Fig. 3B). These characteristics indicated that the NgBR ectodomain is highly

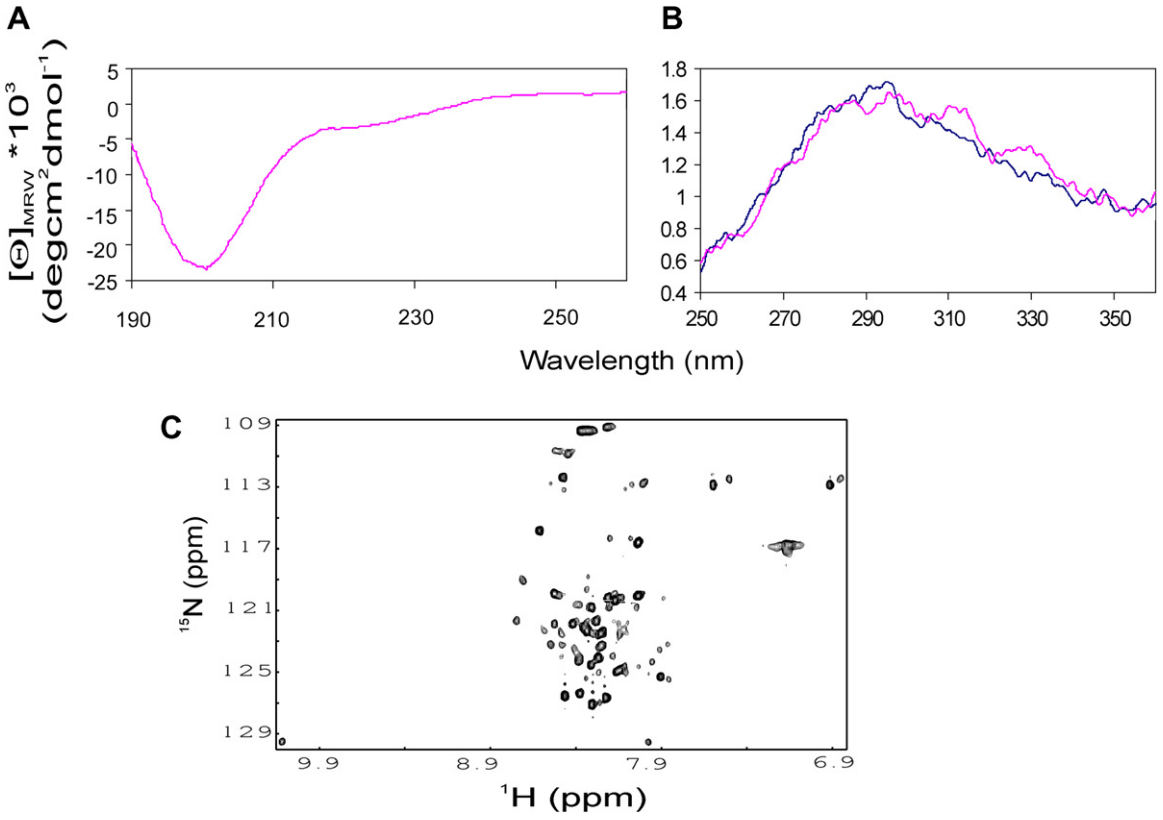

Fig. 3. CD and NMR characterization of the NgBR ectodomain. (A) Far-UV CD spectrum in 20 mM phosphate buffer (pH 6.0) at 25 °C. (B) Near-UV CD spectra in the absence (blue) and presence (pink) of 8 M urea in 20 mM phosphate buffer (pH 6.0) at 25 °C. (C) ^{1}H–^{15}N NMR HSQC spectrum at a concentration of 350 μM acquired in 20 mM phosphate buffer (pH 6.0) at 25 °C.

disordered in the free state. It was also noticeable that there were some extra weak peaks in the HSQC spectrum (Fig. 3C), similar to our previous observation on the Nogo-B N-terminus which was also abundant in proline residues [14]. Therefore, these weak peaks were also likely to result from the *cis–trans* isomerization of the proline residues in the NgBR ectodomain. Similar to the situation we encountered with Nogo-B [14], the extensive presence of the *cis–trans* isomerization made it impossible to achieve NMR sequential assignment of the unstructured and proline-rich NgBR ectodomain.

Structural characterization of NgBR and its cytoplasmic domain

Despite very poor solubility of NgBR and its cytoplasmic domain in salty buffer, they could be easily solubilized in salt-free water to a high concentration of ~450 μM. This thus allowed us to structurally characterize the two recombinant proteins. As shown in Fig. 4A, the far-UV CD spectra of NgBR and its cytoplasmic domain are very similar, with one positive signal at ~190 nm and two negative signals at ~205 and 222 nm, respectively, indicating that

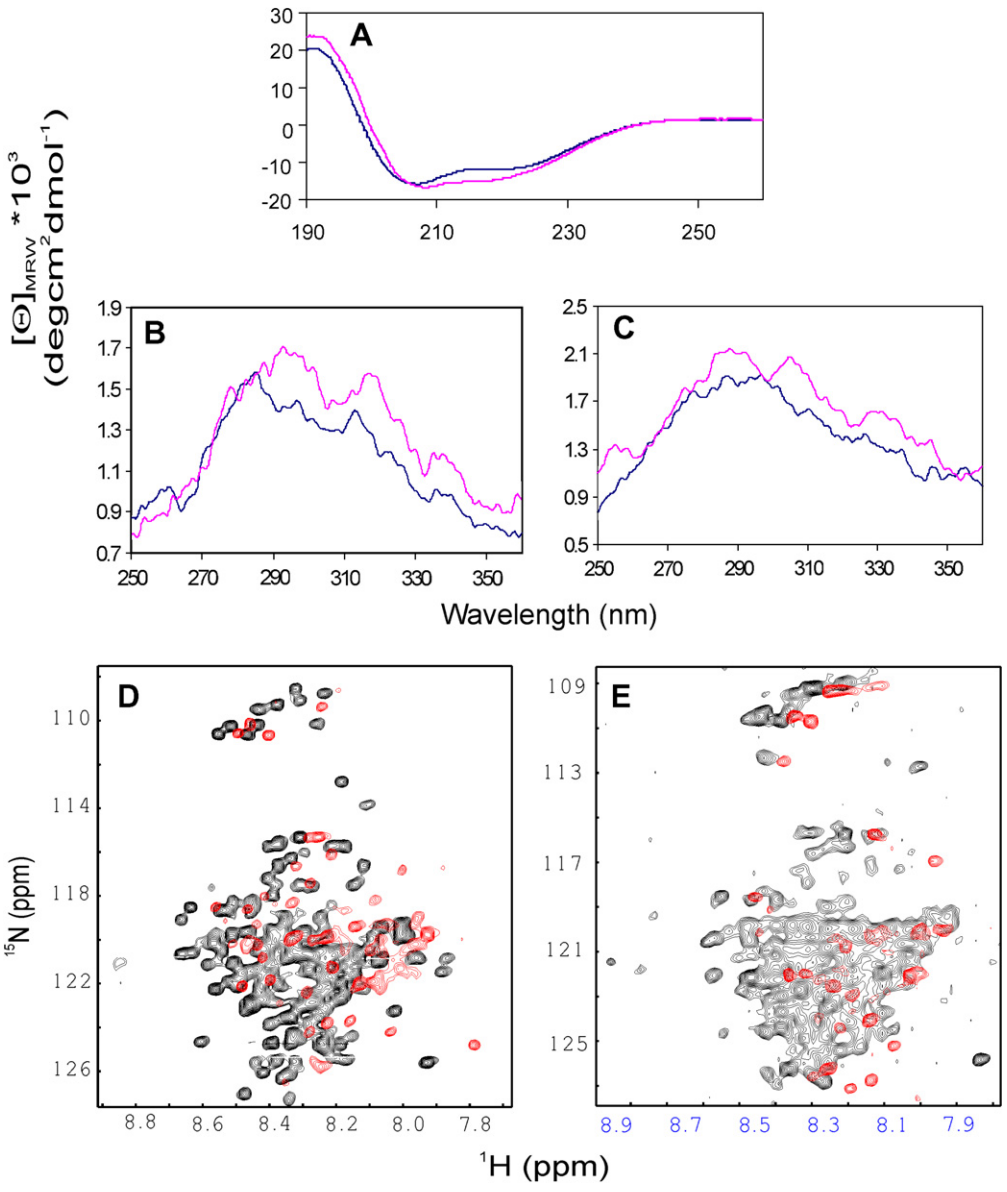

Fig. 4. CD and NMR characterization of the full-length NgBR and its cytoplasmic domain. (A) Far-UV CD spectra of the full-length NgBR (pink) and its cytoplasmic domain (blue) in salt-free water (pH 4.5) at 25 °C. (B) Near-UV CD spectra of the full-length NgBR in the absence (blue) and presence (pink) of 8 M urea in salt-free water (pH 4.5) at 25 °C. (C) Near-UV CD spectra of the NgBR cytoplasmic domain in the absence (blue) and presence (pink) of 8 M urea in salt-free water (pH 4.5) at 25 °C. (D) ^{1}H–^{15}N NMR HSQC spectra of the ^{15}N-labeled cytoplasmic domain in the absence (red) and presence (black) of 8 M urea at a protein concentration of 450 μM acquired in salt-free water (pH 4.5) at 25 °C. (E) ^{1}H–^{15}N NMR HSQC spectra of the ^{15}N-labeled full-length NgBR in the absence (red) and presence (black) of 8 M urea with a protein concentration of 450 μM acquired in salt-free water (pH 4.5) at 25 °C.

both proteins contained high contents of helical structures. Indeed, analysis of the two CD spectra by K2 d [21] indicated that NgBR contained 38% α-helix, 15% β-sheet and 47% random coil while the cytoplasmic domain had 45% α-helix, 23% β-sheet and 32% random coil. On the other hand, very small differences of the near-UV spectra of NgBR (Fig. 4B) and its cytoplasmic domain (Fig. 4C) in the absence and presence of 8 M urea implied that both of them had no tight tertiary packing and as such they were only partially folded.

Indeed, ^{1}H–^{15}N NMR HSQC spectra of NgBR (Fig. 4D) and its cytoplasmic domain (Fig. 4E) again showed very narrow spectral dispersions over both dimensions (~0.8 ppm for ^{1}H and ~20 ppm for ^{15}N), clearly confirming the conclusion that they were only partially folded based on the above CD results. Furthermore, unlike the NgBR ectodomain, in the absence of 8 M urea, only ~45 resonance peaks could be detected for the 250-residue NgBR (Fig. 4D) and ~30 peaks (Fig. 4E) for the 157-residue cytoplasmic domain. On the other hand, introduction of 8 M urea resulted in a sudden appearance of a large amount of HSQC resonance peaks for both NgBR and its cytoplasmic domain. This observation revealed that unlike the NgBR ectodomain which was highly disordered in solution, the cytoplasmic domain was partially folded but a large portion of the molecule was undergoing conformational exchanges on the μs–ms time-scale or/and dynamic aggregation due to the lack of a tight tertiary packing. Consequently, most NMR HSQC resonance peaks were severely broadened and thus became undetectable, which made it impossible to carry out further high-resolution NMR investigation [16,22,23]. In this regard, the NgBR cytoplasmic domain belongs to the second group of the buffer-insoluble proteins based on our previous classification [16].

Discussion

The discovery of the Nogo-B specific receptor NgBR significantly advanced our understanding of the molecular mechanism underlying the diverse functions of Nogo proteins. In particular, the key role of the AmNogoB-NgBR interaction in mediating chemotaxis and 3D tube formation ranks NgBR as an extremely promising target for design of agonists or antagonists of this pathway to modulate vascular remodeling and angiogenesis [7]. Previously we have conducted a systematical characterization of the structural and binding properties of Nogo proteins [6,14–16,24]. In the present study, we initially attempted to determine the structure of NgBR. To our big surprise, the bioinformatics, CD and NMR characterization reveals that the NgBR ectodomain is a member of the intrinsically unstructured protein (IUP) family. Previously, the very unusual properties of the intrinsically unstructured family of proteins have been proposed to be advantageous for special categories of biological functions including signal transduction, regulation, cytoskeletonal organization, protein–DNA recognition, human cancer, endocytosis as well as generating/maintaining membrane structure [10–14,19,20,25–28]. However, the commitment of an intrinsically unstructured protein as the ectodomain of a transmembrane receptor was rarely reported. The ectodomain of the transmembrane β-dystroglycan was characterized to be an intrinsically unstructured protein [29]. However, the known function of the β-dystroglycan ectodomain is to constitute a dystroglycan complex by binding α-dystroglycan which is separated from β-dystroglycan by a post-translational cleavage of a single proprotein at Ser654. To this end, β-dystroglycan is usually not considered to be a transmembrane receptor [30]. Our current finding raises an intriguing question as how the intrinsically unstructured NgBR ectodomain functions. It appears that there may be three possible mechanisms. The first one is that the NgBR ectodomain becomes well-structured upon binding to Nogo-B or other partners. Alternatively it is also possible that unlike the classic receptor ectodomains, the NgBR ectodomain may function more like a scaffold to utilize short motifs to assemble a variety of binding partners over the cell surface. The third one is that similar to β-dystroglycan, the NgBR ectodomain is a component of a multi-component receptor complex [7]. However, if only considering its interaction with Nogo-B, it is very unlikely that upon binding they will form a well-structured complex as both NgBR ectodomain and Nogo-B N-terminus are highly unstructured as well as proline-rich [14]. Certainly, intense studies are demanded to address the implications of this unusual property of NgBR in the future.

It is also valuable to discover that the full-length NgBR and its cytoplasmic domain are highly insoluble in salty buffer but again could be easily solubilized in salt-free water to reach a concentration of 450 μM. Although previously we have demonstrated that salt-free water was able to solubilize all buffer-insoluble proteins we had at that time [15,16], the present result not only provides support but also extends our previous discovery by showing that salt-free water is even capable of dissolving the full-length NgBR carrying a transmembrane fragment. Moreover, the observation that the NgBR cytoplasmic domain is only partially folded supports the previous speculation that it may serve as a scaffold responsible for binding isoprenyl lipids and/or prenylated proteins [7], rather than as a lipid transferase enzyme because in general an enzyme requires a well-robust tertiary fold to implement the catalytic task. It is highly possible that the NgBR cytoplasmic domain becomes well-folded upon binding isoprenyl lipids and/or prenylated proteins.

Acknowledgments

This study is supported by the Biomedical Research Council of Singapore (BMRC) Young Investigator Award R-154-000-217-305 and BMRC Grant R-183-000-097-305 (to J.S.).

References

[1] T. Oertle, M.E. Schwab, Nogo and its paRTNers, Trends Cell Biol. 13 (2003) 187–194.

[2] T. GrandPre, F. Nakamura, T. Vartanian, S.M. Strittmatter, Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein, Nature 403 (2004) 439–444.

[3] L. Acevedo, J. Yu, H. Erdjument-Bromage, R.Q. Miao, J.E. Kim, D. Fulton, P. Tempst, S.M. Strittmatter, W.C. Sessa, A new role for Nogo as a regulator of vascular remodeling, Nat. Med. 10 (2004) 382–388.

[4] Y. Tambe, T. Isono, S. Haraguchi, A. Yoshioka-Yamashita, M. Yutsudo, H. Inoue, A novel apoptotic pathway induced by the drs tumor suppressor gene, Oncogene 23 (2004) 2977–2987.

[5] W. He, Y. Lu, I. Qahwash, X.Y. Hu, A. Chang, R. Yan, Reticulon family members modulate BACE1 activity and amyloid-peptide generation, Nat. Med. 10 (2004) 959–965.

[6] J. Liu, M. Li, X. Ran, J.S. Fan, J. Song, Structural insight into the binding diversity between the human Nck2 SH3 domains and proline-rich proteins, Biochemistry 45 (2006) 7171–7184.

[7] R.Q. Miao, Y. Gao, K.D. Harrison, J. Prendergast, L.M. Acevedo, J. Yu, F. Hu, S.M. Strittmatter, W.C. Sessa, Identification of a receptor necessary for Nogo-B stimulated chemotaxis and morphogenesis of endothelial cells, Proc. Natl. Acad. Sci. USA 103 (2006) 10997–11002.

[8] P. Puntervoll, R. Linding, C. Gemünd, S. Chabanis-Davidson, M. Mattingsdal, S. Cameron, D.M.A. Martin, G. Ausiello, B. Brannetti, A. Costantini, F. Ferrè, V. Maselli, A. Via, G. Cesareni, F. Diella, G. Superti-Furga, L. Wyrwicz, C. Ramu, C. McGuigan, R. Gudavalli, I. Letunic, P. Bork, L. Rychlewski, B. Küster, M. Helmer-Citterich, W.N. Hunter, R. Aasland, T.J. Gibson, ELM server: a new resource for investigating short functional sites in modular eukaryotic proteins, Nucleic Acids Res. 31 (2003) 3625–3630.

[9] D. Frishman, P. Argos, Incorporation of long-distance interactions into a secondary structure prediction algorithm, Protein Eng. 9 (1996) 133–142.

[10] Dosztányi Zsuzsanna, Csizmók Veronika, Tompa Péter, Simon István, The pairwise energy content estimated from amino acid composition discriminates between folded and intrinsically unstructured proteins, J. Mol. Biol. 347 (2005) 827–839.

[11] Dosztányi Zsuzsanna, Csizmók Veronika, Tompa Péter, Simon István, IUPred: web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content, Bioinformatics 21 (2005) 3433–3434.

[12] K. Peng, P. Radivojac, S. Vucetic, A.K. Dunker, Z. Obradovic, Length dependent prediction of protein intrinsic disorder, BMC Bioinform. 7 (2006) 208.

[13] Z. Obradovic, K. Peng, S. Vucetic, P. Radivojac, A.K. Dunker, Exploiting heterogeneous sequence properties improves prediction of protein disorder, Proteins 61 (S7) (2005) 176–182.

[14] M. Li, J. Song, The N- and C-termini of the human Nogo molecules are intrinsically unstructured: bioinformatics, CD, NMR characterization and its functional implications, Proteins: Struct., Funct, Bioinform. 68 (2007) 100–108.

[15] M. Li, J. Liu, J. Song, Nogo goes in the pure water: solution structure of Nogo-60 and design of the structured and buffer-soluble Nogo-54 for enhancing CNS regeneration, Protein Sci. 15 (2006) 1835–1841.

[16] M. Li, J. Liu, X. Ran, M. Fang, J. Shi, H. Qin, J.-M. Goh, J. Song, Resurrecting abandoned proteins with pure water: CD and NMR studies of protein fragments solubilized in salt-free water, Biophys. J. 91 (2006) 4201–4209.

[17] F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, NMRPipe a multidimensional spectral processing system based on UNIX pipes, J. Biomol. NMR 6 (1995) 277–293.

[18] B.A. Johnson, R.A. Blevins, NMRView: a computer program for the visualization and analysis of NMR data, J. Biomol. NMR 4 (1994) 603–614.

[19] P. Romero, Z. Obradovic, X. Li, E.C. Garner, C.J. Brown, A.K. Dunker, Sequence complexity of disordered protein, Proteins 42 (2001) 38–48.

[20] P. Tompa, Intrinsically unstructured proteins, Trends Biochem. Sci. 27 (2002) 527–533.

[21] M.A. Andrade, P. Chacón, J.J. Merelo, F. Morán, Evaluation of secondary structure of proteins from UV circular dichroism using an unsupervised learning neural network, Protein Eng. 6 (1993) 383–390.

[22] J. Song, N. Jamin, B. Gilquin, C. Vita, A. Menez, A gradual disruption of tight side-chain packing, Nat. Struct. Biol. 6 (1999) 129–134.

[23] Z. Wei, J. Song, Molecular mechanism underlying the thermal stability and pH-Induced unfolding of CHABII, J. Mol. Biol. 348 (2005) 205–218.

[24] M. Li, J. Shi, Z. Wei, F.Y. Teng, B.L. Tang, J. Song, Structural characterization of the human Nogo-A functional domains. Solution structure of Nogo-40, a Nogo-66 receptor antagonist enhancing injured spinal cord regeneration, Eur. J. Biochem. 271 (2004) 3512–3522.

[25] V.N. Uversky, Natively unfolded proteins: a point where biology waits for physics, Protein Sci. 11 (2002) 739–756.

[26] H.J. Dyson, P.E. Wright, Intrinsically unstructured proteins and their functions, Nat. Rev. Mol. Cell Biol. 6 (2005) 197–208.

[27] J.J. Ward, J.S. Sodhi, L.J. McGuffin, B.F. Buxton, D.T. Jones, Prediction and functional analysis of native disorder in proteins from the three kingdoms of life, J. Mol. Biol. 337 (2004) 635–645.

[28] K. Gunasekaran, C.J. Tsai, S. Kumar, D. Zanuy, R. Nussinov, Extended disordered proteins: targeting function with less scaffold, Trends Biochem. Sci. 28 (2003) 81–85.

[29] M. Bozzi, M. Bianchi, F. Sciandra, M. Paci, B. Giardina, A. Brancaccio, D.O. Cicero, Structural characterization by NMR of the natively unfolded extracellular domain of beta-dystroglycan: toward the identification of the binding epitope for alpha-dystroglycan, Biochemistry 42 (2003) 13717–13724.

[30] S.J. Winder, The complexities of dystroglycan, Trends Biochem. Sci. 26 (2001) 118–124.