^{1}H, ^{13}C and ^{15}N assignment of the C-terminal domain of GNA2132 from *Neisseria meningitidis*

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Abstract GNA2132 (Genome-derived Neisseria Antigen 2132) is a surface-exposed lipoprotein discovered by reverse vaccinology and expressed by genetically diverse *Neisseria meningitidis* strains (Pizza et al. 2000). The protein induces bactericidal antibodies against most strains of *Meningococccus* and has been included in a multivalent recombinant vaccine against *N. meningitidis* serogroup B. Structure determination of GNA2132 is important for understanding the antigenic properties of the protein in view of increased efficiency vaccine development. We report practically complete ^{1}H, ^{13}C and ^{15}N assignment of the detectable spectrum of a highly conserved C-terminal region of GNA2132 (residues 245–427) in micellar solution, a medium used to improve the spectral quality. The first 32 residues of our construct up to residue 277 were not visible in the spectrum, presumably because of line broadening due to solvent and/or conformational exchange. Secondary structure predictions based on chemical shift information indicate the presence of an all β-protein with eight β strands.

Keywords Antigene · Meningitis · NMR · Structure · Vaccine

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Biological context

Neisseria meningitis is a human pathogen which induces meningitis and sepsis in children and young adults. The bacterium is surrounded by a capsular polysaccharide which defines thirteen different serogroups, five of which (A, B, C, Y, and W135) are the major responsible for disease in humans (Harrison 2006). Conjugate vaccines against serogroups A, C, Y and W135 based on polysaccharides are available or in late phase of development. The same strategy cannot, however, be used for *Meningococcus* B since its capsular polysaccharide has a structural homology with a polysialic acid [α(2–8)N-acetylneuraminic acid], which is present in human cells (Stein et al. 2005). The only vaccines proven to be effective against serogroup B are based on outer membrane vescicles (OMV) purified from the bacterium (Bjune et al. 1991). The main limitation of such vaccines, also called "tailor made", is their strain specificity due to the high variability of PorA, the main antigenic component of the vesicles (Stephens 2007).

We have used a genome-based approach, named "reverse vaccinology", to overcome these limitations and develop a vaccine potentially able to cover the *Meningococcus* B strain diversity (Pizza et al. 2000). This strategy has allowed the identification of novel surface-exposed antigens able to induce bactericidal antibodies (Giuliani et al. 2006), a property known to correlate with protection in humans. Among this is GNA2132, a surface exposed lipoprotein of 48 kDa able to induce bactericidal antibodies and play a protective role in animal models.

Because of its potential importance in vaccinology, GNA2132 is one of the components of a *Meningococcus* B vaccine which has entered phase III clinical trials. Large effort is being dedicated to the definition of the structural

Fig. 1 Quality of the NMR spectrum and secondary structure of the C-terminal domain of GNA2132. **a** ^{1}H-^{15}N HSQC spectrum in 20 mM Tris–HCl, at pH 7, 50 mM NaCl and DPC recorded at 40°C and 700 MHz proton frequency. A close up shows the assignment of the most crowded region. **b** Secondary structure predictions for the sequence of the GNA2132 domain as obtained from TALOS+ (*black arrows*) and from the JPRED webserver (*grey arrows*)
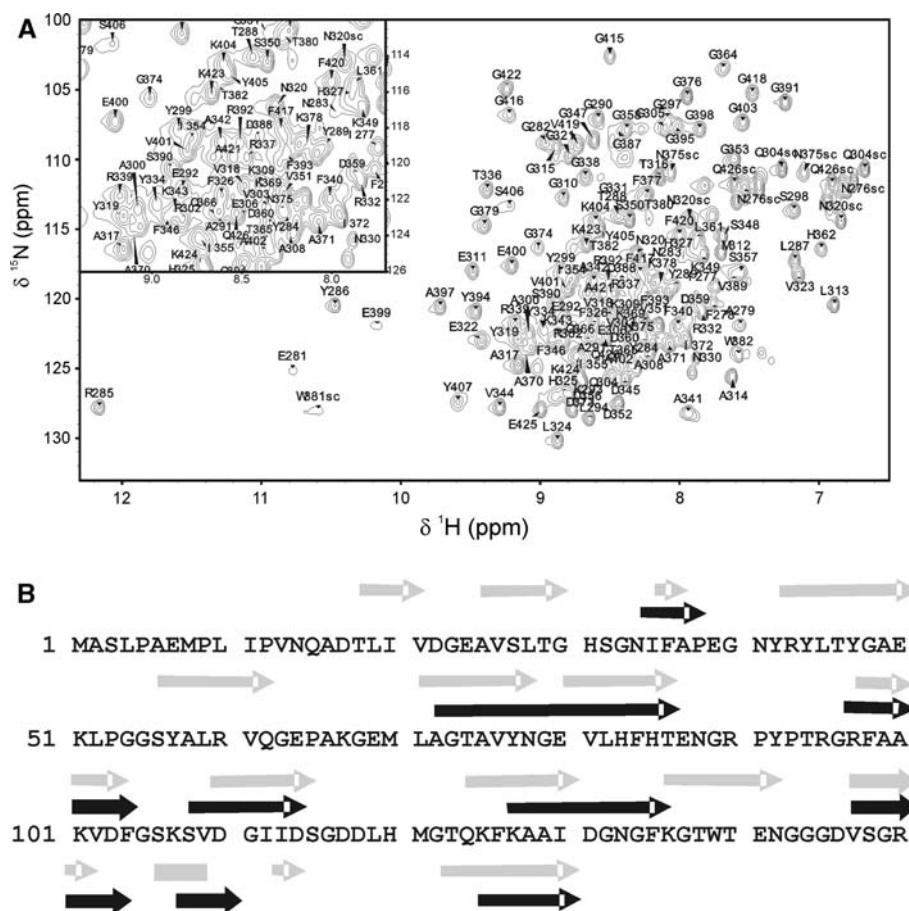
and functional properties of this antigen to better characterize its immune response and potential role in pathogenesis. We have recently shown that GNA2132 is able to bind heparin through an arginine-rich region and is cleaved by two different proteases, human lactoferrin and meningococcal NalP (Serruto et al. 2010). Sequence analysis on a panel of strains representative of the meningococcal diversity has allowed the definition of two regions: an N-terminal domain with several hypervariable regions, and a C-terminal domain highly conserved in sequence (Pizza et al. 2000).

As a starting point towards understanding the antigenic properties of GNA2132 and designing more effective immunogens, we present here the NMR assignment of the C-terminal domain of the protein.

Methods and experiment

Expression and purification

The region 245-427 of the GNA2132 sequence, cloned in a pET21b + vector (Novagen), was expressed in *Escherichia coli* BL21(DE3)-pLysS. The sequence was preceded by an MA sequence added for cloning reasons and followed by a histidine tag (residues LEHHHHHH) for purification purposes. Uniformly ^{15}N,^{13}C-labelled samples were produced by growing cells at 37°C in M9 minimal medium, enriched with 1 g/L ^{15}N-labelled ammonium sulfate and 4 g/L [U-2H,13C]glucose (Cambridge Isotope Laboratories). The protein was induced with 1 mM isopropyl β-D-thiogalactopyranoside and expressed for 4 h. The cell pellet was suspended in 50 mM sodium phosphate buffer at pH 8 and lysed by lysozyme. The GNA2132 domain was purified in two steps by affinity chromatography, using Ni–NTA and cation exchange columns equilibrated in 50 mM sodium acetate at pH 5.5. Sample purity and identity were checked by SDS–PAGE and electrospray mass spectrometry, respectively.

The NMR samples used for assignment typically contained 0.5 mM protein concentrations (95% H_2O, 5% D_2O) in 20 mM Tris–HCl, at pH 7, 50 mM NaCl and in the presence of 30 mM dodecyl-phospho-cholate (DPC). All spectra were recorded at 40°C on a Bruker Avance 700 MHz spectrometer equipped with a cryoprobe. Backbone ^{1}HN, ^{15}N, ^{13}Cα, ^{1}Hα, ^{13}C′, and side chain ^{1}H and ^{13}C resonances were assigned using HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and HNHA

experiments. HCCH-TOCSY, H(CCCO)NH, CC(CO)NH, ^{1}H-^{15}N- and ^{1}H-^{13}C-HSQC-NOESY spectra were also performed for side-chain assignments. Water suppression was achieved by WATERGATE. Data were processed with NMRPipe/NMRDraw (Delaglio et al. 1995) and analyzed with Sparky (Goddard & Kneller, San Francisco, CA). Torsion angles (φ, ψ) and experimentally based secondary structure predictions were carried out for the HN, Hα, N, Cα, Cβ and C′ chemical shifts using TALOS+ (Shen et al. 2009) and CSI (Wishart and Sykes 1994) software. Sequence based secondary structure predictions were obtained using the JPRED website (http://www.compbio.dundee.ac.uk/www-jpred/).

Extent of assignment and data deposition

The GNA2132 domain in aqueous buffer gave rise to well-resolved 1D NMR spectra, which are typical of a well-folded species. However, the presence in the ^{1}H-^{15}N HSQC spectrum of a number of resonances larger than expected suggested the presence of more than one species (data not shown). After initial screening for the best experimental conditions, we noticed that the spectral quality improved drastically by the addition of detergents. The additional species disappeared, for instance, after addition of DPC and the spectrum became of excellent appearance (Fig. 1a). This observation could be of relevance for understanding the functions of the protein in the future.

For the time being, we used DPC for assignment. The construct used spans residues 244-427 of GNA2132. The backbone resonances were fully assigned with the exception of the first 32 residues, of M363, K367, F368 and of the region from Y407 to E413 which could not be identified in the spectrum. These residues are missing because of line broadening likely due to solvent exchange and/or conformational exchange. Of the remaining 142 residues, more than 94% of the backbone resonances and more than 87% of the side chains resonances were unambiguously identified, including the assignment of 90% of the ^{1}H side chain resonances. Chemical shifts were deposited in the BioMagResBank under the access number BMRB 16679.

Evaluation of the obtained Hα, C′, Cα and Cβ chemical shifts by TALOS+ (Shen et al. 2009) and CSI (Wishart and Sykes 1994) indicates that GNA2132 contains eight β-strands (residues 276-279, 314-324, 338-346, 350-355 369-376, 388-394, 399-402, 413-418) in reasonable agreement with sequence structure predictions (Fig. 1b).

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