^{1}H, ^{13}C, and ^{15}N resonance assignment of the TIR domain of human MyD88

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Key words:

Innate immunity, Toll-like receptor, NMR, MyD88, TIR domain

Abstract

Myeloid differentiating factor 88 (MyD88) is one of a critical adaptor molecule in the Toll-like receptor (TLR) signaling pathway. The TIR domain of MyD88 serves

as a protein-protein interaction module and interacts with other TIR-containing proteins such as Mal (MyD88 adaptor-like) and Toll-like receptor 4 to form signal initiation complexes. Here we report the ^{15}N, ^{13}C, and ^{1}H chemical shift assignments of the TIR domain of MyD88. The resonance assignments obtained in this work will contribute to the study of heteromeric TIR-TIR interactions between MyD88 and TIR-containing receptors or adaptors.

Biological context

MyD88 is an essential and universal cytosolic adaptor protein in the signal transduction pathways mediated by members of the IL-1 family, including IL-18, and the Toll-like receptors (TLRs). It consists of an N-terminal death domain, a C-terminal Toll/Interleukin-1 receptor (TIR) domain (~150 amino acid residues), and a small linker segment between them. In innate immune responses, MyD88 plays pivotal roles in bridging between membraneous TLR and downstream kinases, such as the IL-1 receptor-associated kinases (IRAKs), in the cytosol (O'Neill and Bowie, 2007). For example, in the TLR4 pathway, the cytosolic TIR

domain of lipopolysaccharide (LPS)-stimulated TLR4 interacts with the TIR domain of MyD88 (MyD88-TIR). Concurrently, the death domain of MyD88 interacts with the death domain of IRAK4, activating the kinase. This initiates a phosphorylation cascade that eventually activates the transcription factors NF-κB (nuclear factor κB) and AP-1 (activator protein 1) (Akira et al., 2006).

The TIR domains serve as protein-protein interaction modules, which are conserved in the intracellular region of TLRs and also in cytosolic adaptor proteins such as MyD88, Mal (MyD88 adaptor-like), TRIF (TIR domain-containing adaptor inducing IFN-β), TRAM (TRIF-related adaptor molecule), and SARM (sterile α and HEAT Armadillo motifs). The crystal structures of TIR domains of some mammalian membranous receptors (TLR1, TLR2, TLR10, IL-1RAPL) and a bacterial protein (*Paracoccus denitrificans* TIR (PdTIR)) have been reported (Chan et al., 2009; Khan et al., 2004; Nyman et al., 2008; Tao et al., 2002; Xu et al., 2000), in some of which homomeric TIR interfaces were observed. However, the functional relevance of such interactions remains obscure, as the formation of

these homo-dimers of TIR domains had not been observed in solution (Khan et al., 2004; Nyman et al., 2008).

In our previous study, the isolated MyD88-TIR was shown to exist as a monomer, while full-length MyD88 forms a dimer in solution. The dimerization appears to be mediated via homomeric interactions within its death domain (Ohnishi et al., 2009). In that study, we performed NMR titration experiments using ^{15}N MyD88-TIR and characterized a functionally relevant heteromeric TIR-TIR interaction with another TIR domain derived from Mal, which had been shown to enhance LPS-stimulated TLR4 signaling. Interestingly, no interaction has been observed between TLR4-TIR and MyD88-TIR, while Mal has been shown to directly bind TLR4-TIR. Thus, Mal seems to bridge between TLR4 and MyD88. These results exemplified the binding specificity among TIRs and its importance in forming correct signal initiation complexes (Ohnishi et al., 2009).

Here, we describe the NMR assignments of the TIR domain of MyD88. In IL-1R

and TLR signaling, the heteromeric TIR interactions of MyD88 play a central role in the above-mentioned formation of the TLR signalosome. The resonance assignments form a substantial contribution to the study of the heteromeric TIR-TIR interactions between MyD88 and TIR containing receptors or adaptors.

Methods and Experiments

Sample preparation

The portion of the human MyD88 gene encoding the TIR domain (amino acid residues 148-296) was cloned into the vector pGEX-5X-3 digested with restriction enzymes, ECORI and NotI (GE Healthcare). This vector was transformed into *E. coli* BL-21 (DE3) (Novagen). The transformed bacterial cells were cultured at 37°C until the OD_{600} reached approximately 0.5. The cells were then induced with 1.0 mM IPTG and cultured for 16 hours at 25°C. For ^{15}N-or ^{13}C,^{15}N-double-labeling, rich growth media containing stable isotope-enriched nutrients (Silantes) were used. Harvested bacterial cells were once frozen and thawed before the next lysis step. The cells were resuspended in lysis buffer

(20mM Tris, pH 8.0, 400mM KCl, 1mM EDTA, 10mM 2-mercaptoethanol and protease inhibitors) and disrupted by sonication on ice. The supernatant was loaded onto a glutathione Sepharose 4B FF (GE Healthcare) affinity chromatography column. Lysis buffer at 30x the bed volume of this resin was used for the washing step. GST fused MyD88-TIR protein was eluted with elution buffer (50mM Tris, pH 8.0, and 10mM 2-mercaptoethanol, and 10mM reduced glutathione). The GST-tag was cleaved by digestion with Factor Xa (1% of total protein volume) (Haematologic Technologies Inc.). Subsequently, the TIR domain was purified by gel filtration (Sephacryl S-100 HR 26/60 column, GE Healthcare) and cation-exchange chromatography (Mono-S column, GE Healthcare). The gel filtration running buffer was 20 mM potassium phosphate buffer (pH 6.0) containing 100mM KCl, 0.1 mM EDTA and 10 mM DTT. Using this purification protocol, ^{15}N-labeled or ^{13}C, ^{15}N-doubly-labeled TIR domain of MyD88 wild-type proteins was prepared. The yields of these proteins were approximately 1.0 mg from 1 L of rich growth media containing stable isotope-enriched nutrients. For preparing samples where selected amino acids

were not labeled, proteins were over-expressed by the same method described above with a slight modification. The bacterial cells were first cultured in LB media and collected 30 min before IPTG induction by centrifugation. The cells were resuspended and cultured in ^{15}N-enriched M9 media containing unlabelled Gly, Ser and Cys, or Arg alone to a final composition of 100 mg/L for protein expression (The details of this protocol will be published elsewhere by Dr. H. Hiroaki). To improve the sample solubility and stability, the sample buffer was replaced by 20 mM potassium phosphate buffer (pH 6.0) containing 0.1 mM EDTA, 10 mM DTT, 50 mM deuterated L-arginine, and 50 mM deuterated L-glutamic acid. The final concentrations of the protein samples for typical NMR experiments were about 0.3 mM (Golovanov et al., 2004).

NMR spectroscopy

All NMR spectra were recorded at 25°C on a Bruker DRX500 or Avance800 spectrometer equipped with a cryogenic probe. For assignment of backbone ^{1}H, ^{13}C, and ^{15}N resonances, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH and 3D

^{1}H-^{15}N NOESY-HSQC spectra were recorded. For side chain resonance assignment, 2D constant-time ^{1}H-^{13}C HSQC, ^{1}H-^{13}C NOESY-HSQC, HCCH-TOCSY, CC(CO)NNH and HCC(CO)NNH were recorded. All NMR data were processed using NMRPipe (Delaglio et al., 1995), and analyzed using Sparky (Goddard and Kneller, 1999).

Assignments and data deposition

Employment of 50 mM L-arginine and L-glutamic acid in the sample buffer significantly improved the solubility of the sample by approximately three fold, resulting in significantly enhanced NMR signal intensities. Following a standard sequential assignment procedure, 88.6% of the $^{1}H^{N}$, ^{15}N resonances of backbone amide groups (124 out of the 140 non-Pro residues) were assigned (Fig. 1). In addition, 91.3% of Hα (136 out of 149 residues), 93.3% (139 out of 149 residues) of $^{13}C^{\alpha}$, and 91.3% (136 out of 149 residues) of $^{13}C^{\beta}$ resonances were assigned. The sequential correlations of the backbone resonances of 195–200, 202, and 203 were missing. Those residues are involved in the BB-loop region in the

structure of MyD88-TIR. Relatively lower {^{1}H}-^{15}N hetero NOE values of amide groups at the neighboring residues suggested that the region did not adopt a fixed conformation, but had some flexibility (Ohnishi et al., 2009). This might have caused NMR signal broadening at the BB-loop residues. Besides those above-mentioned residues, Thr-185, Asp-186, Tyr-187, and Arg-188 in the turn region were also missing, which could also be due to NMR signal broadening caused by local motions. Nevertheless, substantial numbers of side chain ^{1}H and ^{13}C atoms of those residues have been assigned on the basis of ^{13}C edited NOESY and HCCH-TOCSY spectra. The assigned chemical shifts of the TIR domain of MyD88 have been deposited in the BMRB database under accession number 11078. The coordinates of the TIR domain of human MyD88 have been deposited at the Protein Data Bank under accession code 2z5v.

Acknowledgments

This work was supported in part by the Research and Development Program for New Bio-industry Initiatives (2005-2009) of the Bio-oriented Technology Research Advancement Institution (BRAIN), Japan to NK. This work was also

supported in part by Grants-in-Aid for Scientific Research and the National Project on Protein Structural and Functional Analyses from the Ministry of Education, Science, and Culture of Japan to HO and HT.

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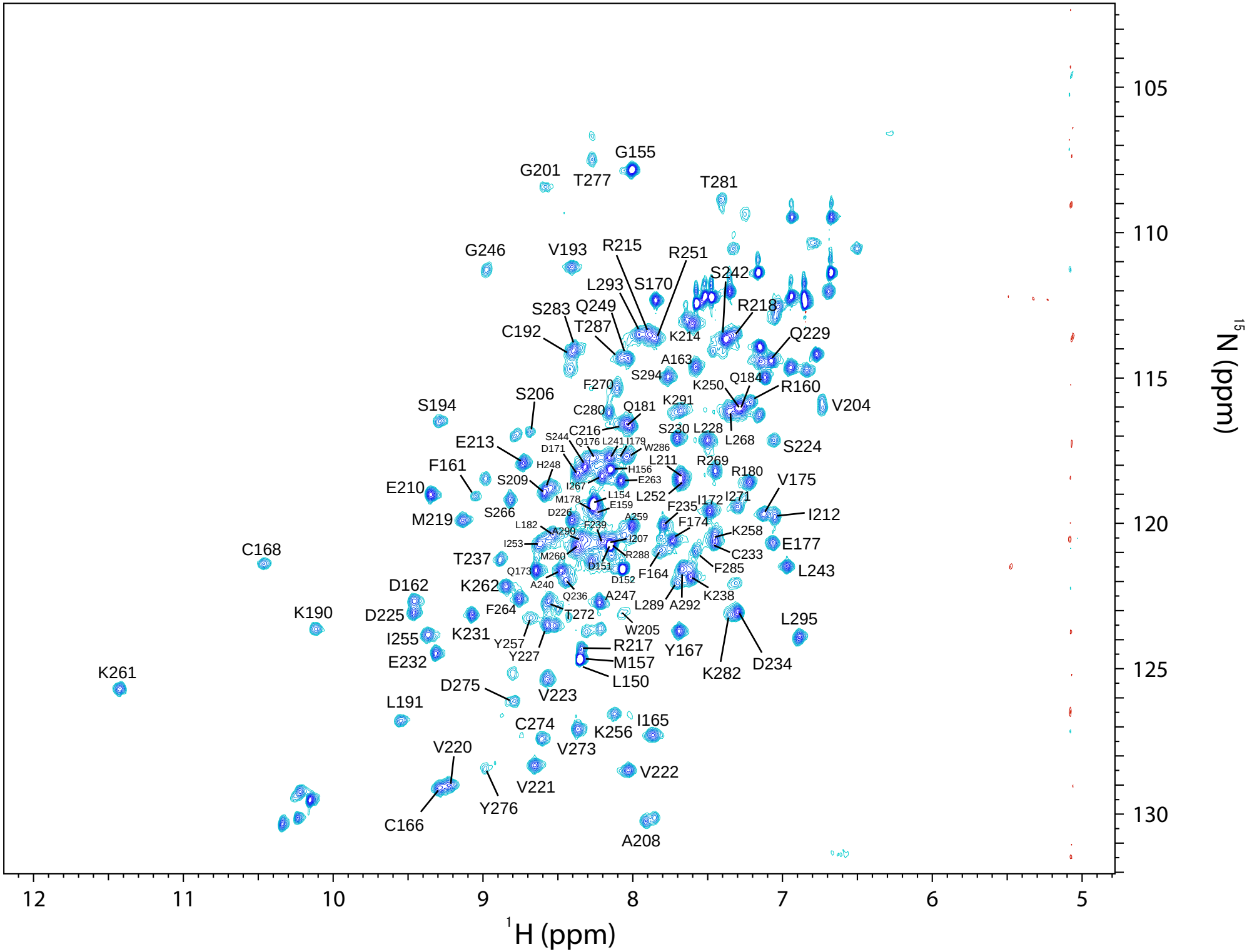
Figure Legend

Fig. 1 ^{1}H, ^{15}N HSQC spectrum of uniformly ^{15}N-labeled TIR domain of human MyD88 in 20 mM potassium phosphate buffer (pH 6.0) containing 0.1 mM EDTA, 10 mM DTT, 50 mM deuterated L-arginine, and 50 mM deuterated L-glutamic acid recorded at 25°C.


G155
G201
T277
T281
G246
V193
R215
R251
S242
L293
S170
S283
Q249
R218
T287
K214
C192
Q229
A163
S294
Q184
F270
K250
R160
S206
S194
K291
V204
C280
Q181
C216
S230
L228
S244
Q176
L241
I179
W286
L268
S224
E213
D171
H248
L211
R269
F161
H156
R180
S209
E263
V175
E210
I267
L154
L252
I172
I271
M178
E159
I212
M219
S266
D226
A259
F235
L182
A290
F239
F174
K258
I253
I207
E177
C168
M260
R288
C233
T237
D151
F285
Q173
D152
F164
L243
D162
K262
A240
Q236
A247
K238
K190
D225
F264
T272
L289
A292
L295
W205
I255
K231
Y257
R217
Y167
E232
Y227
M157
K282
D234
K261
L150
D275
V223
L191
C274
I165
K256
V220
V273
V222
V221
Y276
C166
A208
105
110
115
120
125
130
^{15}N (ppm)
12
11
10
9
8
7
6
5
^{1}H (ppm)