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Type III Effector NleH2 from *Escherichia coli* O157:H7 str. Sakai Features an Atypical Protein Kinase Domain

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Supporting Information

ABSTRACT: The crystal structure of a C-terminal domain of enterohemorrhagic *Escherichia coli* type III effector NleH2 has been determined to 2.6 Å resolution. The structure resembles those of protein kinases featuring the catalytic, activation, and glycine-rich loop motifs and ATP-binding site. The position of helix αC and the lack of a conserved arginine within an equivalent HRD motif suggested that the NleH2 kinase domain's active conformation might not require phosphorylation. The activation segment markedly contributed to the dimerization interface of NleH2, which can also accommodate the NleH1−NleH2 heterodimer. The C-terminal PDZ-binding motif of NleH2 provided bases for interaction with host proteins.

Diarrheagenic enteropathogenic and enterohemorrhagic *Escherichia coli* (EPEC and EHEC, respectively) cause pathologies in humans.[1] EPEC and EHEC inject the type III locus of enterocyte effacement (LEE) and non-LEE effectors to alter the host immune system.[1] Several *E. coli* O157:H7 non-LEE-encoded (Nle) virulence proteins target host NF-κB. The Nle proteins H, denoted NleH1 and NleH2, are among them, sharing 84% identical sequences and having sequences 30 and 27% identical, respectively, to that of *Shigella flexneri* protein kinase effector OspG (Figure S1A of the Supporting Information).[2−4] OspG modulates NF-κB through interaction with host ubiquitin (Ub)-conjugating enzymes that target phospho-IκBα.[2] NleH proteins were shown to autophosphorylate *in vitro*;[5] the molecular details of their activity and their substrates remain uncharacterized. EPEC NleH1 was reported to interact with Bax inhibitor-1 protein, dampening apoptosis during infection,[6] while EHEC NleH1 was reported to interact with ribosomal protein S3 (RPS3), reducing its nuclear abundance.[2] While the mutation of the conserved catalytic K159 or K169 (NleH1 or -2, respectively) is detrimental for NleH inhibitory effects on NF-κB, neither RPS3 nor IKKβ was a substrate of the NleH kinase activity. The CRKL (v-Crk sarcoma virus CT10 oncogene-like protein) was identified as an IKKβ interaction partner and NleH1 kinase substrate, suggesting that the interaction of CRKL with NleH1 facilitates the recruitment of NleH1 to an IKKβ−RPS3−NF-κB complex.[3] NleH1 and -2 have similar roles in the repression of NF-κB through blocking the degradation of IκBα,[7] and NleH1 but not NleH2 binds RPS3.[3] EHEC NleH1 and -2 also interact with each other *in vitro* and *in vivo*.[3] Murine pathogen *Citrobacter rodendium* contains only one NleH homologue analogous to NleH1 in EHEC and EPEC, suggesting that NleH2 may be specific to human pathogens.

We determined the crystal structure of a C-terminal kinase domain (residues 139−303), denoted NleH2$^{139-303}$ (Supporting Information), with four copies arranged in two dimers within the asymmetric unit (buried surface area of ∼3000 Å^2) (Figure 1A,B and Figure S1B of the Supporting Information). In solution, NleH2$^{139-303}$ was present in monomeric and dimeric forms, suggesting that dimerization is concentration-dependent (Figure S2 of the Supporting Information).

PDBeFold[8] and VAST[9] established the structural similarity of NleH2$^{139-303}$ and the mitogen-activated protein kinases interacting kinase 1 [Protein Data Bank (PDB) entry 2HW6[10]], the human Chk1 kinase (PDB entry 2C3K[11]), and a kinase domain of the human Haspin (PDB entry 2WB8[12]). However, NleH2$^{139-303}$ contained only the minimal kinase core (Figure S1D of the Supporting Information), prompting us to conduct a detailed comparative analysis.

The NleH2$^{139-303}$ structure is composed of the N-lobe (residues 139−217) and the C-lobe (residues 218−303) joined by a hinge (residues 213−226) (Figure 1A). The side chains of K169 and E183 are ∼2.5 Å apart, implying that helix αC,[12] i.e., NleH2$^{139-303}$, is in the active state. The lobes create a cleft harboring the potential ATP-, Mg^{2+}-, and substrate-binding sites (Figure 1A). The ATP-binding site encompassed the hinge and extends from the area sandwiched among strands β1, β2, β7, and β8 to a catalytic loop (C-loop; residues 244−254). Residues 150−155 feature a glycine-rich loop (GR-loop) essential for ATP binding. The NleH2 247-FVD-249 sequence corresponds to a typical HRD protein kinase motif. The lack of a conserved Arg in this sequence suggested that NleH2 does not undergo phosphorylation for activation.[13] Adjacent T251

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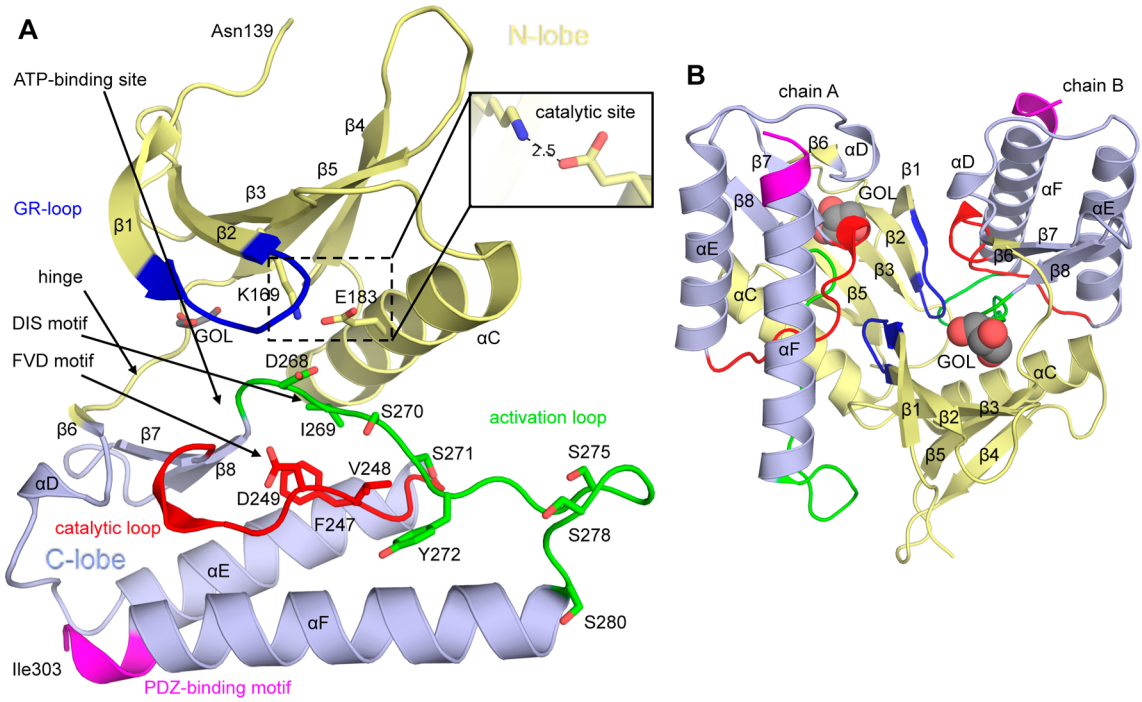

Figure 1. Crystal structure of NleH2$^{139-303}$. (A) Protein kinase fold of NleH2$^{139-303}$. Potential phosphorylation sites (green sticks). A glycerol molecule (GOL) mimics binding of adenosine portion of ATP. (B) NleH2$^{139-303}$ dimer.

substitutes a conserved Lys that usually coordinates the ATP γ-phosphate;[14] the amide nitrogen atoms within the GR-loops of the NleH2$^{139-303}$ dimer may order the γ-phosphate. The NleH2 activation loop (A-loop) is structured, a typical feature of active-state kinases, and its 268-DIS-270 sequence corresponds to a usual DFG Mg^{2+}-binding motif. The NleH2 268-DISSY-271 peptide is, however, not in a three-turn configuration seen in active-state kinases.[13] Further analysis of the kinase structural features such as a regulatory spine[15,16] also pointed to the NleH2$^{139-303}$ conformation corresponding to the active state (Figure S3A of the Supporting Information).[13,16] The A-loop lacked a valid APG motif.[17] The NleH2$^{139-303}$ kinase substrate-binding site is likely located between strand β1 of the N-lobe and the C-loop of the C-lobe extending to an area between the GR-loops within the dimer. The site neighbors the PSD-95/Disc Large/ZO-1 (PDZ)-binding motif (Figure 1A).

The PDZ domains bind class I–III PDZ-binding motifs.[18,19] The class I motif is conserved in NleH (Figure S1A of the Supporting Information) but is not required for NleH2-specific inhibition of the NF-κB luciferase activity.[3] NleH1 interacts via its PDZ motif with the PDZ2 domain of Na^+/H^+ exchanger regulatory factor 2 (NHERF2).[20] The PDZ domains usually bind their motifs in an extended conformation (Figure S3B of the Supporting Information),[21] implying the PDZ-binding motif of NleH2$^{139-303}$ may require a conformational switch to bind NHERF2. The PDZ1–PDZ2 construct of NHERF2 binds chemokine receptor CXCR2;[22] a similar scenario has not been ruled out for NleH.

The NleH2$^{139-303}$ secondary structure elements that are involved in dimerization diverge in sequence from those of NleH1 (Figures S1C and S3C of the Supporting Information). The I277/R267 and N201/G191 substitutions in NleH1 are tolerated in the modeled kinase domain NleH1–NleH2 heterodimer, but not in the NleH1 homodimer (Figure S3D of the Supporting Information), which is in line with NleH1 interacting with NleH2 but not with itself.[3] The formation of NleH2 or NleH1–NleH2 dimers may be alternative strategies for NF-κB disruption, yet understanding the functional applications of NleH oligomerization will require structural characterization of full-length NleH.

During preparation of the manuscript, the crystal structure of the OspG effector in complex with host E2 ubiquitin-conjugating enzyme (E2) charged with Ub became available.[23] It showed structural similarity with NleH2$^{139-303}$ and highlighted the role of helix αF of OspG in Ub binding[24] (Figures S1A and S3E,F of the Supporting Information). Analysis of the OspG–E2–Ub structure suggested that E2 and Ub may cause conformational changes that produce an active form of OspG, increasing its activity.[23] Thus, the NleH (homo/hetero)dimers and/or specific NleH–host complexes may be crucial for adopting an active kinase conformation.

Overall, our analysis revealed atypical kinase features of NleH and proposed models for NleH and NleH–host interactions. OspG and NleH are similar minimal protein kinases with local differences in structure and sequence that account for their specific and diverse functions in the host.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, Table S1, and Figures S1–S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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