Solution Structure and Backbone Dynamics of an Endopeptidase HycI from Escherichia coli

IMPLICATIONS FOR MECHANISM OF THE [NiFe] HYDROGENASE MATURATION

Received for publication, September 29, 2006, and in revised form, November 20, 2006
Published, JBC Papers in Press, December 6, 2006
DOI 10.1074/jbc.M609263200

Fan Yang‡§, Wei Hu‡§, Huimin Xu‡§, Congmin Li†§‡, Bin Xia‡§, and Changwen Jin‡§‡

From the ‡Beijing Nuclear Magnetic Resonance Center, ‡College of Chemistry and Molecular Engineering, and ‡§College of Life Sciences, Peking University, Beijing 100871, China

[NiFe] hydrogenases are metalloenzymes involved in many biological processes concerning the metabolism of hydrogen. The maturation of the large subunit of these hydrogenases requires the cleavage of a peptide at the C terminus by an endopeptidase before the final formation of the [NiFe] metallocenter. HycI is an endopeptidase of the M52 family and responsible for the C-terminal cleavage of the large subunit of hydrogenase 3 in Escherichia coli. Although extensive studies were performed, the molecular mechanism of recognition and cleavage of hydrogenase 3 remains elusive. Herein, we report the solution structure of E. coli HycI determined by high resolution nuclear magnetic resonance spectroscopy. This is the first solution structure of the apo form of endopeptidase of the M52 family reported thus far. The overall structure is similar to the crystal structure of holo-HybD in the same family. However, significant diversity was observed between the two structures. Especially, HycI shows an open conformation at the putative nickel-binding site, whereas HybD adopts a closed conformation. In addition, we performed backbone dynamic studies to probe the motional properties of the apo form of HycI. Furthermore, the metal ion titration experiments provide insightful information on the substrate recognition and cleavage processes. Taken together, our current structural, biochemical, and dynamic studies extend the knowledge of the M52 family proteins and provide novel insights into the biological function of HycI.

Hydrogenases are generally metal-containing enzymes and play a central role in the hydrogen metabolism of many microorganisms. They are involved in many biological processes in which hydrogen molecules are produced and consumed. Based on their metal contents at the active site, metallohydrogenases are classified into two main types, the [Fe] and [NiFe] hydrogenases (1). Escherichia coli has four different [NiFe] hydrogenases, designated as hydrogenases 1–4. Hydrogenases 1–3 are responsible for anaerobic H2 oxidation for energy conserving or involved in H2 production from formate, whereas the function of hydrogenase 4 remains elusive (2–4). Generally, they are heterodimers consisting of small and large subunits. When initially synthesized in cells, the large subunit is in the precursor form lacking the metallocenter and the [NiFe] center is subsequently formed by a complex maturation process. For E. coli hydrogenase 3, seven proteins encoded by genes hypABCDE, hypF, and hycI are involved in the regulation and maturation of the large subunit (HycE) (5, 6). Among them, three proteins responsible for the nickel incorporation play key roles in this process. The HypB protein exhibits GTPase activity and is responsible for nickel delivery (7), the HypC protein acts as a chaperone that interacts with the pre-HycE to form an open conformation for the metal insertion (8), and finally HycI, an endopeptidase, cleaves the C terminus of the pre-HycE. Subsequently, the formation of the metallocenter associated with a conformational change completes the maturation of HycE (6, 9–10).

Previous in vivo and in vitro experiments demonstrated that nickel ion (Ni2+) is essential for the recognition and cleavage of the large subunit by the endopeptidase (11, 12). Furthermore, the C terminus of the pre-large subunit may have interaction with other parts of the protein to form an open conformation amenable to nickel and endopeptidase recognition before it is cleaved (13–15). The crystal structure of the endopeptidase of hydrogenase 2, HybD, bound with Cd2+ was reported (16). Based on the Cd2+-binding site in the crystal structure and mutagenesis experiments, it was established that the highly conserved residues Glu-16, Asp-62, and His-93 in HybD are three key residues involved in the metal binding and play central roles in the cleavage of the C terminus of the large subunit of [NiFe] hydrogenase 2.

Homologous to HybD, HycI belongs to the M52 peptidase family, which is an atypical protease family due to lack of the characteristic motifs of serine, cysteine, zinc, and acid proteases (6). HycI shares only 20% sequence identity with HybD, whereas the putative metal-binding site is highly conserved. To extend our knowledge into the molecular mechanism of hydrogenase maturation, the structure and dynamic features of HycI are essential. Furthermore, the reason why the nickel ion acts as an activator while other bivalent metal ions act as inhibitors remains to be unveiled (11). To address these issues, we have determined the solution structure of apoHycI by high resolu-
tion nuclear magnetic resonance (NMR) spectroscopy, the first solution structure of the apo form of peptidase in the M52 family reported thus far. In addition, we performed the backbone dynamic studies and metal ion titration experiments. The current studies provide novel insights in understanding the molecular mechanisms of the recognition, cleavage, and maturation of the large subunit of [NiFe] hydrogenases.

EXPERIMENTAL PROCEDURES

Cloning and Protein Expression—The E. coli hycI gene was cloned into pET21a (+) expression vector and expressed in E. coli BL21 (DE3) strain (Novagen). The cell culture was grown in 1 liter of Luria Bertani medium, centrifuged, and resuspended in 250 ml of M9 minimal medium at 35 °C with ampicillin and 15NH4Cl in the presence or absence of 13C6 glucose for the preparations of 13C/15N-labeled or 15N-labeled samples, respectively (17). The HycI protein was purified by anion exchange chromatography (Mono Q) and subsequently gel filtration (Superdex-75) using an ÄKTA FPLC system (Amer sham Biosciences). The purity was determined to be >95% as judged by SDS-PAGE. NMR samples were prepared with 1 mM HycI dissolved in 90% H2O/10%D2O buffer containing 50 mM sodium phosphate and 50 mM NaCl (pH 7.0).

NMR Spectroscopy—The NMR experiments were carried out at 25 °C on Bruker Avance 600- and 800-MHz spectrometers equipped with four RF channels and triple resonance probes with pulsed field gradients. The chemical shifts were referenced to internal 2,2-dimethyl-2-silapentanesulfonic acid. Two-dimensional 15N- and 13C-edited heteronuclear single quantum coherence (HSQC)3 and three-dimensional HNCA, HNCO, HNCA/CB, HBHA(CO)NH, CBCA(CO)NH, (H)CCH-COSY, and (H)CCH-TOCSY experiments were performed to obtain the chemical shift assignments of backbone and side chain atoms (18–23). The three-dimensional 15N- and 13C-edited NOESY-HSQC spectra (mixing time 100 ms) were collected to confirm the chemical shift assignments and generate distance restraints for structure calculations (24). All NMR spectra were processed using NMRPipe (25) and analyzed using NMRView (26).

Structure Calculations—The structure calculations were performed using the program package CYANA (27) and refined using AMBER (28). Distance restraints were derived from inter-proton nuclear Overhauser effect (NOE). Dihedral angles (ϕ, ψ) were determined from backbone chemical shifts using TALOS (29). The initial structures were calculated with the CANDID module of the CYANA program (27, 30). The 20 lowest energy structures were selected as models for SANE to extend the NOE assignments (31). Two hundred structures were calculated by CYANA, and the 100 lowest energy structures were used as initial structures and refined using AMBER. Finally, the 20 lowest energy structures were selected to represent the HycI protein. The final structures were calculated using the program packages MOLMOL (32) and PROCHECK_NMR (33).

Structure and Dynamics of E. coli HycI

Titration Experiments of Bivalent Metal Ion—The Ni2+ and Cd2+ titration experiments were performed and monitored by a series of two-dimensional 15N-edited HSQC experiments. The protein was dissolved in 20 mM Tris-HCl buffer, pH 7.0. The metal ions were dissolved in the same buffer. During the titration experiments, the molar ratios of metal ion to protein ranged from 0.1 to 2.0.

Backbone 15N Relaxation Measurements—The backbone 15N relaxation parameters of the longitudinal relaxation rates (R1), transverse relaxation rates (R2), and steady-state heteronuclear [1H]-15N NOE values of HycI were measured using freshly prepared sample (34). The experiments were performed on a Bruker Avance 800-MHz NMR spectrometer at 25 °C. Spectral widths of 11160.7 Hz for 1H and 2676.1 Hz for 15N were used. For the R1 and R2 measurements, 1024 (1H) and 100 (15N) complex data points were collected with 16 transients/ increment and a recycle delay of 2.7 s. The delays used for the R1 experiments were 10 (×2), 100, 300, 500, 800, 1100, 1500, 2000, 2500, and 3190 ms, and those used for the R2 experiments were 8 (×2), 16, 32, 56, 80, 104, 128, and 144 ms. The relaxation rate constants were obtained by fitting the peak intensities to a single exponential function using the nonlinear least squares method as described (35). The [1H]-15N NOE experiments were performed in the presence and absence of a 3-s proton saturation period prior to the 15N excitation pulse and using recycle delays of 2 and 5 s, respectively (36). 32 transients were used for each experiment.

Accession Number—The solution structure of E. coli HycI has been deposited in the Protein Data Bank under the PDB entry 2B1L.

RESULTS

Solution Structure of E. coli HycI—By using the three-dimensional triple resonance NMR spectra, nearly all the chemical shift assignments for backbone atoms were obtained except residues Met-1 and Leu-94. More than 90% of the chemical shift assignments for side chain atoms were obtained with the exception of residues Trp-34, Phe-85, His-90, Met-92, Phe-110, Phe-120, Tyr-121, Tyr-122, Tyr-136, Trp-142, and Phe-148. The two-dimensional 15N-edited HSQC spectrum showing the 1H and 15N resonance assignments of E. coli HycI is shown in Fig. 1.

The HycI structure was calculated using the inter-proton NOE-derived distance restraints in combination with the dihedral angle restraints. The 20 superimposed representative structures, together with the ribbon diagram of the energy-minimized mean structure of HycI, are shown in Fig. 2. The structural statistics are summarized in Table 1.

The overall fold of HycI consists of an α/β sandwich with a twisted five-stranded β-sheet (residues Asp-3–Cys-7 (B1), Trp-34–Asp-38 (B2), Arg-57–Ala-63 (B3), Ile-73–Ile-76 (B4), and Glu-107–Ile-113 (B5)) flanked by five α-helices (residues Ala-18–Ala-28 (α1), Ile-47–Leu-53 (α2), Pro-78–Met-84 (α3), Asn-95–Asp-99 (α4), and Gln-126–Arg-138 (α5)) on two sides. In addition, there are three 310-helices (residues Asp-15–Gly-17 (310–1), Pro-43–Asp-46 (310–2), and Met-86–Thr-88 (310–3) distributed on the surface and proximate to the putative nickel-binding site.

3 The abbreviations used are: HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser effect.
Structure and Dynamics of E. coli HycI

Structural Comparison—Fig. 3A shows the structural comparison of HycI (energy-minimized mean structure) with the crystal structure of E. coli HybD (PDB entry 1CFZ). The two proteins show similar overall packing in the core structural regions. However, significant diversities are observed between them. In particular, HycI is in the apo form, whereas HybD is in the holo form with one Cd$^{2+}$ ion bound/molecule, and the geometry of the three key residues constituting the putative active site is significantly different between the two structures. Based on the sequence alignment and mutagenesis experiments, it was proposed that Asp-16, Asp-62, and His-90 in HycI are the nickel binding residues corresponding to Glu-16, Asp-62, and His-93 in HybD (16). As shown in Fig. 3, B and C, residues Asp-62 and His-90 locate in a loop Thr-89-Leu-94, which is far away from the other two residues (Asp-16 and Asp-62) in HycI. That is unlike the local structure observed in the holo-HybD. Very limited NOE data were obtained for His-90 and nearby residues. The backbone root mean square deviation in this region is 0.9 ± 0.3 Å, which is much larger than that of the secondary structural elements (0.22 ± 0.06 Å). This fact suggests the conformational flexibility in this region and will be discussed below.

Another notable difference is observed around the C-terminal regions. In HycI, the C terminus points to the putative nickel-binding site and does not form regular secondary structures, whereas it forms a short secondary structures in HybD and points away from the protein core structure. In the crystal structure of HybD, a short β-strand from Ile-157 to Pro-158 in the C terminus is formed that is anti-parallel to another short β-strand from Tyr-30 to Ile-31 in the N terminus and is followed by a 3$_{10}$-helix from Arg-159 to Asp-161. It was proposed that the C terminus of HybD involves crystal contacts that extend away from the core structure (16). However, our solution structure of HycI indicates that the C terminus is close to the structural core based on the NOE data. In the three-dimensional $^{15}$N- and $^{13}$C-edited NOESY-HSQC spectra, sufficient NOE data between residues in the segments Ile-73-Asp-77 and Ala-149-Leu-151 were obtained. Different from the crystal structure of HybD, no NOE data could be obtained between the C terminus and the segment Ala-29-Asn-33 in HycI. As discussed by Fritsche et al. (16), the C-terminal conformation of the crystal structure of HybD might be affected by the neighboring molecules and might not be a representation of its conformation in solution. However, this difference may be also due to the metal binding to HybD and the sequence differences between the two enzymes.

Metal Ion Binding—Extensive investigations into the mechanisms of maturation of HycE have been carried out, and it was shown that nickel ion plays a key role in the substrate recognition of endopeptidases (10, 12). We performed the titration experiments using Ni$^{2+}$ and Cd$^{2+}$ monitored by two-dimensional $^{15}$N-edited HSQC spectra. To keep the samples in identical conditions, both the HycI protein and metal ions were dissolved in the 20 mM Tris-HCl buffer at pH 7.0. The initial concentration of protein was 1 mM. During the titration experiments, the metal ion was gradually added into the protein solution with the metal/protein molar ratio ranging from 0.1 to 2.0.

During the titration of Ni$^{2+}$, cross-peaks for several residues in the HSQC spectra showed reduced intensities and even disappeared with the increasing concentration of Ni$^{2+}$, whereas almost no changes in chemical shift were observed for the rest of the residues. As shown in Fig. 4, the missing residues are mostly located in six regions, Val-8 to Asn-10, Asp-16 to Gly-19, Gly-40 to Glu-44, Val-61 to Asp-65, Asp-77 to Leu-97, and the C terminus. Intriguingly, except for the C terminus, the missing residues in the other regions are highly conserved in the sequence alignment among endopeptidases of the M52 family (16). In addition, we repeated the titration experiments at the lower temperature of 10°C. Notably, some peaks that were missing in the HSQC spectra at higher temperature (25°C) reappeared at the low temperature (data not shown). This fact indicates that the missing residues during titration experiments are mostly due to the intermediate conformational exchanges induced by nickel binding, which signifi-

![FIGURE 1. Two-dimensional $^{15}$N-edited HSQC spectrum of the uniformly $^{15}$N-labeled E. coli HycI. The sample was dissolved in a buffer containing 50 mM sodium phosphate and 50 mM NaCl at pH 7.0. The spectrum was collected on a Bruker Avance 800-MHz spectrometer at 25°C. The assignments are annotated by the resonance peaks with the one-letter amino acid code and the sequence number.](Image)
cantly broaden the line width of the cross-peaks and render the signal undetectable.

To take into account the possible paramagnetic effect caused by Ni$^{2+}$ binding, we also performed the titration experiments using diamagnetic Cd$^{2+}$ ion as a control. The results of Cd$^{2+}$ titration experiments were similar to that of Ni$^{2+}$. In addition, besides residues distributed around the six regions that were missing in the Ni$^{2+}$ titration experiments, residues Leu-111, Ile-113, Gln-114, and Met-124 were also missing in the Cd$^{2+}$ titration experiments, suggesting more residues were affected by Cd$^{2+}$ binding than that of Ni$^{2+}$ (Fig. 4).

After the titration experiments, the solution comprising Cd$^{2+}$-HycI complex was loaded onto a gel filtration column equilibrated with the same Tris-HCl buffer used in the titration experiments. The complex was eluted at the same elution volume as the apoHycI, which corresponds to the molecular weight of the monomer. We also examined the elution by the$^{15}$N-edited HSQC spectrum, which was identical to that of the Cd$^{2+}$-HycI complex before gel filtration. This fact excludes the possibility of the aggregation of HycI upon metal binding, which might lead to the peak broadening and missing in the HSQC spectrum.

To test the relative binding affinity of HycI for Ni$^{2+}$ and Cd$^{2+}$, a competition assay was performed. After the protein was saturated with excess Ni$^{2+}$ (5 mM), 2 mM Cd$^{2+}$ was added into the sample. The two-dimensional$^{15}$N-edited HSQC showed an identical spectrum to that of the Cd$^{2+}$-HycI complex, indicative of the substitution of Ni$^{2+}$ by Cd$^{2+}$. In contrast, the HSQC spectrum was unchanged after adding Ni$^{2+}$ into the solution comprising protein saturated with Cd$^{2+}$ (data not shown). These results indicate that Ni$^{2+}$ has lower binding affinity with HycI than that of Cd$^{2+}$, which is likely required for HycI to dissociate from HycE after the C-terminal processing.

**Relaxation Parameters**—To characterize the motional properties and obtain deeper insights into the molecular mechanisms of substrate recognition and cleavage, we determined the$^{15}$N backbone relaxation parameters of HycI, including the longitudinal relaxation rates $R_1$, transverse relaxation rates $R_2$, and heteronuclear Overhauser effect (H$^\text{H}$) $^{15}$N NOE values. In the analysis of the relaxation data, 120 of 156 residues were used. The unanalyzed residues included ten proline residues that have no amide protons, two residues that were unassigned, and...
twenty-four residues that were either overlapped or too weak to be accurately analyzed. The experimentally determined $R_1$, $R_2$, and $\{^1H\} - ^{15}N$ NOE values versus the amino acid sequence are shown in Fig. 5A.

Overall, the entire protein adopts a rigid structure as reflected by the relatively high $\{^1H\} - ^{15}N$ NOE values (>0.75) for most residues in the secondary structures. Residues in the regions from Ser-41 to Ala-43, Asp-79 to Thr-88, Leu-101 to Glu-107, Ile-117 to Met-124, Tyr-136 to Glu-140, and the C terminus show relatively low NOE values (<0.75), indicative of motional flexibility on the picosecond to nanosecond time scales. Notably, residues in the segments Ser-41-Ala-43 and Asp-79-Thr-88 show higher than average $R_2$ values in addition to low NOE val-

FIGURE 3. Structural comparison. A, an overlay of the ribbon diagrams of E. coli HycI (orange red) and E. coli HycD (light yellow). Regions around the putative nickel-binding site are highlighted by a circle and shown in detail in panels B and C. B and C, the local conformations of HycI (B) and HycD (C) describing the putative nickel-binding site. The side chains of the putative nickel binding residues in HycI (Asp-16, Asp-62, and His-90) and those in HycD (Glu-16, Asp-62, and His-93) are labeled and shown in blue. The figures were generated using MOLMOL (32).

FIGURE 4. Metal ion titration. The residues disappeared in the two-dimensional $^{15}N$-edited HSQC spectra of E. coli HycI during titration experiments with the ratios of metal/protein of 0.1 (A) and (D), 0.5 (B) and (E), 1.0 (C) and (F). In the Ni$^{2+}$ titration experiments, the missing residues are mapped onto the HycI structure and shown in yellow in panels A–C. In the Cd$^{2+}$ titration experiments, the missing residues are mapped onto the HycI structure and shown in orange in panels D–F. The first and the last residues of each disappeared segment in Ni$^{2+}$ titration experiments are labeled in panel C. The extra disappeared residues (L111, I113, Q114, and M124) in Cd$^{2+}$ titration experiments are labeled in panel F. The figures were generated using MOLMOL (32).
ues, suggesting fast internal motions on the picosecond to nanosecond time scales and conformational exchanges on the microsecond to millisecond time scales.

Rotational Diffusion Anisotropy—The precise determination of motional anisotropy is crucial to the analysis of NMR relaxation data, particularly for the characterization of conformational exchanges (37). The ratio of the principle components of the inertia tensor of HycI calculated from the solution structure is (1:0.95:0.73), suggesting motional anisotropy. The motional anisotropy can be described by the rotational diffusion tensor. The rotational diffusion tensor was determined following the common procedures by excluding residues with conformational exchanges or internal motions (38). A total of seventy residues were used to define the rotational diffusion tensor. The diffusion tensor for HycI was best described by the axially symmetric model, giving the overall correlation time \( \tau_w = 8.69 \pm 0.04 \) ns and the anisotropy of diffusion tensor \( D_\parallel/D_\perp = 1.11 \pm 0.03 \), which suggests the monomeric state of HycI.

Internal Dynamics—Model-free analysis was performed to extract the dynamic parameters from the experimentally determined relaxation data (39, 40), and the axially symmetric diffusion model was used during the analysis. The calculations were carried out using the experimental data, the uncertainties, and the energy-minimized mean structure as input. Five models with increasing complexity (M1, \( S_2^2 \); M2, \( S_2^2 \), \( \tau_e \); M3, \( S_2^2 \), \( R_{ex} \); M4, \( S_2^2 \), \( \tau_e \), \( R_{ex} \); M5, \( S_2^2 \), \( S_2^2 \), \( \tau_e \), \( R_{ex} \)) were used iteratively to reproduce the experimental data until the confidence reached within 95% (41). The confidence level was estimated using 500 Monte Carlo simulations per run in combination with \( \chi^2 \) and F statistic analysis. The amide bond length was fixed at 1.02 Å, and the \( ^{15}N \) chemical shift anisotropy value of \(-175 \) ppm was used during the calculations. The optimized internal mobility parameters of the generalized order parameter \( S_2^2 \), the fast internal motions on the picosecond to nanosecond time scales \( \tau_e \), and the conformational exchanges \( R_{ex} \) on the microsecond to millisecond time scales are shown in Fig. 5B.

Seventy-eight residues, mainly in the secondary structural elements, could be described by model M1, with an average \( S_2^2 = 0.92 \pm 0.03 \). Nineteen residues mostly near the loop regions were assigned to model M2, with an average \( S_2^2 = 0.86 \pm 0.03 \) and internal motions on the picosecond to nanosecond time scales. Eight residues were assigned to model M3, with an average \( S_2^2 = 0.97 \pm 0.05 \) and conformational exchanges \( R_{ex} \) on the microsecond to millisecond time scales. Twelve residues mainly in the segments Gly-40-Asn-45 and Asp-79-Thr-88 were assigned to model M4 with an average \( S_2^2 = 0.88 \pm 0.04 \), whereas three residues in the C terminus and turns were assigned to model M5, with an average \( S_2^2 = 0.58 \pm 0.03 \).

For a comprehensive overview, the extracted parameters are mapped onto the HycI structure and shown in Fig. 6. Overall, the protein adopts a fairly rigid structure as reflected by the averaged generalized order parameter \( S_2^2 = 0.89 \). However, residues in the segments Gly-40-Asn-45 and Asp-79-Thr-88 were mainly described by M4 with relatively small \( S_2^2 \), showing both significant internal motions on picosecond to nanosecond time scales and conformational exchanges on microsecond to millisecond time scales. The conserved residues Asp-16 and Asp-62 (His-90 is unanalyzed due to signal overlap) are involved in Ni\(^{2+}\) binding and those around the three residues exhibit notable conformational exchanges, indicating that the residues in these regions sample multiple conformational states for Ni\(^{2+}\) insertion.

DISCUSSION

The Structural and Dynamic Basis of Substrate Recognition—We report here the first solution structure, especially the first apo form structure, of the M52 family peptidase. It was previously reported that the precursor of HycE contained nickel when it was directly purified, whereas HycI was in the apo form (10). This result indicated that nickel is inserted into the precursor before its binding with HycI. Therefore, our solution structure of apoHycI provides insightful information in understanding the conformational properties of HycI prior to its recognition of HycE. In the apo form, HycI shows an open binding pocket around the putative nickel-binding site. Moreover, the backbone dynamics is well correlated with the structure and
Structure and Dynamics of E. coli HycI

The Conformational Switch upon Nickel Binding—Our solution structure of the apoHycI is a representation of the conformation prior to the binding with its substrate. In contrast, the crystal structure of HybD bound with a Cd²⁺ might reveal the structure of the endopeptidase during substrate recognition, the first step of the cleavage reaction. Based on the structures of the apoHycI and holo-HybD, we can expect a notable conformational change in HycI upon Ni²⁺ binding (Fig. 3). In the apoHycI, the residue His-90 locates far away from the other two critical conserved residues Asp-16 and Asp-62 and shows an open binding pocket. Upon Ni²⁺ binding, His-90 moves close to Asp-16 and Asp-62 and switches into the closed conformation at the binding site, as observed in the crystal structure of HybD. However, the cross-peaks for residues around the binding site of HycI were missing as shown by the titration experiments (Fig. 3C). It is well understood that the biological function of a protein is strongly dependent on its structure and dynamics. Especially, the protein dynamics plays a critical role in biological processes such as protein-ligand binding, protein-protein interactions, and enzyme catalysis (42–44). The structural and dynamic characterizations of HycI strongly suggest that the open conformation and the dynamic fluctuations of the HycI nickel-binding site are required for the recognition of Ni²⁺ and interaction with HycE.

Implication for the Mechanism of Hydrogenase Maturation—It was postulated that nickel serves as a recognition motif by its interaction with residues His-90 (or His-90 and Asp-16) in HycI (45). In the Ni²⁺ titration experiments, nearly all the cross-peaks for residues around the segment Asp-77-Leu-97 in the HSQC spectrum were missing. In addition, the dynamic data of the apo form of HycI show that residues in the segment Asp-79-Thr-88 are involved in both conformational exchanges on the microsecond to millisecond and fast internal motions on picosecond to nanosecond time scales. These observations strongly suggest that the segment Asp-79-Thr-88 is likely the initial binding site with Ni²⁺-HycE, which may further induce the formation of HycI-HycE complex before the cleavage. In addition, residues in segment Gly-40-Asn-45 show both conformational exchanges and fast internal motions, suggesting its possible role involving the C-terminal cleavage of the pre-HycE.

Concluding Remarks—The NMR structure reveals an open binding pocket around the putative nickel-binding site of HycI. In addition, the backbone dynamics characterizes the motional properties at multi-time scales, which is well correlated with the structure and biological function of HycI. Furthermore, the metal ion titration experiments provide novel insights into the mechanism of substrate recognition and cleavage. The work described here extends the structural and dynamic characterizations of the M52 family, which together with the previous structural and biochemical studies, have established stereochemical insights into the molecular mechanisms of recognition and cleavage of hydrogenases.

Acknowledgments—All NMR experiments were carried out at the Beijing Nuclear Magnetic Resonance Center (BNMRC), Peking University. We thank Xinxin Zhang (BNMRC) and Dr. Xianrong Guo (BNMRC) for kind help during the structure calculations and analysis of dynamic data.
REFERENCES

1. Vignais, P. M., Billoud, B., and Meyer, J. (2001) FEMS Microbiol. Rev. 25, 455–501
2. Ballantine, S. P., and Boxer, D. H. (1985) J. Bacteriol. 163, 454–459
3. Sawers, R. G., Ballantine, S. P., and Boxer, D. H. (1985) J. Bacteriol. 164, 1324–1331
4. Andrews, S. C., Berks, B. C., McClay, J., Ambler, A., Quail, M. A., Golby, P., and Guest, J. R. (1997) Microbiology (Read.) 143, 3633–3647
5. Lutz, S., Jacobi, A., Schsenlog, V., Böhm, R., Sawers, G., and Böck, A. (1991) Mol. Microbiol. 5, 123–135
6. Rossmann, R., Maier, T., Lottspeich, F., and Böck, A. (1993) Eur. J. Biochem. 220, 377–384
7. Menon, A., and Robson, R. L. (1994) J. Bacteriol. 176, 291–295
8. Theodoratou, E., Paschos, A., Magalon, A., Fritsche, E., Huber, R., and Böck, A. (2000) Eur. J. Biochem. 267, 1995–1999
9. Massanetz, C., Fernandez, V. M., and Friedrich, B. (1997) Eur. J. Biochem. 245, 441–448
10. Rossmann, R., Sauter, M., Lottspeich, F., and Böck, A. (1994) Eur. J. Biochem. 213, 110–116
11. Zhang, M., Pradel, N., Mandrand-Berthelot, M.-A., Yu, Z., and Wu, L.-F. (2003) Biochimie (Paris) 85, 575–579
12. Fritsche, E., Paschos, A., Beisel, H.-G., Böck, A., and Huber, R. (1999) J. Mol. Biol. 288, 989–998
13. Marley, J., Lu, M., and Bracken, C. (2001) J. Biomol. NMR 20, 71–75
14. Sattler, M., Schleucher, J., and Griesinger, C. (1999) Prog. Nucl. Magn. Reson. Spectrosc. 34, 93–158
15. Kay, L. E., Ikura, M., and Griesinger, C. (1990) J. Magn. Reson. 87, 620–627
16. Grzesiek, S., and Bax, A. (1993) J. Biomol. NMR 3, 185–204
17. Grzesiek, S., Anglister, J., and Bax, A. (1993) J. Magn. Reson. 101, 114–119
18. Bax, A., Clore, G. M., Driscoll, P. C., Gronenborn, A. M., Ikura, M., and Kay, L. E. (1990) J. Magn. Reson. 87, 201–205
19. Wittekind, M., and Müller, L. (1993) J. Magn. Reson. 101, 201–205
20. Grzesiek, S., and Bax, A. (1993) J. Biomol. NMR 3, 185–204
21. Grzesiek, S., Anglister, J., and Bax, A. (1993) J. Magn. Reson. 101, 114–119
22. Bax, A., Clore, G. M., Driscoll, P. C., Gronenborn, A. M., Ikura, M., and Kay, L. E. (1990) J. Magn. Reson. 87, 620–627
23. Marion, D., Driscoll, P. C., Kay, L. E., Wingfield, P. T., Bax, A., Gronenborn, A. M., and Clore, G. M. (1989) Biochemistry 28, 6150–6156
24. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) J. Biomol. NMR 6, 277–293
25. Johnson, B. A. (2004) Methods Mol. Biol. 278, 313–352
26. Güntert, P., Mumenthaler, C., and Wüthrich, K. (1997) J. Mol. Biol. 273, 283–298
27. Pearlman, D. A., Case, D. A., Caldwell, J. W., Ross, W. R., Cheatham, T. E., III, DeBolt, S., Ferguson, D., Seibel, G., and Kollman, P. (1995) Comp. Phys. Commun. 91, 1–41
28. Cornilescu, G., Delaglio, F., and Bax, A. (1999) J. Biomol. NMR 13, 289–302
29. Herrmann, T., Guntert, P., and Wuthrich, K. (2002) J. Mol. Biol. 319, 209–227
30. Duggan, B. M., Legge, G. B., Dyson, H. J., and Wright, P. E. (2001) J. Biomol. NMR 19, 321–329
31. Koradi, R., Billeter, M., and Wuthrich, K. (1996) J. Mol. Graphics 14, 29–32
32. Laskowski, R. A., Rullmann, J. A., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996) J. Biomol. NMR 8, 477–486
33. Fushman, D., Driscoll, P. C., Kay, L. E., Wingfield, P. T., Bax, A., Gronenborn, A. M., and Guest, J. R. (1997) Biochemistry 36, 3633–3647
34. Fritsche, E., Paschos, A., Beisel, H.-G., Böck, A., and Huber, R. (1999) J. Mol. Biol. 288, 989–998
35. Marley, J., Lu, M., and Bracken, C. (2001) J. Biomol. NMR 20, 71–75
36. Sattler, M., Schleucher, J., and Griesinger, C. (1999) Prog. Nucl. Magn. Reson. Spectrosc. 34, 93–158
37. Kay, L. E., Ikura, M., Tshudin, R., and Bax, A. (1990) J. Magn. Reson. 89, 496–514