



High-pressure NMR analysis on *Escherichia coli* IscU

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Abstract IscU, the iron-sulfur (Fe-S) cluster scaffold protein, is an essential protein for biogenesis of Fe-S clusters. Previous studies showed that IscU manifests a metamorphic structural feature; at least two structural states, namely the structured state (S-state) and the disordered state (D-state), interconverting in a physiological condition, was observed. Moreover, subsequent studies demonstrated that the metamorphic flexibility of IscU is important for its Fe-S cluster assembly activity as well as for an efficient interaction with various partner proteins. Although solution nuclear magnetic resonance (NMR) spectroscopy has been a useful tool to investigate this protein, the detailed molecular mechanism that sustains the structural heterogeneity of IscU is still unclear. To tackle this issue, we applied a high-pressure NMR (HP-NMR) technique to the IscU variant, IscU(I8K), which shows an increased population of the S-state. We found that the equilibrium between the S- and D-state was significantly perturbed by pressure application, and the specific regions of IscU exhibited more sensitivity to pressure than the other regions. Our results provide novel insights to appreciate the dynamic behaviors of IscU and the related versatile functionality.

Keywords IscU, iron-sulfur cluster biogenesis, NMR spectroscopy, high-pressure NMR, metamorphic protein

Introduction

Fe-S cluster is an essential cofactor that mediates various important physiological processes such as electron transport, iron/sulfur delivery, and structural/functional regulation of proteins.¹ Due to the intrinsic toxicity of free iron and sulfide ions, Fe-S cluster biogenesis is tightly regulated by a complex protein network.² It is currently known that *Escherichia coli* employs at least two Fe-S cluster biogenesis mechanisms, among which the ISC (iron-sulfur cluster) system plays a central role as a house-keeping Fe-S cluster biogenesis mechanism.¹

E. coli ISC system is mediated by several highly conserved proteins and their interactions, and the key player among these is IscU, the Fe-S cluster scaffold protein, on which an Fe-S cluster is assembled, and from which the assembled cluster is transferred to other apo-proteins.^{3,4} These versatile roles of IscU are at least partially attributed to its structural diversity. Previous studies showed that IscU has at least two distinct conformations, the S-state (structured) and the D-state (disordered), which interconvert in a physiological condition.⁵ Two structural states exhibit differential preference for the partner proteins. For example, IscS, the cysteine desulfurase that provides sulfurs to IscU for Fe-S cluster biogenesis, prefers to interacting with the D-state of IscU, whereas HscB, the co-chaperone-like protein which guides cluster-bound IscU in the Fe-S cluster transfer pathway, shows stronger interaction with the S-state.¹

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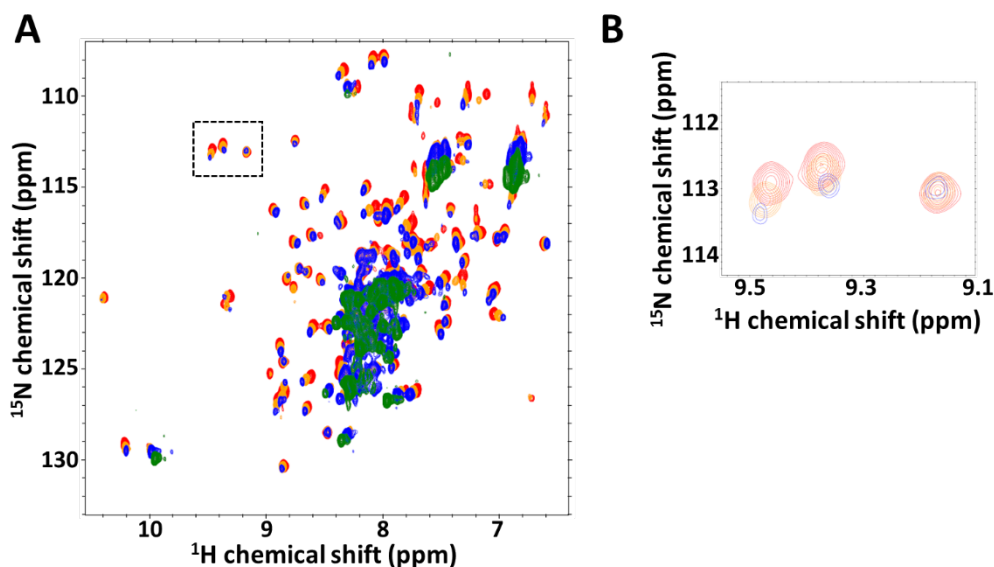


Figure 1. The ^1H - ^{15}N HSQC NMR spectra of $[\text{U-}^{15}\text{N}]$ -IscU(I8K) obtained at various pressure. (A) Four spectra are overlaid for comparison: red, ambient pressure; orange, 500 bar; blue, 1000 bar; green, 2500 bar. Notably, the signals corresponding to the S-state diminished with pressure increase, and the pressure of 2500 bar broadened out most of the S-state signals. The D-state signals exhibited much less sensitivity to pressure. (B) The zoomed-in view of the squared region in the panel A. The signal at around 9.1 ppm (^1H) was marginally perturbed by pressure application, while the other two signals were significantly perturbed by pressure.

These prior studies suggest that the structural flexibility of IscU is an important factor for efficient modulation of the Fe-S cluster biogenesis mechanism. However, it is still elusive to appreciate how IscU maintains its metamorphic feature in a physiological condition. In this work, we employed the HP-NMR technique to investigate the pressure-induced structural changes of IscU. Because pressure application can induce mild denaturation on proteins, HP-NMR is a powerful method to elucidate structural dynamics of proteins in a residue-level.⁶⁻⁸ We applied HP-NMR to the IscU variant whose Ile8 residue was replaced with Lys (I8K); this variant exhibited increased population of S-state, thus being suitable for investigating the effects of pressure on the well-folded state of IscU.

Experimental procedures

The uniformly ^{15}N -labeled ($[\text{U-}^{15}\text{N}]$) protein sample of IscU(I8K) was prepared with the protocol published

previously.^{9,10} The final NMR sample was prepared to 500 μM in a buffer consisting of 20 mM Tris·HCl, 0.5 mM ethylenediaminetetraacetic acid, 5 mM dithiothreitol, and 100 mM NaCl at pH 8. The NMR experiments were conducted with an 800 MHz NMR spectrometer (Bruker) equipped with a cryogenic HCN probe. Pressure was applied during NMR data acquisition with a commercial pressure device (Daedalus Innovations LLC, PA). The temperature was maintained at 298 K. The 2D ^1H - ^{15}N heteronuclear single-quantum coherence (HSQC) spectra of the sample were first collected at ambient pressure, and subsequently with a pressure of 500, 1000, and 2500 bar. After the experiment at 2500 bar, the sample was de-pressurized, and the spectrum was again collected. There was no noticeable spectral difference between two spectra before and after pressurization, confirming the reversibility of IscU(I8K). The chemical shift perturbation (CSP) plot was drawn according to the following equation for each residue of IscU(I8K): $\Delta\delta_{\text{NH}} = [(\Delta\delta_{\text{N}}/5)^2 + (\Delta\delta_{\text{H}})^2]^{1/2}$. Topspin 3 (Bruker) was used for data collection and

processing, and POKY software package was used for data analysis.¹¹

Results and discussion

We monitored the pressure-induced perturbation on the S-state of IscU(I8K) by following the corresponding NMR signals obtained at various pressure conditions. We first collected 2D ^1H - ^{15}N HSQC spectra of $[\text{U-}^{15}\text{N}]$ -IscU(I8K) at ambient pressure, and then obtained the same spectra at 500, 1000, and 2500 bar (Fig. 1A). As expected, we observed that the equilibrium between the S- and D-state is significantly perturbed by pressure; the application of 2500 bar abolished most of the S-state signals (Fig. 1A). This result indicates that the S-state conformation of IscU is more sensitive than the D-

state conformation. This observation is consistent with the previous proposal that the D-state represents a dynamic and heterogeneous structural state.^{2,12} However, it was also notable that the spectrum at 2500 bar exhibited much fewer number of signals than the expected number from its amino-acid sequence. This implies that IscU(I8K) may stabilize a molten globule-like state at a pressurized condition, and it also suggests that the D-state of IscU is, at least partially, different from the fully unfolded state.

Subsequently, we examined the behavior of individual signals corresponding to the S-state during pressure titration. We first noted that some signals showed only minimal movements, while others were significantly perturbed by pressure application (Fig. 1B). To compare the extent of signal perturbations, the CSP plot was drawn for the 2D ^1H - ^{15}N HSQC spectrum of $[\text{U-}^{15}\text{N}]$ -IscU(I8K) at 1000 bar against the same

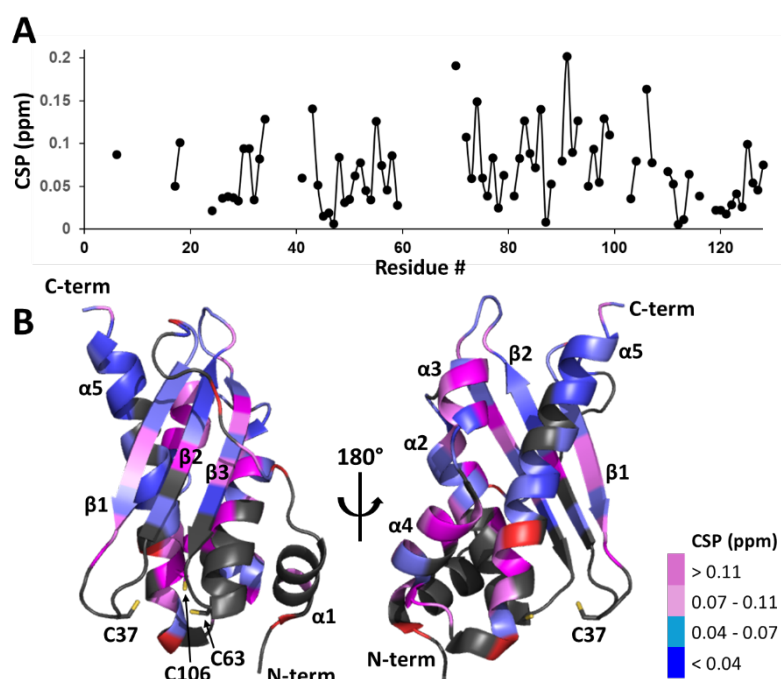


Figure 2. The chemical shift perturbation (CSP) analysis of IscU(I8K) upon pressure application. (A) The CSP plot (the chemical shift of each residue from IscU(I8K) at 1000 bar vs. the chemical shift of the same residue at an ambient condition) was drawn (see ‘Experimental procedures’ for details). (B) The CSP calculation results were marked on the structural model of *E. coli* IscU (PDB 3lv1)¹³ with the following color code: magenta, $\text{CSP} > 0.11$ ppm; pink, $0.07 < \text{CSP} < 0.11$; cyan, $0.04 < \text{CSP} < 0.07$; blue, $\text{CSP} < 0.04$. The red color marked the residues whose signal could be observed at ambient pressure, but not at 1000 bar. The residues, whose signals could not be monitored at any condition due to spectral overlap or missing assignment, were colored black. The highly conserved cysteine residues, C37, C63, and C106, are shown as sticks with yellow tips marking the position of sulfur.

spectrum obtained at ambient condition (Fig. 2A). This result indicated that the regions 24-29, 45-47, 49-50, 112-113, and 116-124 were much less affected by pressurization. These regions include parts of $\beta 1$, $\beta 2$, and $\alpha 5$ (Fig. 2B; the upper side of the structural model near the C-terminal tail¹³), all of which are clustered at the opposite side of the cysteine-rich loops. The cysteine-rich loops, containing highly conserved C37, C63, and C106, constitute the essential active site of coordinating metal ions and mediating an Fe-S cluster assembly, and it was proposed that they remain dynamic in its apo-state to facilitate its physiological activity.¹⁰ We made a consistent observation that the cysteine-rich loops are sensitive to pressure, implying their intrinsic structural mobility. On the other hand, it appears that the $\alpha 5$ region close to the C-terminal tail and its neighboring regions of $\beta 1$ and $\beta 2$ may form a stable core that may exert some resistance against pressure application.

Taken together, the present work demonstrated that a

HP-NMR method is a powerful method to investigate structural dynamics and stability of proteins. Our trial of HP-NMR on IscU(18K) showed that the S-state of IscU is more sensitive to pressure than the D-state. This observation is consistent with the previous reports that the D-state of IscU is highly dynamic, while it still maintains some structural features that is distinctive to a fully unfolded state.^{14,15} In addition, our results provide intriguing evidence that the region around the C-terminal half of $\alpha 5$ may constitute a stable core exhibiting resistance to structural perturbation. This is also consistent with the prior observations that the cysteine-rich loops maintain mobile features for mediating Fe-S cluster biogenesis processes efficiently.¹⁰ More detailed signal analysis of HP-NMR data with additional IscU variants are currently under way, which will contribute to elucidating the heterogeneous structural features of IscU and its relationship with the efficient catalysis of Fe-S cluster biogenesis.

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