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Oxidation-Induced Conformational Change of a Prokaryotic Molecular Chaperone, Hsp33, Monitored by Selective Isotope Labeling

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Abstract : Hsp33, a prokaryotic molecular chaperone, exerts holdase activity in response to oxidative stress. In this study, the stepwise conformational change of Hsp33 upon oxidation was monitored by NMR. In order to overcome its high molecular weight (33 kDa as a monomer and 66 kDa as a dimer), spectra were simplified using a selectively [^{15}N]His-labeled protein. All of the eight histidines were observed in the TROSY spectrum of the reduced Hsp33. Among them, three peaks showed dramatic resonance shifts dependent on the stepwise oxidation, indicating a remarkable conformational change. The results suggest that unfolding of the linker domain is associated with dimerization, but not entire region of the linker domain is unfolded.

Keywords : Hsp33, chaperone, selective isotope labeling, conformational change

INTRODUCTION

Hsp33 is a prokaryotic molecular chaperone protecting cells from severe oxidative stress.[1-2] The expression of Hsp33 is regulated by heat at transcriptional level, but the protein is post-translationally activated by oxidation. Under normal, reducing condition, Hsp33 behaves as a monomer and binds a

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zinc ion via four conserved cysteines at its C-terminal, redox-switch domain. Upon oxidation of the four conserved cysteines, which leads to two intramolecular disulfide bonds, the redox-switch domain becomes unfolded with releasing the zinc ion. Finally, the oxidized Hsp33 forms a dimer with holdase activity that binds folding intermediates as substrates. It has been suggested that the activated Hsp33 can also adopt high-order oligomers which have more potent chaperone activity than the dimeric form.[3] In addition, Ilbert *et al.*[4] have identified an oxidized monomer form of Hsp33, where only two of the four conserved cysteines are oxidized. It is still controversial whether the oxidized monomer form is inactive or just partially active.[4-6] However, it is clear that the smallest active unit of native Hsp33 is an oxidized dimer form. Thus, dimerization seems to be associated with complementing chaperone activity of native Hsp33.

Up to now, several crystal structures of Hsp33 have been solved.[7-11] However, a detailed process of the oxidation-induced activation of Hsp33 is still the subject of much controversy, as the crystal structures contain features that lead to doubts about their physiological relevance. Thus, in the present study, we employed NMR to monitor the oxidation-induced conformational change of Hsp33 in solution. In terms of NMR, the 294-residue protein, Hsp33 is an extremely large molecule, in particular as a dimeric form. Thus, we used a selectively isotope labeled [^{15}N]His-Hsp33, in order to simplify the NMR spectra. Then, we measured NMR spectra of three different forms of Hsp33: reduced monomer, oxidized monomer, and oxidized dimer. Comparison of the NMR spectra provides information regarding the stepwise conformational change of Hsp33 during its activation process.

EXPERIMENTAL

The recombinant *Ec*Hsp33 (Hsp33 from *Escherichia coli*) was prepared in its reduced, zinc-bound form, as described previously.[12] In order to prepare selectively isotope-enriched protein, [^{15}N]His-*Ec*Hsp33, the Hsp33-expressing cells were grown in the M9 minimal medium supplemented with non-labeled amino acids, and prior to induction by IPTG, [^{15}N]histidine was added into the medium at 0.1 mg/ml concentration. Oxidation of 50 μM [^{15}N]His-*Ec*Hsp33 was performed using H_2O_2 as an oxidant at 43°C, according to the method established by Graumann *et al*.[13] Oxidized [^{15}N]His-*Ec*Hsp33 solution was concentrated and applied onto a HiLoad 16/60 SuperdexTM 75 column (43 ml of void volume and 120 ml of total bed volume) pre-equilibrated with a 40 mM HEPES-KOH buffer (pH 7.5), at a 1 ml/min flow rate. Fractions of each eluate were separately pooled, concentrated, and applied onto the same column. This step was repeated several times to completely separate three kinds of oxidized Hsp33: an oxidized oligomer, an oxidized dimer, and a half-oxidized monomer. Apparent molecular weight of each [^{15}N]His-*Ec*Hsp33 species was estimated from elution volume of each eluate, as described previously.[12,14] NMR spectra of approximately 0.2 mM protein were obtained at 313 K on a Bruker Biospin Avance 900 spectrometer equipped with a cryoprobe. Conventional 2D-[^{1}H, ^{15}N]-TROSY spectrum was recorded for each [^{15}N]His-*Ec*Hsp33 species. All NMR spectra were processed using NMRPipe/NMRDraw software and analyzed with NMRView program.

RESULTS AND DISCUSSION

In the gel-filtration analysis, the reduced [^{15}N]His-*Ec*Hsp33 showed an apparent molecular weight (M_{app}) of approximately 38 kDa (data not shown), which is consistent with previous observation[12] and indicative of homogeneous monomeric state in solution. In contrast, oxidized [^{15}N]His-*Ec*Hsp33 solution contained three distinct species with different sizes, each attributable to oxidized oligomer, oxidized dimer, and oxidized monomer, respectively (Figure 1). The first elution (M_{app} > 90 kDa) near the void volume was assignable to oligomeric [^{15}N]His-*Ec*Hsp33. The middle peak (M_{app} ≈ 64 kDa) and the last peak (M_{app} ≈ 42 kDa) could be assigned to dimeric and monomeric form, respectively.

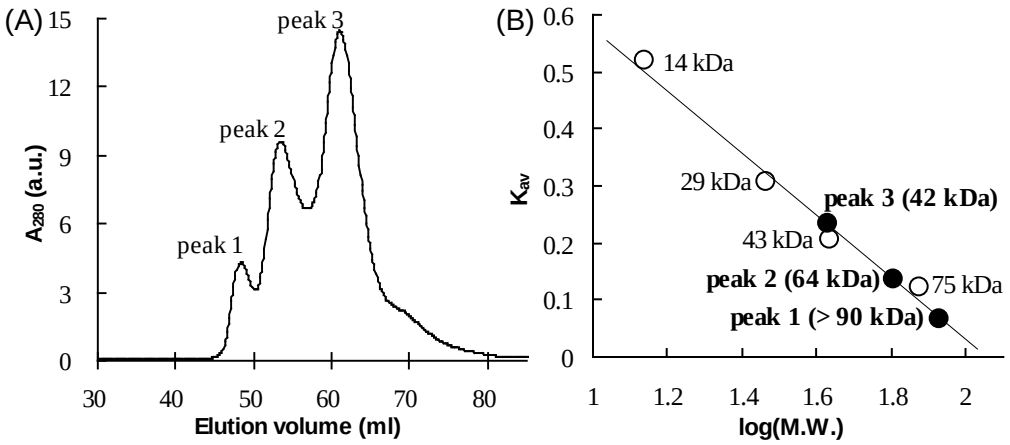

Figure 1. Separation and identification of oxidized [^{15}N]His-*Ec*Hsp33 species. Oxidized [^{15}N]His-*Ec*Hsp33 solution showed three peaks in the elution profile (A) of gel permeation chromatography. Apparent molecular weight was assessed using the elution volume of each eluate (B).

Since the oxidized monomer contains significant portion of unfolding,[4] its hydrodynamic size could be enlarged than expected from the reduced monomer size. NMR spectrum of each oxidized species could be measured independently (Figure 2), because they were not reequilibrated after separation by gel permeation chromatography.

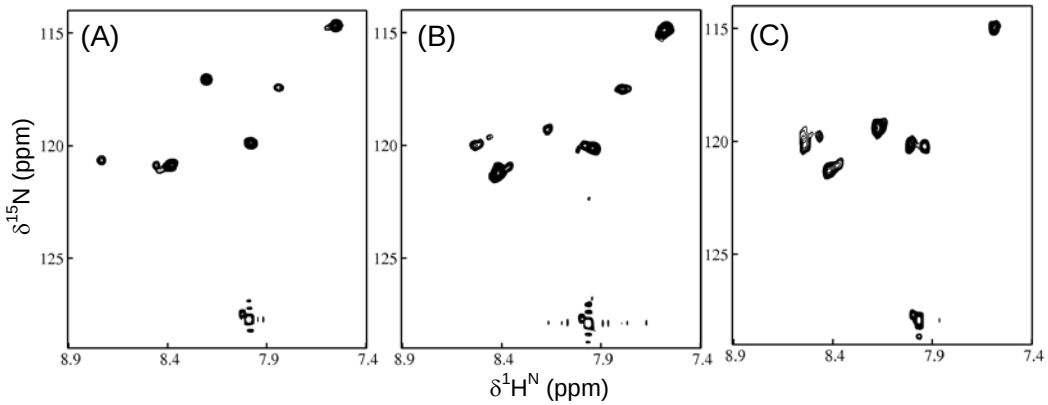

Figure 2. 2D-[^{1}H, ^{15}N]-TROSY spectra of reduced monomer (A), oxidized monomer (B), and oxidized dimer form (C) of the [^{15}N]His-*Ec*Hsp33.

*Ec*Hsp33 has eight histidines (Figure 3A): three in the N-terminal core domain (H6, H10, and H37), two in the middle linker domain (H189 and H216), and three in the C-terminal redox-switch domain (H264, H271, and H294). The 2D-[^{1}H, ^{15}N]-TROSY spectrum of the reduced [^{15}N]His-*Ec*Hsp33 monomer showed eight peaks that exactly equals the number of histidines (Figure 2A). In contrast, oxidized monomer and oxidized dimer form showed nine signals (Figures 2B and 2C), which imply that any one of eight histidines would undergo a slow chemical exchange.

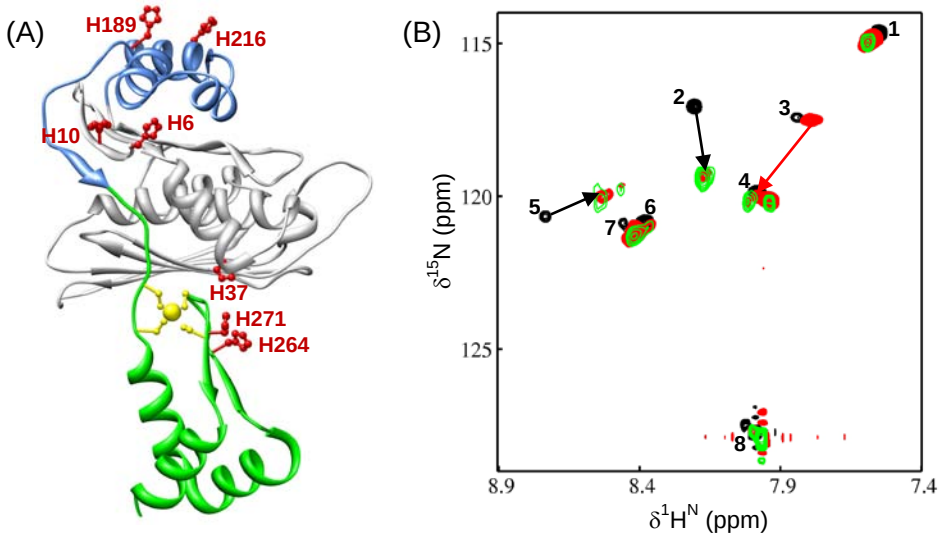

Figure 3. (A) Domain organization and distribution of histidines. A modeled structure of *Ec*Hsp33 at reduced monomer state is presented. The structure was generated by a homology modeling method, using the crystal structure of the *Bacillus subtilis* Hsp33 (PDB code 1VZY)[7] as a template. The N-terminal five and the C-terminal seven residues, which do not match to the 1VZX structure, were not included in the modeled structure. Colors are designated as follows: gray, N-terminal core domain (residues 6 ~ 178); blue, middle linker domain (179 ~ 227); green, C-terminal redox-switch domain (228 ~ 287); yellow, zinc ion and conserved cysteines; red, histidines. (B) Superimposition of 2D-[^{1}H, ^{15}N]-TROSY spectra of [^{15}N]His-*Ec*Hsp33: reduced monomer (black), oxidized monomer (red), and oxidized dimer (green) species, respectively. Each peak of the reduced monomer spectrum is labeled with numbers. Putative assignments of resonance shifts are indicated by arrows for the signals that show relatively large chemical shift perturbation upon oxidation.

As illustrated in Figure 3B, peaks 2 and 5 of the reduced monomer spectrum (black) showed dramatic shifts in the oxidized monomer spectrum (red). It is known that the C-terminal redox-switch domain (green in Figure 3A) totally unfolds upon oxidation. Thus, such a dramatic resonance shift of peaks 2 and 5 is attributable to unfolding process, and the two peaks can be assigned to H264 and H271 residues in the redox-switch domain. In contrast, a noticeable perturbation is not expected for the other histidine, H294, in the redox-switch domain, which is the C-terminal last residue. The peak 8 in Figure 3B can be reasonably assigned to the H294, judging from its position and its extremely high intensity, which are usually observed for the C-terminal residue of proteins.

The peak 3 in Figure 3B was just slightly shifted from the reduced monomer to the oxidized monomer spectrum, but it remarkably shifted in the oxidized dimer spectrum. Ilbert *et al.*[4] have suggested that the linker domain (blue in Figure 3A) is unfolded in the oxidized dimer, but not in the oxidized monomer. Thus, the peak 3 can be assigned to H189 or H216, which are located in the linker domain. Among them, we suggest that the peak 3 originates from H216, rather than H189, since the oxidation-induced unfolding of Hsp33 would be propagated from the C-terminal domain. This means that the region around the H189 is not unfolded in the oxidized, dimeric Hsp33. Likewise, it is inferred that the N-terminal core domain that contains H6, H10 and H37 would not undergo significant conformational change, as no other notable perturbations were observed.

In summary, the oxidation-induced conformational change of Hsp33 was monitored by NMR, using a selectively isotope labeled sample. The results confirm that the conformational change of

Hsp33 upon oxidation occurs in a stepwise manner. The intermediate oxidation, by which only two of the four conserved cysteines are oxidized, results in entire unfolding of the C-terminal redox-switch domain. Then, the linker domain is unfolded upon complement oxidation of the remaining two cysteines. However, the second unfolding does not include the whole region of the linker domain. Thus, it can be conclusively suggested that partial unfolding of the linker domain would be a critical factor for dimerization of Hsp33, as well as for achieving complete activity.

Acknowledgments

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