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Letter to the Editor: Backbone ^{1}H, ^{13}C and ^{15}N resonance assignments for the Mg^{2+}-bound form of the Ca^{2+}-binding photoprotein aequorin

Wakana Ohashi[a], Satoshi Inouye[b], Toshio Yamazaki[a], Yukiko Doi-Katayama[a], Shigeyuki Yokoyama[a] & Hiroshi Hirota[a,*]
[a]*RIKEN Genomic Sciences Center, 1-7-22, Suehiro, Tsurumi, Yokohama, 230-0045, Japan;* [b]*Yokohama Research Center, Chisso Corporation, 5-1 Okawa, Kanazawa, Yokohama, 236-8605, Japan*

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Biological context

The photoprotein aequorin, isolated from the jellyfish *Aequorea aequorea*, emits blue light by an intramolecular reaction upon Ca^{2+} binding (Shimomura et al., 1962). Aequorin consists of apoaequorin (apoprotein) and the peroxide of coelenterazine, which is formed with coelenterazine and molecular oxygen (Head et al., 2000). When mixed with Ca^{2+}, aequorin emits light (λ_{max} = 465 nm), and decomposes into apoaequorin, coelenteramide and CO_2. Apoaequorin is formed by 189 amino acid residues with three EF-hand motifs characteristic of Ca^{2+}-binding sites, and aequorin is a member of the superfamily of EF-hand proteins (Inouye et al., 1985). Recently, the crystal structure of metal-free aequorin was reported, and it revealed that aequorin has four helix-loop-helix EF-hand domains (I–IV) (Head et al., 2000). However, no structural information about the metal-bound form of aequorin is available. Here, we report the backbone resonance assignments of the Mg^{2+}-bound form of aequorin. These assignments will provide information for future NMR-based studies on the metal-induced luminescence mechanism of aequorin.

Material and experiments

The recombinant aequorin used in this study consists of 191 amino acids with the sequence of ANS-residues, instead of the N-terminal V-residue in native aequorin (Inouye et al., 1985, 1989). The ^{13}C, ^{15}N-labeled recombinant apoaequorin was expressed using the piP-HE plasmid in *E. coli* strain BL21 in M9-medium containing ^{13}C-glucose and $^{15}NH_4Cl$. The expressed apoaequorin was converted to aequorin by adding coelenterazine and dithiothreitol, and aequorin was purified as previously described (Inouye et al., 1989, Shimomura and Inouye, 1999). To prepare the ^{15}N-methionine labeled aequorin, the *E. coli* strain B834(DE3) with piP-HE was cultured in LeMaster's medium (LeMaster and Richards, 1985). For NMR analyses, the samples comprised 0.8 mM of uniformly ^{13}C, ^{15}N-labeled aequorin in 10 mM MES (pH 6.6), 3 mM EDTA, 100 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, and 9:1 of H_2O: D_2O. For the ^{15}N-methionine selectively labeled aequorin, the protein concentration was 0.2 mM.

NMR spectra were measured at 293 K on Bruker AVANCE600 and AVANCE800 spectrometers, equipped with pulsed field gradients and triple resonance probes. Resonance assignments were made by analyses of NMR spectra: ^{15}N HSQC, ^{13}C HSQC, HNCA, HN(CO)CA, C(CO)NH, HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO and ^{15}N NOESY-HSQC. To confirm the backbone assignments, ^{15}N-methionine labeled aequorin was used for a ^{15}N HSQC spectrum. All spectra were processed using NMRPipe and analyzed using NMRview and the integrated modules 'Kujira'.

Extent of assignments and data deposition

The triple resonance experimental data were used for the sequence-specific backbone assignments.

*To whom correspondence should be addressed. E-mail: hirota@gsc.riken.jp

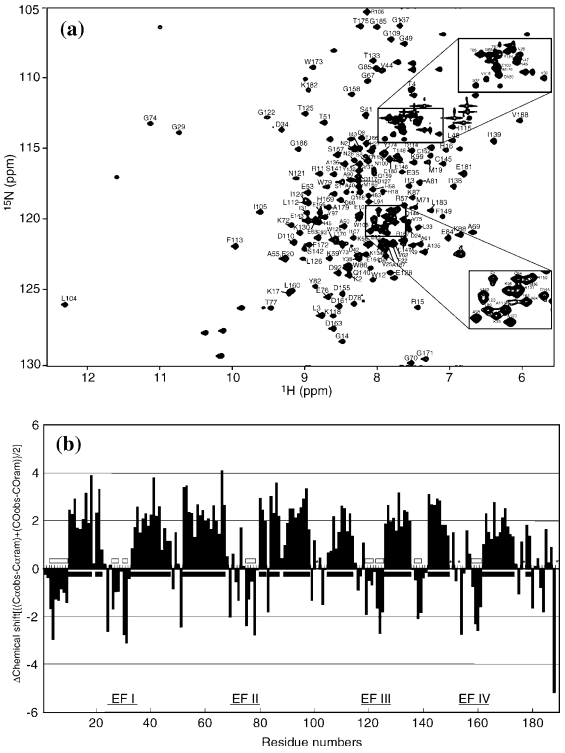

Figure 1. (a) ^{15}N HSQC spectrum of ^{13}C, ^{15}N-labeled Mg^{2+}-bound aequorin with assignments. (b) Averaged secondary shift calculated with Cα and CO chemical shifts. Helical elements and β-strand elements are indicated with filled boxes and open boxes, respectively. Asterisks show uncalculated secondary shift residues. The loop regions of the EF-hands are indicated with a line and the name.

Among the 191 amino acid residues of recombinant aequorin, the amino acid sequence from 2 to 189 in native aequorin corresponds to that from 4 to 191 and the numbering of the amino acid residues follows that of native aequorin sequence in this paper. Backbone resonance assignments of the Mg^{2+}-bound aequorin were obtained for 179 residues from 183 non-proline residues in the native sequence (97.8%) (Figure 1). The unassigned residues were V151, D153, I154 and D177. D177 is located prior to proline. In the crystal structure of the metal-free form of aequorin, V151, D153 and I154 are located at the end of an α-helix and the loop of an EF-hand motif (EF-hand IV). The main reason for the lack of these resonance assignments might be the line-broadening caused by the structural flexibility in this region. The Cβ chemical shift value of M38 was slightly out of the usual range. To confirm the resonance assignment of the methionine, we measured the ^{15}N HSQC spectrum of Mg^{2+}-bound, ^{15}N-methionine labeled aequorin. Five signals were observed in the ^{15}N HSQC spectrum, and the chemical shifts of these signals were identical to those of the methionine residues assigned in this study. From the α(Cα) and O(CO) chemical shifts, the secondary shifts were calculated. In the crystal structure, the regions of P10 to L23 and W79 to A98 formed α-helical structures for EF-hand I and EF-hand II, respectively. The secondary shifts of M19, F22, W79, I83 and K88 were slightly lower than those for a typical α-helical structure, suggesting that α-helical regions were partially disordered. The α-helical structure of the EF-hand III region (I105-D114 and L126-A135) was somewhat short, as compared with the corresponding region in the crystal structure (L104-V116 and L126-A136, respectively). In addition, the secondary shifts suggested that the β-strand region of EF-hand II was expanded slightly. Although there are some local differences, most of the structural elements agree well with the crystal structure of metal-free aequorin (PDB ID, 1EJ3). Thus, we concluded that the global structure of the Mg^{2+}-bound aequorin is similar to that of the metal-free form of aequorin. Further NMR investigations will provide not only structural information about metal-bound aequorin in solution but also valuable insights into the dynamic features of its light emission and regeneration mechanisms. The chemical shifts have been deposited in the BioMagResBank, with the accession number 6418.

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