Solution Structural Studies of GTP: Adenosylcobinamide-Phosphateguanylyl Transferase (CobY) from Methanocaldococcus jannaschii

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Abstract

GTP:adenosylcobinamide-phosphate (AdoCbi-P) guanylyl transferase (CobY) is an enzyme that transfers the GMP moiety of GTP to AdoCbi yielding AdoCbi-GDP in the late steps of the assembly of Ado-cobamides in archaea. The failure of repeated attempts to crystallize ligand-free (apo) CobY prompted us to explore its 3D structure by solution NMR spectroscopy. As reported here, the solution structure has a mixed α/β fold consisting of seven β-strands and five α-helices, which is very similar to a Rossmann fold. Titration of apo-CobY with GTP resulted in large changes in amide proton chemical shifts that indicated major structural perturbations upon complex formation. However, the CobY:GTP complex as followed by 1H-15N HSQC spectra was found to be unstable over time: GTP hydrolyzed and the protein converted slowly to a species with an NMR spectrum similar to that of apo-CobY. The variant CobYG153D, whose GTP complex was studied by X-ray crystallography, yielded NMR spectra similar to those of wild-type CobY in both its apo- state and in complex with GTP. The CobYG153D:GTP complex was also found to be unstable over time.

Introduction

Coenzyme B12 (a.k.a. adenosylcobalamin or AdoCbi) is the largest, non-polymeric molecule with biological activity. AdoCbi belongs to the broadly distributed family of cyclic tetrapyrrole molecules known as ‘The Pigments of Life’, which includes hemes, factor F430, and chlorophylls [1]. The core ring structure of AdoCbi (a.k.a. the corrin ring) contains a cobalt ion chelated by pyrrolic nitrogens. On the upper (beta) face of the ring, a covalent bond links 5’-deoxyadenosine (Ado) and the Co ion. This unique organometallic bond is critical to the function of the coenzyme. The lower (alpha) face of the ring features a nucleotide loop tethered to a
substituent of the ring via a phosphodiester bond. Two features unique to the nucleotide loop are the alpha-N-glycosidic bond between the base and ribosyl moiety, and the diversity in the base [2]. ‘Cobamide’ is the term used to refer to complete B_{12}-like molecules, regardless of their base. The best known cobamide is cobalamin, which contains 5,6-dimethylbenzimidazole as its base.

The assembly of the nucleotide loop evolved differently in bacteria and archaea. In both domains, the pathway starts with the synthesis of AdoCbi-P, which is then converted to AdoCbi-GDP, the so-called activated corrin ring. The difference between the way archaea and bacteria synthesize AdoCbi-GDP lies in the guanylyl transferase that transfers the GMP moiety of GTP to AdoCbi-P. Bacteria use a bi-functional kinase/guanylyl transferase enzyme (CobU, EC 2.7.7.62) [3–5], whilst archaea evolved CobY (E.C. 2.7.7.62), a guanylyl transferase that lacks kinase activity [6]. Crystal structures of CobU in its apo form and in complex with GMP are available (PDB 1C9K [5] and 1CBU [7], respectively). The crystal structure of CobYG153D in complex with GTP is also available (PDB 3RSB) [8], but efforts to crystallize the apo-forms of CobY or CobYG153D were unsuccessful.

Results of biochemical experiments performed during the course of this work revealed that two subunits of apo-CobY bind one GTP molecule with a binding constant of $K_b = 2.0 \times 10^{-5} \text{M}^{-1}$ and a dissociation constant of $K_d = 5.0 \times 10^{-6} \text{M}$, but apo-CobY failed to bind GTP analogues, such as GMP-PNP, GMP-PCP or even GDP [9]. CobY binds GTP first before binding AdoCbi-P 200 [9]. The Ado moiety of the corrinoid is required for binding, but the order of binding is clear. The G153D variant of CobY (CobYG153D) crystallized in the presence of GTP and led to the determination of the 3D structure of the complex by X-ray crystallography at a resolution of 2.8 Å [8]. Repeated failed attempts to crystallize the apo-CobY protein prompted us to explore solution NMR spectroscopy as a means for determining the structure of apo-CobY and its complex with GTP. To aid in answering how CobY binds GTP and is involved in transferring the GMP moiety to AdoCbi-P, we conducted structural studies using nuclear magnetic resonance (NMR) spectroscopy. We report here the solution structure of apo-CobY, which has allowed comparison with the X-ray structure of CobYG153D. We also present NMR studies of CobYG153D and interactions of the proteins with GTP.

Materials and Methods

Protein production and sample preparation

[U-^{15}N]-CobY, [(U-^{13}C,^{15}N)-CobY, and [U-^{13}C,^{15}N]-CobYG153D protein samples containing 196 amino acids (residues 1–196) used NMR studies were produced in minimal medium according to the protocol described previously [10], except that E. coli BL21-CodonPlus (DE3)-RIL (Stratagene) was used for protein production, and cultures were grown in Erlenmeyer flasks. The M.jannaschii cobY gene was expressed from plasmid pCobY14 [9]. Proteins were purified as previously reported [9] with the following modifications. Cell-free extract was applied to a 5 mL HiTrap phenyl (high-sub) FF column (GE Healthcare) equilibrated with tris (hydroxymethyl) aminomethane hydrochloride buffer (50 mM Tris-HCl, pH 8.0 at 4°C) containing 55 g/L (NH_4)_2SO_4. Protein was eluted at a flow rate of 5 mL/min with a linear gradient to 100% Tris-HCl buffer. CobY-containing fractions were concentrated and dialyzed against Tris-HCl buffer. Protein purity was assessed as previously reported [9] and found to be >95% homogeneous (data not shown). Ion exchange chromatography was therefore omitted.

[U-^{13}C,^{15}N]-CobY protein used for structure determination was further dialyzed against 50 mM deuterated Tris buffer (pH 8.0 at 4°C) containing 50 mM NaCl, 5 mM dithiothreitol (DTT) and 10 mM MgCl_2. To prevent bacterial growth, 0.2% NaN_3 was added to all samples and proteins were stored at 4°C.

Abbreviations: Apo-CobY, wild-type, ligand-free CobY; CobYG153D, G153D variant of CobY; BMRB, Biological Magnetic Resonance Data Bank; DTT, dithiothreitol; HSQC, heteronuclear single-quantum coherence; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy; PDB, Protein Data Bank; rmsd, root mean square deviation; $\tau_c$, rotational correlation time; TALOS, torsion angle likelihood obtained from shifts.

Competing Interests: The authors have declared that no competing interests exist.

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NMR Data Collection and Analysis

All NMR spectra were recorded at the National Magnetic Resonance Facility at Madison (NMRFAM) on Varian VNMRS (600 MHz, 800 MHz and 900 MHz) spectrometers equipped with triple-resonance cryogenic probes. The temperature of the sample was regulated at 40°C. Sequence specific backbone resonance assignments were conducted for CobY using a series of 2D and 3D heteronuclear NMR spectra. NMR data were collected for both CobY containing 2.0 mM [U-13C,15N] protein dissolved in NMR buffer with 50 mM Tris, 5 mM DTT, 50 mM NaCl, 10 mM MgCl2, 95% H2O, 5% D2O. Raw NMR data were processed with NMRPipe [11] and analyzed using the programs XEASY [12] and NMRFAM-SPARKY [13]. 2D 1H-15N HSQC and 3D HNCO data sets were used to identify the number of spin systems, and these identifications plus 3D HNCACB and 3D CBCA(CO)NH data sets were used as input to the PINE server [14] to determine sequence specific backbone resonance assignments. In addition, backbone resonance assignments were confirmed on the basis of 3D 15N-edited 1H-1H 3D-NOESY. 2D 1H-13C HSQC, 3D HBHA(CO)NH, 3D HC(CO)NH, 3D C(CO)NH experiments were used to assign the side chain and HB and HA resonances. 3D 15N-edited 1H-1H NOESY (100 ms mixing time), and 3D 13C-edited 1H-1H NOESY (120 ms mixing time) experiments were used to derive the distance constraints to determine the three dimensional structure of protein [15]. Standard pulse sequences were used to record steady state [1H]-15N NOE and 15N relaxation (T1, T2) data [16]. To determine the 15N T1 values, multiple interleaved NMR spectra were recorded with relaxation delays of 10, 100, 200, 400, 600, 800, 1000, 1200, and 1400 ms. To determine 15N T2 values, multiple interleaved NMR spectra were recorded with delays of 10, 30, 50, 70, 90, 110, and 150 ms. Relaxation rates were calculated by least-squares fitting of peak heights versus relaxation delay to one single exponential decay by using NMRFAM-SPARKY. The reported error estimates are standard deviations derived from fitting the data. Steady-state [1H]-15N NOE values were calculated from the ratio of peak heights in a pair of NMR spectra acquired with and without proton saturation. The signal-to-noise ratio in each spectrum was used to estimate the experimental uncertainty.

Structure calculation and analysis

For the structure calculation, 15N resolved 1H-1H 3D NOESY and 13C resolved 1H-1H 3D NOESY spectra were used to derive the intra molecular distance restraints. TALOS+ software [17] was used to derive backbone dihedral angle restraints ϕ and ψ from 1H, 15N, 13CA, 13CB, 13C chemical shifts. CYANA (version 3.0) [18] was used for automated NOESY peaks assignments and structure calculation. NOESY peaks assigned automatically by CYANA were used as a guide to further refine the structure. Programs MOLMOL [19] and PYMOL [20] were used, respectively, to calculate the root mean square deviation (rmsd) and for graphical analysis. The PSVS server [21] was used to check the quality of the structure.

Results and Discussion

Optimization of NMR sample conditions

By optimizing the buffer composition and temperature, we discovered conditions that led to sharp and uniform signals in the 1H-15N HSQC spectrum (Fig 1A) and good triple-resonance and NOESY data, as needed for a successful structure determination. The final conditions were: 2 mM protein in 50 mM TRIS buffer pH 7.0 containing 50 mM NaCl and 10 mM MgCl2. Data were collected at 40°C.
The solution NMR structure of apo-CobY was determined from 3246 distance constraints from NOESY spectra and 220 angle constraints derived from chemical shifts by using the TALOS+ program [17]. Two hundred refined structures were generated, and the best 20 conformers, those with lowest energy that showed the fewest constraint violations with CYANA

![2D 1H-15N NMR Spectra of CobY and its GTP Complex.](A) 1H-15N HSQC spectrum of apo-CobY. Amide peaks are labeled with their residue assignments. (B) Overlay of the 1H-15N HSQC spectrum of apo-CobY (red) with that of the CobY:GTP complex (blue).

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**Structure of apo-CobY**

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were chosen for additional water bath refinement using PONDEROSA-C/S [22] assisted Xplor-NIH [23].

Statistics for the solution structure (Table 1) are indicative of its high quality. The average number of constraints per residue was 17.6, and, of these, an average of 4.2 per residue were long-range constraints. The root mean square deviation (rmsd) for backbone heavy atoms was $< 1.0 \text{ Å}$ overall and $\sim 0.6 \text{ Å}$ for backbone heavy atoms in regular secondary structure. Of the

| Table 1. Statistics Describing the NMR Solution Structure of Wild-type apo-CobY. |
|-------------------------------------------------|-----------------------------|
| Constraints                                      | Description                | Value                |
| Conformationally restricting distance constraints |                               | (number)             |
| Intraresidue $[i = j]$                          |                             | 1049                 |
| Sequential $(i-j) = 1$                          |                             | 903                  |
| Medium Range $[1 < (i-j) \leq 5]$               |                             | 470                  |
| Long Range $(i-j) > 5$                          |                             | 824                  |
| Total                                           |                             | 3246                 |
| Dihedral angle constraints (number)              |                             |                      |
| $\varphi$                                       |                             | 110                  |
| $\psi$                                          |                             | 110                  |
| Constraints per residue (average number)         |                             |                      |
| Total                                           |                             | 17.6                 |
| Long-range                                      |                             | 4.2                  |
| CYANA [18] target function (Å)                   |                             | 6.60±0.43            |
| Average rmsd to the mean coordinates of water refined CNS coordinates (Å) | |                      |
| regular secondary structure elements, backbone heavy |                             | 0.62±0.08 $^a$      |
| regular secondary structure elements, all heavy atoms |                             | 0.94±0.08 $^b$      |
| backbone heavy atoms                             |                             | 0.66±0.07 $^b$      |
| all heavy atoms                                  |                             | 1.05±0.09 $^b$      |
| Validation parameters                           |                             |                      |
| PROCHECK [24] rawscore ($\varphi$ and $\Psi$/all dihedral angles) | | -0.7/-0.12 $^c$    |
| PROCHECK Z-scores ($\varphi$ and $\Psi$/all dihedral angles) | | 0.04/-0.71 $^c$    |
| MOLPROBITY [25] raw score/Z-score                |                             | 34.76/-4.44 $^c$    |
| Ramachandran plot summary: ordered residue ranges (%) |                             |                      |
| most favored regions                             |                             | 91.5                 |
| additionally allowed regions                     |                             | 8.2                  |
| generously allowed regions                       |                             | 0.2                  |
| Average number of distance constraint violations per CYANA conformer | |                      |
| $0.2–0.5 \text{ Å}$                             |                             | 0.0                  |
| $> 0.5 \text{ Å}$                               |                             | 0.0                  |
| Average number of dihedral-angle constraint violations per CYANA conformer | |                      |
|                                               |                             | 0.0                  |

$^a$ Residues: $2–7$ ($\beta_1$), $22–24$, $27–29$, $29–39$($\alpha_1$), $44–49$ ($\beta_2$), $54–64$ ($\alpha_2$), $70–74$ ($\beta_3$), $80–90$ ($\alpha_3$), $95–99$ ($\beta_4$), $101–104$, $107–123$ ($\alpha_4$), $129–135$($\beta_5$), $149–157$($\beta_6$), $166–170$($\beta_7$), $175–177$, $181–195$ ($\alpha_5$).

$^b$ Residues: $2–7$, $21–135$, $148–195$.

$^c$ Residues: $2–7$, $20–25$, $27–102$, $106–139$, $149–152$, $154–160$, $163–176$, $179–194$. 

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backbone torsion angles, 91% were in the most favored and 8% were in additionally allowed regions of the Ramachandran plot. The PROCHECK [24] Z-scores for backbone / all atoms were −0.04 / −0.71.

The structure consists of a mixed α/β-fold (Fig 2). The seven β-strands (A-G) consist of residues β_{A}(D2−M6), β_{B}(N44−T49), β_{C}(I70−D74), β_{D}(P95−S99), β_{E}(A129−M133), β_{F}(P151−V157), and β_{G}(E167−V170). The five α-helices (I-V) consist of residues α_{I}(L29−K39), α_{II}(P54−Y64), α_{III}(Y80−Y90), α_{IV}(K108−K123), and α_{V}(T180−K192). The orientations of the β-strands make up a twisted β-sheet (Fig 2A, 2B, and 2C); six of the seven β-strands are arranged in parallel fashion: β_{C}(↑) β_{B}(↑) β_{A}(↑) β_{D}(↑) β_{E}(↑) β_{C}(↑). In addition, a short and stable β-hairpin is located between residues I22 and L29, and a short anti-parallel β-sheet-like structure is formed by residues D101-N104 and I175-N177. Four α-helices (I, II, IV, and V) are arranged on one side of the β-sheet, whereas one α-helix (IV) is on the other side of the β-sheet. α-Helix I is in contact with β-strands A and B, whereas α-helix II is in contact with β-strands B and C. α-helices I and II also contact one other; α-helix III contacts half of the β-sheet (β-strands C, B, A, D, and F); and α-helix IV contacts the other side of the β-sheet (β-strands A, D, F, E, and G). The loops connecting the secondary structural elements (α_{1}−β_{A}, α_{4}−β_{D}, β_{E}−β_{B}, and β_{C}−α_{5}) are highly flexible and unstructured. Resonance assignments could
not be obtained for residues in some of these loops because of exchange broadening, which led to the disappearance of the amide peaks. The backbone rmsd plotted against the amino acid sequence (Fig 2D) shows that the polypeptide chain is flexible between residues 8–20 and 133–153. The C-terminal helix is also relatively dynamic as determined from heteronuclear NOE values. The coordinates were deposited in the Protein Data Bank (PDB) with accession code 2MZB, and the chemical shifts were deposited in Biological Magnetic Resonance Data Bank (BMRB) with accession code 25482.

Structural homologues of apo-CobY

We used the software programs DALI [26] and ProFunc [27] to search for structural homologues of apo-CobY. The five most similar structures, all determined by X-ray crystallography, contained mononucleotide binding domains with a canonical Rossmann fold (Fig 3). Cytidinyl monophosphate 2-keto-3-deoxy-manno-octonic acid synthetase (CMP:Kdo) from *Escherichia coli* (PDB 1H7F, Z-score 15.8, rmsd 2.6 Å, seq ID 14%) is involved in the synthesis of lipopolysaccharides that are toxic to Gram-negative bacteria [28]. Glucose-1-phosphate cytidylyl
transferase from *Salmonella typhi* (PDB 1WVC, Z-score 15.2, rmsd 3.3 Å, seq ID 18%) catalyzes the transfer of a CMP moiety from CTP to glucose 1-phosphate [29]. Cytidine transferase from *Streptococcus pneumonia* (PDB 2VSI, Z-score 15.6, rmsd 3.0 Å, seq ID 13%) is involved in the synthesis of cytidine-5'-diphosphate (CDP)-ribitol from ribitol 5-phosphate and CTP [30]. 2-C-methyl-D-erythritol 4-phosphate cytidylyl transferase from *Thermotoga maritima* (PDB 1VPA, Z-score 15.3, rmsd 3.1 Å, seq ID 18%) catalyzes the formation of diphosphocytidyl-2-C-methyl-D-erythritol from CTP and 2-C-methyl-D-erythritol 4-phosphate. N-acetylneuraminic cytidylyl transferase from *Neisseria meningitides* (PDB 1EYR, Z-score 15.3, rmsd 3.0 Å, seq ID 17%) catalyzes the reaction of CTP and N-acetylneuraminic to form CMP-N-acetylneuraminic and bisphosphate [31]. A structure-based multiple sequence alignment performed by PROMALS3D [32] revealed several highly-conserved residues (Fig 4): A3, I5, A7, R13, K19, G26, (K/R)27, (D/E)58, T180, D183, and L184. These residues appear to play important roles in nucleotide binding. In particular, residues R13 and K19 directly coordinate the phosphate group of GTP in the X-ray structure of CobYG153D [8].

![Fig 4. Structure-based Multiple Sequence Alignment of Wild-type apo-CobY with the Five Most Structurally Similar Proteins.](https://doi.org/10.1371/journal.pone.0141297.g004)
All these structures share < 20% sequence identity with CobY and additionally contain HTH motifs and/or a β-hairpin structure that facilitates the formation of homodimers (Fig 4).

Dynamics of ligand free CobY

One of the advantages of NMR spectroscopy over X-ray crystallography is its ability to provide information about the dynamic properties of proteins in solution. For CobY we accomplished this by measuring nitrogen spin-lattice ($T_1$) and spin-spin ($T_2$) relaxation times and heteronuclear NOEs ($^{15}$N NOEs) for backbone amide resonances. As shown in Fig 5, we found that the relaxation parameters are fairly similar throughout the polypeptide chain, indicating uniform overall protein dynamics. The only exceptions were inflexible loops connecting regular secondary structure elements, in particular $\alpha_1$-$\beta_A$, $\alpha_4$-$\beta_D$, $\beta_E$-$\beta_F$, and $\beta_G$-$\alpha_5$; these regions yielded weak electron density in the X-ray studies of CobY. The average $T_1$ (~750 ms) and $T_2$ (~70 ms) values are those expected for a monomeric protein of ~23 kDa and are consistent with the 3D NMR solution structure. In addition, the rotational correlation times ($\tau_c$) of the amide protons calculated from $T_1$ and $T_2$ revealed an average $\tau_c = 9.9 \pm 0.7$ ns (Fig 5) consistent with monomeric protein in solution. By contrast, the X-ray structure of the CobYG153D:GTP complex was modeled as a weak dimer [8].

Comparison of the NMR structure of apo-CobY and the X-ray structure of the CobYG153D:GTP complex

The solution structure of apo-CobY and the crystal structure of CobYG153D:GTP complex (PDB 3RSB) [8] exhibit similar 3D folds (structures superimposed in Fig 6A). The elements of regular secondary structure (consisting of 67 amino acid residues) superimposed with an average rmsd = 0.83Å. The X-ray structure has a relatively low resolution (2.8 Å), and electron density was not identified for residues 8–11, 74–81, 126–127, and 192–196. Perhaps the largest structural differences are in $\alpha$-helix-III, which is structured in the NMR solution structure but unstructured in the X-ray structure (Fig 6B). Although isothermal titration calorimetry (ITC) studies suggested one GTP molecule per two units of CobY [9], the X-ray structure of the CobYG153D:GTP complex was modeled as a dimer with one GTP molecule bound to each subunit. The ITC results were obtained with active enzyme that may have been turning over during the experiment. Our NMR experiments with both CobY and CobYG153D are consistent with a 1:1 complex.

Complex formation of CobY with GTP

To probe the effect of added GTP on apo-CobY, we titrated a sample of $^{15}$N-labeled apo-CobY with GTP and followed the chemical shifts in a series of $^1$H-$^{15}$NHSQC spectra. Several amide cross peaks exhibited large perturbations (Fig 6D–6F), indicating that significant conformational changes accompanied the formation of the GTP complex. $^1$H-$^{15}$N HSQC spectra acquired following the addition sub-stoichiometric amounts of GTP (not shown), exhibited two sets of peaks, one corresponding to free CobY and one to the CobY:GTP complex; this indicates a slow off rate for GTP dissociation.

Two factors appear to be responsible for these chemical shift changes: (i) GTP-induced ordering of the binding domain and (ii) electrostatic interactions between the ligand and protein backbone. In the X-ray structure of the CobYG153D:GTP complex, the tri-phosphate group of GTP is in the proximity of the region of the protein (residues 10–18) that appears disordered in the apo-enzyme both in X-ray crystal data, which lacked electron density for these residues, and in the solution spectra, which lacked signals from these residues as attributed to exchange broadening. The overlaid expansions of three regions of the $^{15}$N HSQC spectra of
Fig 5. Dynamics of apo-CobY as Represented by Residue-specific Backbone Amide Spin-lattice ($T_1$) and Spin-spin ($T_2$) Relaxation Times, Heteronuclear NOE ($^{15}$N NOE) Values, and Correlation Times. The flexible regions of the polypeptide chain are circled in red; the vertical red box represents missing resonances from residues that are assumed to be flexible; and the horizontal red box indicates the C-terminal helix, which appears to be more flexible than other secondary features as indicated by the smaller $^{15}$N NOE values. The $\tau_c$ values were calculated from measured $T_1$ and $T_2$ values by using the formula, $\tau_c = 1/4\pi\nu_0 (\sqrt{6} (T_1/T_2) – 7)$. The average $\tau_c$ value (9.9 ± 0.7 ns) indicates that CobY is monomeric in solution under the NMR sample conditions.

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CobY and CobYG153D:GTP complex (Fig 6D–6F) indicate that the amide protons of A7, G8, K55, G79, G153 shift significantly upon GTP binding. The weighted chemical shift perturbations (CSPs) mapped on 3D structure of CobYG153D (Fig 6C) show that some are close to the GTP binding site and others are distant.

Comparison of CobT and CobYG153D

Comparison of the 1H-15N HSQC spectra of [U-15N]-CobY and [U-15N]-CobYG153D (Fig 7A) indicates that the chemical shift differences are small. The sample of 15N-labeled CobYG153D was saturated with GTP, and the resulting spectrum was compared to that of apo-CobYG153D (Fig 7B). The chemical shift perturbations upon GTP binding are very similar to those observed with wild-type CobY (Fig 1B).

The differences in the chemical shifts of CobY and CobYG153D are plotted as a function of residue number in Fig 8A and are mapped onto the 3D structure of the protein in Fig 8B. As

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expected, atoms in residues near residue 153 (the substitution site) exhibit the largest chemical shift differences.

The GTP complexes with wild-type CobY and CobYG153D proved to be unstable in solution. NMR spectra taken over time (not shown) indicated each complex converted to an unknown intermediate state over a period of about 24 hours (most probably the "switch-off" GDP

![Fig 7. Comparison of $^1$H-$^{15}$N HSQC Spectra of CobY and CobYG153D. (A) Spectrum of apo-CobY (red) overlaid with that of apo-CobYG153D (blue). (B) Spectrum of apo-CobYG153D (blue) overlaid with that of the CobYG153D:GTP complex (purple).](doi:10.1371/journal.pone.0141297.g007)

![Fig 8. Representation of Differences in the Chemical Shifts of CobY and CobYG153D. (A) Weighted rmsd of amide proton chemical shift differences ($0.5 [\Delta \delta(1HN)^2 + 0.2 \Delta \delta(15N)^2]]^{1/2}$) plotted as a function of the amino acid residue number. (B) Mapping of the weighted rmsd chemical shift differences onto the 3D NMR structure of CobY. Color code: (red, yellow, green, cyan) spectrum of chemical shift differences from largest to small; (blue) no significant chemical shift difference.](doi:10.1371/journal.pone.0141297.g008)
complex) and then converted over a period of days to species with spectra resembling those of the apo-proteins. Repeated attempts to make a stable GTP-CobY complex by reducing the temperature and saturating with GTP were unsuccessful. The instability of the complexes prevented us from determining their solution structures.

Conclusions

Solution NMR studies of apo-CobY yielded a 3D structure of high quality with a fold is similar to that of the low resolution X-ray structure of the CobYG153D:GTP complex [8]. We found CobY to be monomeric in solution in both its apo- and GTP-bound forms, whereas the X-ray structure of the CobYG153D:GTP complex was modeled as a homodimer. Other differences may reflect problems in tracing the chain in the X-ray map.

It is known that complexes of GTPases with GTP are conformationally flexible to allow for the conversion of GTP to GDP and transfer of the phosphate group. The proposed two-state mechanism has been extensively studied for small GTPases, such as Ras, RhoA, and Sec4 [34]. The active “switch-on” state has GTP bound, whereas the inactive “switch-off” state has GDP bound. Titration studies of CobY followed by NMR spectroscopy revealed that CobY forms a tight 1:1 complex with GTP. However, the complex was found to degrade over time, which prevented the determination of its solution structure.

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Author Contributions

Conceived and designed the experiments: KKS MO WMW JCE-S. Performed the experiments: KKS MO MT WMW. Analyzed the data: KKS MT. Wrote the paper: KKS MO MT WMW JCE-S JLM.

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