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Supplementary information

Structure of sirohydrochlorine ferrochelatase SirB: The last of the structures of the class II chelatase family

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name	"Ancestral" CbiX ^s (or CfbA)	СЫХ	SirB	СыК	HemH
Structure					
Organization	Homodimer	monomer	monomer	monomer	monomer
Active site	Central dimer interface	N-terminal domain	N-terminal domain	C-terminal domain	C-terminal domain
Physiological function	Co-chelatase (Ni-chelatase)	Co-chelatase	Co-chelatase	Co-chelatase	Fe-chelatase

Supplementary Figure 1. Summary of evolutionarily related class II chelatase family proteins, and the SirB structure determined in this work. The cobalt-sirohydrochlorin chelatase CbiX^S,^{1, 2} which has recently been characterised as nickel chelatase CfbA^{3, 4} involved in the biosynthesis of coenzyme F430 in methanogenic archaea, is regarded as an "ancestral" class II chelatase. SirB^{5, 6} and CbiX⁷ are also cobalt-chelatases catalysing the formation of cobalt-sirohydrochlorin, and are classified as class II chelatases having metal-binding active sites at their N-terminal domains. In contrast, HemH⁸⁻¹¹ and CbiK^{2, 12, 13} have their metal-binding active sites at the C-terminal domains. These two chelatases, HemH and CbiK, however, play different roles: HemH, for the biosynthesis of heme, using iron and protoporphyrin IX; CbiK, for the biosynthesis of cobalt-sirohydrochlorin. The protein structures were depicted using PyMOL (Schrödinger, LLC) and rainbow-colored from the N-terminus (blue) to the C-terminus (red).



Supplementary Figure 2. Model structures of Co^{2+} -bound SirB with sirohydrochlorin (SHC), protoporphyrin IX, and uroporphyrin I. These docking models were compared with the crystal structures of CbiX^S with cobalt-SHC (PDB ID: 2XWQ),² CbiK with cobalt-SHC (PDB ID: 2XWP),² HemH with copper-containing *N*-methyl mesoporphyrin (PDB ID: 1C9E),¹⁴ and human ferrochelatase with heme (PDB ID: 3HCN).¹⁴ Tetrapyrroles were shown as stick models. In human ferrochelatase, iron and sulphur of the [2Fe-2S] cluster are shown as yellow and brown spheres, respectively.

Bacillus_subtilis_SirB Bacillus_megaterium_SirB Fictibacillus_solisalsi_SirB Anoxybacillus_vitaminiphilus_SirB Salimicrobium_halophilum_SirB Bacillus_megaterium_CbiX Fictibacillus_solisalsi_CbiX Anoxybacillus_vitaminiphilus_CbiX Synechocystis_sp_CbiX Trichodesmium_erythraeum_CbiX Streptomyces_coelicolor_CbiX Magnetococcus_marinus_CbiX	MHKKLTKEV 	1 10 MKQAILYVCH MKQAILYVCH MKQAILYVCH MKQAILYVCH MQAILYICH MGGULYVSH MKAILWVCH MKAILWVCH MKAILWVCH TTPPALLIAGH TTPPALLIAGH MSDIL	20 SRVKKAQOEAAAF SRVKEGADQAVAF SRIKAGTEKAREF SRIARACQEAVAF SRIERARQEAVSO SRIEETROEVERV SRDPEGNDQIREF SRDPEGNDQIREF SRDPEGNQIEVERF TRDDGRGEFFLDF TRDDGRGEFFLDF TRDDGRGEFFLDF SRHAKGNREVEKF	30 40 LEGCKAHISVP.VQBISTIELQE LERCKKNLDVP.IQEVGPLELAS AEQCMRHIDVP.IQEVGPLELAS VERCKKKIDVP.IQEVGPLELAS VVDANSGVNTP.LQEMGFLELAS VKDANSGVNAT.LHEUCYLEIAS ISTMKHDWDASILVETCFLEPER IDEIRTKVDSELLVETCFLEPER IAQLEKKIESSPIVETCYLEPER IAQLEKKIESSPIVETCYLEPE VAQYQALDHSR.PVIPCFLEITE VAAYQALDHSR.PVIPCFLEITE VAAYQALDHSR.PVIPCFLEITE PAEMWQKRHGDW.RVELCFIEFAE
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Bacillus_subtilis_SirB Bacillus_megaterium_SirB Fictibacillus_solisalsi_SirB Anoxybacillus_vitaminiphilus_SirB Salimicrobium_halophilum_SirB Bacillus_megaterium_cbix Fictibacillus_solisalsi_cbix Anoxybacillus_vitaminiphilus_cbix Synechocystis_sp_Cbix Trichodesmium_erythraeum_cbix Streptomyces_coelicolor_cbix Magnatococcus_marinus_cbix	210 220 230 EIEREVQKLKAHNPNVYLSSYIGFHPHVKNAF EIREELEQLSTDAQOFILANYLGYHDJCAHIL GMKKAIAEQARE.TGGELILCEALGYHPVLGEVL KLKKLKLNLSASDKOFILCNYLGYHPJLQEVL SIREKLSAVNVA.VKNOFICCNQLGSHALMQRAL HEREVGAKE.EGKWVDTAHVLDNHFFIIEIL RLEEMVKQYKMOHENIEFKLAGYFGFHPKLQTI RLEEMVEOFRGQYGVGVDFQLAKYYGFHPKLGTI RLEEMVEOFRGQYGVGVDFQLAKYYGFHPKLEAIF KIFTITEEQRATFPEIEIQSLSEMGIQFELALV KIFDITAQQQEQYPDISMTCLPEIGAHFTLLELL RVKHQTEEWAAAHPETDVRSADVIGFEBLIDLV RIRQVARLERQYFTIAFALGGYFGFEBIFDLL	240 LNRVRBTAANSI SHOVKILLSSK LSRVDBAQQR YRVNBLVSSP AERVVRSVHSVH KERABEGLSGE IERVQCALADE REREIETOLGO BREIETOLGO WERVEBAVGGD DHEVGEALTGV	250 EG. QFDFDGGSYA N. QYDVYRYA V. EKLTQHV D. EFVSPRGA P. FPTTEKGEVH WK. M. NCDTCQYR WK. M. NCDCQYR VA. M. NCEMCKFR K. M. NCEMCKFR E.R. M. NCDSCVYR SHQPLMECDGCKYR.	260 SA.AH
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Supplementary Figure 3. Amino acid sequence alignments of SirB and CbiX, using Clustal omega.¹⁵ The number of amino acid residues of *Bacillus subtilis* SirB are shown on the alignments. His10, Glu43, and His76 of *B. subtilis* SirB, which are ligands for cobalt/iron substrate binding, are strictly conserved in both SirB and CbiX. The figure was made using ESPript 3.¹⁶ White letters on a red background indicate strictly conserved amino acid residues. Red letters in blue boxes indicate well-conserved amino acids.



Supplementary Figure 4. Overall structures of (A) metal-free SirB and (B) Co^{2+} -bound SirB. (C) Superimposition of active sites of metal-free and Co^{2+} -bound SirB. Co^{2+} and water molecules are shown in pink and small red spheres, respectively. The amino acid side chains ligating to the Co^{2+} ions are shown as stick models.



Supplementary Figure 5. Comparison of metal-binding sites between metal-free SirB and Co^{2+} -bound SirB. (A) Metal-free SirB and (B) Co^{2+} -bound SirB at the Co1 and Co2 sites; (C) Metal-free SirB and (D) Co^{2+} -bound SirB at the Co3 and Co4 sites; (E) Metal-free SirB and (F) Co^{2+} -bound SirB at the Co5 site.



Supplementary Figure 6. The geometry of the Co1 site. Green circles with arrows indicate plausible $2F_0$ - F_c electron density corresponding to waters or solvents, although the density was too small to model anything. The geometry appears to be octahedral with slight distortion, not tetrahedral. The $2F_0$ - F_c electron density and Co-anomalous difference maps were contoured at 1 σ and at 4 σ , respectively.

Co ²⁺ -bound SirB			
Site	<i>B</i> -factor (Å ²)		
Col	68.5		
Co2	128.3		
Co3	82.8		
Co4	102.8		
Co5	119.8		

Supplementary Table 1. The *B*-factor of each metal site of Co^{2+} -bound SirB. The occupancy of each metal was 1.0.

Supplementary Table 2. List of primers. The *NcoI* and *XhoI* restriction sites are underlined.

Primer	Sequence
NcoI-BsSirA-F	5'-ATA <u>CCATGG</u> GGAAAGTATATATTGTAGGAG-3'
XhoI-BsSirC-R	5'-
	GTG <u>CTCGAG</u> GATCCTTATTTCTTTCTTCTCATCTGAT
	AAAGCC-3'
His6-BsSirB-F	5'-
	ATGGGCAGCAGCCATCATCATCATCATCACAGCAG
	CATGAAACAAGCAATTTTATATGTC-3'
BsSirA-R	5'-TTTTACAACGCCTCGCTTAAATC-3'

	SirB (metal-free)	
Data collection		
Temperature (K)	100	
Wavelength (Å)	1.000	
Resolution range ^(a)	39.6-2.0 (2.1-2.0)	
Space group	<i>P</i> 2 ₁	
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	69.7 53.8 80.4	
α, β, γ (°)	90 105.4 90	
Total reflections ^(a)	258734 (24477)	
Unique reflections ^(a)	38718 (3817)	
Redundancy ^(a)	6.7 (6.4)	
Completeness (%) ^(a)	99.7 (99.1)	
$I/\sigma_I^{(a)}$	20.2 (2.55)	
$R_{\rm sym}$ (%) ^{(a), (b)}	7.5 (77.2)	
$CC_{1/2}^{(a)}$	1.00 (0.84)	
Refinement		
Resolution range (Å) ^(a)	31.4-2.0 (2.1-2.0)	
$R_{ m work}$ (%) ^{(a), (c)}	18.5 (26.4)	
$R_{\rm free}$ (%) ^{(a), (d)}	21.6 (30.1)	
Rmsd bond length (Å) ^(e)	0.005	
Rmsd bond angle (°) ^(e)	1.06	
Clashcore, all atoms	4.9 (98th percentile)	
Average <i>B</i> -factor ($Å^2$)	38.0	
PDB ID	5ZT8	

Supplementary Table 3. Data collection and refinement statistics.

^{*a*}The values in parentheses are for the highest resolution shell. ${}^{b}R_{sym} = \sum |I - \langle I \rangle| / \sum I$, where *I* is the intensity of each reflection. ${}^{c}R_{work} = \sum ||F_{o}| - k|F_{c}|| / \sum |F_{o}|$, where F_{o} and F_{c} are the observed and calculated structure factor amplitudes, respectively. ${}^{d}R_{free}$ was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. ${}^{e}rmsd$, root mean square deviation.

	Co ²⁺ -bound SirB	Co ²⁺ -bound SirB
		(Co peak)
Data collection		
Temperature (K)	100	100
Wavelength (Å)	1.000	1.605
Resolution range ^(a)	41.8-2.9 (3.0-2.9)	41.8-3.1 (3.2-3.1)
Space group	<i>P</i> 2 ₁	<i>P</i> 2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	65.0 52.1 75.5	65.0 52.2 75.6
α, β, γ (°)	90 112.1 90	90 112.1 90
Total reflections ^(a)	66609 (6910)	42871 (390)
Unique reflections ^(a)	10156 (1024)	7555 (250)
Redundancy ^(a)	6.6 (6.7)	5.7 (1.5)
Completeness (%) ^(a)	99.8 (100.0)	86.9 (29.7)
$I/\sigma_I^{(a)}$	12.68 (1.8)	13.7 (1.1)
$R_{\rm sym}$ (%) ^{(a), (b)}	11.0 (102.0)	9.0 (57.9)
CC _{1/2} ^(a)	1.00 (0.76)	1.00 (0.66)
Refinement		
Resolution range $(Å)^{(a)}$	32.4-2.9 (3.0-2.9)	
$R_{ m work}$ (%) ^{(a), (c)}	24.5 (35.9)	
$R_{\rm free}$ (%) ^{(a), (d)}	27.6 (41.3)	
Rmsd bond length (Å) ^(e)	0.007	
Rmsd bond angle (°) ^(e)	1.53	
Clashcore, all atoms	12.0 (96th percentile)	
Average <i>B</i> -factor (Å ²)	88.7	
PDB ID	5ZT7	

^{*a*}The values in parentheses are for the highest resolution shell. ${}^{b}R_{sym} = \sum |I - \langle I \rangle|/\sum I$, where *I* is the intensity of each reflection. ${}^{c}R_{work} = \sum ||F_{o}| - k|F_{c}||/\sum |F_{o}|$, where F_{o} and F_{c} are the observed and calculated structure factor amplitudes, respectively. ${}^{d}R_{free}$ was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. ${}^{e}rmsd$, root mean square deviation.

	Co ²⁺ -bound SirB	Co ²⁺ -bound SirB
	(Co inflection)	(Co remote)
Data collection		
Temperature (K)	100	100
Wavelength (Å)	1.606	1.566
Resolution range ^(a)	41.8-3.1 (3.2-3.1)	41.8-3.1 (3.2-3.1)
Space group	<i>P</i> 2 ₁	<i>P</i> 2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	65.0 52.2 75.6	65.0 52.2 75.6
α, β, γ (°)	90 112.1 90	90 112.1 90
Total reflections ^(a)	42871 (390)	42987 (433)
Unique reflections ^(a)	7555 (257)	7613 (283)
Redundancy ^(a)	5.7 (1.5)	5.6 (1.5)
Completeness (%) ^(a)	87.0 (30.5)	87.4 (33.4)
$I/\sigma_I^{(a)}$	13.7 (1.1)	13.8 (1.1)
$R_{\rm sym}~(\%)^{(a),~(b)}$	9.0 (57.9)	9.2 (62.9)
CC _{1/2} ^(a)	1.00 (0.66)	1.00 (0.58)
Refinement		
Resolution range $(Å)^{(a)}$		
$R_{ m work}$ (%) ^{(a), (c)}		
$R_{\rm free}$ (%) ^{(a), (d)}		
Rmsd bond length (Å) ^(e)		
Rmsd bond angle (°) ^(e)		
Clashcore, all atoms		
Average <i>B</i> -factor (Å ²)		
PDB ID		

^{*a*}The values in parentheses are for the highest resolution shell. ${}^{b}R_{sym} = \sum |I - \langle I \rangle|/\sum I$, where *I* is the intensity of each reflection. ${}^{c}R_{work} = \sum ||F_{o}| - k|F_{c}||/\sum |F_{o}|$, where F_{o} and F_{c} are the observed and calculated structure factor amplitudes, respectively. ${}^{d}R_{free}$ was calculated as the R_{work} for 5% of the reflections that were not included in the refinement. ${}^{e}rmsd$, root mean square deviation.

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