Supplemental Information for:

"The Effect of Metal on the Biochemical Properties of the *Helicobacter pylori* HypB, a Maturation Factor of [NiFe]-Hydrogenase and Urease" A. M. Sydor, J. Liu, and D. B. Zamble

Primer Name	Primer Sequence
HpHypB forward	⁵ 'AAGGAAC CATATG AGCGAACAACGACAAG ³ '
HpHypB reverse	⁵ 'TCGCTA CTCGAG TTAAAACGAATGCGTGG ³ '
C106A, H107A forward	⁵ CACCACCGGCGAAGCAgcCgcTTTGGAAGCGAGCATG ³
C106A, H107A reverse	⁵ CATGCTCGCTTCCAAAgcGgcTGCTTCGCCGGTGGTG ³

Table S1: PCR primers used for cloning and mutagenesis.^a

^aRestriction enzyme sites are shown in bold. Mutations are shown in lowercase.

Protein	Added Metal	Elution Volume	Calculated MW	Relative Peak	Assignment
		(mL)	(kDa)	Area ^b	
	Аро	16.9 ± 0.2	28.1 ± 2.6	100	Monomer
	0.5 eg. Ni(II)	15.9 ± 0.1	44.7 ± 1.6	76	Dimer
	0.5 cq. 10(11)	17.2 ± 0.1	23.0 ± 0.7	24	Monomer
WT <i>Нр</i> НурВ	1 eq. Ni(II)	15.7 ± 0.1	50.9 ± 1.9	100	Dimer
	1 eq. Zn(II)	15.6 ± 0.1	52.7 ± 2.7	3.5	Dimer
		17.0 ± 0.1	25.8 ± 1.0	96.5	Monomer
	1 eq. Zn(II) + 1	15.86 ± 0.01	46.2 ± 0.2	5	Dimer
	eq. Ni(II)	17.34 ± 0.02	21.9 ± 0.2	95	Monomer
C106A, H107A	Аро	17.2 ± 0.1	23.8 ± 0.8	100	Monomer
<i>Нр</i> НурВ	1 eq. Ni(II)	17.1 ± 0.1	24.5 ± 0.8	100	Monomer
	1 eq. Zn(II)	17.11 ± 0.01	24.6 ± 0.1	100	Monomer

Table S2: Summary of gel filtration chromatography results of WT and mutant HpHypB with added metals.^a

^aErrors are standard deviations of at least two replicates. The expected molecular weights are: WT *Hp*HypB monomer: 27.2 kDa; WT *Hp*HypB dimer: 54.4 kDa; C106A, H107A *Hp*HypB monomer: 27.1 kDa. ^bThe relative peak areas varied by <3.5 % of the total peak area.

Added ligend	Elution Volume	Calculated MW	Relative Peak	Assignment
Auteu nganu	(mL)	(kDa)	Area ^b	Assignment
5 eg CTP	15.9 ± 0.2	45.4 ± 4.0	8	Dimer
3 сц. 011	17.2 ± 0.3	24.0 ± 3.2	92	Monomer
5 eq. CDP	16.0 ± 0.1	43.9 ± 1.7	9.5	Dimer
5 tq. ODI	17.4 ± 0.1	21.3 ± 0.7	90.5	Monomer
1 eg Ni(II) + 5 eg CDP	15.8 ± 0.2	48.0 ± 4.4	81.5	Dimer
1 cq. 10(11) + 5 cq. 0D1	17.21 ± 0.04	23.4 ± 0.4	18.5	Monomer
1 og Nj(II) + 5 og CTP	16.03 ± 0.04	42.4 ± 8.0	84	Dimer
1 cq. m(n) + 5 cq. 011	17.29 ± 0.03	22.4 ± 0.3	16	Monomer
5 eq. GTP + 400 µM GTP	16.8 ± 0.1	58.4 ± 3.5	31.5	Dimer
in MP	18.34 ± 0.04	27.6 ± 0.6	68.5	Monomer
5 eq. GDP + 400 µM GDP	16.99 ± 0.01	54.2 ± 0.4	27	Dimer
in MP	18.3 ± 0.1	28.1 ± 2.0	73	Monomer
5 eq. GTP + 400 µM GTP	18.33 ± 0.04	27.9 ± 0.5	100	Monomer
in MP + 1 eq. Zn(II)				
5 eq. GDP + 400 µM GDP	18.225 ± 0.007	29.3 ± 0.1	100	Monomer
in MP + 1 eq. Zn(II)				

Table S3: Summary of gel filtration chromatography results of WT *Hp*HypB with added nucleotides.^a

^aErrors are standard deviations of at least two replicates. The expected molecular weights are: WT *Hp*HypB monomer; 27.2 kDa; WT HpHypB dimer; 54.4 kDa. MP indicates that the nucleotide was included in the mobile phase. ^bThe relative peak areas varied by <3.5 % of the total peak area.

	High-Affinity Site			Poly-His Regio	n				
	10	20	30	40	50	60	70	80	
			[]		[]	[]			
H. pylori	MSEQ						RQESL	QNNPNLSK - K	18
H. hepaticus	MNLS						R N E R L	ENNPNLNK - K	18
M. jannaschii	MHL							V G	5
E. coli	MCTTCGCGEG	NLYIEGDE	HNPHSAFRSA	PFAPAARPKM	KITGIK AP	EFTPSQTEEG	DLHYGHGEAG	THAPGMSQRR	76
B. japonicum	MCTVCGCSDG	KASIEHAHDH	HHDHGHD H	DHGHDGHHHH	HHGHDQDHHH	HHDHAHGDAG	LLDCGANPAG	QKIAGMSSDR	78

Walker A Motif									
	90	100	110	120	130	140	150	160	
			[]						
H. pylori	DVKIVEKILS	KNDIKAAEMK	ERYLKEGLYV	LNFMSSPGSG	KTTMLE-NLA	DFKD-FKFCV	VEGDĹQTNRĎ	ADRLRKKGVS	96
H. hepaticus	SIQIVSKILS	QNDLKAQELR	EKYKQLGVYV	INLMSSPGSG	KTTLLE-SLS	AFKD - FTFCV	LEGDLQTNRD	AQRLEAKGVS	96
M. jannaschii	VLDIAKDILK	ANKRLADKNR	KLLNKHGVVA	FDFMGAIGSG	KTLLIEKLID	NLKDKYKIAC	IAGDVIAKFD	AERMEKHGAK	85
E. coli	MLEVEIDVLD	KNNRLAERNR	ARFAARKQLV	LNLVSSPGSG	KTTLLTETLM	RLKDSVPCAV	IEGDQQTVND	AARIRATGTP	156
B. japonicum	IIQVERDILG	KNDRLAADNR	ARFRADEVLA	FNLVSSPGAG	KTSLLVRAVS	ELKDSFAIGV	IEGDQQTSND	AERIRATGVP	158

	Conserved CH Motif			Walker B Motif	Switch II Mot	tif			
	170	180	190	200	210	220	230	240	
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H. pylori	AHQITTGEAC	HLEASMIEGA	FDLLKDEGAL	EKSDFLIIEN	VGNLVCPSSY	NLGAAMNIVL	LSVPÉGDDKV	LKYPÍMFMCÁ	176
H. hepaticus	AYQITTGEAC	HLEASMIETA	LCELQKQCDM	QQMDYLFIEN	VGNLVCPASY	DVGASLNIVL	LSTPEGDDKI	LKYPTMFMCA	176
M. jannaschii	VVPLNTGKEC	HLDAHLVGHA	LEDL N L	DEIDLLFIEN	VGNLICPADF	DLGTHKRIVV	ISTTEGDDTI	EKHPGIMKTA	161
E. coli	AIQVNTGKGC	HLDAQMIADA	APRL PL	DDNGILFIEN	VGNLVCPASF	DLGEKHKVAV	LSVTEGEDKP	LKYPHMFAAA	232
B. japonicum	AIQVNTGKGC	HLDAAMVGEA	YDRL PW	LNGGLLFIEN	VGNLVCPAAF	DLGEACKIVV	FSTTEGEDKP	LKYPDMFAAS	234

Nucleotide Binding

	Motif							
	250	260	270	280	290	300		
	· · · · · · · ·							
H. pylori	DAVIISKADM	VEVFNFRVSQ	VKEDMQKLKP	EAPIFLMSSK	DPKSLEDFKN	FLLEKKRENY	Q S T H S F	242
H. hepaticus	DAVIV <mark>SKAD</mark> M	MQYFGFSLTR	VKEDLGQLKA	NVPLFLTSNK	ERESIAKVRD	FIEQKRAQNY	QSHHQF	242
M. jannaschii	DLIVI <mark>NKID</mark> L	ADAVGADIKK	MENDAKRINP	DAEVVLLSLK	TMEGFDKVLE	FIEKSVKE	V K	221
E. coli	SLMLL <mark>NKVD</mark> L	LPYLNFDVEK	CIACAREVNP	EIEIILISAT	SGEGMDQWLN	WLETQRC	A	290
B. japonicum	SLMLI <mark>nkid</mark> l	ASVLDFDLAR	TIEYARRVNP	KIEVLTLSAR	TGEGFAAFYA	WIRKRMAATT	PAAMTAAE	302

Figure S1. Amino acid alignment of HpHypB with homologs. The specific strains are as follows: *Escherichia coli* K12 substr. MG1655, *Helicobacter pylori* 26695, *Helicobacter hepatics* ATCC 51449, *Bradyrhizobium japonicum* USDA110, and *Methanocaldococcus jannaschii* DSM 2661. All sequences were retrieved from the NCBI database and aligned using ClustalW (1).

REFERENCES

1. Higgins, D. G., and Sharp, P. M. 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73:237-244.