Supplemental figures, figure legends and tables

DiGeorge Critical Region 8 (DGCR8) Is a Double-Cysteine-Ligated Heme Protein

Ian Barr[1], Aaron T. Smith[2], Rachel Senturia[1], Yanqiu Chen[1], Brooke D. Scheidemantle[1], Judith N. Burstyn[2,3], Feng Guo[1,3]

From [1] Department of Biological Chemistry, David Geffen School of Medicine, Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA, and

[2] Department of Chemistry, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA

[3] Address correspondence to: Feng Guo, Ph.D., 611 Charles E. Young Dr. E., 202 Boyer Hall, Los Angeles, CA 90095. Fax: 310-206-7286; E-mail: fguo@mbi.ucla.edu. Or Judith Burstyn, 1101 University Avenue, Madison, Wisconsin 53706. Fax: F608-262-6143; E-mail: burstyn@chem.wisc.edu.

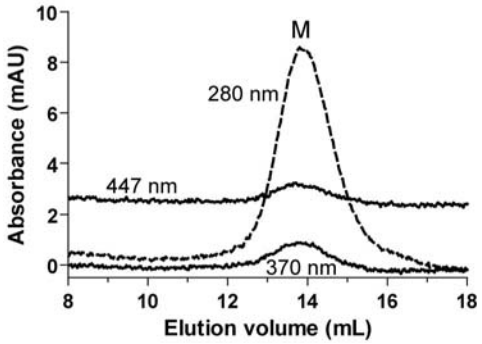

Figure S1. Monomeric P351A did not bind heme.

Size exclusion chromatogram of 4 μM heme-free P351A monomer incubated with equimolar ferric heme. Monomeric heme-free P351A was purified following the procedure as described (1), except with an additional round of size exclusion chromatography to remove the residual dimers. The 447-nm curve was nudged upwards to improve clarity. The monomer peak is labeled as "M". A 370-nm absorbing species co-eluted with the P351A monomer; this species most likely represents non-specifically bound heme, and may constitute 15% of the heme that was added in the incubation.

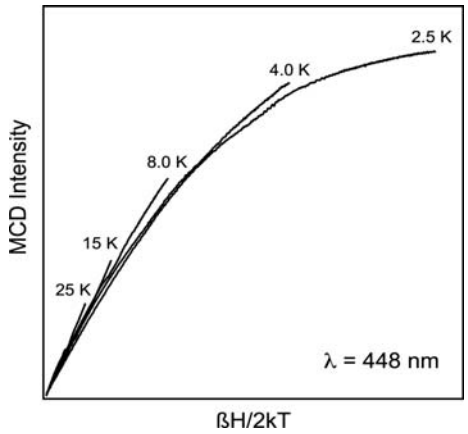

Figure S2. Magnetic saturation curves taken for the frog DGCR8 HBD-His$_6$.

The field dependence of the MCD intensity at 448 nm was recorded at 2.5, 4.0, 8.0, 15 and 25K. The curves were normalized to the most intense data point (2.5K, 7T). The nesting of these curves is consistent with a low spin Fe(III), S=1/2 state for the heme iron.

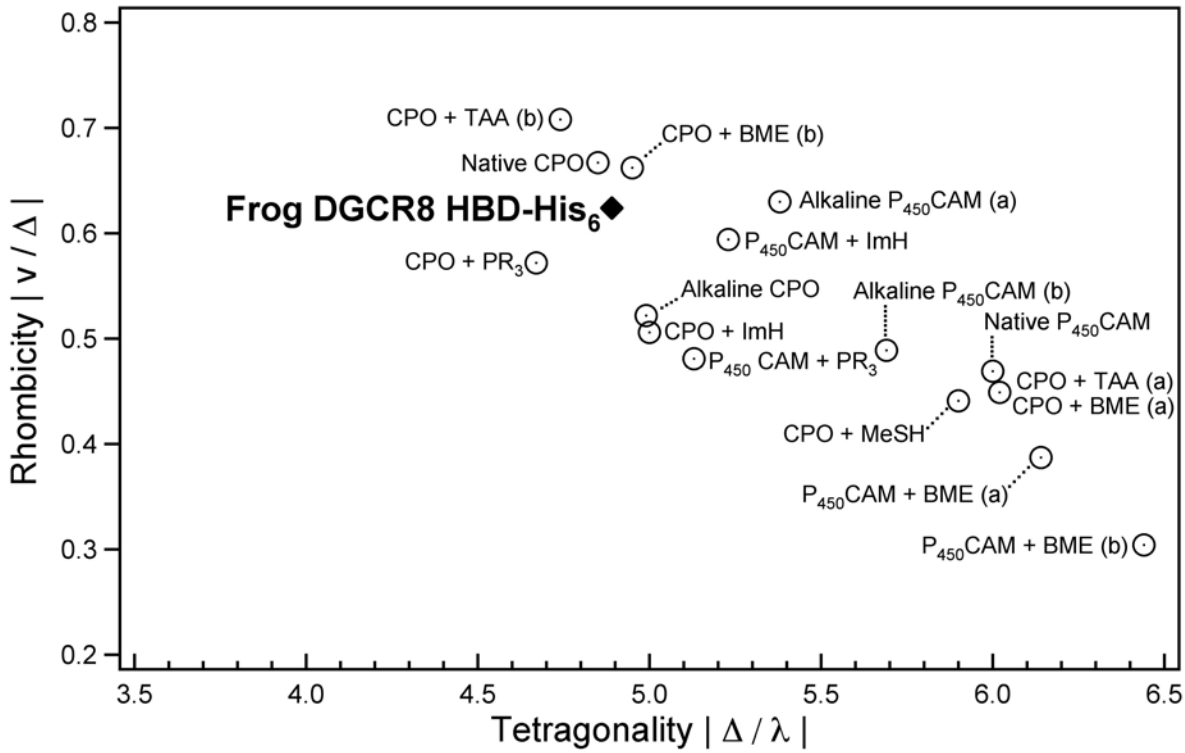

Figure S3. Blumberg-Peisach correlation diagram showing the position of ferric frog DGCR8 HBD-His_6 among other sulfur- and phosphorus-donor complexes of ferric chloroperoxidase and cytochrome $P450_{CAM}$.

The plotted values of rhombicity ($|v/\Delta|$) versus tetragonality ($|\Delta/\lambda|$) show that the environment of the Fe(III) heme in frog DGCR8 HBD-His_6 is most similar to that of native chloroperoxidase to which exogenous thiols or phosphines have been added. $|v/\Delta|$ and $|\Delta/\lambda|$ were calculated directly from the EPR *g* values presented in reference (2). The following improper axis system was used, as outlined in reference (3): $g_x = -g_y$; $g_y = g_z$; and $g_z = -g_x$. Rhombicity and tetragonality values were each calculated as outlined in (7) after the improper axis transformation was applied: $V/\lambda = (g_x/(g_z + g_y)) + (g_y/(g_z - g_x))$; $\Delta/\lambda = |(g_x/2(g_z + g_y)) + (g_z/(g_y - g_x)) - (g_y/2(g_z - g_x))|$; $V/\Delta = (V/\lambda) / (\Delta/\lambda)$. The following abbreviations are used: CPO: chloroperoxidase; $P450_{CAM}$: cytochrome $P450_{CAM}$; TAA: thioacetic acid; BME: β-mercaptoethanol; ImH: imidazole; MeSH: methanethiol; PR_3: bis(hydroxymethyl)-methyphosphine. "(a)" and "(b)" in the diagram refer to different sets of multiple, overlapping rhombic signals that are observed for a single sample.

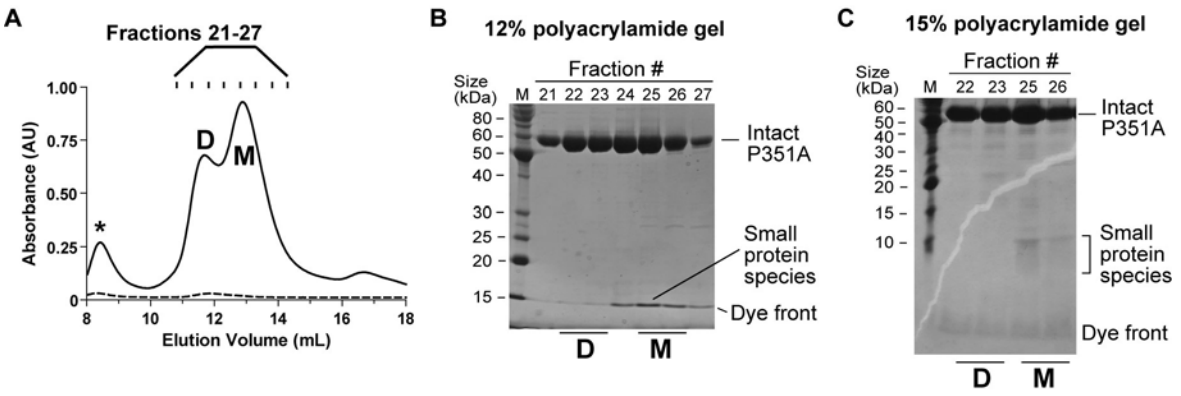

Figure S4. The "monomer" fractions of NC1 P351A expressed in *E. coli* may consist of heterodimers.

The NC1 P351A mutant was expressed in *E. coli* and purified using the procedure described in the main text. (*A*) Size exclusion chromatogram in the last step of the purification procedure. "D" and "M" indicate the peaks assigned to be dimers and monomers, respectively. The asterisk indicates an impurity (mostly nucleic acids) from the bacterial extract. The SDS-12% (*B*) and 15% (*C*) polyacrylamide gels of the chromatographic fractions, stained with Coomassie Brilliant Blue G-250, showed small protein species. These species co-migrated with the dye front on the 12% gel (*B*) and were resolved into a smear with the most intensity centered at around 10 kDa (*C*). Western blotting using an anti-DGCR8 antibody (M.G. and M.F., data not shown) indicated that the small protein species are fragments of DGCR8, thus might have originated from proteolytic cleavage of a NC1 subunit during bacterial expression or purification. Similar observations were made for the wild-type NC1 protein as well.

Supplemental Tables

Table S1. Electronic absorption peaks and spin states of native DGCR8 proteins and of sulfur- and phosphorus-donor complexes of ferric chloroperoxidase and cytochrome $P450_{CAM}$.

Fe(III) proteins and ligands	pH	Soret [d]		α/β	Spin state	Ref
		Hyper blue	Hyper red			
Human DGCR8 NC1 Native	8.0	366	450	556	LS[a]	This work
Frog DGCR8 HBD Native	8.0	366	451	557	LS	This work
Chloroperoxidase						
Native	3-7	399		514/542	LS	(4)
Thiol/thiolate						
H_2S	6-7	369	449	NR[b]	LS	(4)
H_3CSH	6.0	372	455	NR	LS	(4)
$HO(CH_2)_2SH$	6.0	380	455	NR	HS/LS[c]	(4)
$H_3CH_2COC(O)CH_2SH$	6.0	371	454	556	LS	(4)
Phosphine						
$(HOCH_2)_2PCH_3$	6.0, 6.5	376	450	553	LS	(5)
Thioether						
H_3CSCH_3	3-6	417		NR	HS/LS	(4)
Disulfide						
H_3CSSCH_3	3-6	417		NR	HS/LS	(4)
Cytochrome $P450_{CAM}$						
Native (camphor-free)	7.0	417		536/569	LS	(6)
Thiol/thiolate						
H_2S	7.0	390	467	564	LS	(6)
$H_3C(CH_2)_2SH$	9.1	377	466		LS	(6)
$HO(CH_2)_2SH$	8.2	376	464	558	LS	(6)
$ClC(CH)_4CSH$	7.0	381	463	560	LS	(6)
Phosphine						
$(HOCH_2)_2PCH_3$	7.3	375	446	553	LS	(5)
Thioether						
H_3CSCH_3	7.0	424		538/570	LS	(6)
Disulfide						
H_3CSSCH_3	7.0	418		536/568	LS	(6)

[a] LS: low-spin; HS: high-spin. [b] NR: not reported. [c] HS/LS indicates that the protein exists as an admixture of high- and low-spin states. [d] Hyper red and hyper blue refer to the two components of the split Soret. A single value indicates the absence of a hyperporphyrin spectrum.

Table S2. EPR parameters (*g* values) of the native Frog DGCR8 HBD and of sulfur- and phosphorus-donor complexes of ferric chloroperoxidase and cytochrome P450$_{CAM}$.

Fe(III) protein	pH	g_z	g_y	g_x	Ref
Frog DGCR8 HBD Native	8.0	2.60	2.27	1.84	This work
Chloroperoxidase					
Native	6.0	2.62	2.26	1.83	(2)
	9.5	2.54	2.28	1.85	(2)
Thiol/thiolate					
H_3CSH	3.0	2.45	2.27	1.91	(2)
$H_3CH_2COC(O)H_2CSH$	6.0	2.37	2.25	1.94	(2)
		[*2.43*[a]	*2.26*[a]	*1.91*[a]]	(2)
$H_3CC(O)SH$	3.0	2.66	2.26	1.82	(2)
		[*2.44*		*1.91*]	(2)
$HO(CH_2)_2SH$	6.0	2.61	2.26	1.84	(2)
		[*2.44*		*1.91*]	(2)
Phosphine					
$(HOCH_2)_2PCH_3$	7.0	2.59	2.28	1.82	(5)
Thioether					
H_3CSCH_3	NR[b]	2.62	2.26	1.83	(7)
Disulfide					
H_3CSSCH_3	NR	2.62	2.26	1.83	(7)
Cytochrome P450$_{CAM}$					
Native (camphor-free)	5.5-9	2.44	2.25	1.91	(6)
Thiol/thiolate					
$H_3C(CH_2)_2SH$	7.0	2.41	2.24	1.92	(6)
		[*2.34*		*1.94*]	(6)
$HO(CH_2)_2SH$	7.0	2.42	2.27	1.92	(6)
		[*2.38*	*2.27*	*1.93*]	(6)
		[*2.52*	*2.27*	*1.90*]	(6)
$(C_6H_5)CH_2SH$	6-9.5	2.39	2.25	1.94	(6)
		[*2.44*	*2.25*	*1.92*]	(6)
Phosphine					
$(HOCH_2)_2PCH_3$	7.0	2.51	2.28	1.86	(5)
Thioether					
H_3CSCH_3	7.0	2.50	2.27	1.89	(6)
Disulfide					
H_3CSSCH_3	7.0	2.42	2.25	1.92	(6)

[a] Values in brackets represent minor signals present. In most cases, the relationship of the major signal to the minor signal was not explored. [b] NR: not reported.

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