

Are Free Radicals Involved in IspH Catalysis?

An EPR and Crystallographic Investigation

Supporting Information

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Table S1. ^{17}O hyperfine coupling constants of oxygen-containing ligands bound to the aconitase Fe_a site (the unique 4th iron). Units: MHz.

Sample Ligand bound to Fe _a	Substrate/inhibitor- free aconitase	Substrate (<i>cis</i> -aconitate) bound aconitase	<i>Trans</i> -aconitate bound aconitase	Nitro- isocitrate bound aconitase
HO ⁻ (solvent)	$A_{iso} = 10.3$	-	-	-
H ₂ O (solvent)	-	$A_{iso} = 8.6$	$A_{iso} = 9.3$	$A_{iso} = 8.6$
Hydroxyl group	-	-	-	$A_{iso} = 9.5$
Carboxyl group	-	$A_y = 15$ (β carboxyl)	-	$A_y = 13$ (α carboxyl)
References	1	1; 2	1	1-3

Table S2. \mathbf{g} tensors of various iron-sulfur proteins and model systems.

	Enzymes	comments	g_1	g_2	g_3	g_{iso}	Δg	references
[Fe ₄ S ₄] ⁺ enzymes	<i>Aquifex aeolicus</i> IspH	ligand-free (major component)	2.040	1.916	1.865	1.940	0.175	4
		ligand-free (minor component)	2.040	1.935	1.840	1.938	0.200	
		with pyridine diphosphate inhibitor	2.041	1.921	1.853	1.938	0.188	5
	<i>E. coli</i> IspH	ligand-free (reconstituted)	2.037	1.921	1.921	1.960	0.116	6
	<i>E. coli</i> IspG	ligand-free	2.04	1.90	1.90	1.95	0.140	7
	<i>E. coli</i> fumarase	ligand-free, S=1/2 component	2.03	1.94	1.88	1.95	0.15	8
		with sodium fumarate	2.035	1.92	1.815	1.92	0.22	
	aconitase (from bovine heart)	ligand-free	2.06	1.93	1.86	1.95	0.2	9
		citrate bound	2.04	1.85	1.78	1.89	0.26	
	endonuclease III Fe-S center		2.04	1.94	1.94	1.97	0.1	10
	Pyruvate formate-lyase activating enzyme	ligand-free	2.02	1.94	1.88	1.947	0.14	11,12
		SAM bound	2.01	1.88	1.87	1.92	0.14	
	Benzoyl CoA reductase	cluster I	2.017	1.938	1.908	1.954	0.109	13
		cluster II	2.05	1.93	1.93	1.971	0.123	
		cluster III	2.03	1.995	1.965	1.997	0.065	
	DNA polymerase CysB motif		2.04	1.93	1.93	1.97	0.11	14
ferredoxins	<i>Bacillus polymyxa</i> ferredoxin		2.06	1.93	1.88	1.96	0.180	15
	<i>Bacillus stearothermophilus</i> ferredoxin		2.06	1.93	1.89	1.96	0.170	16
	<i>Chromatium</i> ferredoxin		2.04	1.93	1.93	1.97	0.110	17 18
	<i>Clostridium pasteurianum</i> ferredoxin		2.06	1.92	1.88	1.95	0.180	19
	<i>Desulfovibrio gigas</i> ferredoxin		2.07	1.94	1.92	1.98	0.150	
	<i>Rhodospirillum rubrum</i> ferredoxin		2.03	1.94	1.94	1.97	0.090	20
	<i>R. gelatinosa</i> high potential iron-sulfur protein	super-reduced, [4Fe-4S] ⁺	2.04	1.92	1.92	1.96	0.120	21
synthetic [Fe ₄ S ₄] ⁺	[Fe ₄ S ₄ (SCH ₂ Ph) ₄] ³⁻		2.04	1.93	1.93	1.97	0.110	22
	[Fe ₄ S ₄ (SPH) ₄] ³⁻ in solution		2.06	1.93	1.93	1.97	0.130	23
	[Fe ₄ S ₄ (SCH ₂ CH ₂ OH) ₄] ³⁻		2.05	1.93	1.93	1.97	0.115	24
	[Fe ₄ S ₄ (S-p-C ₆ H ₄ Br) ₄] ³⁻ polycrystalline		2.06	1.93	1.89	1.96	0.170	25,26
	[Fe ₄ S ₄ (S-p-C ₆ H ₄ Br) ₄] ⁵⁻ in solution		2.04	1.92	1.92	1.96	0.120	26
oxidized high	<i>R. gelatinosa</i> HiPIP		2.11	2.03	2.03	2.06	0.080	27
	<i>Chromatium vinosum</i>	the major component	2.12	2.04	2.02	2.06	0.100	28

potential iron-sulfur proteins (HiPIP)	HiPIP								
	<i>Chromatium vinosum</i> HiPIP	the minor component	2.13	2.07	2.04	2.08	0.090		
	<i>Ectothiorhodospira halophila</i> iso-II HiPIP	single component	2.145	2.034	2.024	2.068	0.121	29	
	<i>Rhodoferax fermentans</i> HiPIP		2.114	2.033	2.033	2.060	0.081	30	
	<i>E. halophila</i> HiPIP I	the major isomer	2.14	2.03	2.03	2.07	0.110		31
	<i>E. halophila</i> HiPIP I	the minor isomer	2.10	2.06	2.03	2.06	0.070		
	<i>Rhodococcus tenuis</i> HiPIP		2.11	2.03	2.03	2.06	0.080	32	
	<i>Rhodophila globiformis</i> HiPIP		2.12	2.03	2.03	2.06	0.090	21	
	<i>Thiobacillus ferrooxidans</i>		2.127	2.034	2.034	2.065	0.093	33	
	<i>Halorhodospira halophila</i> isoprotein I	major component (91%)	2.1435	2.0350	2.0295	2.0693	0.1140		34
synthetic HiPIP models	<i>Ectothiorhodospira vacuolata</i> isoprotein I	major component (70%)	2.1090	2.0287	2.0287	2.0555	0.0803		
	<i>Ectothiorhodospira vacuolata</i> isoprotein II	major component (70%)	2.112	2.038	2.025	2.0583	0.0870		
	<i>Rubrivivax gelatinosus</i>	major component (64%)	2.1127	2.0305	2.0305	2.0579	0.0822		
	(NBu ₄) ₂ [Fe ₄ S ₄ (SR) ₄]	in toluene	2.10	2.05	2.03	2.06	0.070	35	
	$[(C_2H_5)_4N]_2$		2.142	2.013	2.004	2.053	0.138		36
[4Fe-4S] enzymes With unsaturated ligands	[Fe ₄ S ₄ (SCH ₂ Ph) ₄]	γ -irradiated single crystal	2.146	2.009	2.003	2.053	0.143		
			2.101	2.039	2.023	2.054	0.078		
			2.070	2.026	2.018	2.038	0.052		
	<i>Aquifex aeolicus</i> IspH		2.125	2.015	2.000	2.047	0.125	4	
			2.080	2.010	2.000	2.030	0.080	4	
			2.112	2.011	2.011	2.045	0.101		37
			2.085	2.011	2.011	2.036	0.074		
			2.087	2.015	2.000	2.034	0.087	Fig. S1A	
	<i>Aquifex aeolicus</i> IspH E126Q mutant		2.059	2.007	2.007	2.024	0.052	Fig. S1B	
			2.088	2.006	1.995	2.030	0.093	Fig. S1C	
<i>E. coli</i> IspH	<i>E. coli</i> IspH		2.145	2.011	1.990	2.049	0.155	Fig. S1D	
			2.135	2.008	1.985	2.043	0.150	Fig. S1E	
			2.059	2.002	1.982	2.014	0.077	Fig. S1F	

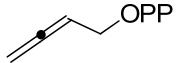
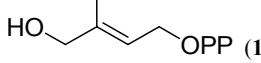
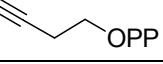
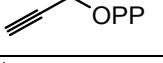
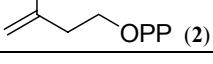
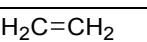
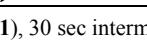
			2.085	2.015	2.001	2.034	0.084	Fig. S1G
			2.099	2.008	2.008	2.038	0.091	Fig. S1H
<i>E. coli</i> IspG			2.090	2.010	1.975	2.025	0.115	38
			2.088	2.012	2.003	2.034	0.085	Fig. S1I
			2.090	2.012	1.995	2.032	0.095	38
			2.065	1.995	1.975	2.012	0.090	38
	Benzoyl-CoA reductase		2.120	2.017	2.017	2.051	0.103	13
	nitrogenase		2.123	1.978	1.949	2.017	0.174	39
			2.123	1.998	1.986	2.036	0.137	
IspH reaction intermediates trapped with the wild type enzyme	<i>Aquifex aeolicus</i> IspH	HMBPP (1), 30 sec intermediate	2.172	2.010	1.994	2.059	0.178	Fig. S1J
	<i>E. coli</i> IspH	HMBPP (1), 30 sec intermediate	2.172	2.011	1.996	2.060	0.176	Fig. 3A
		iso-HMBPP (5), 30 sec intermediate	2.171	2.005	2.005	2.060	0.166	Fig. 6D
		FMBPP (4), 30 sec intermediate	2.172	2.011	1.996	2.060	0.176	Fig. 3B
	<i>Aquifex aeolicus</i> IspH E126A mutant	HMBPP (1, unreactive)	2.122	1.997	1.975	2.031	0.147	4
IspH reaction intermediates trapped with E126A/E126Q mutants	<i>Aquifex aeolicus</i> IspH E126Q mutant	HMBPP (1, unreactive)	2.122	1.999	1.963	2.028	0.159	Fig. S1K
		iso-HMBPP (5, unreactive), comp. I	2.091	2.007	1.994	2.031	0.097	Fig. S1L
		iso-HMBPP (5, unreactive), comp. II	2.091	1.994	1.994	2.026	0.097	
	<i>E. coli</i> IspH E126Q mutant	HMBPP (1, unreactive)	2.132	2.003	1.972	2.036	0.160	Fig. 1A
		FMBPP (4, unreactive)	2.132	2.003	1.972	2.036	0.160	Fig. 1C
		iso-HMBPP (5, unreactive), comp. I	2.091	1.999	1.999	2.030	0.092	Fig. 6C
		iso-HMBPP (5, unreactive), comp. II	2.091	1.999	1.982	2.024	0.109	
IspG reaction intermediate	<i>E. coli</i> IspG	reaction intermediate "X"	2.092	2.018	1.999	2.036	0.093	38
	<i>T. thermophilus</i> IspG	reaction intermediate "X"	2.087	2.019	2.000	2.035	0.087	40
Ferredoxin: Thioredoxin Reductase	FTR from spinach	reaction intermediate	2.11	2.00	1.98	2.03	0.13	41
		NEM-modified	2.11	2.00	1.98	2.03	0.13	42
		super oxidized	2.090	2.040	2.020	2.05	0.07	

Table S3. Data collection and refinement statistics.

IspH: <i>iso</i> -HMBPP (5)	
Crystal parameter	
Space group	P2 ₁ 2 ₁ 2 ₁
Celldimensions	
<i>a, b, c</i> (Å)	70.6; 80.8; 113.1
Molecules per AU ^a	2
Data collection	
Beam line	SLS, X06SA
Wavelength (Å)	1.0
Resolution range (Å) ^b	10-1.9 (2.0-1.9)
Unique reflections ^c	98052
Completeness (%) ^b	99.6 (99.1)
<i>R</i> _{merge} (%) ^{b,d}	6.8 (49.0)
I / σ (I) ^b	11.6 (2.5)
Refinement	
Resolution (Å)	15-1.9
<i>R</i> _{work} / <i>R</i> _{free} ^e	0.179 / 0.221
No. atoms	
Protein	4773
Ligand	28
FeS-Cluster	16
Water	518
<i>B</i> -factors	29.3
R.m.s. deviations ^f	
Bond lengths (Å)	0.023
Bond angles (°)	2.3
Ramachandran (%) ^g	98.2/1.8/0.0
PDB accessioncode	4EB3

^a Asymmetric unit^b values in parenthesis of resolution range, completeness, *R*_{merge} and I/σ (I) correspond to the last resolution shell^cFriedel pairs were treated as identical reflections^d $R_{\text{merge}}(I) = \sum_{hkl} \sum_j |[I(hkl)_j - I(hkl)]| / [\sum_{hkl} I_{hkl}]$, where $I(hkl)_j$ is the jth measurement of the intensity of reflection hkl and $\langle I(hkl) \rangle$ is the average intensity^e $R = \sum_{hkl} | |F_{\text{obs}}| - |F_{\text{calc}}| | / \sum_{hkl} |F_{\text{obs}}|$, where R_{free} is calculated without a sigma cutoff for a randomly chosen 5% of reflections, which were not used for structure refinement, and R_{work} is calculated for the remaining reflections^f Deviations from ideal bond lengths/angles^g Number of residues in favoured region / allowed region / outlier region

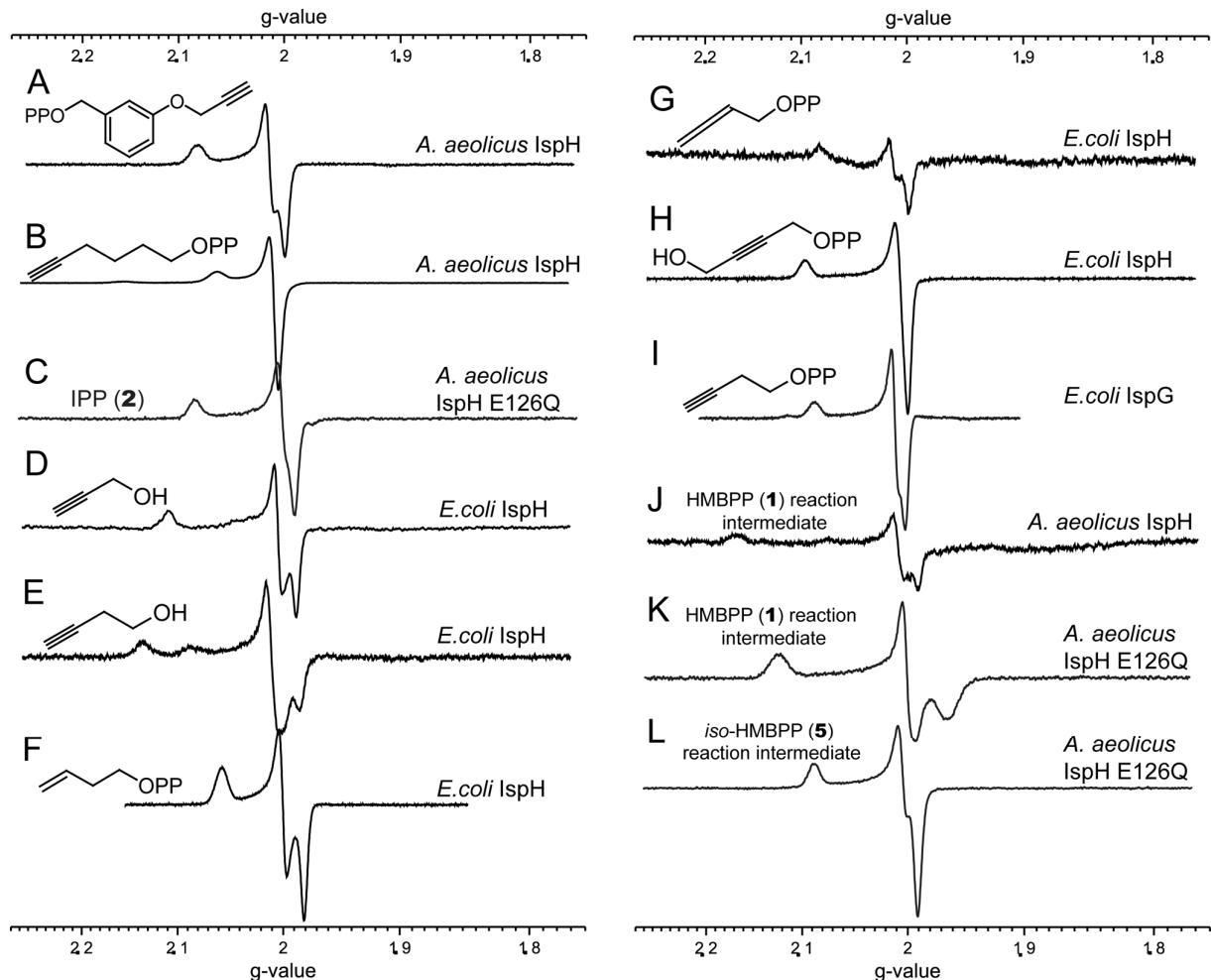


Figure S1. Previously unpublished X-band EPR spectra of IspH/IspG with alkene/alkyne ligands, and *A. aeolicus* IspH reaction intermediates.

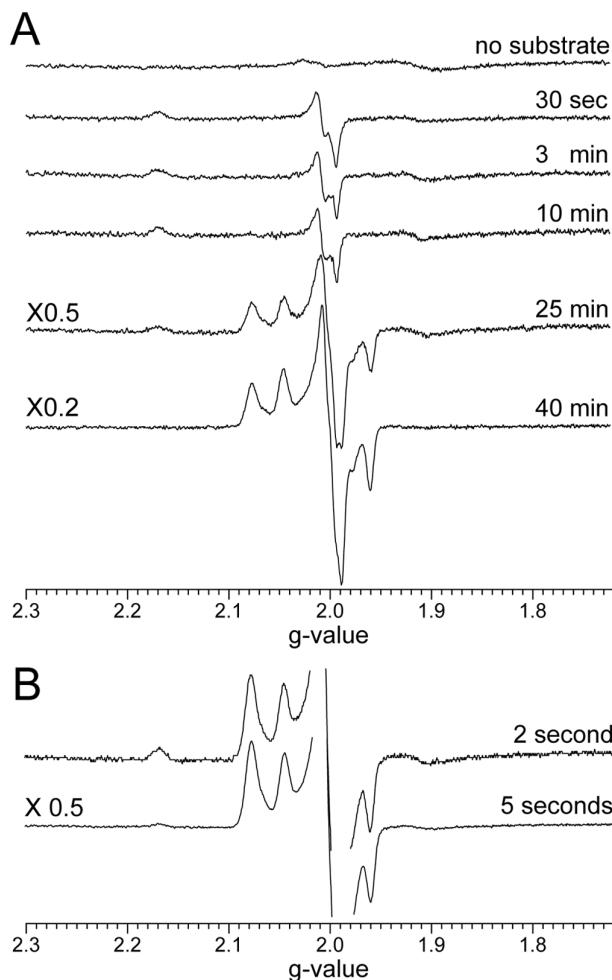


Figure S2. X-band EPR spectra of *E.coli* IspH with **1** (HMBPP) after different reaction times. (A), reaction in the absence of any electron mediator. IspH protein concentration was 0.15 mM; sodium dithionite was added to 30 equivalents; substrate **1** was added to 10 equivalents. The reaction was freeze-quenched at 30 seconds and 3, 10, 25 and 40 minutes. At the first three time points, only the reaction intermediate signal was observed; at 25 min, a mixture of the intermediate and products-bound IspH was observed; at 40 min, only products-bound IspH signal was observed. The top spectrum is the EPR spectrum of IspH with 30 equivalents of sodium dithionite and without substrate **1**. (B), reaction in the presence of 1 equivalent of methyl viologen. Sodium dithionite was added to 120 equivalents and substrate **1** was added to 50 equivalents. The reaction was freeze-quenched at about 2 and 5 seconds.

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