

The SOD1 Inhibitor, LCS-1, Oxidizes H₂S to Reactive Sulfur Species, Directly and Indirectly, Through Conversion of SOD1 to an Oxidase

Kenneth R. Olson ^{1,2,*}, Tsuyoshi Takata ¹, Kasey J. Clear ³, Yan Gao ¹, Zhilin Ma ^{1,2}, Ella Pfaff ^{1,2}, Karthik Mouli ⁴, Thomas A Kent ⁴, Prentiss Jones, Jr. ⁵, Jon Fukuto ⁶, Gang Wu ⁷ and Karl D. Straub ^{8,9}

¹ Department of Physiology, Indiana University School of Medicine South Bend, South Bend, IN 46617, USA

² Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA

³ Department of Chemistry and Biochemistry, Indiana University South Bend, South Bend, IN 46615, USA

⁴ Institute of Biosciences and Technology, Texas A&M Health Sciences Center-Houston Campus, Houston, TX 77030, USA

⁵ Toxicology Department, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI 49007, USA

⁶ Department of Chemistry, Sonoma State University, Rohnert Park, CA 94928, USA

⁷ Department of Internal Medicine, McGovern Medical School, University of Texas, Houston, TX 77030, USA

⁸ Central Arkansas Veteran's Healthcare System, Little Rock, AR 72205, USA

⁹ Departments of Medicine and Biochemistry, University of Arkansas for Medical Sciences, Little Rock, AR 72202, USA

* Correspondence: kolson.1@nd.edu; Tel.: +574-631-7560; Fax: +574-631-7821

Running Head: Sulfur Metabolism by LCS-1

***Address correspondence to:**

Kenneth R. Olson, Ph.D.
Indiana University School of Medicine -South Bend
Raclin Carmichael Hall
1234 Notre Dame Avenue
South Bend, IN 46617
Phone: (574) 631-7560
Fax: (574) 631-7821
e-mail: olson.1@nd.edu

Inorganic sulfur speciation

The percent ionization of inorganic RSS (H_2S_{1-6}) was calculated at pH increments from 3 to 10 using Henderson Hasselbach equations (eqs. 1, 2) and the dissociation constants in **Table**

1. In this case with S_1 as an example,

$$\text{pH} - \text{pK}_{a1} = \text{Log}_{10}[\text{HS}^-]/[\text{H}_2\text{S}] \quad (\text{eq. 1}),$$

$$\text{pH} - \text{pK}_{a2} = \text{Log}_{10}[\text{S}^{2-}]/[\text{HS}^-] \quad (\text{eq. 2}).$$

At any specified pH, if X equals $\text{antilog}_{10}(\text{pH} - \text{pK}_{a1})$ and Y equals $\text{antilog}_{10}(\text{pH} - \text{pK}_{a2})$ then,

$$X = [\text{HS}^-]/[\text{H}_2\text{S}] \quad (\text{eq. 3}),$$

and,

$$Y = [\text{S}^{2-}]/[\text{HS}^-] \quad (\text{eq. 4}),$$

Solving eqs. 3 and 4 for H_2S and S^{2-} ,

$$[\text{H}_2\text{S}] = [\text{HS}^-]/X \quad (\text{eq. 5}),$$

$$[\text{S}^{2-}] = [\text{HS}^-]*Y \quad (\text{eq. 6}).$$

Letting the total sulfur species,

$$\text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-} = 1 \quad (\text{eq. 7}),$$

and substituting for H_2S and S^{2-} (eqs. 5 and 6),

$$\text{HS}^-/X + \text{HS}^- + \text{HS}^-*Y = 1 \quad (\text{eq. 8}),$$

HS^- is then,

$$\text{HS}^- = 1/(1/X + 1 + Y) \quad (\text{eq. 9}),$$

and HS^- can be calculated at any pH using X and Y for that pH. Knowing HS^- , H_2S and S^{2-} can then be calculated at that pH by rearranging eqs. 3 and 4 and the percent of each species can be calculated from the total $\text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$.

Table 1. pK_{a1} and pK_{a2} of catenated sulfur species^a.

Species	pK _{a1}	pK _{a2}
S ₁	7.0	>14
S ₂	5.0	9.7
S ₃	4.2	7.5
S ₄	3.8	6.3
S ₅	3.5	5.7
S ₆	3.2	5.2

a, From [1]

References

1. Kamyshny, A., Jr.; Goifman, A.; Rizkov, D.; Lev, O., Formation of carbonyl sulfide by the reaction of carbon monoxide and inorganic polysulfides. *Environ. Sci. Technol* **2003**, 37, (9), 1865-1872.

Supplemental Figure Captions

Supplemental Figure S1. Absorption spectra of LCS-1 reactions with H₂S and effects of SOD, cysteine (Cys) and hydrogen peroxide (H₂O₂), reactant concentrations are shown above each panel. (A-D) Spectra of individual reactants, (E- K) Spectra with DMSO spectrum subtracted. Scale to right indicates elapsed time, H₂S and Cys were added after 10 s, arrows mark absorption peaks and wavelength (nm). H₂S did not affect the LCS-1 spectrum.

Supplemental Figure S2. LC-MS/MS analysis of products after 30 min incubation of 300 μ M H₂S with 10 μ M LCS-1 and 0.1 μ M SOD1 without or with 1 μ M catalase (Cat). Cat slightly decreased H₂S₂₋₄ and H₂SO₃, increased H₂S₂O₃, whereas H₂S, H₂S_{5,6} and HS_{4,5}OH were unaffected. Mean +SE, n=3 replicates; *, $p < 0.05$; ***, $p < 0.001$ versus without Cat.

Supplemental Figure S3. Effects of LCS-1-thiol adducts on H₂S oxidation by SOD1. (A,B) Effects of 30 min pre-incubation with GSH (A) or Cys (B) on polysulfide production (SSP4 fluorescence) from oxidation of 300 μ M H₂S by 10 μ M LCS-1 and 0.1 μ M SOD1. Pre-incubation with equimolar (10 μ M) GSH increased polysulfides compared to LCS-1-SOD1, whereas 50 μ M GSH almost completely inhibited it, polysulfide production was inhibited by both 10 μ M and 50 μ M Cys. Little polysulfides were produced by 30 min preincubation with GSH or Cys in the absence of either SOD1 or LCS-1. Mean +SE, n=4 wells; ***, $p < 0.001$ versus H₂S-LCS-1-SOD (no adduct); #, $p < 0.05$, ###, $P < 0.001$, 10 μ M versus respective 50 μ M GSH or Cys.

Supplemental Figure S4. Effects of LCS-1-SH adducts on H₂S oxidation by SOD-1. (A)

Preincubation of 10 μ M LCS-1 or 10 μ M LCS-1 plus 0.1 μ M SOD1 for 10 min with either 10 μ M or 50 μ M H₂S followed by 300 μ M H₂S and SSP4 compared to LCS-1 plus SOD pre-incubated for 0 or 10 min without H₂S. (B) H₂S (10 μ M or 50 μ M) was incubated in covered containers with 10 μ M LCS-1 without or with 0.1 μ M SOD for 30 min, the covers were then removed for 1 h to let un-reacted H₂S dissipate and 0.1 μ M SOD1 added, the LCS-1-SH adduct without SOD1 was then incubated with SOD1 for another 30 min before adding H₂S and SSP4. These samples were compared to controls preincubated for similar intervals but without H₂S. All H₂S adducts decreased polysulfide production. Bar graphs summarize results at 140 min (A) or 120 min (B), line graphs show responses over time. Mean \pm SE, n=4 wells per treatment; ***, $p < 0.001$ versus respective LCS-1 plus SOD1 without H₂S; ##, $p < 0.01$, ###, $p < 0.001$, 10 μ M H₂S versus 50 μ M H₂S preincubation.

Supplemental Figure S5. Effects of duration of incubation of either 1 μ M (A) or 10 μ M (B)

LCS-1 with 0.1 μ M SOD1, prior to addition of 300 μ M H₂S, on polysulfide production (SSP4 fluorescence). LCS-1 was incubated with SOD1 0, 30, 60, 90, or 120 min before addition of H₂S and SSP4 and fluorescence measured over an additional 130 min. The onset of polysulfide production was slower with 1 μ M LCS-1 than with 10 μ M LCS-1 but duration of LCS-1 incubation with SOD1 did not significantly affect polysulfide production suggesting that LCS-1 rapidly reacts with SOD1. Mean \pm SE, n=4 wells per treatment.

Supplemental Fig. S6. (A-C) Effects of LCS-1 and ATN-244 on SOD1 inhibition using the nitro blue tetrazolium assay. (A) Standard curve of SOD1 inhibition, approximate IC₅₀ was 0.06

μM SOD and nearly complete inhibition was obtained with 1 μM SOD. **(B-C)** ATN-244 concentration-dependently inhibited SOD1 with nearly complete inhibition at 10 μM . Conversely, LCS-1 only inhibited SOD1 by approximately 15% and there was little difference between 0.1 and 100 μM LCS-1. **(D)** LCS-1 inhibition of SOD1 using the WST1 assay. Ten and 20 μM LCS-1 inhibited SOD1 by 22 and 35%, respectively but they were not significantly different from each other. Mean \pm SE, $n=4$ (nitro blue assay) or $n=3$ (WST1 assay) wells; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ versus SOD alone.