SUPPORTING INFORMATION

This section contains additional EPR spectral data, including the reaction of 4-thia-DL- and D-lysine with 5,6-LAM, data showing biphasic burst consumption of 4-thia-D-lysine in reaction with 5,6-LAM.

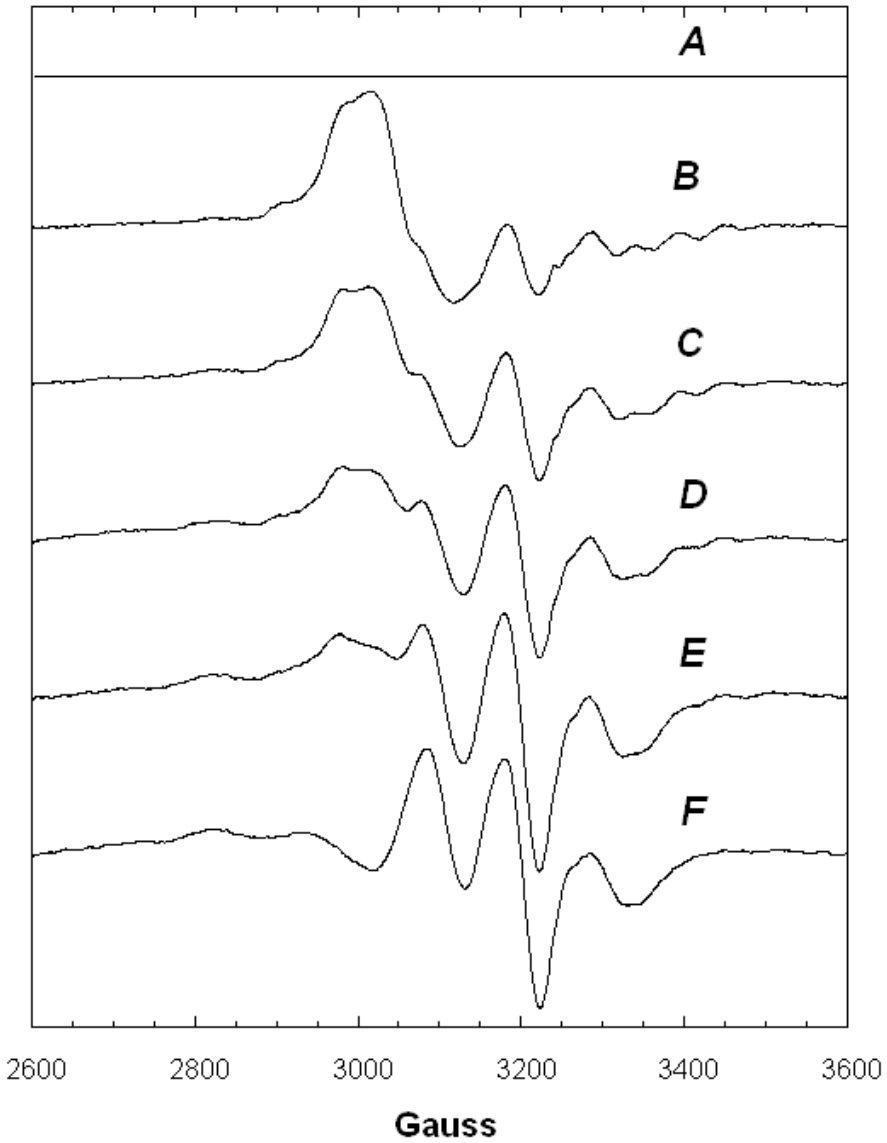


Figure S1. Time-dependent radical formation in the reaction of 5,6-LAM with 4-thia-DL-lysine. Spectra acquired at X-band (9.23-9.70 GHz) and normalized for intensity. The reaction mixture contained 20 mM 4-thia-DL-lysine, 120 μM PLP, and 50 μM 5,6-LAM in 0.25 mL of 100 mM K^+-EPPS buffer, pH 8.5. Reaction was initiated by addition of 120 μM adenosylcobalamin, and the samples were frozen at 77 K after various reaction times: **A**, before the addition of adenosylcobalamin; **B**, 10 s; **C**, 20 s; **D**, 30 s; **E**, 40 s; **F**, 2 min. All the EPR spectra were obtained at 77K. Instrument settings: microwave frequency, 9.1 GHz; modulation amplitude, 6.3 G; microwave power, 5 mW. The detailed procedures are described in Experimental Procedure.

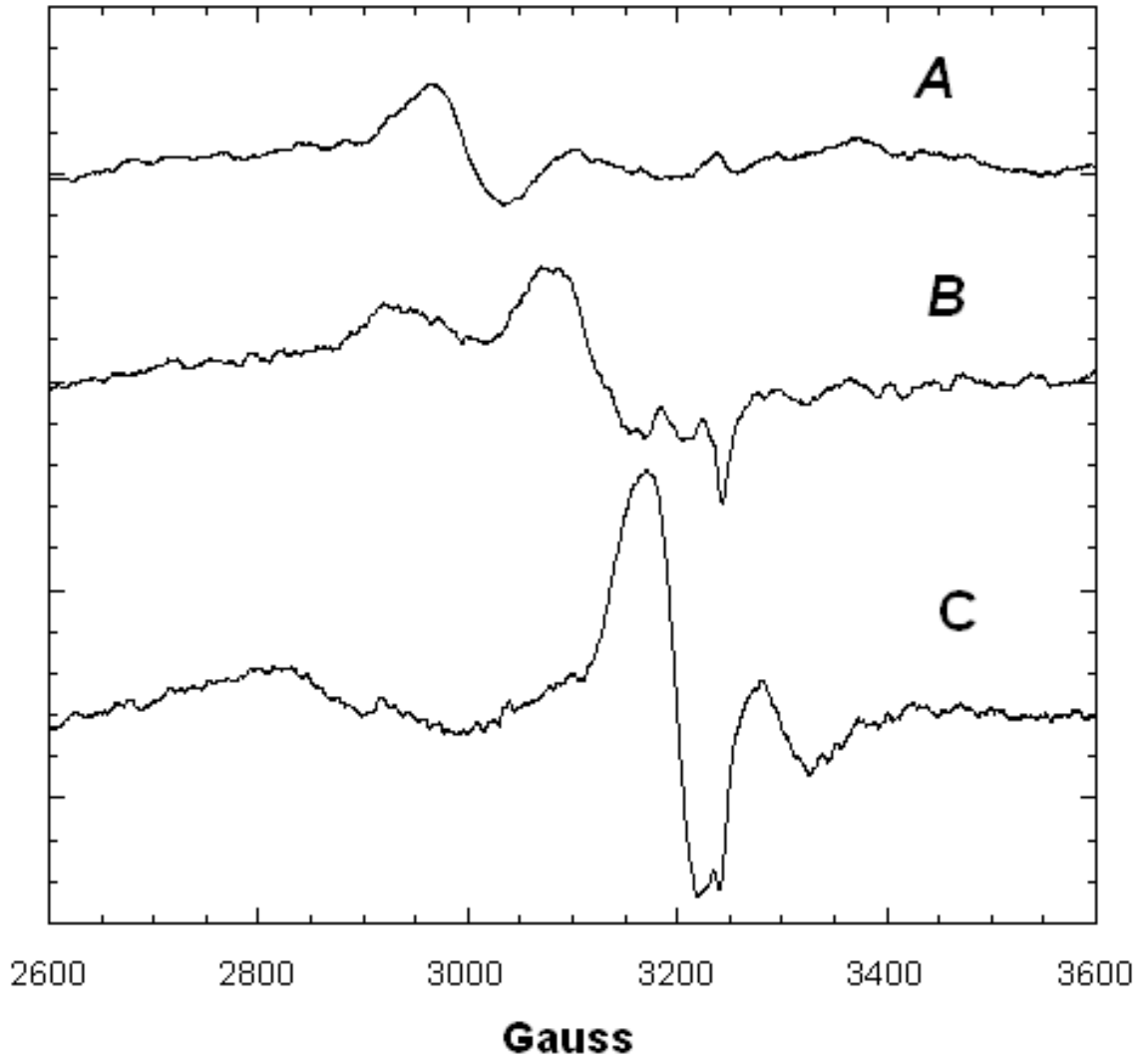
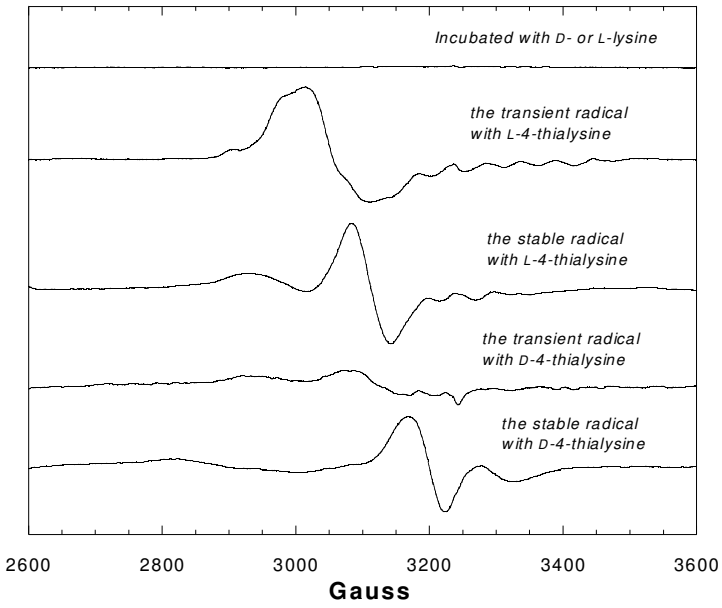


Figure. S2 EPR spectra of radical triplets in reaction of 4-thia-D-lysine with 5,6-LAM. Because of the fast rate of the reaction of 4-thia-D-lysine, samples were prepared for EPR analysis by the rapid-mix freeze-quench method. The spectra were recorded at **A**, 250 ms; **B**, 2 s; and **C**, 10 s. The samples were prepared using an Update Instruments Rapid mixing apparatus equipped with an Update Model 745 Syringe-RAM Controller. Prior to assembling the instrument for a run, the syringes and timing tubes were held in the Coy anaerobic chamber for two days. The enzyme, cofactors, substrate, and buffer were made anaerobic by repeated evacuation and purging with oxygen-free argon. Reactant solutions were drawn into the syringes in the anaerobic chamber and then moved to the rapid mix apparatus. One syringe contained 0.2 mM 5,6-LAM, 0.25 mM PLP, 20 mM 4-thia-D-lysine, and 0.1 M K^+EPPS (pH 8.5) in 1.0 mL, and the other contained 1.0 mL 0.25 mM adenosylcobalamin in 0.1 M K^+EPPS buffer (pH 8.5).. The mixed and aged samples were quenched in isopentane at –140 °C as described [Beinert, H., Hansen, R. E., and Hartzell, C. R. (1976) *Biochim. Biophys. Acta 423*, 339-355].

A



B

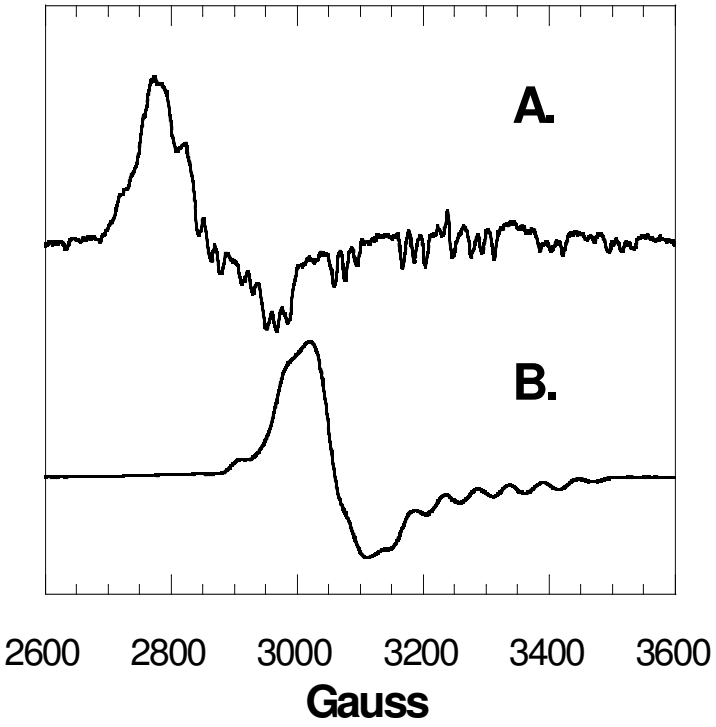


Figure S3. EPR spectra in reactions of 4-thia-D- and 4-thia-L-lysine with 5,6-LAM. **A**, Reaction of 5,6-LAM with adenosylcobalamin, PLP, and D- or L-lysine gives no EPR spectrum (top). Reactions of 4-thia-D- or 4-thia-L-lysine give different transient and persistent spectra. **B**, The upper spectrum is of cob(II)alamin bound at the active site of 5,6-LAM. The lower spectrum is of the transient cob(II)alamin-4-thia-L-lysine triplet.

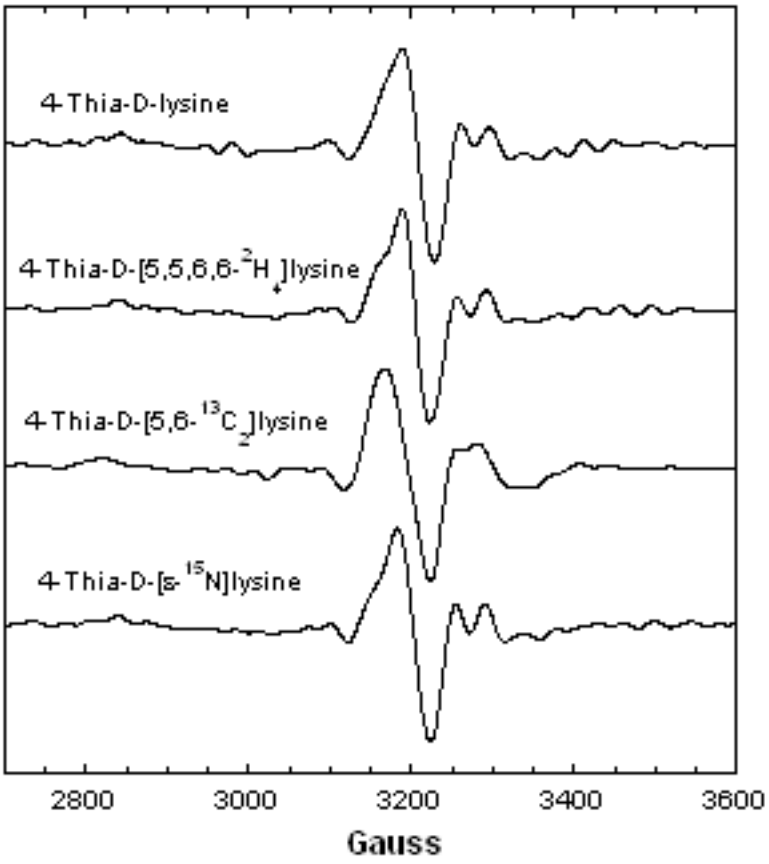


Figure S4. Isotope edited EPR spectra of the persistent radical with 4-thia-D-lysine. EPR spectra at 9.1 GHz of the persistent radical with 100 μM activated 5,6-LAM, adenosylcobalamin, and PLP reacting with 20 mM 4-thia-D-lysine without and with ^{2}H, ^{13}C, or ^{15}N-labeling.

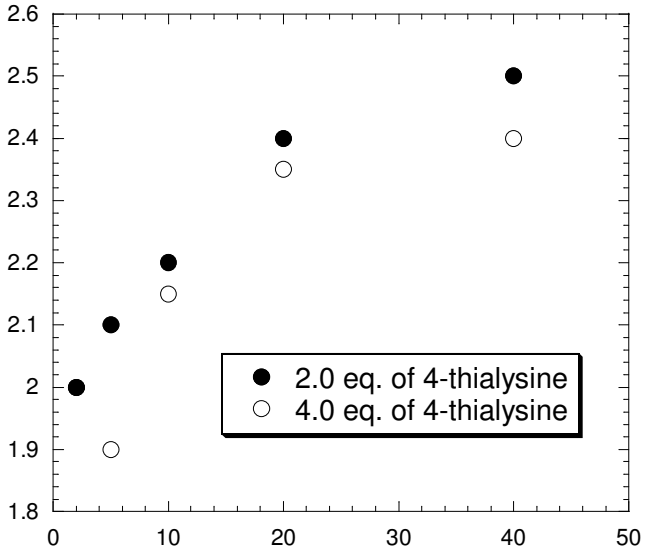


Figure S5. Reversibility of suicide inhibition by 4-thia-L-lysine.

The reaction mixtures consisted of 25 μM 5,6-LAM (9 IU mg^{-1}), 50 μM adenosylcobalamin, 25 μM PLP with 50 μM or 100 μM 4-thia-L-lysine for 30 min decreased the specific activity to 2 IU mg $protein^{-1}$. The solutions were diluted 25-fold and assayed for activity at 2, 5, 10, 20, and 40 min after dilution. The significance of the activity increases during 40 min after dilution is discussed in the text.

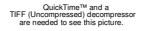


Figure S6. Biphasic decomposition of 4-thia-D-lysine in reaction with 5,6-LAM.

The reaction mixture consisted of 100 μM 5,6-LAM, 100 μM 4-thia-D-lysine, 200 μM PLP, 150 μM adenosylcobalamin, amd 100 mM EPPS buffer at pH 8.5. The reaction was initiated by addition of adenosylcobalamin. Aliquots were removed at indicated times and quenched in acidic solutions containing a known amount of D-lysine as an internal standard. Quenched samples were derivatized with PITC and analyzed by HPLC.