Supplemental Data

Experimental Procedures

Reagents- The following were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO): ampicillin, DEAE-Sephacel, Ultrogel AcA-44, β-mercaptoethanol, PLP, bovine serum albumin, succinyl-CoA, ALA-hydrochloride, α-ketoglutaric acid, α-ketoglutarate dehydrogenase, HEPES-free acid, MOPS, tricine, thiamine pyrophosphate, NAD^+, and the bicinchoninic acid protein determination kit. Glucose, glycerol, glycine, disodium ethylenediamine tetraacetic acid dihydrate, ammonium sulfate, magnesium chloride hexahydrate, and potassium hydroxide were acquired from Fisher Scientific (Pittsburgh, PA). Sodium dodecyl sulfate polyacrylamide gel electrophoresis reagents were acquired from Bio-Rad. *Xba* I, *Bam* HI restriction enzymes, Vent DNA Polymerase, and T_4 DNA ligase were from New England Biolabs (Ipswich, MA). Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA).

Mutagenesis- Site-directed mutagenesis for the S254A and S254T mouse ALAS variants was performed on the whole plasmid pGF23 using a previously described method (Miyazaki and Takenouchi, 2002). The mutagenic oligonucleotides for S254A and S254T were: 5'-GAG ACT GTT CAT <u>GCC</u> ATG GAT GGT GCC-3' and 5'-GAG ACT GTT CAT <u>ACC</u> ATG GAT GGT GCC-3', respectively, with the introduced codon substitutions underlined. The PCR-generated DNAs were sequenced between the *Blp* I and *Bam* HI restriction enzyme sites to confirm the presence of the intended mutation. The products were then digested with *Blp* I and *Bam* HI and subcloned into pGF23 digested similarly.

Steady-state kinetic characterization of ALAS variants. Steady-state activity of ALAS was measured at 30°C by coupling the production of coenzyme A to the reduction

of NAD^+ by α-ketoglutarate dehydrogenase and monitoring the increase in absorbance at 340 nm. The assay conditions were 20 mM HEPES, pH 7.5, 1 mM NAD^+, 3 mM $MgCl_2$, 250 μM thiamine pyrophosphate, 0.25 units of α-ketoglutarate dehydrogenase, 5-200 mM glycine, 1-30 μM succinyl-coenzyme A, and 5 μM of ALAS (or ALAS variants). To determine K_m and V_m values, the concentrations of the substrates were varied (in matrixes of six glycine and six succinyl-CoA concentrations) and the observed rates were fitted to the Michaelis-Menten equation using the nonlinear regression analysis software program SigmaPlot (Systat Software Inc.). Values of k_{cat}, k_{cat}/K_m^{Gly} and k_{cat}/K_m^{SCoA} were calculated by dividing the fitted values of V_m, V_m/K_m^{Gly} and V_m/K_m^{SCoA} by the concentration of the enzyme.

References

Miyazaki K., Takenouchi M. (2002) Creating random mutagenesis libraries using megaprimer PCR of whole plasmid. *Biotechniques* **33**, 1033-1034, 1036-1038.