Supporting online material

Directed evolution of mutant TyrRS enzymes. The general procedures for the positive and negative selections have been reported previously\textsuperscript{14}. Briefly, a combination of plasmid pBK-lib-m(14) and pBK-lib(7) was transformed into competent \textit{E. coli} DH10B harboring the plasmid pRep(2)/YC (12). The transformed cells were grown in 500 ml of GMML medium (1 × M9 minimal media with 1% glycerol, 0.3 mM leucine, 1 mM MgCl\textsubscript{2}, 0.1 mM CaCl\textsubscript{2} and 0.5% NaCl) containing 40 µg/ml tetracycline, 50 µg/ml kanamycin, 68 µg/ml chloramphenicol, and 1 mM 2 for 60 hours at 37°C. Plasmids (pBK) were purified from surviving cells and transformed into \textit{E. coli} DH10B harboring pLWJ17B3(7) to start the negative selection. Cells were then plated onto LB (Luria-Bertani) plates containing 40 µg/ml chloramphenicol, 50 µg/ml kanamycin, and 0.02% L-arabinose and incubated at 37°C for 8 hours. Plasmids pBK were purified from surviving cells and used for the subsequent positive and negative selections. After five rounds of positive and four rounds of negative selections, three candidate pairs of orthogonal tRNA-synthetases that conferred substrate-dependent chloramphenicol resistance were isolated and sequenced.

Expression and characterization of mutant myoglobin. DH10B cells containing pBAD/JYAMB-4TAG(12) and pS1-90 were grown in a 500 ml GMML culture containing kanamycin, tetracycline, 0.02% L-arabinose, 5 µM FeCl\textsubscript{3}, and 0 or 1 mM of 2. The cells were pelleted, lysed, and the proteins were purified by affinity chromatography with Ni\textsuperscript{2+}-NTA beads under native conditions. Proteins were analyzed by 12% SDS-PAGE and silver-stained. Aliquots of purified proteins were subject to high resolution mass spectrometric analysis. Matrix-assisted laser desorption ionization (MALDI) with a time-of-flight (TOF) mass spectrometer (Voyager DE-STR, Applied Biosystems, Foster
City, CA) was used to measure the molecular weight of the protein. Protein samples were desorbed and ionized upon irradiation from a 337 nm nitrogen laser. Sinapinic acid was used as the MALDI matrix. Lectin binding and glycosyltransferase reactions were carried out following the established protocols (22, 23).

*On-blot galactosyltransferase labeling assay.* Wild type myoglobin (1 µg) and Gly4→1 mutant myoglobin (1 µg) were resolved by 12% SDS-PAGE and transferred to a PVD membrane. The membrane was then treated with bovine milk galactosyltransferase (1 U), UDP-[H3]galactose (0.5 µCi) and calf intestinal alkaline phosphatase (1 U) for 24 hours at room temperature. After extensive washes, the membrane was exposed to X-ray film using Enhanced autoradiography. The result was shown as follows:

Only the glycomyoglobin was labeled; no detectable signal was observed for the wild-type myoglobin.

Acknowledgments

This work was funded by a grant from Department of Energy (DOE, DE-FG03-00ER45812) and a grant from NIH (GM44154).