SUPPLEMENTARY MATERIALS

Sulfide binding properties of truncated hemoglobins.

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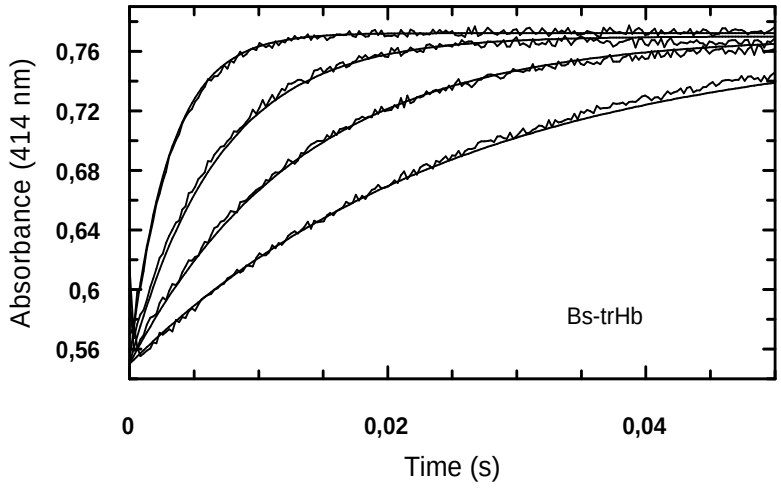
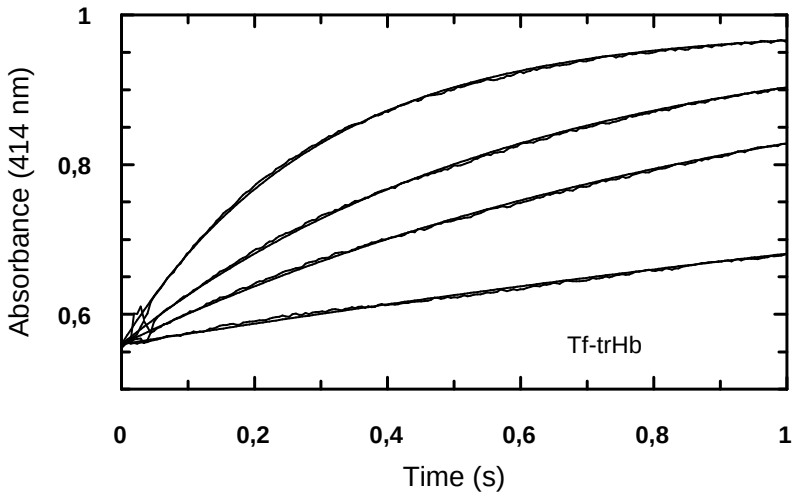
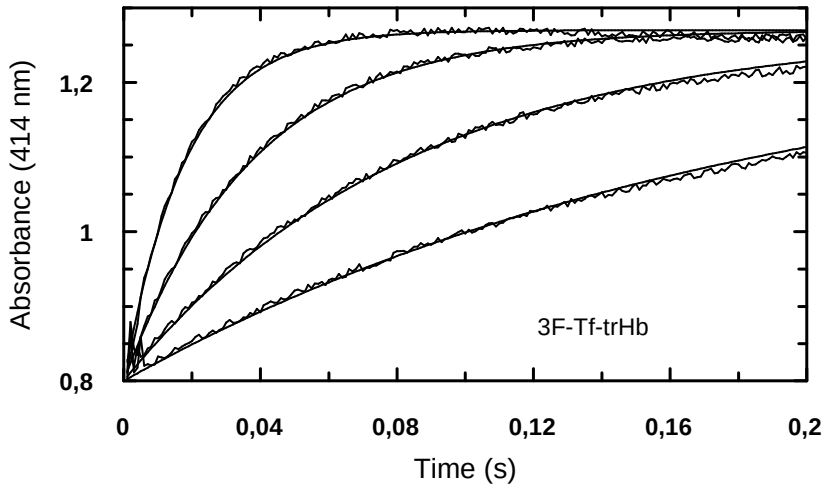
Azide binding kinetics

Azide binding kinetics were carried out by mixing the hemoglobin solutions (Bs-trHb, 6.2 μM; Tf-trHb, 7 μM; 3F-Tf-trHb, 9.5 μM) with increasing azide concentrations (from top to bottom, 1 mM, 0.5 mM, 0.025 mM and 0.125 mM), in 0.1 M phosphate buffer at pH 7.0 and 25 °C. Each curve was fitted to a single exponential with the standard software provided by Applied Photophysics. Second order plot yielded k_{N3}- values of $2.9\pm0.22\times10^{5}$ $M^{-1}s^{-1}$ for Bs-trHb; for $3.4\pm0.2\times10^{3}$ $M^{-1}s^{-1}$ Tf-trHb, $5.5\pm0.4\times10^{4}$ $M^{-1}s^{-1}$ for 3F-Tf-trHb.

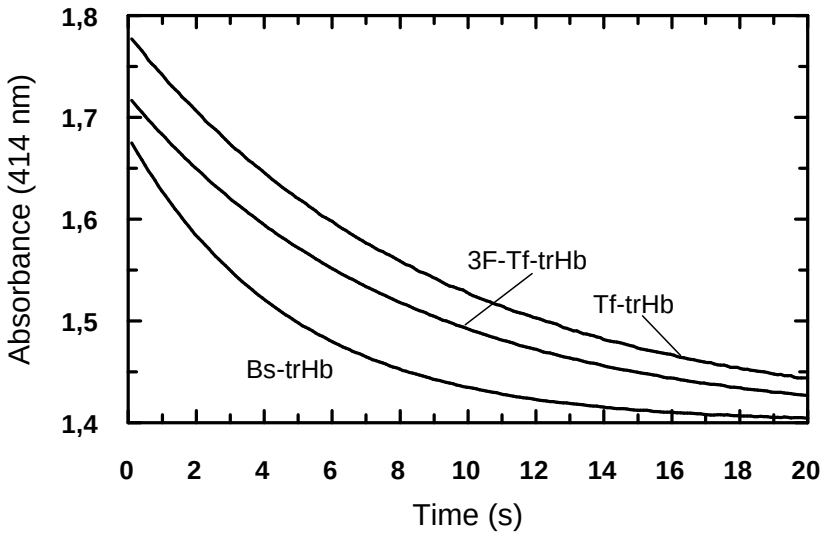






Azide dissociation kinetics

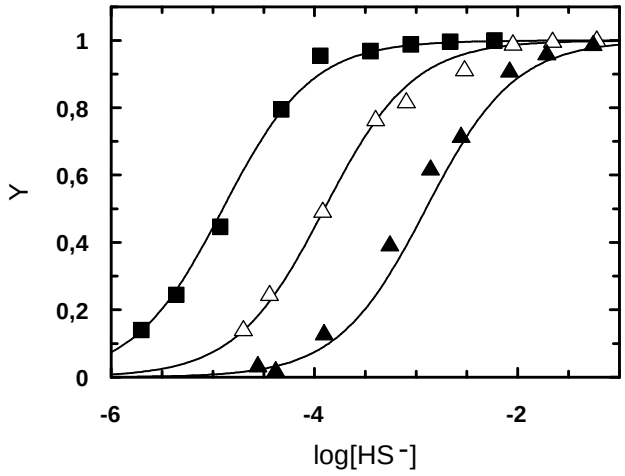
Proteins were saturated with 10 mM sodium azide and mixed with 1 mM sodium sulfide solutions in a stopped flow apparatus. The signal decrease at 414 nm (the peak of the azide adduct) is a first order process corresponding to azide release. Control experiments at 425 nm (not shown) reveal a rise in absorbance with identical time course. Experiments were carried out in 0.1 M phosphate buffer at pH 7.0 and 25 °C. First order rates rates were 0.21± 0.03 s^{-1} for Bs-trHb 0.11± 0.02 s^{-1} for Tf-trHb and 0.125 ± 0.02 s^{-1} for 3F-Tf-trHb.



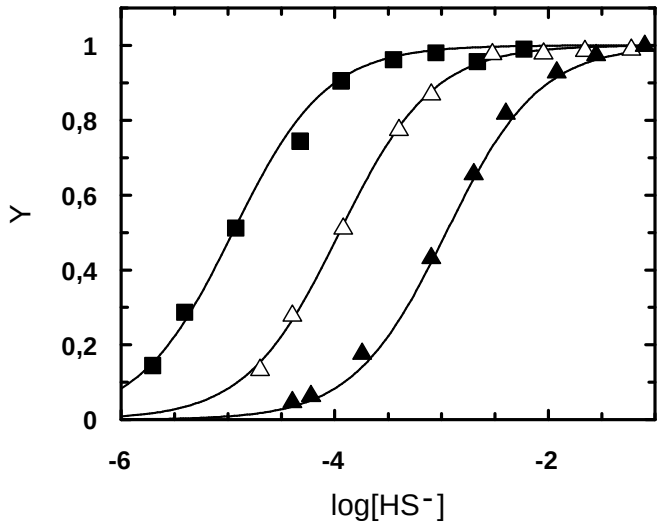
Azide/sulfide equilibrium displacement titrations

Protein solutions (3 ml, 10-12 μM) containing 1 mM (black triangles), 0.1 mM (white triangles) or 0.001 mM (black squares) sodium azide in 0.1 M phosphate buffer at pH 7.0 were placed in a 1 cm quartz cuvette in a HP 8453 diode array spectrophotometer equipped with magnetic stirring and a Peltier thermostatted cell holder. A few μl of a sodium sulfide nonhydrate stock solutions (10 mM or 0.1 mM) were added and the absorption spectrum was recorded after 5 minutes. Absorbance changes were transformed into fractional saturation by standard procedures. Experimental data sets relative to each protein were fitted simultaneously to simple ligand binding curves (see Materials and methods) by a least squares fitting method based on the Matlab program (South Natick, MA, USA).

Bs-trHb



Tf-trHb



3F-Tf-trHb