

The Heme Iron Nitrosyl Complex of MauG Reveals an Efficient Redox Equilibrium Between Hemes with Only One Heme Exclusively Binding Exogenous Ligands[†]

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EXPERIMENTAL PROCEDURES

General. MauG was isolated as described earlier (1). All reagents and solvents were purchased from commercial sources and were used without further purification unless otherwise noted. Nitric oxide (NO) was purchased from Sigma-Aldrich.

Preparation of Nitrosyl Complexes of MauG. Tris·HCl (50 mM, pH 7.5) buffer was made anaerobic by bubbling with argon over night. Sodium dithionite (8.7 mg) in a sealed vial was made anaerobic by repeated cycles of vacuum and flushing with argon. The dithionite stock solution was prepared by adding 1 ml of the anaerobic Tris-HCl buffer to the sodium dithionite vial using a gas-tight syringe. The dithionite concentration was determined by using potassium hexacyanoferrate(III) as reagent and methylene blue as indicator (2). Appropriate amount of dithionite was added to MauG under O₂-free conditions to reduce the enzyme.

Nitric oxide was introduced through a gas-tight syringe to the headspace of the quartz EPR tubes or UV-vis cuvettes containing the ferrous enzymes prepared under anaerobic conditions. An argon flush was maintained above samples to protect them from oxidation by O₂ and to minimize an anomalous EPR signal near $g = 2$ which derives from NO.

EPR Spectroscopy. The low temperature EPR spectra were recorded at 9.6 GHz frequency using a Bruker EMX spectrometer and a high sensitivity cavity at 9.38 GHz. Both spectrometers

were equipped with an Oxford Instruments liquid helium cryostat. Temperature was controlled by a digitalized Oxford temperature controller. A Bruker ER035M Gauss Meter was used during measurements to assist the *g*-value determination.

References Cited

1. Wang, Y., Graichen, M. E., Liu, A., Pearson, A. R., Wilmot, C. M., and Davidson, V. L. (2003) MauG, a novel diheme protein required for tryptophan tryptophylquinone biogenesis, *Biochemistry* 42, 7318-7325.
2. de Groot, D. C. (1967) Titrimetric determination of sodium dithionite with potassium hexacyanoferrate(III) as reagent and methylene blue as indicator, *Fresenius' Journal of Analytical Chemistry* 229, 335-339.