



Case Presentation

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FTIR Investigation of Ligand Photodissociation and Migration Dynamics in Inducible Nitric Oxide Synthase

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Abstract

Background: Inducible nitric oxide synthase (iNOS) is a heme enzyme that produces nitric oxide (NO) from L-arginine. Ligand dynamics within the heme pocket influence catalytic activity and product release.

Methods: A experimental manuscript framework is presented using Fourier transform infrared (FTIR) photolysis difference spectroscopy and temperature-derivative spectroscopy (TDS) to examine ligand behavior in the oxygenase domain of iNOS. Carbon monoxide (CO) and NO were used as spectroscopic probes of the heme environment at cryogenic temperatures.

Results: The study framework reproduces the principal observations reported for iNOS ligand dynamics: CO generated defined photoproduct states with characteristic infrared bands, whereas photodissociated NO did not produce comparably resolved trapped photoproduct bands. TDS indicated a dominant transient rebinding state rather than multiple stable secondary docking sites.

Conclusion: FTIR spectroscopy combined with cryogenic photolysis and TDS can provide quantitative information about ligand binding, migration, and rebinding in heme proteins.

Keywords: iNOS; FTIR spectroscopy; nitric oxide; carbon monoxide; ligand photodissociation; heme; temperature-derivative spectroscopy

Introduction

Inducible nitric oxide synthase is a homodimeric heme enzyme involved in nitric oxide production during inflammatory and immune responses. The oxygenase domain contains the heme active site, while the reductase domain provides electrons required for catalysis.

Infrared spectroscopy offers a sensitive method for examining ligand interactions with heme proteins. Carbon monoxide is particularly useful because its stretching vibration responds to the local electrostatic environment and can therefore serve as a probe of the active site. NO is physiologically relevant but presents additional experimental challenges because its infrared signal is weaker and its photochemistry differs from that of CO.

This manuscript follows the scientific organization and principal findings of the published FTIR study while replacing its author and institutional information with details.

Materials and Methods

The iNOS oxygenase domain was expressed in a bacterial expression system and purified by affinity chromatography using a standard recombinant-protein workflow. For spectroscopic measurements, purified protein

was prepared in a cryosolvent and supplemented, where indicated, with L-arginine, N-hydroxy-L-arginine, or tetrahydrobiopterin.

CO-ligated samples were prepared under a carbon-monoxide atmosphere following reduction of the heme iron. NO-ligated samples were prepared under oxygen-free conditions and exposed to NO. Samples were placed between calcium-fluoride windows in a cryogenic cell.

Infrared transmission spectra were recorded over the ligand-sensitive mid-infrared region. Photodissociation was induced with 532-nm laser illumination. Difference spectra were calculated from measurements obtained before and after illumination. Temperature-derivative spectroscopy was then used to monitor ligand rebinding as the sample temperature was increased.

Results

CO-bound iNOS displayed distinct heme-bound stretching bands with more than one active-site substate, consistent with structural heterogeneity within the heme environment. Addition of the H4B cofactor altered the distribution of CO stretching frequencies but did not eliminate the underlying heterogeneity.

After photodissociation, CO generated well-defined photoproduct bands within the protein matrix. These features are consistent with transiently trapped ligand orientations stabil by interactions with the surrounding protein.

In contrast, photodissociated NO did not produce comparably well-resolved photoproduct bands. The absence of sharply defined NO docking signatures suggests that NO occupies less specific transient configurations after photolysis, a behavior that may facilitate eventual release from the protein.

TDS analysis was consistent with a dominant kinetic rebinding pathway and did not reveal a series of stable secondary docking sites comparable to those described in some other heme proteins. The experimental framework also allows investigation of ligand-associated changes in the heme-thiolate environment.

Discussion

The contrasting behavior of CO and NO illustrates how ligand chemistry and protein environment jointly determine photodissociation and migration. CO can be stabil in defined orientations, making its infrared bands particularly informative about transient docking sites. NO appears to interact less specifically with the protein interior, potentially supporting efficient product release during catalysis.

FTIR photolysis difference spectroscopy is especially useful when combined with cryogenic trapping because protein and solvent motions are strongly reduced, allowing otherwise short-lived photoproduct states to be observed. TDS adds a kinetic dimension by monitoring the temperature dependence of rebinding.

These observations contribute to a molecular understanding of iNOS function and may inform studies of inhibitor binding, ligand recognition, and regulation of nitric oxide release.

Conclusion

FTIR spectroscopy and temperature-derivative spectroscopy provide complementary approaches for resolving ligand dynamics in iNOS. CO serves as a sensitive local probe of the heme environment, whereas NO displays less-defined photoproduct behavior. Together, these approaches offer mechanistic insight into ligand migration and rebinding in heme enzymes.

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