Mössbauer Studies of a Non-Heme Iron-Nitrosyl Center in a Biosynthetic Model of Nitric Oxide Reductases in Myoglobin Containing Zn-Protoporphyrin IX

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Introduction

Nitric oxide reductases (NORs) are a family of enzymes that play important role in N_2 cycle by catalyzing the reduction of nitric oxide to nitrous oxide ($2NO + 2H^+ + 2e^- \rightarrow N2O + H2O$) [1]. NORs active site consists of a heme and a non-heme Fe(II) (Fe_B) binuclear center [1]. Since they are large membrane proteins it is difficult to study their mechanism. Using biosynthetic protein design approach we have reported a functional and structural model of NOR, Fe_BMb1, by redesigning the distal pocket of a small, and easy to purify protein myoglobin (Mb) [2]. One critical barrier to study the mechanism is elucidating the electronic properties of Fe_B-nitrosyl complex due to the difficulty in obtaining pure FeB-NO complex owing to the interference from the high affinity heme site. To overcome this barrier, we have replaced the high affinity heme with isostructural zinc protoporphyrin IX (ZnPP) and studied the reconstitution of the Fe_B site and the resulting Fe_B-NO complex using structural and various spectroscopic studies.

Experimental

Samples were prepared at UIUC. Protein purification and ZnPP reconstitution, and $^{57}FeCl_2$ synthesis was done according to literature methods [3]. Mössbauer samples were prepared anaerobically in 50 mM Bis-Tris pH 7.3, by reconstitution of the Fe_B site of Fe_BMb1 using aliquots of ^{57}Fe(II) up to 1.0 eq., followed by formation of the nitrosyl complex using excess DEA NONOate as the NO donor. Final concentrations of the sample were ~4-5 mM. Mössbauer data were collected at various temperatures and various magnetic field strengths at the National High Magnetic Field Laboratory. Data were analyzed using a custom built software at Knox College.

Results and Discussion

The sample 57Fe-ZnPPFeBMb-NO contained two species, as shown in Figure 1. A HS S = 2 ferrous species (green lines) which is the starting material accounting up to 67% of the total area, and another HS S = 3/2 $\{FeNO\}^7$ complex (blue lines) accounting up to 33% of the total area. The parameters extracted from the fits are shown in Table 1.

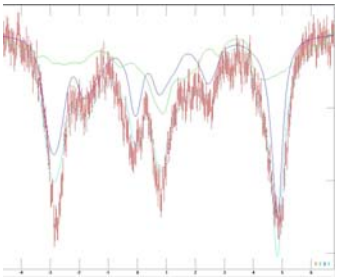

Figure 1. Mössbauer spectrum of Fe(II)-ZnPP-Fe_BMb1-NO. T = 4K.

Table 1. Parameters obtained from fitting of the Mössbauer data. The numbers in parenthesis indicate the unreacted starting material.

Sample	δ_{Fe} (mm/s)	ΔE_Q (mm/s)	%	η	D (cm^{-1})	E/D	S
^{57}Fe-ZnPPFe$_B$Mb1-NO	0.69 (1.12)	–1.91 (–2.68)	33 (67)	0.30 (1.0)	10.6 (4.7)	0.065 (0.27)	3/2 (2)

Conclusions

We have been able to obtain the S=3/2 $\{FeNO\}^7$ non-heme iron nitrosyl complex in engineered Mb protein ^{57}Fe-ZnPPFe$_B$Mb1-NO. However, low occupancy (33%), and poor signal to noise calls for re-preparing the samples at higher concentrations and collect data for the new samples.

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References

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