# STRUCTURAL MOBILITY IN HUMAN MANGANESE SUPEROXIDE DISMUTASE

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## Introduction

Human manganese superoxide dismutase (MnSOD) is a homotetramer of 22 kDa subunits, containing dimeric and tetrameric interfaces, that catalyzes the conversion of superoxide anion radicals to $O_2$ and $H_2O_2$ [1]. We have investigated conformational mobility at these interfaces by measuring H/D exchange kinetics and $^{19}$F NMR spectra, both being excellent methods for analyzing local environments and side chain conformational flexibility in proteins. We have prepared human MnSOD in which all nine tyrosine residues in each subunit are replaced with 3-fluorotyrosine. The $^{19}$F NMR spectrum of this enzyme showed five sharp resonances that have been assigned by site-specific mutagenesis to replace each 3-fluorotyrosine with phenylalanine. We have used $^{19}$F NMR to estimate conformational mobility at the dimeric and tetrameric interfaces of fluorinated MnSOD, and mass spectrometry to determine rates of H/D exchange kinetics in MnSOD. Taken together, these studies show that residues at the dimeric interface, such as Tyr169, have significantly less conformational freedom or mobility than do residues at the tetrameric interface, such as Tyr45.

## Experimental

The $^{19}$F NMR spectra of fluorinated samples were recorded on a Bruker Avance 500 spectrometer. A $^{1}$H 5mm TXI probe tuned for $^{19}$F at 470 MHz was employed. Temperature was varied from 17$^{o}$ C to 62$^{o}$ C by the flow of heated nitrogen gas. Spectra were acquired by averaging 4000 scans with a scan rate of 8000 per hour.

## Results and Discussion

The $^{19}$F labels in fluorinated MnSOD serve as conformational probes of the enzyme and its interfaces. Five sharp and distinct $^{19}$F resonances were observed for fluorinated MnSOD, all for side chains greater than 13 Å from the paramagnetic manganese. These were assigned by site-specific mutation replacing individual 3-fluorotyrosine residues with Phe. The four 3-fluorotyrosine residues we were unable to detect (residues 34,165,166,176) are buried and within 9 Å of the paramagnetic manganese; we assume this caused sufficient broadening that we cannot observe them. We emphasize here the properties of fluorinated Tyr45 and 169 which are located in the tetrameric and dimeric interfaces respectively. Tyr45 showed a large increase in chemical shift as temperature increased, moving toward the position of the $^{19}$F peak for denatured enzyme, and showed a large decrease in linewidth as temperature increased. These features characterize a region at the tetrameric interface with increased conformational and dynamic mobility as temperature increases. The $^{19}$F resonance of fluorinated Tyr169 was notable because it did not change appreciably in linewidth or chemical shift over the temperature range from 17 $^{o}$ to 57 $^{o}$C.

## Conclusions

The data appear best interpreted that motional freedom upon increasing temperature is much more apparent for fluorinated Tyr45 at the tetrameric interface that for fluorinated Tyr169 at the dimer interface. This conclusion is also reached from the H/D exchange experiments showing residue 45 of human MnSOD in a region of very rapid exchange and with a deuteration level > 90% in the experiments. Taking residue 45 as a reporter for the tetrameric interface, the data indicate differential conformational effects with the region of the tetrameric interface having more conformational mobility than that of the dimeric interface.

## Acknowledgements

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## References

[1] Hsu, J.-L., *et al.*, Journal of Biological Chemistry, **271**, 17687-17691 (1996).