FLUORINE-19 NMR REVEALS REGIONS OF THERMAL STABILITY IN HUMAN MANGANESE SUPEROXIDE DISMUTASE

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Introduction

Fluorine spin labeling of proteins allows for the observation of localized nuclei within a polypeptide. Fluorine spectra are typically highly resolved compared to the very broad multi-peaked proton spectra of the same compound. ^{19}F is in 100% natural abundance and is a ½ spin nucleus. We sought to identify resonances for the 3-fluorotyrosines within manganese superoxide dismutase (MnSOD). Human MnSOD is a homotetramer with 22 kDa subunits. The structure is a dimer of dimers showing a dimeric interface between subunits and a tetrameric interface between dimers. We have shown that a reporter tyrosine at the dimeric interface is much less affected by increasing temperature than a reprter tyrosine at the tetrameric interface. These experiments take advantage of a large body of research in the literature on the NMR properties of fluorine-labeled biological molecules [1]. There is a high sensitivity of fluorine chemical shifts to structure of proteins (up to 8 ppm); this is in contrast to the range of proton chemical shifts of ± 0.3 ppm under similar situations [1].

Experimental

^{19}F labeled MnSOD was expressed in SOD-deficient *E. coli* QC774. Growth media contained 3-fluorotyrosine purchased from ICN Biomedicals Inc. Protein was expressed in the soluble fraction and purified by anion exchange chromatography. Amino acid analysis and MALDI-TOF mass spectrometry were used to estimate that 80% of all tyrosines were replaced with 3-fluorotyrosine. There is no evidence that fluorine becomes incorporated at other positions to a measurable extent. The protein crystallized readily and a crystal structure at 1.8 X resolution showed the positions of the fluorinated tyrosine residues. The structures of the fluorinated and unfluorinated enzymes were closely superimposable with a root-mean-square deviation in all Cα atoms of 0.3 X. Seven site-directed mutants were made: Y9F/Y11F, Y34F, Y45F, Y166F, Y169F, Y176F, Y193F. Wild type constructs contained in a pTrc99A plasmid were used as a template for mutagenesis. All mutants were expressed in QC774 E. *coli* cells that do not express MnSOD. Spectra of ^{19}F labeled samples were collected on a Bruker Avance 500 MHz Spectrometer using a ^{1}H 5mm TXI probe tuned to 470 MHz for fluorine resonance measurements. Sample concentrations were 1 mg/ml in 20 mM phosphate pH 7.8. Temperature effects were measured incrementally in five degree K units from 290 to 335.

Results and Discussion

^{19}F NMR has been successful in identifying resonances with different responses to an increase in temperature from 17 ^{o}C to 62 ^{o}C in wild-type MnSOD in which all nine tyrosines in each of the four subunits are replaced with 3-fluorotyrosines. These spectra identify regions of the enzyme that respond differently to an increase in temperature. In particular, Tyr 169 showed very little change in either chemical shift or line width while Tyr 45 showed large changes over the temperature range studied. Tyr 169 is located along the dimeric interface and Tyr 45 crosses the tetrameric interface. We conclude that there is significantly greater structural and dynamic change at the tetrameric interface and very little at the dimeric interface, as measured by these reporter tyrosines, when temperature increases. These results enhance our understanding of catalysis and function of MnSOD, in particular the role of the subunit interfaces and why the enzyme is a tetramer.

Acknowledgements

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References

[1] Lau, E. Y. and Gerig, J. T. Origins of fluorine NMR chemical shifts in fluorine-containing proteins. *J. Am. Chem. Soc.* **122**, 4408-4417 (2000).