Structural Study Of Human Peroxiredoxin 6

W. Tang (UF, Biochemistry and Molecular Biology); A. Kuznetsova (UF, Chemistry); S. Feinstein (UPenn, Med School); A. Fisher (UPenn, Med School); J.R Long (UF, Biochemistry and Molecular Biology)

Introduction

Peroxiredoxin 6 (Prdx6) is a 25kDa bifunctional enzyme that plays a key role in lung anti-oxidant defense. Since phospholipids are a major structural component of all cell membranes, their peroxidation represents a severe threat to cellular integrity. Prdx6 can directly reduce phospholipid hydroperoxides (PLOOH) through its peroxidase activity to the much less toxid hydroxyphospholipid (PLOH), which is further reduced to phospholipid (PL). This reaction is important for preventing the chain reaction of lipid peroxidation and for membrane repair following an oxidative stress. Our previous studies have shown that Prdx6 also has phospholipase A_2 (PLA_2) activity. This bifunctionality as well as the use of glutathione instead of thioredoxin as the physiological reductant distinguishes it from 2-Cys Prdxs. It was proposed that binding of the phosphorylcholine head group of the peroxidized phospholipid to a surface docking site (H26, S32, W33) that overlaps with phospholipase catalytic site (H26, S32, D140) results in insertion of the peroxidized sn-2 fatty acid into the hydrophobic pocket of peroxidase catalytic site generating proximity of peroxide group to conserved C47 that is buried at the base of hydrophobic narrow pocket and is a part of triad essential for the peroxidatic function of the enzyme [1, 2]. However, at the molecular level, the exact mechanisms of phospholipid binding and enzymatic reaction remain unknown. Hence, it is our primary interest to study the conformation of Prdx6 by means of high resolution NMR, and hopefully we will be able to gather enough information to answer the question.

Experimental

All the Prdx6 samples are expressed and purified at UPenn, and then transferred to UF for NMR experiments. Our previous studies have demonstrated that Prdx6 is stable over a pH range of 5.5-8. In order to have optimal NMR sensitivity and physiologic relevance to the pH environment of the plasma membrane:cytoplasmic interface, we have decided to maintain our sample at pH 6. All the NMR experiments are done on a 600MHz magnet with a cryoprobe at AMRIS facility.

Results and Discussion

A ^{1}H-^{15}N TROSY spectrum collected for a uniformly ^{15}N labeled Prdx6 sample at pH6 shows 221 distinguishable resonances. The dispersion of the peaks is consistent with a well-folded protein. Assignment of all the peaks will be our next focus.

Conclusions

Our preliminary experiments have proven the feasibility of high resolution NMR in our study of Prdx6. We are currently recording 2D/3D NMR spectra on uniformly 13C{15N} labeled Prdx6 samples for full assignment and the structural calculation.

Acknowledgements

NHMFL user program and NIH R01 HL102016.

References

[1] Fisher, A.B., *et al*., Antioxid. Redox Signal, **15**, 831-(2011).
[2] Choi, H.J., *et al*., Nat. Struct. Biol., **5**, 400-406 (1998).

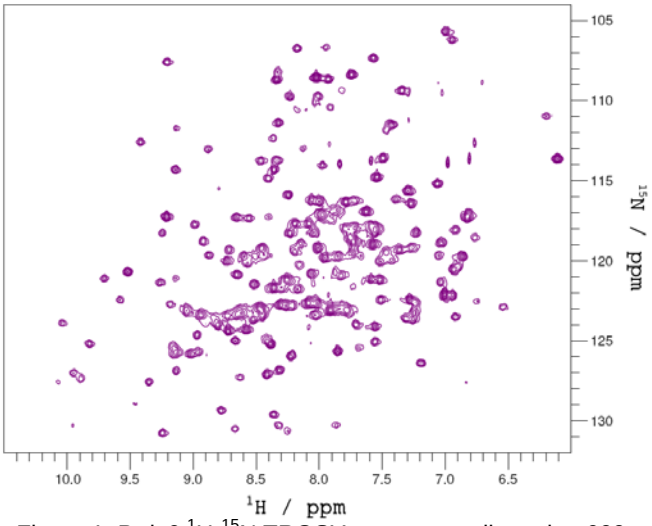

Figure 1. Prdx6 ^{1}H-^{15}N TROSY spectrum collected at 600 MHz using ~1 mg of protein and a 5 mm cryoprobe.