

SUMMARY AND CONCLUSION

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In this thesis, the results on structure-function relationship of lipoxygenase 1 from soybean have been presented. The solution conformation of the enzyme has been followed by various spectroscopic methods and activity measurements. The thermal stability of the enzyme has been followed by thermal inactivation kinetics and mid point of thermal inactivation temperature. The study has led to the following conclusions.

1. LOX 1 used in this study was essentially purified by the method of Axelrod et al (1981). Introduction of an additional step in the purification improved the specific activity from 110 to 150 units mg^{-1} protein. The purity of the preparation was ascertained by SDS-PAGE, and sedimentation velocity measurements. The homogeneity of the preparation was exceeded 95% and it has a sedimentation coefficient of 5.0 ± 0.2 S.

2. The enzyme has been found to be rich in hydrophobic amino acids, specially the aromatic amino acids. The ratio of hydrophilic amino acids to hydrophobic amino acids was 1.14. The average hydrophobicity index, according to Nozaki and Tanford scale being 1.14 kcal. The hydropathy plot suggested the presence of hydrophobic clusters in the molecule and the grand average hydropathy value of -3.2 would explain its solubility behaviour. Of the two domains, the N-terminal domain being more hydrophobic.

3. The overall folding of the molecule was compact at pH 9.0 with the accessibility of cysteine and tryptophan residues being minimal. The

tryptophan residues were in a hydrophobic environment. The fractional accessibility of fluorophores for dynamic quenchers like potassium iodide and acrylamide were low.

4. The solution conformation of the enzyme was characterized by the presence of 29% α -helix, 14% β -bend, 12% β -sheet and 4-5% aperiodic structure, and found to be in good agreement with the recently reported X-ray structure and secondary structure presented based on amino acid sequence data. The fine structure of the enzyme was characterized by well resolved near UV CD bands at 293, 288, 283, 277 and 266 nm.

5. The non-ionic surfactants like Brij 35, Tween-20 and the anionic surfactants cholic acid, deoxycholic acid and taurocholic acid enhanced the relative enzyme activity upto 200% at low concentrations and at higher concentrations they reversibly inhibited the enzyme activity. The inhibition caused by SDS and CTAB were irreversible. The inhibition of the enzyme activity followed the order CTAB > Tween-20 > SDS > Brij-35 > DOC > taurocholic acid > cholic acid. The changes in the kinetic behaviour of the LOX 1 in different surfactant solutions could be explained in terms of altered V/K ratio.

6. Brij-35, DOC, and cholic acid induced α -helix formation at higher concentration; SDS and taurocholic acid induced the formation of aperiodic structure at high concentrations; Tween-20 and CT AB promoted the β -structure. Aromatic chromophores were perturbed to different extent in these surfactant solutions.

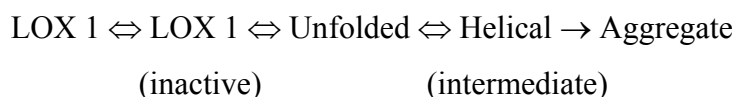
7. LOX 1 got inactivated irreversibly at 54°C at pH 9.0; 44°C being the midpoint of thermal inactivation temperature. The kinetics of thermal inactivation followed the first order kinetics between 36°C and 46°C. The irreversible inactivation of the enzyme involved the oxidation of cysteine residues resulting in intermolecular cross-linking. The non-ionic surfactants, Brij-35 and Tween-20 enhanced the thermal stability by strengthening the hydrophobic interaction around the cysteine residues.

8. Monohydric alcohols inhibited the activity of LOX 1. The inhibition was reversible and of mixed type. Monohydric alcohols increased K_m and decreased V_{max} . The state of the substrate did not affect the type and extent of inhibition. The inhibition by monohydric alcohols could be directly attributed to the binding of alcohols at the substrate binding/catalytic site.

9. Monohydric alcohols at higher concentrations induced more ordered structure in the enzyme molecule. The equilibrium constants obtained for native to helical intermediate(s) were in millimolar range and increased with carbon chain length of the alcohols. The free energy change for the transition was small, but positive in nature.

10. Alcohols decreased the thermal stability of LOX 1. The activation enthalpy (ΔH^*) and activation entropy (ΔS^*) of thermal inactivation increased in presence of alcohols.

11. The following minimal scheme has been proposed to account for the interaction:



12. The unfolding of LOX 1 by urea and GdmCl was characterized by non-superposable equilibrium transition curves for (i) enzyme activity; (ii) ellipticity values at 222 nm; and (iii) relative fluorescence intensity at 332 nm. The transition curves have more than one plateau region suggesting the presence of intermediates in the unfolding. Kinetic measurements supported the presence of stable intermediates during unfolding.

13. Upto 1 M urea concentration, there was no significant loss in activity although loss in secondary structure was ~20%. At 4.0 M urea concentration, there was complete loss of activity with a midpoint concentration of 2.5 M urea.

14. The loss in secondary structure was biphasic. The first transition had a midpoint concentration of 1.2 M, while the second transition which was completed at 8.0 M had a midpoint concentration of 3.5 M urea.

15. The changes in relative fluorescence intensity and shift in emission maximum were completed at 8.0 M urea. The Stern-Volmer constant for acrylamide and potassium iodide did not change upto 4.0 M urea, but increased at higher concentrations.

16. The reactivity of the sulfhydryl groups to Ellman's reagent increased during the course of unfolding.

17. The unfolding was irreversible and complex because of the multi-domain nature. The apparent irreversibility could be related to aggregation during unfolding which precluded the determination of thermodynamic parameters.

18. LOX 1 molecule was stable in the pH range 4-10. The irreversible inactivation above pH 10 was due to the oxidation of cysteine residues. The irreversible inactivation below pH 4.0 was due to irreversible conformational change in the molecule. LOX 1 was more stable in acid pH.

19. There were thirteen tryptophan residues in the LOX 1 molecule. Under non-denaturing conditions, the accessibility of tryptophan residues was pH dependent and at optimum pH of enzyme activity (pH 9.0) only one tryptophan residue was accessible; with the decreasing pH from 9 to 2, the accessibility increased.

20. Modification of the accessible four tryptophan residues, under non-denaturing conditions at pH 4.0, with N-bromosuccinimide resulted in complete loss of activity, modification of one of the residues being critical for activity.

21. Modification of tryptophan residues did not affect the substrate binding affinity. Presence of substrate during modification did not affect the extent of modification. The kinetic lag in the fatty acid oxidation was enhanced due to modification.

22. Modification of the surface exposed tryptophan residues did not affect the structural integrity and conformation of the enzyme molecule.

23. The apparent stability of the enzyme molecule decreased due to modification of accessible tryptophan residues.

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