SUMMARY AND CONCLUSIONS

The salient features of this investigation are summarized as follows

1. Horsegram has been identified to be the richest source of lipoxygenase activity reported in plants so far. Horsegram seed has 1800 U/g of lipoxygenase activity with a specific activity of 37 U/mg compared to 150 – 350 U/ g in the case of other plant sources. Soyabean, the richest source of lipoxygenase activity so far, has 350 U/ g.
2. The lipoxygenase activity from horsegram is highly thermostable. The activity could be extracted with sodium phosphate buffer, (pH 6.0). The extract, after centrifugation to recover the supernatant, retained 50% activity after heating it for 100 min at 90°C.
3. Horsegram has at least two proteins with lipoxygenase activities – HGLOX1 and HGLOX2. Both these proteins exhibit characteristics that are unusual.
4. HGLOXI could be purified to homogeneity by conventional chromatography using gel filtration and ion exchange chromatography to a final yield of 25% and 4.1 fold purity. The protein has a specific activity of 154 U/ mg protein and a molecular weight of 110, 000.
5. HGLOX1 activity in horsegram was unusual compared to other plant lipoxygenases in several characteristics:
    - i The protein is a multisubunit protein – a dimer of two unidentical subunits as determined by mass spectrometry. All other plant lipoxygenases are reported to be single polypeptide chains.
    - ii The pH optimum is in the acidic range of 4 – 5.0. Only potato lipoxygenase is reported to have an acidic pH optimum.

iii The enzyme is highly thermostable retaining 50% activity on incubation at 85.5°C for 30 minutes. Enthalpy (ΔH^*), free energy ΔG^* and entropy ΔS^* of thermal inactivation was 49 kcal.mol^{-1}, 25.8 kcal.mol^{-1} and 66.1 cal.mol^{-1}.K^{-1} respectively at 78°C.

iv HGLOXI was found to have weak cooxidizing activity for β-carotene and was inhibited by classical lipoxygenase inhibitors like NDGA and ETYA.

v 4-nitrocatechol, an iron chelating substance did not inhibit lipoxygenase activity.

vi The protein has manganese as the prosthetic group and is devoid of iron.

vii The protein has no cross- reactivity with antibodies raised against soya lipoxygenase -1.

viii HGLOXI was rich in β-structure and did not match with other plant or animal lipoxygenases. The near UV CD structure did resolve well.

ix The amino acid sequence and N- terminal sequence have no similarities to the reported sequences of plant or animal lipoxygenases.

x The amino acid composition, N-terminal sequence, mass spectra, haemagglutination activity, presence of manganese and carbohydrate contents confirmed the identity of HGLOX1 as seed lectin.

xi The lectin characteristics of the protein were very similar to that reported earlier. Haemagglutination activity of the protein is 45 U/ mg protein. The lectin activity is inhibited by N-acetyl galactosamine – an inhibitor of haemagglutination activity of *Dolichos biflorus* lectin.

xii The lectin and LOX activity were located in different loci of the molecule. However, the fractionated subunits exhibit only weak LOX activity (6- 8 U/ mg protein) and haemagglutination activity.

5. The second lipoxygenase activity in horsegram, HGLOXII could be purified to homogeneity by gel filtration and affinity chromatography to a final yield of 0.4% and a specific activity of 22 U/ mg protein. The molecular weight of the protein was 34 kDa as determined by SDS-PAGE and size exclusion HPLC.
6. HGLOXII resembled plant lipoxygenases in several aspects.
   - i It is a monomeric protein active in the acidic pH range 4.5 – 5.
   - ii It had iron (0.48 μg/ mg protein) as the prosthetic group like other plant lipoxygenases.
   - iii The activity could be inhibited by NDGA and 4-nitrocatechol – classical lipoxygenase inhibitors.

7. HGLOXII had several unusual features that differed from other plant lipoxygenases.
   - i It is rich in β-structure unlike other lipoxygenases. The near UV CD spectra is not well resolved.
   - ii The products of the reaction with linoleic acid are 9 and 13-hydroperoxides in the ratio 25: 75, respectively.
   - iii The enzyme is highly thermostable with a midpoint for thermal inactivation at 74°C.
   - iv The amino acid composition did not match with any reported lipoxygenases. The N-terminal sequence of the protein had no cross reactivity with antibodies raised against soy lipoxygenase 1.