Summary, Concluding Remarks and Future Prospects
SUMMARY

The results presented in this thesis can be summerized as follows:

1. It has been shown that the ARSs from both EW and RL are capable of catalyzing a time and enzyme concentration dependant release of PPI.

2. The control RL enzyme is less active (20-30%) compared to the EW enzyme.

3. The PPI release activity of the enzymes from both the systems has been shown to be independant of the presence or absence of aminoacids.

4. tRNA on the other hand has a profound effect in the RL system and brings about a time dependant increase in PPI release which is not inhibited by fraction C.

5. No such tRNA dependant increase in PPI release is seen in the earthworm system.

6. The isolated inhibitor (fraction C) does not bind to the active ARSs from RL.

7. On the contrary, it binds to E.coli tRNA and reduces its aminoacid acceptor activity to 50%

8. The low aminoacid acceptor activity (25%) of the EW tRNA is not abolished by extensive dialysis, whereas heat denaturation, renaturation
and dialysis increases the aminoacid acceptor activity of the EW tRNA (60-65%).

9. Similar results are obtained in a 'mixed tRNA assay' where the inhibitory activity of the EW tRNA is measured.

10. The earthworm enzyme seems to slowly regains its aminoacylation activity upon gel filtration on Sephadex G-100. The spectral properties and RNA content of the separated peaks show the presence of a 'nucleic acid' component in them. No such component is seen in the rat liver enzyme.

11. It has been shown that polyacrylamide gel electrophoresis is unable to separate the inhibitor from the enzyme fraction.

12. It has been shown that the inhibitor is not localized in any one of the subcellular fractions of the EW, namely nuclei, mitochondria, microsome, ribosome or the supernatant.

13. The inhibition shown by the earthworm subcellular fraction in a 'mixed enzyme assay' was not an artifact was proved by the noninhibitory effect of the subcellular fractions from the control RL system.

14. The results obtained with the size exclusion chromatography has shown that fraction C is not a pure compound but is a mixture of heterogenous molecular weight components.
15. The mixture seems to contain a range of compounds ranging in molecular weight from 730-1490 with 2 predominant molecular species with molecular weight 1240 and 890.

CONCLUDING REMARKS AND FUTURE PROSPECTS

The results presented in this thesis, together with previous work from this laboratory have shown the presence of a potent endogenous aminoacylation inhibitor in earthworm *Lampito mauritii*. This inhibitor which copurifies with ARSs was shown to be a small molecular weight, heat stable, organic molecule. The present study on molecular exclusion chromatography confirms the molecular weight (730-1490) and heterogeneity of the compound earlier observed [Sivaram, 1985].

The studies on the site of action of the inhibitor, clearly indicate catalytic activity of the enzyme in the first partial reaction of aminoacylation there by suggesting the second partial reaction to be the site of inhibition. The observation, that the tRNA dependent induction of the PPI release is not affected by the inhibitor, indicates that the inhibitor does not prevent the tRNA - enzyme interaction. These results suggest that the inhibitor may not act at the level of tRNA binding but may play a crucial role at a post tRNA binding sequence of aminoacylation.

The lack of affinity of the inhibitor for the ratliver enzyme, but its high affinity for *E.coli* tRNA, and restoration of aminoacylation capacity to otherwise inactive earthworm tRNA, upon release of the inhibitor, confirms the
fact that the inhibitor acts by binding to the tRNA rather than the enzyme. This data is in confirmation with the earlier observation that, tRNA at very high concentration competes out the inhibition. Analysis of the above results suggests that the inhibitor may bring about its action either by binding to the common CpCpA sequence at the 3' end of the tRNA or it may bind to a crucial nucleotide sequence of the tRNA needed for aminoacylation in general.

The high affinity of the inhibitor for the tRNA, and its lack of affinity for the ARSs, suggest that in earthworm system the copurification of the inhibitor with the enzyme fraction, may be due to the coelution of the inhibitor bound tRNAs at this salt concentration.

The requirement of drastic treatment, like heat denaturation, for the release of the inhibitor from the tRNA, explains the difficulties encountered in the preparation of an inhibitor free enzyme. Analysis of the peaks obtained, after gel filtration on Sephadex G-100, suggests that this technique in the long run may help in the preparation of an active enzyme, free of the inhibitor. The data on spectral properties and RNA content of the peaks indicate that the earthworm enzyme is associated with a 'nucleic acid', which coelutes with the high, medium and low molecular weight components.

The failure to localize the inhibitor to any subcellular fraction indicates that the inhibitor may not be compartmentalized in vivo. More work is needed in this direction to clarify this aspect. Obviously an inhibitor at the level of translation and at the very first step of protein biosynthesis would be of great interest for further research which would include purification and chemical characterization of the inhibitor.