5.0 SUMMARY

Buffalo plasma $\alpha_2$M was purified to an apparent homogeneity by ammonium sulphate fractionation and gel filtration chromatography. The protein was purified 35-fold with an yield of about 61%. The molecular weight determined by gel filtration and SDS-PAGE was 660 KDa. SDS-PAGE in presence of thiol reductant dissociated the protein into quarter subunits with a molecular weight of 165 KDa. The stokes radius of buffalo $\alpha_2$M calculated from gel filtration data was 85$^0$ Å.

The purified buffalo $\alpha_2$M migrated as a single band on polyacrylamide gel electrophoresis and showed an increased mobility after reaction with trypsin. Methylamine caused only a small change in electrophoretic mobility. Trypsinization of the methylamine treated preparation completed the transformation to the fast form.

Studies of methylamine revealed that buffalo $\alpha_2$M has thiol esters of unequal reactivity. Two of the four thiol esters appeared recalcitrant to methylamine treatment. The carbohydrate composition of the purified protein was 7.8% dry weight of the molecule. The amino acid composition of buffalo $\alpha_2$M appeared typical of $\alpha_2$Ms except for the deficient in proline and aspartic acid and higher content of alanine. Buffalo $\alpha_2$M exhibited good immunological cross reactivity against human and goat $\alpha_2$M.

Sodium thiocyanate at 1.2M or higher concentration dissociated the native buffalo $\alpha_2$M into half molecules consisting of two disulphide bonded subunits. Methylamine treatment rendered the molecule more resistant to dissociation than native $\alpha_2$M. The observed fluorescence change indicates that conformational alteration occurs gradually on exposure to sodium thiocyanate.
The physiological zinc concentration of buffalo plasma was about 18-20 \( \mu \text{M} \). \( \alpha_2 \text{M} \) pre-treated with up to 30\( \mu \text{M} \) zinc retained most properties of native \( \alpha_2 \text{M} \), while \( \alpha_2 \text{M} \) treated with 200\( \mu \text{M} \) zinc exhibited an irreversible loss in activity, although it displayed the characteristic proteolysis and methylamine induced alterations in electrophoretic mobility.

Trypsin treatment resulted in a significant decrease in intrinsic fluorescence of buffalo \( \alpha_2 \text{M} \) whereas methylamine caused only marginal alterations. The magnitude of conformational changes occurring on methylamine and trypsin treatment were markedly higher in case of the \( \alpha_2 \text{M} \) pretreated with 200 \( \mu \text{M} \) zinc. The changes in the CD spectrum of buffalo \( \alpha_2 \text{M} \) were also very small on methylamine treatment whereas loss in ellipticity was remarkable on treatment with Sepharose-linked trypsin. Treatment of buffalo \( \alpha_2 \text{M} \) with 200 \( \mu \text{M} \) zinc resulted in significant alteration in the CD spectrum also after treatment with methylamine or trypsin. Prolonged incubation with high concentration of the metal ion caused the dissociation of \( \alpha_2 \text{M} \) into half molecules.