PURIFICATION OF ∝-GALACTOSIDASE FROM HUMAN PLACENTA

Gamini Vithanachchi and C. Deepal Mathew
Faculty of Medicine, University of Colombo.

∝-Galactosidase is a lysosomal enzyme present in both plant and animal tissues. The enzyme hydrolyses ∝-D-galactoside linkages. In human tissues it acts on glycosphingolipids. The defficiency of ∝-galactosidase is an inborn error, that leads to the Fabry's disease by the accumulation of ceramide trihexosides. A major problem in the study of ∝-galactosidase is the presence of two enzymes, ∝-galactosidase and an aminidase, which can act on the artificial substrate 4-Methyl umbelliferyl- ∝-D-galactoside. In this study we have purified the ∝-galactosidase by affinity chromatography.

In our studies the human placenta was homogenized and the extract was purified by acidification, 40% - 80% ammonium sulphate fractionation and affinity chromatography. The affinity gel, Sepharose-4B lysine galocturonate was prepared by adding lysine to the CNBr activated sepharose 4B and binding galacturonic acid in the presence of 1-Ethyl-3-(3-dimethyl aminopropyl) carbodimide. The galacturonic acid content in the gel was 12.12μ moles/ml. The affinity column separates the aminidase from ∝-galactosidase. The purified ∝-galactosidase showed a times purity of 16827. The enzyme was shown to be homogeneous by polyacrylamide gel electrophoresis.