E-16 **LARGE SCALE PURIFICATION OF ∝-GALACTOSIDASE FROM COCONUT KERNEL**

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∝—Galactosidase from cocount kernel has been purified to homogenity and characterized. Yield was only 12% and the amount of purified enzyme obtained was low. The limiting step was gel filtration. The amount of purified enzyme obtained was insufficent for a detailed study of the properties of ∝-galactosidase. This paper describes attempts at purifying ∝-galactosidase on a large scale.

Partially purified ∝-galactosidase obtained from $(NH_4)_2SO_4$ fractionation was dialysed and separated on DEAE Sephadex. The specific activity was 8,019 milliunits/mg protein and yield was 56%. As this enzyme was not pure further purification was attempted by affinity chromatography.

The spacer arm lysine was bound to CNBr activated Sepharose 4B and the D-galacturonic acid was covalently linked to the lysine using carbodimide. The ∝-galactosidase from DEAE-Sephadex was purified on Sepharose—lysine-galacturonic acid gel. The specific activity of pure ∝-galactosidase was 20,000 milliunits/mg protein and the yield was 48%. However, the capacity of the gel was low due to the high inhibitory constant of galacturonic acid ($Ki = 1.5 \times 10^{-2}M$). Galactose-O-Carboxyanilide was synthesized whose Ki value was found to be $8.3 \times 10^{-4}M$. This was coupled as in the case of D-galacturonic acid to the Sepharose-lysine gel. Due to high affinity, the adsorbed ∝-galactosidase had to be eluted out with the substrate. Further studies were not carried out as the ligand had broken down either due to bacterial action or acidity of the medium. Work is progressing to use galactosamine as the affinity ligand.