ABSTRACT

Cholesterol determination is routinely performed in hospitals as a diagnostic test for some pathological state such as artherosclerosis or jaundice etc. In the present time determination of cholesterol by chemical reagents has been replaced by enzymatic reagents. The assay system requires cholesterol esterase, cholesterol oxidase, and peroxidase. As a result the enzymatic method which is superior to chemical methods in all aspect is rather expensive. The major objective of this project was to find suitable source of cholesterol oxidase so that enzymic reagent for cholesterol determination could be produced locally.

Screening for the enzyme source was carried out in various kind of vegetable and in microorganism. Cholesterol oxidase was found in one of 276 isolates of actinomycetes. The morphology and culturing condition of the isolate has been investigated.

Evaluation of the suitability of the enzyme produced from this isolate of actinomycete was carried out by partial purification of the enzyme, using DEAE-cellulose chromatography and gel filtration. The partially purified enzyme was studied for its physical and biochemical properties, as followings : molecular weight is 34,000, Km is 1.47×10^{-4}M, the enzyme is specific for 3β-hydroxysterols with double bond at C-5, stability to heating is up to 45°C, stability at -10°C for one month with 5% loss of activity, optimum pH is around 6.5 and optimum temperature is at 40°C.

The partially purified enzyme was compared with commercial cholesterol oxidase in the determination of serum cholesterol. Correlation ($r = .987$) between the two source of enzyme was statistically significant.

From this study it was concluded that cholesterol oxidase by actinomycete is very suitable in all aspects to be used as a diagnostic enzyme.