SUMMARY AND CONCLUSION
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Cat fish (*Tachysurus thalassinus*) contains considerable amount of the enzyme cholesterol esterase in the body organs of which the liver has maximum concentration, 126 units/g. wet weight of liver protein.

A method to purify the enzyme from the liver has been developed. And the purity obtained was 300 fold. The method consists of the following stages.

1. Extraction with phosphate buffer
   The homogenised liver was extracted with ice cold 0.1 M McIlvaine phosphate buffer, pH 7.0 and filtered to remove fat.

2. Ammonium sulphate fractionation
   The extract which contained the protein was fractionally precipitated with ammonium sulphate at 0.75 saturation. The precipitate containing the enzyme was dissolved in 0.1 M buffer.

3. Desalting
   The dissolved protein was desalted using Sephadex G-25.

4. DEAE cellulose ion exchange chromatography
   The cholesterol esterase solution obtained after Sephadex G-25 column chromatography was subjected to DEAE cellulose column chromatography under potassium chloride gradient elution using UV monitor and a fraction collector to get a partially purified enzyme.
5. **Sephadex G-100 column chromatography.**

A portion of the more concentrated solution of cholesterol esterase from the DEAE cellulose chromatography was subjected to Sephadex G-100 column chromatography. The fraction gave only one peak showing that it was a pure protein. This was further confirmed.

The cholesterol esterase of cat fish on Sephacryl S-200 chromatography gave only one peak. On SDS polyacryl amide gel electrophoresis it gave only one band. Molecular weight determination by gel filtration on Sephadex G-100 and SDS poly acrylamide gel electrophoresis gave an average value of 86,000 daltons.

The enzyme showed no light absorbance in the visible range. Maximum absorption was at 280 nm. The ratio of absorbance at 280 nm to 260 nm was about 1.192.

Amino acid analysis revealed that it contains more of serine along with arginine, leucine, lysine etc. The serine plays a major role in the properties and uses of the enzyme. The enzyme contains 22% basic and 30% acidic amino acid residue. The hydrophobic residues is about 40%.

Cholesterol esterase isolated from cat fish has got an optimum pH 6.2 for reaction and optimum temperature, 37°C. It was also stable in the pH range of 5.5 to 7.4 and temperature range of 0-45°C.
Studies with substrates showed that the enzyme is not so specific towards any substrates. However it showed a differential rate of activity with different substrates. The maximum rate of reaction was with cholesterol linolate followed by cholesterol linolate, cholesterol oleate, cholesterol palmitate, cholesterol stearate, cholesterol laurate and cholesterol butyrate.

A number of metallic and non-metallic ions were inhibitors of cholesterol esterase. The inhibition of aldehydes and ketones were in the order of methyl ethyl ketone acetone acetaldehyde formaldehyde.

The inhibition of cation was in the order of \( \text{Mg}^{++} > \text{Cu}^{+} > \text{Zn}^{++} > \text{Mn}^{++} > \text{Mg}^{++} > \text{Ca}^{++} > \text{Ba}^{++} > \text{Sn}^{++} \). And inhibition of anion was \( \text{F}^{-} > \text{Cl}^{-} > \text{Br}^{-} > \text{I}^{-} \).

It is also found that FCHB and DFP also were inhibitors of cholesterol esterase. None of this was found to be total inhibitors.

Cholesterol esterase was immobilised using polyacrylamide gel and by chitosan. This immobilised enzyme retained 60-70% of activity of the original enzyme. It was also observed that the immobilised enzyme is more stable than the nature enzyme and also can be stored at \(-20^\circ\text{C}\) for 3 months without significant loss in its activity.
FLOW SHEET OF PURIFICATION OF CHOLESTEROL ESTERASE FROM CAT FISH LIVER

(All operations done at 0 -4 °C)

Minced liver

Extract with 10 volumes of phosphate pH 7.0 buffer

Centrifuge -> Residue discarded

Add ammonium sulphate 75 % saturation, kept overnight.

Filterate -> Residue discarded

Centrifuge -> Add ammonium sulphate kept overnight

Filtrate discarded -> Residue dialysed

esterase solution

Sephadex G-25 chromatography

Deionised lipase solution

DEAE cellulose chromatography

Partially purified esterase solution

Sephadex G-100 chromatography

Pure cholesterol esterase
CONCLUSION

An attempt was made to find out whether a cheap resource like fish can be utilised for the extraction of the enzyme cholesterol esterase. The method developed would be useful for large scale preparation of the enzyme which has got wide application in the pharmaceuticals and biotechnology.

The immobilisation method using chitosan is also useful for preparing a stable and active enzyme than the natural enzyme.

Isolation and purification of cholesterol esterase was a difficult task. Even though methods have been developed on its preparation a stable enzyme could not be produced due to various reasons. In this study a method is perfected to isolate the enzyme from a cheaper resource and the same was stabilised by immobilising with chitosan, a byproduct from the crustacean shell. The properties of pure native and immobilised cholesterol esterase were also studied.