MOLECULAR WEIGHTS OF CHOLINESTERASES FROM THE INDIAN HONEY BEE APIS INDICA, HOUSE FLY MUSCA DOMESTICA AND THE DESERT LOCUST SCHISTOCERCA GREGARIA

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ABSTRACT

The molecular weights of Cholinesterases (ChE's) from the head homogenates of honey bee, house fly and the desert locust were determined by gel filtration on Sephadex G-200. The results indicate that both honey bee and locust ChE's have a molecular weight of around 82,000 while the house fly ChE has a molecular weight of 165,200. In all the runs, the acetyl-ChE came as a first peak indicating that the enzyme is also present as a very high polymer with a molecular weight running to millions.

Cholinesterase (ChE) of insects has been a subject of great interest from toxicological and kinetic points of view. Efforts to date to purify the enzyme from insect sources have only been moderately successful. As a consequence of the lack of success in the purification trials, there is a paucity of knowledge on the molecular properties of this enzyme. The present report describes experiments aimed at the estimation of molecular weights of ChE's from the Indian honey bee, house fly and the desert locust.

ChE's used in the present investigation were obtained from the heads of honey bee, house fly and the desert locust. The tissues were homogenized in an all-glass Potter-Elvehjem type homogenizer with ice-cold phosphate buffer of pH 7.0 (NaCl = 26.3 g, KH₂PO₄ = 3.85 g, NaOH = 1.0 g and water to make 1 litre). The homogenates were centrifuged for 10 minutes at 6,000 rpm in a refrigerated centrifuge at 0°C. The supernatants were collected and used in the gel filtration studies.

A 2.5 cm × 45 cm K 25/45 column (Pharmacia Fine Chemicals, Uppsala, Sweden) was set with Sephadex G-200 (Pharmacia Fine Chemicals) which was previously swollen in phosphate buffer. The buffer used for homogenization was diluted ten times with 0.5 M NaCl and used in the columns. The dead volume (V₀) of the column was determined before each experiment by running Blue Dextran-2000. The column was standardized by running proteins of known molecular weights like γ-globulin (human), serum-albumin (bovine) and myoglobin; and was found to obey the regression equation proposed by Determann and Michel (1966). The volume of homogenate put on to the column was 2 ml for each experiment. An automatic siphoning system was employed to collect 3 ml fractions.

ChE activity in the fractions was estimated using acetylthiocholine bromide (ATChBr) (Ellman et al., 1961; and Smissaert, 1964), indophenyl acetate (IPA) (Kramer and Gamsen, 1958) and ortho-nitrophenyl acetate (ONPA) (Main et al., 1961) as substrates.

Figure 1 shows the ChE activity of various fractions collected from the run using honey bee homogenate. A large portion of the enzyme eluted out at or near the dead volume (84 ml). There was a second peak when the elutes were tested with IPA and ONPA as substrates; but this was not discernible with ATChBr. The molecular weight of ChE in peak II was calculated to be 82,040.

Figure 2 depicts the pattern of distribution of enzyme activity, in the run with house fly homogenate, when determined using IPA as substrate. As with the honey bee, here also the first peak came out at the dead volume (84 ml) of the column. The molecular weight of ChE in the second peak was found to be 165,200.

Figure 3 gives the pattern of ChE activity in locust homogenate when the fractions were tested for the enzyme with IPA as the substrate. It is clear from the figure that in this case also there were two peaks—the first almost at the dead volume and the second around the 43rd fraction. The molecular weight of locust ChE eluted out as the second peak amounted to 82,410.

It is apparent from a perusal of the literature relating to the molecular weight of ChE that the enzyme appears in many polymer forms or aggregates; this is clearly indicated for eel ChE in the reports of Lawler (1963) and Kromzner and Wilson (1964). The results of the present studies also give credence to the above assumption. It can be presumed that the monomer unit of ChE has a molecular weight around 82,000. It is clear from the results that the ChE's from honey bee and the desert locust exist as their basic units in the elutes of the second peak while the enzyme.

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from house fly in the same peak seems to be a dimer with a molecular weight of 165,000. Krysan and Chadwick (1966) reported the molecular weight of house fly ChE to be approximately equal to 160,000.

Evidence has also been obtained from the present investigations that the honey bee ChE in the second peak is not of the 'true ChE'

![Graph](image)

**FIG. 1.** Pattern of ChE activity from the run with honey bee homogenate.

![Graph](image)

**FIG. 2.** Distribution of ChE activity in the run with house fly homogenate when determined using IPA as substrate.

There were distinct peaks at the dead volume in all the three insect species, indicating clearly that the enzyme is also present as a high polymer with a molecular weight running to millions. Kunkee and Zweig (1953) also reported the molecular weight of ChE from *Apis mellifera* to be "in millions"; their enzyme preparation could well be considered as an agglomerate of basic units and to be type because the second peak did not emerge with ATChBr as the substrate. Hence, this peak may be comprised solely of aliphatic and aromatic esterases. It can also be inferred that most of the acetyl-ChE in the homogenates came out in the first peak.

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FIG. 3. Pattern of ChE activity in locust homogenate when the fractions were tested for the enzyme with IPA as substrate.


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