THE CATALYTIC PROPERTIES OF THE ISOENZYMES OF GLUTATHIONE TRANSFERASE FROM FRUIT BAT, *Eidolon helvum* (Kerr) LIVER

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ABSTRACT

This study investigated the physiochemical and catalytic properties of the isoenzymes of glutathione transferase (GST) in the fruit bat *(Eidolon helvum*, Kerr) liver, with a view to having an understanding of the survival strategy of this animal, which feeds on various plant materials many of which contain toxic secondary metabolites.

A 15% homogenate (15 g in 85 ml buffer) of a fresh liver sample, obtained from live bats, was prepared in 10 mM Tris-HCI buffer pH 8.0, containing 1 mM EDTA, 1 mM 2-mercaptoethanol and 10% glycerol, using a warring blender. The crude homogenate was centrifuged at 20,000 rpm for 30 minutes at 4°C using a Beckman ultracentrifuge to obtain a cloudy supernatant. The supernatant was further centrifuged at 45,000 rpm for 1 hr to obtain a clear crude enzyme extract. The extract was purified on DEAE-Sephacel anion exchange chromatography and CM-TrisacryI cation exchange chromatography, followed by gel filtration on Sephadex G-100. Determination of native and subunit molecular weights of the purified enzyme was carried out by gel filtration on Sephadex G-100 and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) respectively. In order to classify the various purified GST isoenzymes obtained, the specific activities towards various substrates [1,2-dichloro-4-nitrobenzene (DCNB), 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(p-nitrophenoxy)propane (EPNP), ethacrynic acid, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-CI) and p-nitrophenyl acetate (p-NPA)] were determined by monitoring the enzyme-catalyzed conjugation of the various substrates with reduced glutathione (GSH} in a spectrophotometer under standard conditions. The apparent Michealis-Menten constant (K_m) and maximal velocity (V_{max}) for GSH, and some of the utilizable electrophilic substrates, were also determined by varying the concentrations of the substrates while keeping that of GSH constant and

vice-versa. The data also obtained were analyzed by double reciprocal plot. Similarly, the effect of the inhibitors: cibacron blue, hematin, and S-hexylglutathione, which could be used to discriminate between the different GST classes were studied by assaying the enzyme at different concentrations of the inhibitors.

The results showed that the DEAE-Sephacel anion- exchange chromatography resolved the crude enzyme extract into two major peaks of activity designated as A and B which were separately pooled. Further purification of pool A on CM-Trisacryl cation-exchange chromatography resolved it into four peaks of activity which were designated, following the established convention, as AA, AB, AC and AD; AA being the isoenzyme that eluted at the highest ionic strength and AD, the lowest. However, further purification of pool B on this column gave only one peak of GST activity, which was designated as isoenzyme B. The native molecular weight of the isoenzymes estimated from a calibration curve ranged from 40 to 44 kDa. The subunit molecular weights of each of the isoenzymes estimated by SDS-PAGE were between 21 to 23 kDa. On the basis of this, the native isoenzymes could therefore be said to be dimeric proteins. The fruit bat GST isoenzymes conjugated CDNB, NBD-CI and p-NPA to varying degrees with GSH, but could not utilize ethacrynic acid, DCNB and EPNP. Hematin inhibited all the isoenzymes to the same extent, but isoenzymes AB, AC and AD were more sensitive to cibacron blue than isoenzymes AA and B.

In conclusion, the occurrence of GST in multiple forms with different catalytic properties could assist fruit bats in detoxication of various allelochemicals encountered during feeding.