SEPARATION OF THE ISOENZYMES OF RAT LIVER GLUTATHIONE TRANSFERASE BY CHROMATOFOCUSING

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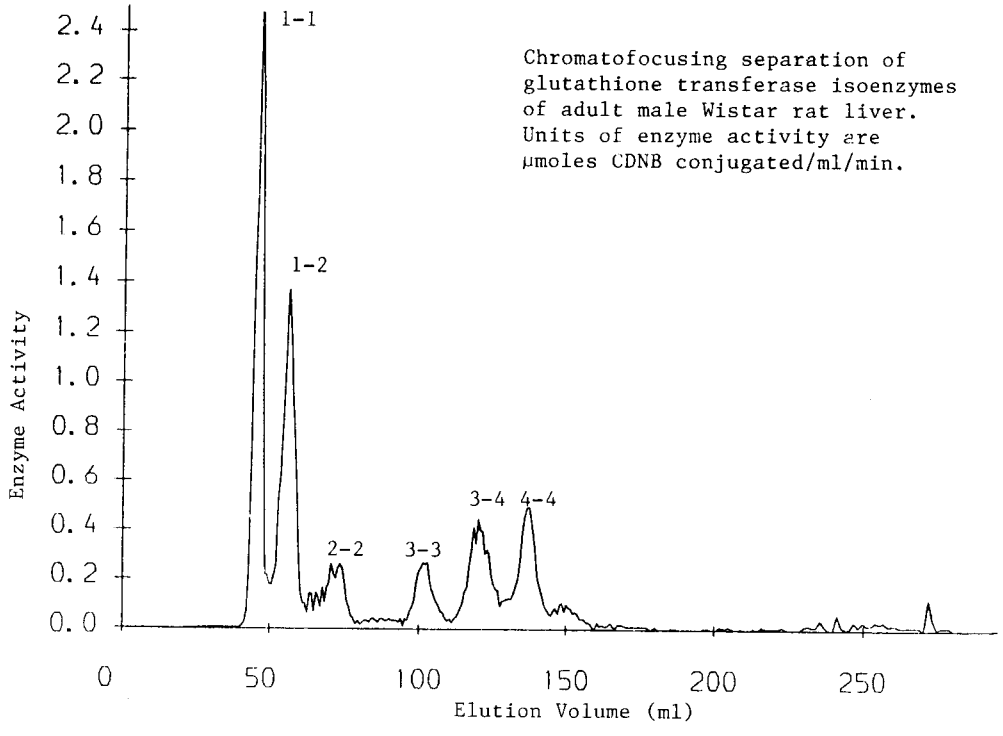
The glutathione transferases are a family of isoenzymes which catalyse the conjugation of a variety of electrophilic xenobiotics to glutathione. As toxic metabolites of drugs and of carcinogens can act as electrophilic substrates, the enzymes therefore have an important role in detoxication. Rat liver is known to contain several glutathione transferases, each enzyme having its own distinct substrate specificities (Mannervik & Jensson, 1982). As we are developing new fluorogenic substrates for this enzyme system, of which monobromobimane (mBrB) is an example (Hulbert & Yakubu, 1983), it became necessary to study andoptimise methods of separation of the glutathione transferase isoenzymes. The figure shows a separation by chromatofocusing of the major glutathione transferases present in male Wistar rat liver. Cytosolic fraction (100,000g supernatent containing 30 mg total protein) was applied to a column (55 cm x 5 mm)of gel PBE 118 (Pharmacia, 10 ml bed volume) and eluted with successive ampholyte solutions over the pH range 10.5-4.0. Enzyme activity was measured using the general substrate 1-chloro-2,4-dinitrobenzene (CDNB). The isoenzymes have been characterised by their individual substrate specificies using a range of substrates; the new nomenclature (Jakoby et al, 1984) is employed. These purified isoenzymes have been examined for their ability to conjugate mBrB, and we can report that mBrB is a versatile general substrate for all the major isoenzymes.



Chromatofocusing separation of glutathione transferase isoenzymes of adult male Wistar rat liver. Units of enzyme activity are μmoles CDNB conjugated/ml/min.

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