MERCAPTURIC ACID BIOSYNTHESIS : THE SEPARATE IDENTITIES OF GLUTATHIONE-S-ARYL CHLORIDE TRANSFERASE AND LIGANDIN

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SUMMARY : Glutathione-S-aryl chloride transferase (E.C.2.5.1.13) and ligandin have similar molecular weights, but are different electrophoretically and immunologically and can be separated by isoelectric focusing. Glutathione-S-aryl chloride transferase has been obtained as a by-product of ligandin purification. The different roles of glutathione-S-aryl chloride transferase and ligandin in excretion by the mercapturic acid pathway are discussed.

INTRODUCTION :

Evidence to support a claim that GSH-aryl transferase * (1) and ligandin, a major protein of cytosol (2), are identical has recently been submitted (3, 4). If this is the case glutathione conjugation becomes one more item in a long list of properties attributed to ligandin (2) and implies that ligandin catalyses the first essential step in the excretion of aryl precursors by the mercapturic acid pathway (1).

In the present work it is demonstrated that GSH-aryl transferase and ligandin are separate proteins. Nevertheless they may both play a role in mercapturic acid excretion and this is discussed.

MATERIALS AND METHODS :

Unfractionated cytosol : A 1 : 1 homogenate of male Wistar strain rat livers in 0.25M sucrose was prepared and centrifuged at 100,000 x g for 60 min. The supernatant fraction was then studied by either isoelectric focusing or gel filtration. In the former case the cytosol was applied to an LKB Uniphor isoelectric focusing column containing a sucrose gradient and Ampholines with a range from pH7 to pH10 (LKB Produkter AB, Sweden). In the latter case the cytosol was applied to a 90 x 2 cm column of Sephadex G 100 equilibrated with 0.01M sodium phosphate buffer, pH 7.4 and 0.2M with respect to NaCl.

* Abbreviation : GSH-aryl transferase = glutathione-S-aryl chloride transferase.

Ligandin : The preparation of ligandin was similar to that originally described except that a step involving zone electrophoresis was replaced by isoelectric focusing between pH 7 and pH 10 (5).

Estimation of ligandin : Ligandin was estimated by radial immunodiffusion in agarose gels impregnated with antiserum (6). Antiserum was raised to pure ligandin in the rabbit.

Estimation of GSH-aryl transferase : Activity was assayed using 3 : 4 - dichloronitrobenzene as a substrate (7). Activity towards bromsulphophthalein, a postulated substrate for GSH-aryl transferase (1) was also measured (8). A unit of activity was the conjugation of 1μmole of substrate per min. at 37°C. Protein was determined by the Lowry method using bovine serum albumin as a standard (9).

Polyacrylamide gel electrophoresis : GSH-aryl transferase preparations and ligandin were compared by polyacrylamide gel electrophoresis in sodium dodecylsulphate, 8M urea (10).

RESULTS :

Isoelectric focusing of fresh unfractionated cytosol : Although neither gave rise to single components, the GSH-aryl transferase and ligandin of fresh cytosol were separated in one isoelectric focusing run (see Fig. 1). GSH-aryl transferase showed peaks at pH 7.6 and pH 8.0 and a shoulder at pH 8.4, while ligandin showed a peak at pH 9.5 and a shoulder at pH 9.1. Seventy three per cent of the GSH-aryl transferase activity in the original cytosol was recovered after 72 h isoelectric focusing.

Gel filtration of fresh unfractionated cytosol : GSH-aryl transferase and a bromsulphophthalein binding component, assumed to be ligandin, had the same elution volume when fresh unfractionated cytosol was submitted to gel filtration on Sephadex G 75 (3). A similar experiment was made in the present work but with two differences. Firstly, the more highly resolving Sephadex G 100 was used and secondly ligandin was estimated using a specific antiserum. No difference could be detected between the elution volumes of GSH-aryl transferase and ligandin.

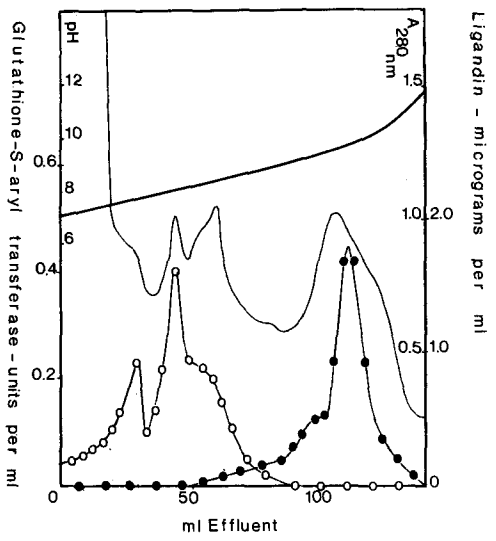

Figure 1. Isoelectric focusing of rat liver cytosol between pH 7 and pH 10. pH... ▬ ; optical absorbance at 280 nm... —— ; units of GSH-aryl transferase per ml effluent...o—o—o ; micrograms ligandin per ml effluent... ●—●—●

GSH-aryl transferase preparations : GSH-aryl transferase is present in ligandin preparations made in this laboratory prior to isoelectric focusing when it then separates as several components with isoelectric points in the range pH 7.5 to pH 8.5. It can be further purified by additional isoelectric focusing followed by passage through a Sephadex G 100 column. The several isoelectric forms of such preparations show conjugating activity not only to 3 : 4 - dichloronitrobenzene but also to bromsulphophthalein. The specific activities obtained are 4.1 units per mg for 3 : 4 - dichloronitrobenzene and 1.4 units per mg for bromsulphophthalein. These activities represent purifications of 100 fold over cytosol for both substrates.
On polyacrylamide gel electrophoresis in sodium dodecylsulphate, 8M urea these protein preparations show a high degree of protein purity. One component predominates with a mobility similar to that of the ligandin subunit (2), however minor components are also present (see Fig. 2).
Although the principal component resembles ligandin, immunological tests show the complete absence of ligandin itself.

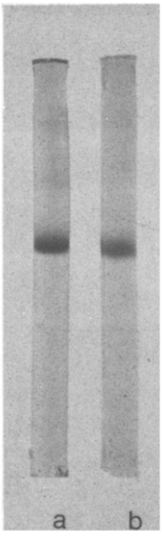

Figure 2. Polyacrylamide gel electrophoresis of a GSH-aryl transferase preparation and ligandin in sodium dodecylsulphate, 8M urea. a = GSH-aryl transferase, b = ligandin. The anode is at the bottom of the gels.

DISCUSSION :

It has been shown that GSH-aryl transferase and ligandin are different electrophoretically and immunologically and are therefore different proteins. GSH-aryl transferase preparations have been made with higher specific activities than others previously described (7, 11) and with an apparent high degree of protein purity. Even so these preparations have only 100 times the specific activity of cytosol. There are three possible explanations for this situation. GSH-aryl transferase may be an abundant protein of the cytosol. The predominant protein component in GSH-aryl transferase preparations may represent enzyme protein which has lost activity during purification. A minor component might represent the active molecule. These three possibilities are being investigated.

GSH-aryl transferase is named because of its ability to conjugate aryl chlorides with glutathione. However there is evidence that the same enzyme catalyses the conjugation of glutathione to other substrates including nitroquinoline-N-oxide, certain nitrofurans and the cholephilic agent bromsulphophthalein which also binds to ligandin (1, 11). The

present results which show both that activity towards both 3 : 4 - dichloronitrobenzene and bromsulphophthalein is parallel in isoelectric focusing experiments, and that the degree of purification of activity towards both substrates is similar, support previous evidence that these two substrates are conjugated with glutathione by the same enzyme. Other substrates from among those mentioned above will be studied in future experiments.

By bringing about the conjugation of certain precursors with glutathione, GSH-aryl transferase has a clear-cut role in one of the excretory pathways of the liver. The conjugate which results from this reaction may be either excreted as such, or further metabolized and excreted as a mercapturic acid (1).

Ligandin may also be involved in excretion by the mercapturic acid pathway. Since it binds azodye carcinogen metabolites covalently through a cysteinyl residue (2), it could give rise to a mercapturic acid precursor in the course of its normal catabolism. The possibility that mercapturic acids might arise from protein precursors was once a matter of debate which was not entirely resolved (1).

Ligandin also binds an azodye carcinogen glutathione conjugate non-covalently. This is another example of ligandin's characteristic affinity for organic anions (2). Whether or not this binding is a significant event in the subsequent excretion of the conjugate or its further metabolism is not known.

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