USE OF IMMOBILIZED ENZYMES TO STUDY MECHANISMS OF DRUG METABOLISM

Catherine Fenselau

Department of Pharmacology and Molecular Sciences
Johns Hopkins University, Baltimore
MD 21205, U.S.A.

Protein solubilized from hepatic microsomes from rabbit, monkey and man has been immobilized on cyanogen bromide-activated beaded Sepharose (1). Among many proteins present, we have demonstrated that UDP-glucuronyl transferase, glutathione transferase, P-450 oxygenase and epoxide hydratase activities may be supported by the appropriate cofactors (2-4). This immobilized cell-free system has the advantages that enzyme activities can be sustained for long periods of time and that protein may be removed from the reaction by simple centrifugation. This system provides an effective technique for synthesis of small amounts of phase I and II metabolites, as well as for studying mechanisms of metabolism from a bioorganic point of view. Preliminarily this *in vitro* system appears to provide comparisons of species differences in metabolism which are relevant to differences *in vivo*.

Examples will be presented of studies of the metabolism of thalidamide, clofibrate and indomethacin, and the anti-tumor agents cyclophosphamide and phenylalanine mustard. The immobilized system has been used to produce unstable intermediates by P-450 oxygenation and to trap these by conjugation with glucuronic acid or glutathione (4, 5). Acyl-linked glucuronides have been synthesized and shown to alkylate albumin (6-8). This provides a chemical mechanism for drug immunogenicity (9). Glutathione conjugates formed from the alkylating mustards provide a chemical mechanism for induced drug-resistance (10). A new conjugation reaction has been characterized for glutathione, in which cyclic aziridinium and quaternary ammonium groups are displaced by glutathione, under enzyme catalysis (11).

References

1. Parikh, I., MacGlashan, D.W. and Fenselau, C., Immobilized glucuronyl-transferase for synthesis of conjugates. *J. Med. Chem.*, 19: 296 (1976).
2. Fenselau, C., Pallante, S. and Parikh, I., Solid phase synthesis of drug glucuronides by immobilized glucuronyl-transferase. *J. Med. Chem.*, 19: 679 (1976).
3. Lehman, J.P., Ferrin, L., Fenselau, C. and Yost, G.S., Simultaneous immobilization of cytochrome P-450 and glucuronyl-transferase for synthesis of drug metabolites. *Drug Metab. Disp.*, 9: 15 (1981).
4. Pallante, S., Lisek, C.A., Dulik, D.M. and Fenselau, C., Glutathione conjugates: Immobilized enzyme synthesis and characterization by fast atom bombardment mass spectrometry. *Drug Metab. Disp.*, 14: 313 (1986).
5. Fenselau, C. and Yelle, L., Analysis of glucuronides, sulfates and glutathione conjugates by mass spectrometry. In G. Paulson, ed., "Chemistry of Conjugates Xenobiotics," Amer. Chem. Soc., Washington, DC, 1985.
6. Stogniew, M. and Fenselau, C., Electrophilic reactions of acyl-linked glucuronides: Formation of clofibrate mercapturate in humans. *Drug Metab. Disp.*, 10: 609 (1982).
7. van Breemen, R.B. and Fenselau, C., Reaction of 1-O-acyl glucuronides with 4-(p-nitrobenzyl)pyridine. *Drug Metab. Disp.*, 14: 197 (1986).
8. van Breemen, R.B., Fenselau, C., Mogilevsky, W. and Odell, G.B., Reaction of bilirubin glucuronides with serum albumin., *J. Chromatogr.*, in press.
9. Smith, P.C., McDonagh, A.F. and Benet, L.Z., Irreversible binding of Zomepirac to plasma protein in vitro and in vivo. *J. Clin. Invest.*, 77: 934 (1986).
10. Dulik, D.M., Fenselau, C. and Hilton, D., Characterization of Melphalan-GSH adducts whose formation is catalysed by glutathione transferase. *Biochem. Pharm.*, 1986, in press.
11. Dulik, D.M. and Fenselau, C., Conversion of Melphalan to 4-(glutathionyl)-phenylalanine: A novel mechanism for conjugation by glutathione-S-transferases. *Drug Metab. Disp.*, submitted.