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ENZYME PROFILES OF MAMMALIAN BILE

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SUMMARY

The activities of subcellular marker enzymes in bile and liver homogenate from several mammalian species have provided information on the specificity of protein release during bile formation.

The presence of significant amounts of the plasma membrane enzymes alkaline phosphodiesterase I and leucyl-β-naphthylamidase in bile, in addition to alkaline phosphatase and 5′-nucleotidase, and the relative absence of intracellular enzymes lends support to the view that bile salt liberation from the hepatocyte is accompanied by a partial solubilization of the plasma (canalicular) membrane without extensive damage to the whole hepatocyte.

The sequence of some of the biochemical events which occur during the formation of bile, a secretion which contains large amounts of biological detergents, is uncertain. It has been suggested [1] that the large amounts of lecithin and unesterified cholesterol in bile may be obtained from the bile canalicular membrane during (or after) the passage of bile salts out of the hepatocyte. If this solubilisation occurs, other components of the bile may also reflect, to some extent, the composition of the membrane.

The plasma membrane enzymes 5′-nucleotidase and alkaline phosphatase have been identified in bile for some time (see for example refs 2 and 3). We have now attempted to extend this observation to other plasma membrane enzymes, notably leucyl-β-naphthylamidase and alkaline phosphodiesterase I. In addition, we have studied other enzymes chosen because their subcellular localizations are established in liver to the extent of their use as biochemical "markers" in cell fractionation experiments. The relationship of the specific activities of all of these enzymes in bile to those in the liver homogenate may therefore provide information on the specificity of protein release during bile formation and the contribution of various subcellular organelles to it.

Bile was obtained from freshly killed (ox, sheep, pig) or cannulated (rat) individuals and chilled. Liver homogenates from the donor animals were prepared in ice-cold 1 mM $NaHCO_3$. Two samples of human bile were included in this survey, obtained from patients who had undergone biliary drainage following cholecystectomy. These specimens were generously provided by Dr H. G. Sammons of the Depart-

TABLE I

RATIOS OF THE SPECIFIC ACTIVITIES OF ENZYMES IN BILE IN RELATION TO THE SPECIFIC ACTIVITIES IN THE LIVER HOMOGENATE OF THE DONOR ANIMAL

Values for the percentage inhibition in bile were obtained by the incubation of the liver homogenate (sheep) with an excess (9 vols) of bile, prior to assay and also, in some cases, with varying concentrations of mixed bile salts. ND = Not determined.

Enzyme	Subcellular localisation	Percentage inhibition by biliary components	Ratio of specific activities (bile to homogenate)														
			Sheep						Pig			Ox		Rat			
			A	B	C	D	E	F	A	B	C	A	B	A	B	C	D
5′-Nucleotidase	Plasma membrane	0	0.2	8.5	2.0	3.8	5.0	17	0	0	0.5	17	11	1.0	1.1	0.9	3.8
Alkaline phosphatase	Plasma membrane	30–40	17	26	16	23	18	46	15	3.5	4.0	6.0	6.5	0.2	0.2	0.2	0.3
Alkaline phosphodiesterase I	Plasma membrane	30	8.7	6.0	17	7.4	1.6	2.3	2.3	2.0	1.0	0.4	0.2	0.1	0.1	0.1	0.2
L-Leucyl-β-naphthylamidase	Plasma membrane	80	0.2	1.4	0.3	0.2	0.1	0.2	2.7	1.6	0.6	0	0	0.6	0.4	0.6	0.6
Mg^{2+}-ATPase	Plasma membrane and intracellular	0	0.1	0.5	0.9	1.0	0.6	3.5	0	0	ND	0.4	0.2	ND	0.2	0.1	0.3
Aryl esterase	Endoplasmatic reticulum	20	ND	ND	ND	ND	0.5	1.4	0.2	0.3	0.5	1.3	1.0	0.3	1.7	1.3	1.8
Succinate dehydrogenase	Mitochondria	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aryl sulphatase	Lysosomes	0	1.0	0	0.7	1.2	1.4	1.0	0.5	0.2	0.1	0.8	0.6	0.6	0.3	1.0	1.9
Acid phosphatase	Lysosomes	0	0	0	0	0	0	0	0	0	0	0.2	0.4	0.1	0.1	0	0
Lactate dehydrogenase	Cytosol	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0
Alcohol dehydrogenase	Cytosol	0	0	0	0	0	0	0	0	1.5	0	0	0	0	0	0	0

ment of Clinical Chemistry, East Birmingham Hospital. The biles and liver homogenates were stored in small aliquots at −20 °C until required. This storage did not affect the activities of the enzymes studied. Protein was estimated according to the method of Lowry et al. [4].

The following enzyme activities were assayed both in the biles and in the appropriate liver homogenates (the putative subcellular localisation is given in brackets in each case): 5′-nucleotidase (plasma membrane), EC 3.1.3.5 [5], alkaline phosphatase (plasma membrane), EC 3.1.3.1 [6], alkaline phosphodiesterase I (plasma membrane), EC 3.1.4.1 [7], L-leucyl-β-naphthylamidase (plasma membrane), EC 3.4.11.1 [8], Mg^{2+}-ATPase (plasma membrane and intracellular), EC 3.6.1.3 [9], acid phosphatase (lysosomal), EC 3.1.3.2 [10], aryl sulphatase (lysosomal), EC 3.1.6.1 [11], succinate dehydrogenase (mitochondrial), EC 1.3.99.1 [12], aryl esterase (endoplasmic reticulum), EC 3.1.1.2 [11], lactate dehydrogenase (cytosol), EC 1.1.1.27 [13], and alcohol dehydrogenase (cytosol), EC 1.1.1.1 [14].

In order to assess the possible enrichment of an enzyme during bile formation, the activities of the enzymes are expressed as the ratio of the specific activity in bile to that in the liver homogenate of the donor animal in each case (Table I). Possible inhibition of these enzymes by biliary components was assessed by their assay in homogenates (of sheep), in the presence of excess (9 vols) bile or, in some cases, a range of concentration of mixed bile salts. (Values recorded in the table have not been adjusted for inhibition.)

Table II give a comparison of the specific activities in the human bile samples with those of the other species in this survey.

The appearance in the bile of all the species tested (with minor variations) of significant amounts of alkaline phosphodiesterase I, plus to a lesser extent leucyl-β-naphthylamidase (which was up to 80 % inhibited by bile salts) together with 5′-nucleotidase and alakaline phosphatase, indicates that the release of several plasma

TABLE II

SPECIFIC ACTIVITIES OF ENZYMES IN BILE

Specific activities were calculated in μmol product formed/mg protein per h except for L-leucyl-β-naphthylamidase, which is given in $A_{560\ nm}$/mg protein per h. Values for sheep, ox, pig and rat are means.

Enzyme	Sheep	Pig	Ox	Rat	Human	
					A	B
5′-Nucleotidase	3.0	0.1	16	0.3	0.8	1.4
Alkaline phosphatase	10	1.4	2.0	0.02	0.6	0.5
Alkaline phosphodiesterase I	3.7	0.4	0.4	0.1	0.03	0.2
L-Leucyl-β-naphthylamidase	0.6	0.5	0	0.3	2.1	3.4
Mg^{2+}-ATPase	2.1	0	1.5	0.2	0	0.2
Aryl esterase	94	53	2.9	50	1.0	0
Succinate dehydrogenase	0	0	0	0	0	0
Aryl sulphatase	0.7	0.1	0.04	0.2	0	0.03
Acid phosphatase	0	0	0.1	0.1	0	0
Lactate dehydrogenase	0	0.3	0	0	0	0
Alcohol dehydrogenase	0	0.2	0	0	0	0

membrane protein components is a consistent feature of bile salt secretion. That this release is preferential rather than representing cell shedding, breakage or dissolution is indicated by the almost total absence, in bile, of the intracellular enzymes acid phosphatase, succinate dehydrogenase, lactate dehydrogenase and alcohol dehydrogenase (which are little affected by biliary components). Polypeptide profiles of the bile samples used in these studies were obtained using polyacrylamide gel electrophoresis in sodium dodecylsulphate [15] and the specificity of protein release into the bile was confirmed by the small number of bands as compared with gels of the appropriate liver homogenates. For each species the differences in the degree of enrichment between each enzyme in the bile indicate that these membrane proteins may not be released, possibly by solubilisation, to the same extent. The consistent presence of aryl sulphatase and aryl esterase in bile is anomalous, however, indicating either a further complexity in the protein release into bile or that the putative subcellular localisations of these activities may not be absolute.

Enhanced serum levels of 5′-nucleotidase and alkaline phosphatase are well known as a diagnostic feature of cholestatic jaundice [16]. Serum leucyl-β-naphthylamidase levels have occasionally been studied in this condition [17]. With the recognition that this enzyme and also alkaline phosphodiesterase I are biliary enzymes, further opportunities for the biochemical investigation of liver diseases present themselves. Moreover, the processes of bile formation may represent a useful system for the study of the effects of detergents on biological membranes in relation to the possible associations of individual lipid and protein components within them.

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REFERENCES

1 Small, D. M. (1970) Adv. Int. Med. 16, 243–264
2 Coleman, R. (1973) in Form and function of phospholipids (Ansell, G. B., Dawson, R. M. C. and Hawthorne, J. N., eds), 2nd edn, pp. 345–375, BBA Library 3, Elsevier Scientific Publishing Company
3 Price, C. P., Hill, P. G. and Sammons, H. G. (1972) J. Clin. Pathol. 25, 149–154
4 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
5 Michell, R. H., and Hawthorne, J. N. (1965) Biochem. Biophys. Res. Commun. 21, 333–338
6 Connock, M. J., Elkin, A. and Pover, W. R. (1971) Histol. J. 3, 11–22
7 Brightwell, R. and Tappell, A. L. (1968) Arch. Biochem. Biophys. 124, 325–332
8 Goldberg, T. A. and Rutenburg, A. M. (1958) Cancer 11, 283–291
9 Quigley, J. P. and Gotterer, G. S. (1969) Biochim. Biophys. Acta 173, 456–468
10 Hubscher, G. and West, G. R. (1965) Nature 205, 799–800
11 Shephard, E. H. and Hubscher, G. (1969) Biochem. J. 113, 429–440
12 Pennington, R. J. (1961) Biochem. J. 80, 649–654
13 Wroblewski, F. (1955) Proc. Soc. Exp. Biol. Med. 90, 210–213
14 Racker, E. (1950) J. Biol. Chem. 184, 313–319
15 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
16 Righetti, A. B-B. and Kaplan, M. M. (1972) Gastroenterol. 62, 1034–1039
17 Batsakis, J. G., Kremers, B. J., Thiesen, M. M. and Shilling, J. M. (1968) Am. J. Clin. Path. 50, 485–490