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Plasma membrane associated actin in liver of normal and tumour-bearing rats

Catherine M. Clark and George A.J. Goodlad

Department of Biochemistry and Microbiology, University of St. Andrews, St. Andrews, Fife, KY16 9AL (U.K.)

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A study was made of the association of actin with different plasma membrane fractions from liver of normal rats and from the enlarged liver of rats bearing a Walker 256 carcinoma where a decrease in the state of polymerisation of cytoplasmic actin has been previously observed. As estimated by the DNAase I inhibition assay, actin constituted approx. 7% and 3%, respectively, of the protein of membrane fractions enriched in lateral or bile-canalicular domains, but only trace amounts were found in the sinusoidal fraction. [^{3}H]Cytochalasin B binding indicated the presence of 20 and 13 pmol of high-affinity binding sites per mg protein in lateral and bile-canalicular fractions, but none in the sinusoidal. K_d for cytochalasin B binding was of the order of 1 nM for lateral and bile-canalicular fractions. Polypeptide profiles obtained by SDS/urea/polacrylamide gel electrophoresis of non-ionic detergent-insoluble residues differed for all three fractions although some proteins, including actin, occurred as major components of both bile-canalicular and lateral regions. Tumour growth had no effect on the actin content, high-affinity cytochalasin B binding or polypeptide profiles of the three membrane fractions.

Introduction

The growth of the Walker 256 carcinoma in the rat acts as a mitogenic stimulus to the host liver [1,2]. The resulting increase in liver cell proliferation is accompanied by a decrease in the proportion of actin in a filamentous form suggesting an impairment in the polymerisation of cytoplasmic actin [3]. While the actin microfilament system in non-muscle cells is closely associated with the plasma membrane and is believed to modify its biochemical and morphological properties [4,5] it is equally evident that assembly of cytoplasmic microfilaments can be altered through interaction with plasma membrane proteins initiating important events such as differentiation and cell division [6,7]. This diversity of interactions between microfilaments and plasma membrane may be effected by specialised domains of the plasma membrane. The plasma membrane of the mammalian liver can be divided into three distinct domains; the sinusoidal, the lateral and the bile-canalicular which exhibit considerable specialisation with respect to absorption, response to external stimuli, secretion, intercellular communication and attachment to neighbouring cells or the extracellular matrix. While the presence of actin has been demonstrated in plasma membrane preparations of rat liver [8–10] its relative abundance in the different domains, its association with other membrane proteins and its relationship to the cytoplasmic microfilament system remain to be established.

In an attempt to obtain some insight into the nature of possible interactions between the liver plasma membrane and cytoplasmic actin we have prepared plasma membrane fractions enriched in one or other of the three domains from liver of

control and tumour-bearing rats and examined the nature of any association of actin with these fractions.

Experimental

Materials. Rat skeletal muscle actin was prepared according to Haverberg et al. [11]. Bovine serum albumin, cytochalasin B, dihydrocytochalasin B, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′,*N*,*N*′-tetracetic acid (EGTA), *p*-nitrophenyl-5′-thymidylate, concanavalin A, 5′-adenosine monophosphate, deoxyribonuclease I (EC 3.1.21.1), phenylmethanesulphonyl fluoride (PMSF) and Nonidet P-40 were obtained from Sigma Chemical Co. Ltd. *Escherichia coli* RNA polymerase (EC 2.7.7.6), bovine serum albumin and trypsin inhibitor were obtained from Boehringer Corporation (London) Ltd., [4(n)-^{3}H]cytochalasin B (17 Ci/mmol) was obtained from Amersham International p.l.c.

Animals. Female rats of the Wistar strain of fasting body weight 180–200 g were inoculated in the dorsal region with a suspension of Walker 256 carcinoma cells and maintained with weight-paired controls under conditions described previously [12]. Tumour-bearing animals along with controls were killed in the fasting state 9–10 days after tumour implantation when the mean tumour weight was 14.6 g.

Preparation of liver membrane fractions. Plasma membrane fractions enriched in either bile-canalicular or lateral domains of rat liver were prepared essentially as described by Wisher and Evans [13] and a fraction enriched in the sinusoidal domain as described by Touster et al. [14]. In both procedures the centrifugation time using discontinuous gradients was restricted to 3 h so that the total preparation time was less than 6 h. Aliquots of the initial homogenate and the final membrane fractions were frozen immediately in liquid nitrogen and kept in a frozen state until used for analysis.

Analysis of plasma membrane fractions. Activities of 5′-nucleotidase (EC 3.1.3.5) [15], phosphodiesterase I (EC 3.1.4.1) [13], glucose-6-phosphatase (EC 3.1.3.9) [16] and succinate dehydrogenase (EC 1.3.99.1) [17] were estimated by published methods. The actin content of the membrane fractions was estimated using the DNAase I inhibitor assay [18]. Protein was estimated according to Lowry et al. [19] using bovine serum albumin as standard.

[^{3}H]Cytochalasin B binding assays. These were carried out on isolated membrane preparations as described by Lin and Lin [20]. In some cases assays were performed in the presence of 0.7 M glucose or $7 \cdot 10^{-5}$ M dihydrocytochalasin B. The data were graphed as Scatchard plots [21]. Radioactivity counting was carried out using a dioxan-based scintillator.

Extraction of plasma membrane fractions with non-ionic detergent. Membrane fractions (1 mg protein) were suspended in 1 ml 10 mM sodium phosphate, pH 7.2 containing 0.145 M NaCl, 1 mM PMSF, 0.5–1.5% (v/v) Nonidet P-40, 1 mM Ca^{2+} and 1 mM Mg^{2+} and incubated for 30 min at 0°C. In some experiments the incubation was carried out in the absence of calcium ions by omitting these from the incubation and adding EGTA to a final concentration of 1 mM. The insoluble material remaining was obtained as a pellet by centrifugation at $100\,000 \times g_{av}$ at 0°C for 1 h. 5′-Nucleotidase and protein concentrations of the residue were estimated as described above.

SDS-Polyacrylamide gel electrophoresis. Detergent-insoluble residues were incubated in the presence of 1%(w/v) sodium dodecyl sulphate, 1%(w/v) mercaptoethanol, 8 M urea, 1 mM PMSF in 10 mM sodium phosphate buffer, pH 7.0 for 2 h at 37°C and electrophoresed in 5%(w/v) polyacrylamide gels according to Weber and Osborn [22]. Gels were stained for protein with 0.25%(w/v) Coomassie blue.

Results and Discussion

The distribution of the recognised plasma membrane marker enzymes 5′-nucleotidase and phosphodiesterase I in the three fractions was similar to that reported by Wisher and Evans [13] (Table I). The specific activities of glucose-6-phosphatase and succinate dehydrogenase, markers for endoplasmic reticulum and mitochondria, were similar to those observed by other workers [13,14].

The reductions in 5′-nucleotidase and phosphodiesterase I activities observed in whole homogenates from liver of tumour-bearing rats were reflected in the decreased specific activities of the

TABLE I

DISTRIBUTION OF ENZYMIC ACTIVITIES IN MEMBRANE FRACTIONS FROM LIVER OF CONTROL AND TUMOUR-BEARING RATS

The mean weights of livers from control and tumour-bearing rats were 3.44 ± 0.07 (S.E.) and 4.33 ± 0.08 g per 100 g initial body weight. Enzyme activities are expressed as μmol substrate reacted per mg protein per h. Figures in parenthesis represent number of preparations. Results for 5'-nucleotidase and phosphodiesterase I are given as mean ± S.E., those for glucose-6-phosphatase and succinate dehydrogenase as average of two experiments. * Differences between values from tumour-bearing rats and corresponding controls statistically significant ($P < 0.01$, Student's t-test). ** Difference between value for tumour-rats and corresponding value for control animals statistically significant ($P < 0.02$, Student's t-test).

		Membrane fraction			
		Whole homogenate	Bile-canalicular	Lateral	Sinusoidal
5'-Nucleotidase	Control	0.92 ± 0.07(7)	88.5 ± 5.0(6)	22.5 ± 2.0(8)	17.2 ± 1.3(6)
	Tumour-bearing	0.58 ± 0.07(7) *	61.9 ± 7.5(6) **	17.6 ± 1.4(8)	10.9 ± 1.0(6) *
Phosphodiesterase I	Control	2.62 ± 0.01(7)	162.3 ± 6.9(6)	47.9 ± 5.6(8)	53.0 ± 3.7(6)
	Tumour-bearing	2.13 ± 0.06(7) *	107.3 ± 8.7(6) *	38.7 ± 3.2(8)	31.7 ± 1.7(6) *
Glucose-6-phosphatase	Control	4.3(2)	2.1(2)	3.2(2)	4.2(2)
Succinate dehydrogenase	Control	6.0(2)	0.6(2)	1.8(2)	1.2(2)

two enzymes found in bile-canalicular and sinusoidal fractions from these animals (Table I) but no significant alterations in activities were observed in preparations of lateral domain.

The actin contents of the three domains, estimated by the DNAase I inhibitor assay are shown in Table II. In the absence of 0.75 M guanidine-HCl no actin was estimable, suggesting that all the actin present was in a polymerised form. Actin constituted 7% of the total membrane protein of the lateral fraction, 2–3% of the protein of bile-canalicular fraction but only trace amounts were detectable in the sinusoidal fraction. Tumour growth had no significant effect on actin concentration in any of the fractions.

In the human erythrocyte cytochalasin B binds with high affinity to two sites on the plasma membrane, a sugar transport site and a motility-related site. The latter has been shown to be the 'barbed' or fast-growing ends of actin filaments which occur as part of a cytoskeletal matrix [23,24].

Fig. 1 shows that cytochalasin B bound with high affinity to both the lateral and the bile-canalicular membrane fractions but not to the sinusoidal fraction. The presence of 0.7 M glucose did not alter the binding characteristics indicating the absence of cytochalasin-sensitive sugar transport sites. The high affinity binding was completely inhibited by $7 \cdot 10^{-5}$ M dihydrocytochalasin B, confirming that only actin-binding sites were involved since this cytochalasin derivative binds only to this type of high affinity site [25]. Scatchard plot analysis (Table III) showed that the K_d for the reaction between the drug and its binding sites on the bile-canalicular and lateral membranes was of the order of 1 nM, indicating a very high affinity for cytochalasin and its receptors. Bile-canalicular and lateral fractions from normal rats contained, respectively, 13 and 20 pmoles binding sites per mg membrane protein.

TABLE II

ACTIN CONTENT OF LIVER MEMBRANE FRACTIONS FROM CONTROL AND TUMOUR-BEARING RATS

Actin was estimated by the DNAase I inhibitor assay [18]. Values are expressed as mean ± S.E. Figures in parenthesis represent number of preparations.

Membrane fraction	μg actin per mg membrane protein	
	Control	Tumour-bearing
Bile-canalicular (7)	27 ± 2	22 ± 3
Lateral (7)	67 ± 6	68 ± 4
Sinusoidal (6)	3 ± 1	3 ± 1

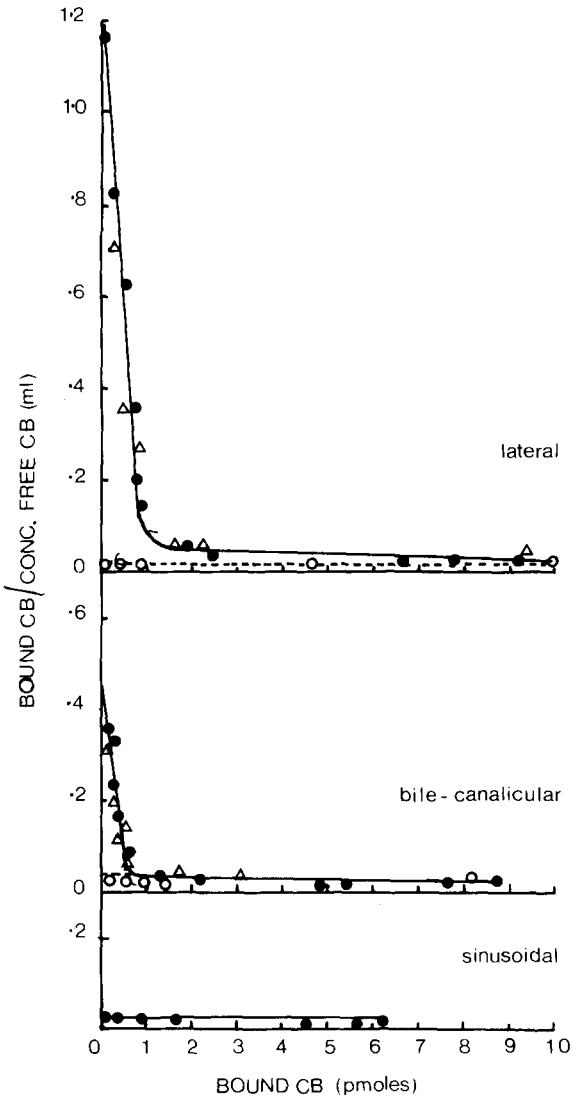

Fig. 1. Scatchard plots of the binding of cytochalasin B (CB) to plasma membrane fractions (50 μg protein) from liver of control rats. Fractions were assyed in the absence (●) or presence of 0.7 M glucose (△) or $7 \cdot 10^{-5}$ M dihydrocytochalasin B (○).

The ratio of pmoles actin to pmoles binding site suggests that actin is present as short filaments containing 49 and 83 molecules G-actin, respectively, in bile-canalicular and lateral fractions. These would appear to be longer than the actin oligomers associated with erythrocyte membranes where a figure of 10–17 monomers per binding site has been reported [26].

There was no alteration in the concentration of binding sites in either the bile-canalicular or lateral membrane preparations from tumour-bearing rats and the affinity of cytochalasin for these membranes was similar to that observed in control preparations.

Treatment of plasma membranes of different cell types, with non-ionic detergents causes solubilisation of lipids and integral membrane proteins and leaves an insoluble residue of cytoskeletal proteins and associated membrane components. Actin has been shown to be a component of such residues prepared from erythrocytes and lymphocytes [27–29], and was also shown to be a constituent of an insoluble residue remaining after extraction of a non-fractionated rabbit-liver membrane preparation with 1 or 2% Triton X-100 [9]. In the present work 65% of the protein from lateral and sinusoidal regions of rat liver membrane and 60% of that of the bile-canalicular region were extracted with solutions containing 0.5–1.5% Nonidet P-40 while approx. 70% of the 5′-nucleotidase activity was retained in the detergent-insoluble residues.

The polypeptide profiles obtained by polyacrylamide gel electrophoresis of the detergent-insoluble residues of plasma membrane fractions from liver of normal rats is shown in Fig. 2. A major band which co-electrophoresed with a purified sample of rat skeletal muscle actin was pre-

TABLE III

BINDING OF CYTOCHALASIN B TO HIGH AFFINITY BINDING SITES IN MEMBRANE FRACTIONS FROM LIVER OF CONTROL AND TUMOUR-BEARING RATS

Data, corrected for binding to low affinity sites, are expressed as mean ± S.E. Figures in parenthesis represent number of preparations.

Membrane Fraction	Number of binding sites (pmol/mg protein)		K_d (nM)	
	Control	Tumour-bearing	Control	Tumour-bearing
Bile-canalicular (3)	13 ± 1	11 ± 1	1.4 ± 0.09	1.6 ± 0.24
Lateral (4)	20 ± 2	19 ± 1	0.8 ± 0.9	1.2 ± 0.03

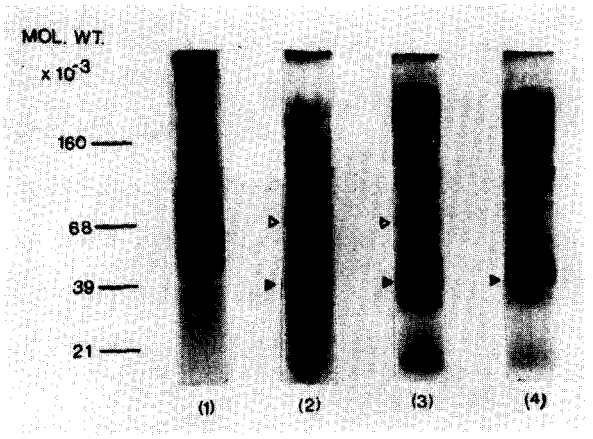

Fig. 2. SDS-polyacrylamide gel electrophoresis of Nonidet P-40-insoluble residues of rat liver plasma membrane fractions. Track I, sinusoidal preparation; track 2, bile-canalicular preparation; track 3, lateral preparation; track 4, lateral preparation extracted with detergent in absence of Ca^{2+} ions. Samples were analysed by electrophoresis on 5% polyacrylamide gels and stained with Coomassie blue. Closed arrowheads indicate the position of migration of rat muscle actin; open arrowheads indicate the position of a band of apparent molecular weight 68000 which was absent when Ca^{2+} ions were omitted from the extraction medium. The position of the marker proteins (*E. coli* RNA polymerase, bovine serum albumin and trypsin inhibitor) are shown.

sent in preparations from the bile-canalicular and lateral domains but was absent from preparations from the sinusoidal fraction. Although bands corresponding to molecular weights 227000, 124000, 98000, 83000, 68000, 33000 and 19000 were present in preparations from both bile-canalicular and lateral fractions, the relative intensities of some of these differed in the two preparations, those corresponding to proteins of molecular weights 124000 and 83000 being particularly prominent in the bile-canalicular preparations. Major protein bands of molecular weights of 208000 and 160000 were unique to lateral preparations while prominent bands with molecular weights of 170000 and 150000 were only observed in bile-canalicular preparations. The detergent-insoluble residue from the sinusoidal fraction showed few, if any, protein components common to the residues from the other two fractions. The polypeptide composition of the insoluble residue remaining after extraction of lymphocyte plasma membrane with 1% Nonidet P-40 was shown to be dependent on the presence of Ca^{2+} ions. In the absence of Ca^{2+} ions, proteins of molecular weights 68000, 55000, 3300 and 28000 were extractable by the detergent solutions [30]. When liver membrane fractions were extracted with Nonidet P-40 in the presence of 1 mM EGTA but absence of added Ca^{2+} ions, the disappearance of the 68000 molecular weight protein from insoluble residues of the lateral (Fig. 2) and bile-canalicular fractions (not shown) was observed but no other alterations in polypeptide profiles were apparent. Preparations of the three liver membrane domains from the tumour-bearing rat gave an electrophoretic pattern which showed no apparent differences from those from control animals (data not shown).

The low amounts of actin present in the sinusoidal fraction, the lack of cytochalasin binding and the absence of actin from the detergent-insoluble residue suggest that if there is any interaction between cytoskeletal actin and this membrane fraction it must be of a different nature from those occurring at the other two domains. Indeed any such association of actin with the sinusoidal region might well be expected to be of a more labile form since it is mainly at this surface that external stimuli interact with receptors to induce motile and proliferative responses of the cell.

The results obtained in the present work indicate that the alteration in the state of polymerisation of actin in the cytoplasm of the liver of the rat in response to the growth of the Walker 256 carcinoma [3] is not associated with any marked change in plasma membrane-associated actin.

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