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Rapid Report

Actin and actin-binding proteins in plasma membranes derived from Walker 256 ascites or solid tumour cells

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Plasma membranes from Walker 256 carcinoma cells grown ascitically or as a solid tumour were examined with respect to actin content, [^{3}H]cytochalasin B-binding and the binding of ^{125}I-labelled G-actin to membrane proteins separated by SDS-PAGE. Differences were observed both in cytochalasin B-binding to membrane actin and affinity of ^{125}I-labelled G-actin for specific membrane proteins.

Changes in cytoplasmic monomeric and filamentous actin pools have been demonstrated in cells undergoing alterations in shape and motility [1]. The cell membrane plays a significant role in this since many of the changes can be effected by the binding of extracellular ligands to cell surface receptors [2]. Tumour cells have the ability to adapt to varying environmental conditions when growing in different locations with associated changes in cell composition and membrane properties [3–6]. When Walker 256 carcinoma cells which have been growing ascitically are induced to grow as a solid tumour there is a 20% reduction in cytoplasmic actin and a much greater proportion of actin is in the unpolymerised form [4]. Actin is associated in significant amounts with the plasma membrane of many cell types, where it is thought to play a role in membrane–cytoskeletal interactions [2]. To investigate any alterations in membrane actin which might accompany changes in cytoplasmic actin observed in Walker tumour cells, we have made a study of actin and actin-binding proteins in the Walker cell plasma membrane when cells are grown ascitically or in the solid form.

A suspension of Walker 256 carcinoma cells grown as an ascitic tumour was injected into adult female Wistar rats, either intraperitoneally for the production of ascites tumours or subcutaneously in the dorsal region for the production of solid tumours. Ascitic fluid was aspirated after 5–7 days and treated as described by Church et al. [7] for the isolation of tumour cells. Solid tumours were removed after 10 days and viable portions washed with 1 mM $NaHCO_3$. Purified plasma membranes were obtained by centrifugation in the discontinuous sucrose gradient described by Church et al. [7], material appearing at the 35–39% interphase being collected.

As previously found [6] nuclei from ascitic and solid tumour preparations showed a similar DNA content, 19 ± 1.8 (S.E.) and 21 ± 1.1 pg per nucleus, respectively, in keeping with the aneuploid character of the Walker tumour [8] and indicating that both preparations were essentially homogeneous. Gel electrophoresis of plasma membrane proteins was carried out under denaturing conditions according to Laemmli [9] using a 5–20% (w/v) SDS-polyacrylamide gradient gel. Fig. 1 shows that only minor differences were apparent in the two membranes. The details of these and of enzyme levels in the two membranes have been published elsewhere [6].

Table I shows that the amount of actin per mg protein estimated by the DNase-I inhibition assay [10] was the same in preparations from both ascitic and solid tumours. Extraction of these membranes with 0.5% (v/v) Nonidet P-40 resulted in the retention of 80% of this actin in the extracted membranes, suggesting that this actin may be part of a membrane cytoskeleton as postulated for other tissues [11–14].

Cytochalasin B binds with high affinity to the 'barbed' or fast-polymerising ends of actin filaments. [^{3}H]Cytochalasin B binding assays were carried out as

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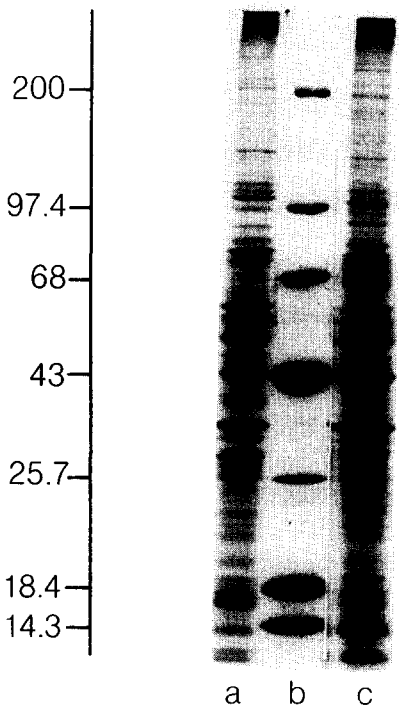

Fig. 1. Plasma membrane proteins from ascitic (a) and solid (c) tumours separated by SDS-PAGE on 5–20% (w/v) polyacrylamide gradients. Molecular masses (in kDa) of marker proteins (b) are indicated on the left margin.

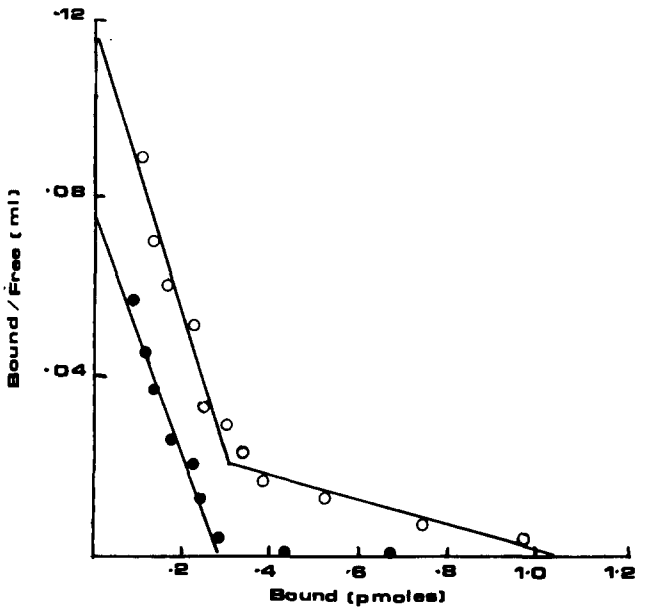

Fig. 2. Scatchard plot analysis of the binding of [^{3}H]cytochalasin B to plasma membranes (50 μg protein) from ascitic (•) and solid (○) tumours. The concentration of [^{3}H]cytochalasin B was in the range $1.8 \cdot 10^{-9}$ M to 10^{-6} M. Data were corrected for non-specific binding of radioactivity. Protein was estimated by the method Lowry et al. [26].

described by Lin and Lin [15] on isolated plasma membranes. All assays were performed in the presence of 0.7 M glucose to saturate any sugar transport sites which may be present since these also bind with high affinity to cytochalasin B [16]. Ascitic and solid tumour cell membrane preparations bind cytochalasin with high affinity. Scatchard plot analysis of [^{3}H]cytochalasin B binding to tumour cell membranes is shown in Fig. 2. The membranes from ascitic cells possessed a single class of high-affinity binding site while there was evidence in the solid tumour of two classes of binding site. In the presence of $7 \cdot 10^{-5}$ M dihydrocytochalasin B binding of [^{3}H]cytochalasin B was completely inhibited (results not shown) confirming that actin-binding sites were involved since this cytochalasin derivative competes only for such sites [15].

Analysis of the data from Fig. 2 (Table I) indicates high-affinity binding sites in both membranes of similar K_d (3–4 nM) and capacity (7 pmol per mg membrane protein). In the solid tumour cell membrane a second class of binding site was also identified with a $K_d = 32$ nM and a binding capacity of 20 pmol per mg membrane protein. Purified F-actin has been shown to bind to cytochalasin B with high affinity ($K_d = 5$ nM). However, when F-actin is reacted with cytochalasin B in the presence of tropomyosin and troponin, an additional class of binding site of lower affinity is generated as a result of conformational changes [17]. The additional binding sites observed in the membrane from the solid tumour cells might suggest a modification of the actin in this membrane arising from interaction with other proteins.

Rat muscle actin was isolated [18] and further purified by gel filtration on Sephadex G-150 [19]. ^{125}I-labelled G-actin was prepared by the method of Bolton and Hunter [20] and used in gel overlay experiments

TABLE I

Dissociation constants (K_d) and concentration of binding sites (n) of cytochalasin B-binding to tumour plasma membranes

K_d and n were obtained from Scatchard plot analysis. Results are expressed as means ± S.E. of four experiments. n.d., not detected. Total membrane actin was estimated by the method of Blikstad [10] in the presence of 0.75 M guanidine HCl.

Membrane source	Actin content (pmol/mg protein)	High-affinity site I		High-affinity site II	
		K_d (nM)	n (pmol/mg protein)	K_d (nM)	n (pmol/mg protein)
Ascitic	1598 ± 183	3.8 ± 0.3	7 ± 0.4	n.d.	n.d.
Solid	1514 ± 207	3.1 ± 0.3	7 ± 0.7	32 ± 3	20 ± 2

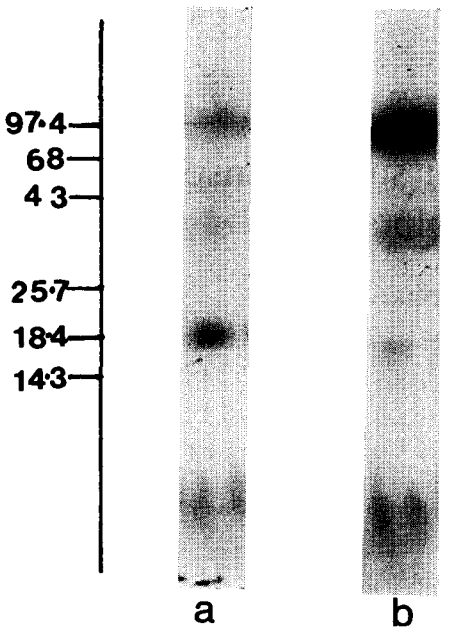

Fig. 3. Autoradiograph of ^{125}I-labelled G-actin overlay of plasma membrane proteins renatured after separation by SDS-PAGE on a 5–20% (w/v) polyacrylamide gradient gel. Ascitic membrane (a), solid membrane (b). This autoradiograph is one of three replications of this experiment.

according to Snabes et al. [21]. ^{125}I-labelled G-actin bound with high affinity to only four proteins in the plasma membranes as shown by bands corresponding to 95 kDa, 45 kDa, 32 kDa and 18 kDa (Fig. 3). Comparison of the results with the two membrane preparations indicated a more intense labelling of the 95 kDa protein in the membrane from cells grown as a solid tumour and of the 18 kDa protein in the membrane from ascitic cells. Identification of actin-binding proteins by this technique can serve only as an indication of possible anchorage sites for membrane or cytoplasmic actin. Proteins which bind to either G- or F-actin have been identified in the plasma membrane of other cell types [22–25] and at least one has been shown to be an actin barbed-end capping protein [25]. We have at present no information on the relationship between membrane-associated actin and the actin-binding proteins identified in the membrane. The differences between the two membranes in the relative intensity of labelling of the 95 kDa and 18 kDa proteins may, however, provide a basis for further investigation, both in relation to the appearance of the additional cytochalasin B-binding sites in the plasma membrane from cells of the solid tumour and in the differences in the state of polymerisation of actin observed previously in the solid and asitic tumour cell [4].

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