INTERACTION OF CYTOPLASMIC PROTEINS WITH LIPOSOMES AND THEIR CELL SPECIFICITY

Kozo UTSUMI, Eiji OKIMASU, Yoshiki TAKEHARA*, Sadahiro WATANABE*, Masanobu MIYAHARA and Yasuki MOROMIZATO*

Department of Medical Biology, Kochi Medical School, Kochi 781–51 and **Department of Anatomy, Okayama University Medical School, Okayama 700, Japan*

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1. Introduction

Numerous studies have shown that the plasma membrane has a fundamental role in the regulation of cell metabolism [1–4]. Attention has been focussed on the transmembrane control mechanism concerning the interaction between plasma membrane and cytoskeletal structures [2–12]. For the transfer of biological information from external surface to cytoplasm, 3 steps are postulated:

(i) Interaction of surface receptors with ligands at the external cell surface;
(ii) Transmission of the information across the plasma membrane;
(iii) Modulation of cytoplasmic components at the inner surface of the membrane.

For example, cell agglutination by concanavalin A (con A) is accompanied by the redistribution of con A receptors on the cell surface from a random pattern to form a cap [2], and microtubules and microfilaments are involved in the transmembrane control of redistribution of lectin receptor sites in the cell membrane [2,3]. Therefore, the interaction of cytoplasmic proteins with the plasma membrane through their lipophilic nature and affinity to the membrane-integrated proteins have an important role for the regulatory mechanism of the transmembrane control system in cells.

Here we report our attempts to determine the existence of an interaction between cytoplasmic proteins and artificial lipid vesicles, liposomes. We find that cytoskeletal components such as tubulin, actin and α-actinin have a strong tendency to associate with liposomes and that this characteristic, which may be altered by several physiological conditions, is responsible for their interaction with lipid components of liposomes.

2. Materials and methods

Polymorphonuclear leukocytes (PMN) of guinea pig, Ehrlich ascites tumour cells (EATC), human platelets and rat brain and liver were studied.

The interactions of liposomes with cytoplasmic proteins of various cells or purified proteins were carried out by modification of the method in [13]: Cells or tissues were suspended in hypotonic saline solution, 10 mM NaCl–15 mM phosphate buffer (pH 6.75)–1 mM $MgCl_2$, and homogenized with a Teflon–glass homogenizer at 4°C. The homogenate was centrifuged for 60 min at 100 000 × *g* and the supernatant fraction was kept at 4°C until required.

Actin and tubulin were purified from rat muscle and brain as in [14] and [15], respectively.

Liposomes were prepared as follows: 15 mM dimyristoyl phosphatidylcholine (DMPC) was dissolved in chloroform–methanol (2:1, v/v) in the presence or absence of 15 mM cholesterol, 1.5 mM cetylamine and 1.5 mM dicetylphosphate. Aliquots (5 ml) of these lipid solutions were evaporated in test tubes using a Taiyo concentrator (TC-8). Following the addition of 0.1 M NaCl–20 mM phosphate buffer (pH 6.75) (5 ml) the tube was agitated on a vortex mixer for 10 min at 25°C. The multilayered liposomes were then sonicated using a sonifier (Branson, type 185) for 10 min at 25°C. The interaction of cytoplasmic proteins from the homogenates with liposomes was carried out as follows: The liposomes (15 μmol phospholipid/ml) were incubated with cytoplasmic or purified proteins (10 mg protein/ml) at 37°C for 30 min

in the presence of a proteinase inhibitor, TPCK. After incubation, the liposome–protein mixture was mixed with equal volume of 1.10 density Ficoll solution containing 0.1 M NaCl–20 mM phosphate buffer (pH 6.75). The mixture (2 ml) was layered within a discontinuous Ficoll density gradient of the following densities: 1.08, 1.05 (sample), 1.04, 1.03, 1.01 and 1.007. All the Ficoll densities were made with 0.1 M NaCl–20 mM phosphate buffer (pH 6.75). The liposomes which had interacted with cytoplasmic or purified proteins floated upward through this gradient to the interface between 1.03 and 1.01 on centrifugation at 100 000 × *g* for 60 min at 30°C. In some experiments, the floated liposomes were washed once through layers of 1.05–1.03 specific gravity of Ficoll by centrifugation at 100 000 × *g* for 60 min.

SDS–polyacrylamide gel electrophoresis (PAGE) was done as in [16]. All samples were boiled for 2 min in 1% SDS containing 1% 2-mercaptoethanol. Proteins were fixed and stained in 0.25% Coomassie blue, 10% glacial acetic acid, 50% methanol mixture for 12 h.

Molecular weights were determined by comparison with R_F-values of RNA polymerases (Seikagaku Kogyo, Tokyo).

Protein concentrations were determined as in [17] and the phospholipid content of liposomes by the method in [18].

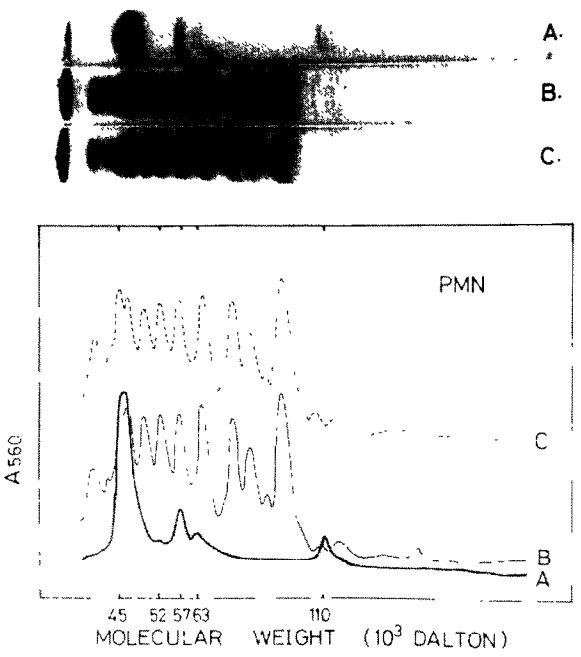

Fig.1. SDS-PAGE analysis of liposome-associated cytoplasmic proteins of guinea pig polymorphonuclear leukocytes. PMN cytoplasmic fraction was incubated with DMPC–cholesterol–cetylamine liposomes for 30 min at 37°C. The liposome fraction was isolated by Ficoll density gradient centrifugation following the method described: (A) Liposome-associated proteins; (B) non-associated proteins; (C) total cytoplasmic proteins.

3. Results and discussion

3.1. *Association of cytoplasmic proteins to liposomes*

As described by many investigators, tubulin exists in the plasma membrane and has a lipophilic nature [13,19,20]. The nature of membrane association is also observed in several cytoskeletal elements [21–24]. The nature of membrane association of tubulin was confirmed [13] by the interaction of purified tubulin and liposomes. Also we have examined the interaction between liposomes and cytoplasmic proteins of various cells or purified cytoskeletal proteins, by a modification of the method in [13]. After the incubation of cytoplasmic proteins of PMN with liposomes, consisting of DMPC–cholesterol–cetylamine, for 30 min at 37°C certain proteins such as cytoskeletal elements of the cytoplasm associate with the liposomes and may be separated from other proteins by density gradient centrifugation. The separated proteins were M_r ($\times 10^{-3}$): 45, 52, 55 and 110, as determined by SDS–PAGE (fig.1,2). These proteins were identical to puri-

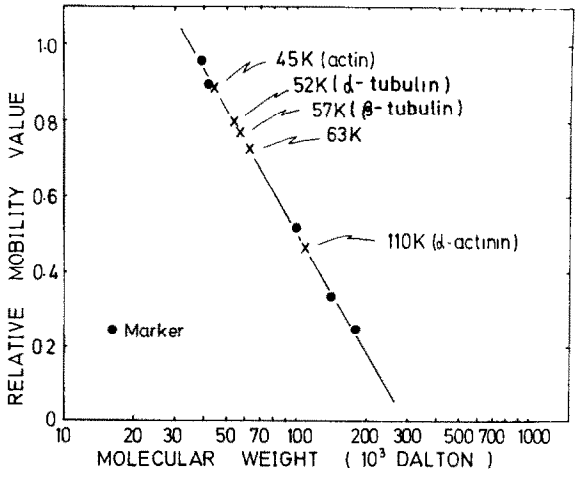

Fig.2. Molecular weight of liposome-associated proteins. Molecular weights were determined by SDS–PAGE using RNA-polymerases as standard proteins. The 45, 52, 55 and 110 × 10^3 M_r proteins coincide with the M_r of actin, α- and β-tubulins and α-actinin, respectively.

fied actin, tubulin and α-actinin, respectively, as regards behavior on SDS–PAGE. The purified actin also associated with liposomes and floated upward through a Ficoll density gradient as observed with cytoplasmic proteins. Furthermore, we confirmed the association of purified tubulin to liposomes as in [13].

3.2. *Tissue specificity on membrane associating character of cytoplasmic proteins*

The proteins of contractile and cytoskeletal elements, actin, myosin, tropomyosin, α-actinin, tubulin, prokeratin, vimentin, gelsolin, actinogelin and desmin have been detected in several cells. Most of these elements have the ability to associate with biological membranes as assessed by electron microscopy. Various amounts of these proteins are found in different cells: a large amount of tubulin is found in brain, actin in platelets and PMN and vimentin in lens-forming cells [24].

With all the cells or tissues examined, these 45, 52, 55 and 110 × 10^3 M_r proteins were detected in liposome fraction following separation from the liposome-protein mixture. The amounts of protein associated with liposomes are cell specific: a large amount of 45 × 10^3 M_r protein but a small amount of 52 × 10^3 and 55 × 10^3 M_r proteins were in liposome fraction from PMN or platelet homogenate. But a larger amount of tubulin and a small amount of actin were recovered from brain tissue homogenate as shown in fig.1, fig.3a, fig.4. In common with other cell types it was possible to demonstrate the association of 45, 52, 55 and 110 × 10^3 M_r proteins from liver and Ehrlich ascites tumour cells, with liposomes. However, several minor unidentified proteins were associated with liposomes and separated as shown in fig.3b,c.

3.3. *Various factors affecting the interaction between liposomes and cytoplasmic proteins*

The interaction between the cytoplasmic proteins and liposomes was affected by lipid composition of liposomes. In the case of DMPC liposomes, the affinity of cytoplasmic proteins was rather non-specific and the amount of protein associated with the liposomes was remarkably decreased by addition of cholesterol. The reduced association to DMPC–cholesterol liposomes was reversed by the addition of 1/10 vol. cetylamine but not by dicetylphosphate (fig.4, table 1). In this case, the associated proteins were rather specific for cytoskeletal proteins. Furthermore, preliminary experiments on the interaction between cytoplasmic

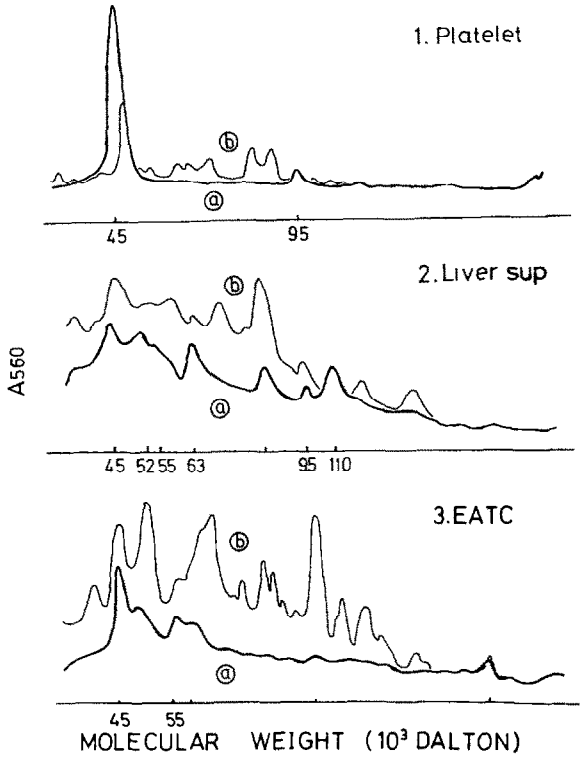

Fig.3. SDS–PAGE patterns of liposome-associated proteins of platelets, liver and Ehrlich ascites tumour cells. Cytoplasmic proteins were reacted with liposomes as in the case of PMN. All the experimental conditions were as in fig.1: (1) human platelets; (2) rat liver; (3) Ehrlich ascites tumour cells; (a) non-associated proteins; (b) liposome-associated proteins.

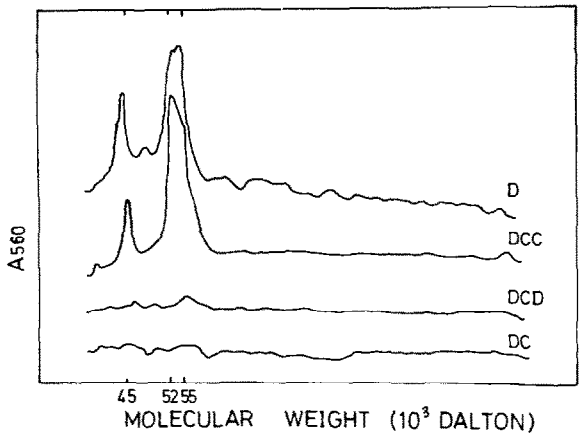

Fig.4. Effect of liposome components on the association of cytoplasmic proteins of rat brain tissue to liposomes. Experimental conditions were as in fig.1. Liposome components were changed as indicated in the figure. DMPC–cholesterol–cetylamine–dicetylphosphate (1:1:0.1:0.1). *Abbreviations:* D, DMPC; DC, DMPC + cholesterol; DCC, DMPC + cholesterol + cetylamine; DCD, DMPC + cholesterol + dicetylphosphate.

Table 1
Effect of liposome components on the association of cytoplasmic proteins of rat brain tissue to liposomes

Liposome lipid	Protein (μg)	Ratio
	Phospholipid–P (μg)	
Dimyristoyl phosphatidylcholine (DMPC)	8.48	4.42
DMPC + cholesterol + cetylamine	8.18	4.26
DMPC + cholesterol + dicetylphosphate	2.54	1.32
DMPC + cholesterol	1.92	1.00

Experimental conditions were as in fig.4

proteins of brain and inside-out vesicles of erythrocytes show that many of cytoplasmic proteins, including cytoskeletal proteins, associate with the inside-out vesicles. From the data obtained in this experiment, it is suggested that the cytoplasmic proteins especially cytoskeletal elements have the necessary characteristics to associate with inner surface of plasma membrane and various cytoplasmic organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and lysosomes. The ability of these proteins to interact with these biological membranes is possibly altered by several membranous and cytoplasmic factors. Therefore, the cytoplasmic proteins which have the ability to associate with biological membranes may have an important role in regulating cytoplasmic metabolism and cell movement.

Acknowledgements

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