Acta Medica Okayama

Volume 35, *Issue* 4 1981 *Article* 1

OCTOBER 1981

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Abstract

In order to get precise information about the movement of plasma membrane proteins in cap formation, cyto- and bio-chemical analyses were made of the plasma membranes from non-capped areas of Ehrlich ascites tumor cells (EATCs) exposed to concanavalin A (Con A). Blebs formed by treatment with cytochalasin B (CB) of the non-capped areas of cells having a cap were isolated and used as the plasma membranes from non-capped areas (ConA-CB-bleb fraction). This bleb fraction was compared with a bleb fraction prepared from cells without ConA-treatment (CB-bleb fraction). Cytochemical analysis of ConA-CB-bleb fraction revealed a decreased in conA binding sites (ConA-BS) compared to the CB-bleb fraction. SDS polyacrylamide slab gel electrophoresis also revealed a decrease in the major components of ConA-BS of the ConA-CB-bleb fraction. The minor components of ConA-BS showed no distinct quantitative difference between the ConA-CB-bleb and CB-bleb fractions. NA+ K+-adenosine triphosphatase (ATPase), 5' nucleotidase (5'ND) and gamma-glutamyl transpeptidase (gamma-GTP) did not show any decrease in activity in the ConA-CB-bleb fraction, but the activity of D+-stimulated phosphatase (K-Pase) was decreased. The findings indicate that there are two types of plasma membrane glycoproteins in EATCs; one includes those participating in cap formation due to ConA, e.g. the major components of ConA-BS and K-Pase, and the other, those not participating in such cap formation, e.g. some minor components of ConA-BS, ATPase, 5'ND and gamma-GTP, which keep their places without moving.

KEYWORDS: concanavalin A, cytochalasin B, capping, bleb, biochemical analysis.

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*PMID: 6117190 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 35, (4), 221—234 (1981)

MOVEMENT OF PLASMA MEMBRANE PROTEINS OF EHRLICH ASCITES TUMOR CELLS IN RELATION TO CAP FORMATION INDUCED BY CONCANAVALIN A: A STUDY ON THE NON-CAPPED AREAS

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Received March 6, 1981

Abstract. In order to get precise information about the movement of plasma membrane proteins in cap formation, cyto- and bio-chemical analyses were made of the plasma membranes from non-capped areas of Ehrlich ascites tumor cells (EATCs) exposed to concanavalin A (ConA). Blebs formed by treatment with cytochalasin B (CB) of the non-capped areas of cells having a cap were isolated and used as the plasma membranes from non-capped areas (ConA-CB-bleb fraction). This bleb fraction was compared with a bleb fraction prepared from cells without ConA-treatment (CB-bleb fraction). Cytochemical analysis of ConA-CB-bleb fraction revealed a decrease in ConA binding sites (ConA-BS) compared to the CB-bleb fraction. SDS polyacrylamide slab gel electrophoresis also revealed a decrease in the major components of ConA-BS of the ConA-CB-bleb fraction. The minor components of ConA-BS showed no distinct quantitative difference between the ConA-CB-bleb and CB-bleb fractions. Na^+, K^+-adenosine triphosphatase (ATPase), 5'nucleotidase (5'ND) and γ-glutamyl transpeptidase (γ-GTP) did not show any decrease in activity in the ConA-CB-bleb fraction, but the activity of K^+-stimulated phosphatase (K-Pase) was decreased. The findings indicate that there are two types of plasma membrane glycoproteins in EATCs; one includes those participating in cap formation due to ConA, *e.g.* the major components of ConA-BS and K-Pase, and the other, those not participating in such cap formation, *e.g.* some minor components of ConA-BS, ATPase, 5'ND and γ-GTP, which keep their places without moving.

Key words : concanavalin A, cytochalasin B, capping, bleb, biochemical analysis.

It is generally believed that the capping phenomenon or the aggregation of plasma membrane proteins as seen on EATCs exposed to ConA is closely related to the function of the cytoskeletal system like microfilaments and microtubules linked to the receptors (1-6). Some plasma membrane proteins other than the receptors, however, may also be involved as seen in the co-capping of some histocompatibility antigens together with the surface immunoglobulins of lymphocytes exposed to anti-surface immunoglobulin antibodies (5, 7, 8). These findings are morphological observations and the precise changes in membrane components have not been detailed yet. Bichemical analyses may serve to make these changes clear, though it is not always easy to get a pure fraction of the special cell

surface areas.

Recently, Condeelis succeeded in isolating the ConA-caps of *Dictiosterium discoideum amoebae*. Biochemical analysis revealed a quantitative increase in cap-associated actin and myosin during the cap formation and a decrease in them with the completion of the process (9).

Quite recently, I have succeeded in getting the plasma membrane fraction of non-capped areas of EATCs having ConA-caps in a highly purified state suitable for biochemical analysis. EATCs form caps in response to treatment with ConA (3) and form blebs or zeiotic knobs by treatment with CB on their surfaces (10-12). And also EATCs having a ConA-cap form blebs on non-capped areas by incubating with CB without demolishing the caps (13). These blebs can be dislodged from the cells easily by scraping with a teflon-glass homogenizer. With these isolated blebs, the plasma membrane fraction of the non-capped areas is obtained by density gradient centrifugation.

In this paper it is reported that by the ConA-cap formation, the non-capped area of EATC plasma membrane loses most of the major components of ConA-BS and K-Pase activity without any decrease in the quantity of the minor components of ConA-BS and the activities of ATPase, 5'ND and γ-GTP.

MATERIALS AND METHODS

EATCs harvested 8-12 days after intraperitoneal inoculations of 2 x 10^6cells in Swiss mice were used. Freshly obtained ascites was centrifuged and the sedimented cells were washed with cold Mg^{++}- and Ca^{++}- free Dulbecco's phosphate buffer saline (PBS) (14), by repeated centrifugations, six times at 500 rpm for 5 min, by which contaminated erythrocytes were removed completely.

The freshly prepared EATCs resuspended in PBS, 1-2 x 10^7 cells /ml, were used for the preparation of bleb fractions. The blebs formed on EATCs exposed to CB were isolated from the cells by scraping with a teflon-glass homogenizer. The cell debris was

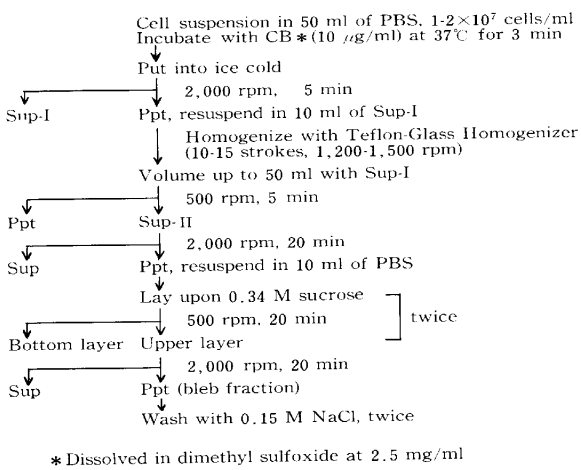

Fig. 1. Procedure used for the preparation of the bleb fractions.

removed by weak centrifugation and the bleb fraction was purified by sucrose gradient centrifugation (CB-bleb fraction) (Fig. 1).

The blebs formed by CB on the ConA-treated EATCs (ConA-CB-blebs) were obtained with the cells incubated with ConA (50 μg/ml) at 37°C for 15 min by the method above. But with ConA-treatment, about 40% of EATCs did not form caps (3). These cells also formed blebs by the later CB-treatment. Therefore, these blebs contaminating the ConA-CB-bleb fraction were adsorbed to Sephadex-G-25 by adding Sephadex powder, 0.5 g, to Sup II in Fig. 1, left standing for about 5 min to be sedimented and the supernatant was separated by sucking gently with a pipette. At each step, wet preparation of the bleb fractios were observed with a light microscope to ensure the process. Thus about 500 μg protein of the bleb fraction was obtained from 10^8 EATCs.

Plasma membranes of native EATCs having neither caps nor blebs (native membrane; NM) were prepared by the method described by Brunette and Till (15). This fraction served as a native control.

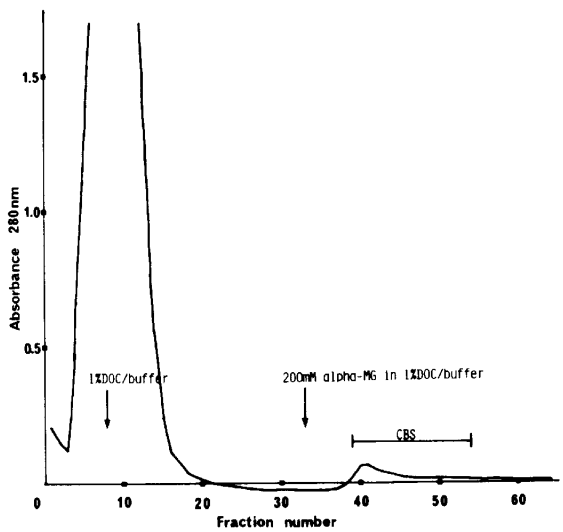

Fig. 2. Elution pattern obtained by ConA-Sepharose 4B affinity chromatography on the DOC-solubilized NM fraction. Each 3.0 ml was fractionated and the fractions eluted by 0.2 M α-methyl D-glucoside (α-MG) in DOC-buffer were collected for CBS.

ConA-BS were prepared with a NM fraction by affinity chromatography using ConA-Sepharose 4B (Pharmacia Fine Chemicals) packed into 1.6Øx 9 cm column according to a modified method of Nachbar *et al.* (16). Before use, the column was equilibrated with 1% sodium deoxycholate (DOC) in 10 mM Tris-HCl (pH 7.8) (DOC-buffer). The NM fraction dissolved in DOC-buffer was let through the column. The column was washed with DOC-buffer and then eluted with 0.2 M α-methyl D-glucoside (α-MG) dissolved in DOC-buffer (Fig. 2). The fractions eluted by α-MG were collected and concentrated to one-hundredth their volume using a macrosolute concentrator, Minicon-B (collected ConA-BS; CBS). About 1 mg of CBS was obtained from 40 mg protein of NM fraction.

Electron-microscope observations were made on the EATCs exposed to ConA (50 μg/ml) at 37°C for 15 min followed by CB-treatment (10 μg / ml) at 37°C for 3 min and also the cells exposed to CB only. The fractionated blebs and NM were also observed

under the electron-microscope. Samples were fixed with 2% glutaraldehyde in PBS, post-fixed with 1% osmium tetraoxide in 0.1 M phosphate buffer (pH 7.4), dehydrated and embedded in Epon-812. ConA-BS were visualized on the samples fixed with glutaraldehyde by the horseradish peroxidase-diaminobenzidine (HRP-DAB) method described by Bernhard and Avrameas (17). All observations were carried out without lead and uranium stains except the NM fraction.

SDS polyacrylamide slab gel electrophoresis was performed using a 7.5% lower gel in 0.375 M Tris-HCl (pH 8.8) and a 3% upper gel in 0.125 M Tris-HCl (pH 6.8) according to the method described by Laemmli and associates (18). ConA-CB-bleb and CB-bleb fractions were treated with a hypotonic solution (10 mM NaCl), centrifuged at 10,000 rpm for 20 min and the sedimented membrane fractions were used. Other materials; NM fraction, CBS and a standard, were used without any special treatment. These samples were dissolved in a SDS (1%)-glycerol (10%) mixture, put in a boiling water bath for 2 min, cooled and centrifuged at 30,000 rpm for 1 hr. The supernatants were obtained discarding unsoluble sediments. After electrophoresis, the gels were fixed and stained with Coomassie blue for protein and with PAS for carbohydrate. The stained gels were scanned at 560 nm for Coomassie blue and 545 nm for PAS. As a standard, myosin-B prepared from skeletal muscles of rats (19), α-actinin from skeletal muscles of fowls (20) and ConA were mixed roughly equally in protein concentration and used. One hundred μg protein of the samples in 20 μl was used for Coomassie blue stain and 300 μg protein in 60 μl for PAS stain.

The assays of ATPase (21), 5'ND (22), K-Pase (23) and glucose-6-phosphatase (G6Pase) (24) were made under the conditions reported (Table 1). For the measurement of inorganic phosphate released, the method of Fiske and Subbarow (25) was used for ATPase, and the method of Baginski and associates (26) for 5'ND and G6Pase. The activity of 5'ND was estimated as the difference between the activites of 5'AMPase and 2'AMPase (22). For the measurement of K-Pase activity, p-nitrophenyl phosphate was used as a substrate and the released p-nitrophenol was estimated at 420 or 405 nm. The activities of γ-GTP and acid phosphatase (ACP) were measured with the kits for sale,

TABLE 1. CONDITIONS OF THE ENZYME ASSAYS

	Medium	Time & volume
ATPase [3,6,1,3]	3 mM ATP, 150 mM NaCl, 15 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 20 mM Tris-HCl(pH 7.1),	60 min 1.0 ml
K-Pase	3.7 mM p-nitrophenyl phosphate, 25 mM KCl, 3.7 mM $MgCl_2$, 30 mM Tris-HCl(pH 8.0),	10-60 min 1.0 ml
5'ND [3,1,3,5]	3 mM 2'AMP or 5'AMP, 8 mM $MgCl_2$, 80 mM glycine-NaOH(pH 8.7),	180 min 0.3 ml
γ-GTP [2,3,2,2]	4 mM γ-glutamyl-3-carboxy-4-hydroxy anilide, 50 mM glycylglycine, 95 mM Tris-HCl(pH 8L5),	60-120 min 0.4 ml
G6Pase [3,1,3,9]	25 mM glucose-6-phosphate, 62.5 mM sucrose, 0.25 mM EDTA, 25 mM cacodylic acid-NaOH(PH 6.5).	60-120 min 0.4 ml
ACP [3,1,3,2]	5 mM phenyl phosphate disodium 44 mM citric acid-NaOH(pH 4.85)	60 min 2.0 ml

Yatolon Inc. Japan; a modified method of Orlowski and associates (27) for γ-GTP and that of Kind and King (28) for ACP. Protein concentration was measured by the method of Lowry and collaborators (29).

The activities of these enzymes were measured in ConA-CB-bleb fraction, CB-bleb fraction, NM fraction and untreated native EATCs. The influence of CB and ConA on the activities of ATPase, 5'ND, K-Pase and γ-GTP was studied adding CB (10 μg / ml) and ConA (50 μg / ml) to their assay systems.

RESULTS

Under an electron-microscope, EATCs were round in shape and had many microvilli on their surfaces (3, 12). By incubatini with CB at 37°C for 3 min, a number of blebs appeared all around the cell surfaces. In the samples stained by the HRP-DAB method, ConA-BS appeared as electron-dense deposits on the cell membrane being distributed uniformly on the cell surfaces as well as on the blebs (Fig. 3-a). Dimethyl sulfoxide, which was used as the solvent for CB, had no ill effect on the bleb formation and on the distribution of ConA-BS.

ConA-treatment of EATCs at 37°C for 15 min induced cap formations on about 60% of the treated cells (3). In those cells having caps, ConA-BS accumulated in the capped areas. When the cells having caps were treated with CB, blebs formed on their surfaces except the capped areas, but the bleb formation did not induce any change in the location of ConA-BS. That is, the capped areas had dense deposits of reactive products and the non-capped areas had only small deposits including the surface of the blebs (Fig. 3-b).

The bleb fractions obtained from these cells consisted of many blebs, few EATCs and a small number of membranous vesicles as observed under a phase contrast microscope (Fig. 4-a). Under an electron-microscope, the isolated blebs consisted of plasma membranes, ribosomes and a few fibrous structures, but other membranous components such as mitochondria, micorosomes and lysosomes were rarely encountered. Subfractionation studies of the bleb fractions by SDS polyacrylamide disc gel electrophoresis and enzymatic analysis revealed that the contaminated membranous vesicles were not of organelles but of fragmented plasma membranes. No distinct morphological differences were observed between ConA-CB-blebs and CB-blebs. Cytochemical studies with the HRP-DAB method, however, revealed a marked positive reaction of ConA-BS on CB-blebs but a poor reaction on ConA-CB-blebs (Fig. 4-c,d).

The electron-microscope observations of NM fraction or the plasma membrane separated directly from native EATCs showed cytoplasmic membrane contaminated with some ribosomes and a few filamentous structures. But other organelles like mitochondria, lysosomes and microsomes were rarely encountered. The pictures were essentially the same as those of the bleb fractions obtained from the cells affected by CB (Fig. 4-b).

With electrophoresis, ConA-CB-bleb, CB-bleb and NM fractions had many Coomassie blue stained bands (Fig. 5-a and 6-a). CBS had two main bands of

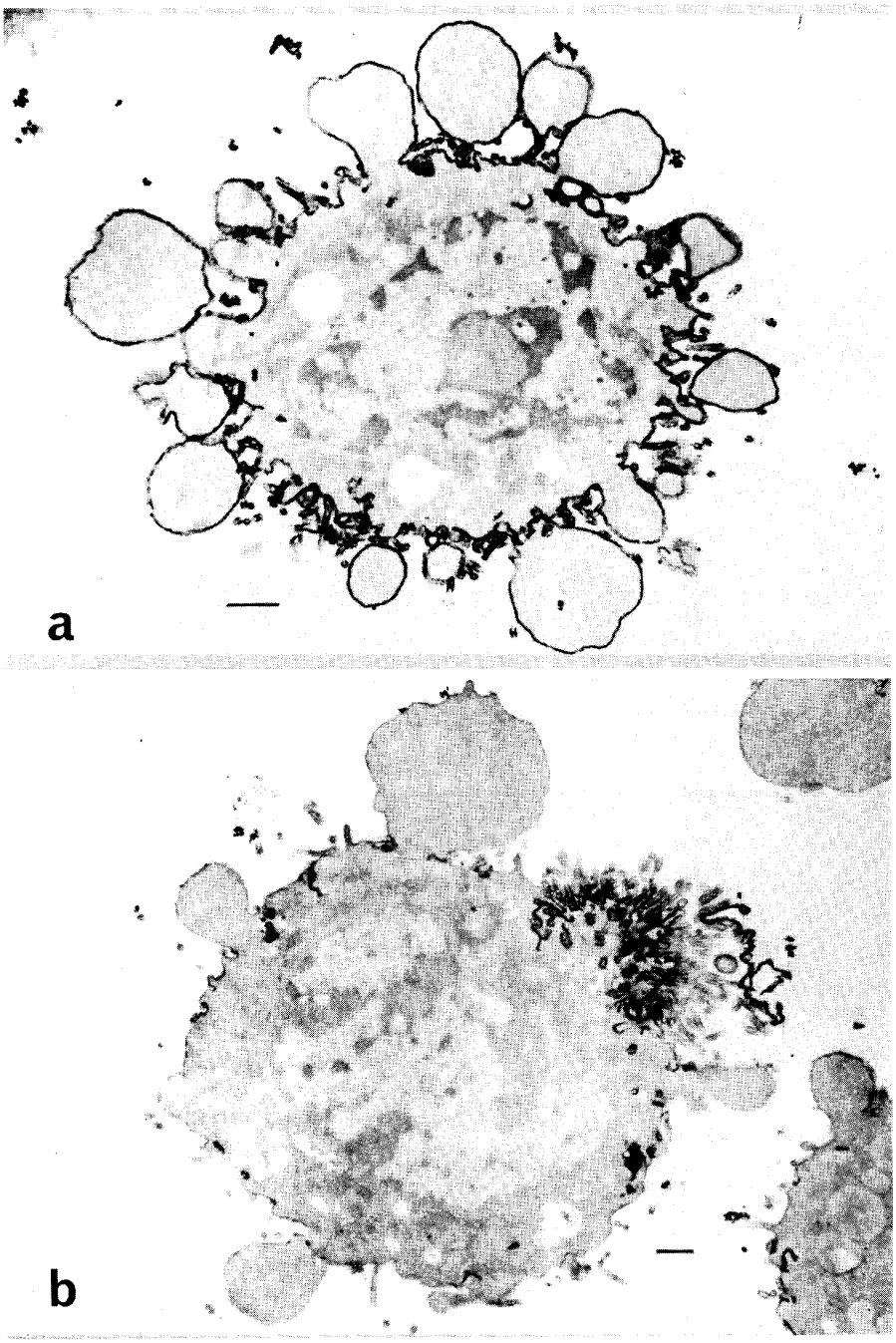

Fig. 3. Electron-micrographs of EATC having CB-blebs (a) and one having ConA-CB-blebs (b). ConA-BS were visualized by the HRP-DAB method. The cells were photographed without lead and uranium stainings. Bar = 1μm.

about 140,000 and 120,000 dalton (arrows in Fig. 5 and 6), and several minor bands including those of about 200,000, 170,000, 100,000, 80,000, 70,000, 55,000 and 48,000 dalton (Fig. 5-a-C and 6-a-C). With PAS stain, the two major bands appeared distinctly and the minor bands as broad ones (Fig. 5-b-C and 6-b-C). The NM fraction showed a similar electrophoretic pattern to that of the CB-bleb fraction. The pattern of ConA-CB-bleb fraction was nearly identical with that of CB-bleb fraction except the two bands comparable to the major bands of CBS; these bands were faint in ConA-CB-bleb fraction, while they appeared clear in CB-bleb fraction and NM fraction (Figs. 5 and 6). The bands comparable to the minor bands of CBS gave no differences among ConA-CB-bleb, CB-bleb and NM fractions. CBS had a ConA-band, which will be due to the release of ConA from ConA-Sepharose 4B. A distinct ConA-band was also observed in ConA-CB-bleb fraction which had only poor ConA-BS electronmicroscopically (Fig. 5-a and 6-a).

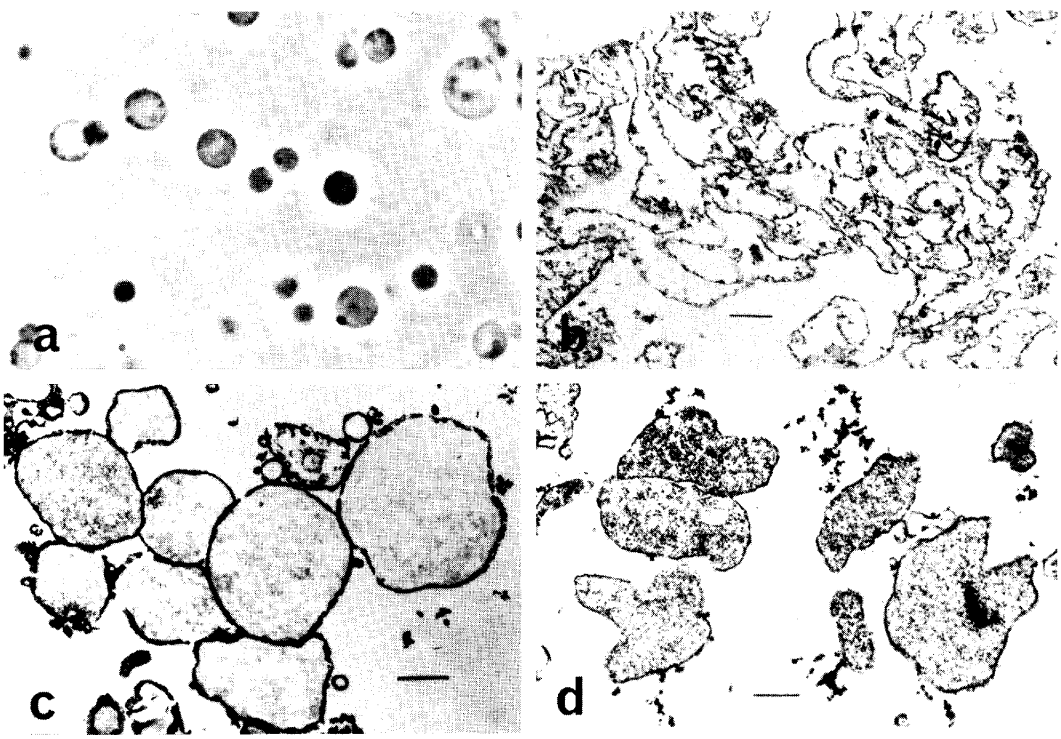

Fig. 4. Pictures of the bleb fractions (a, c, d) and NM fraction (b). a; phase contrast micrograph of purified bleb fraction (CB-bleb fraction). x 1,500. b; electron-micrograph of NM fraction, stained with lead and uranium. Bar = 1μm. c; electron-micrograph of CB-bleb fraction taken without lead and uranium stainings. ConA-BS were stained as in Fig. 3. Bar = 1μm. d; electron-micrograph of ConA-CB-bleb fraction stained as in c. Bar = 1μm.

Enzymatic analysis revealed that the activities of ATPase, 5'ND and γ-GTP were nearly equal in intensity among ConA-CB-bleb, CB-bleb and NM fractions. But K-Pase activity of ConA-CB-bleb fraction appeared lower than that of CB-bleb fraction by about 40%. The activities of G6Pase, a microsomal enzyme, and ACP, a lysosomal one, were nearly the same in all fractions (Table 2). The activities of the enzymes measured on untreated native EATCs were lower than or nearly equal to those measured on the plasma membrane fractions, e.g. NM fraction; one-tenth in ATPase, one-half in K-Pase, one-third in γ-GTP and near-

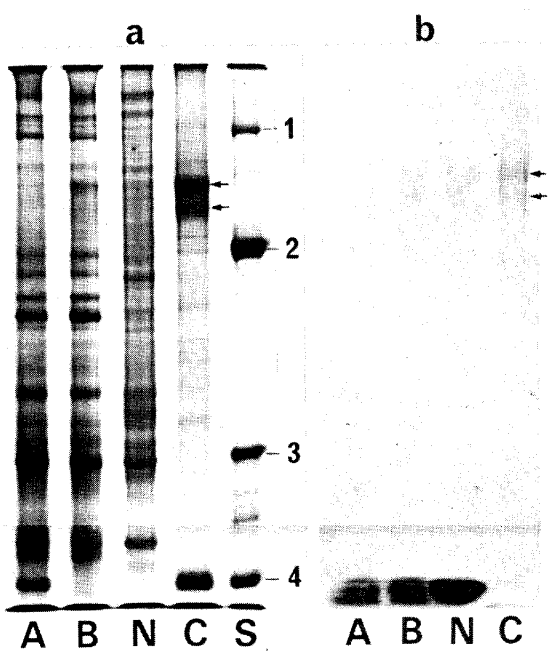

Fig. 5. (above) Patterns of SDS polyacrylamide slab gel electrophoresis on ConA-CB-bleb fraction (A), CB-bleb fraction (B), NM fraction (N), CBS (C) and standard (S). a; Coomassie blue stain. b; PAS stain. 1; myosin, 220,000 dalton. 2; α-actinin, 95,000 dalton. 3; actin, 43,000 dalton. 4; ConA, 27,000 dalton. Arrows indicate the major components of ConA-BS.

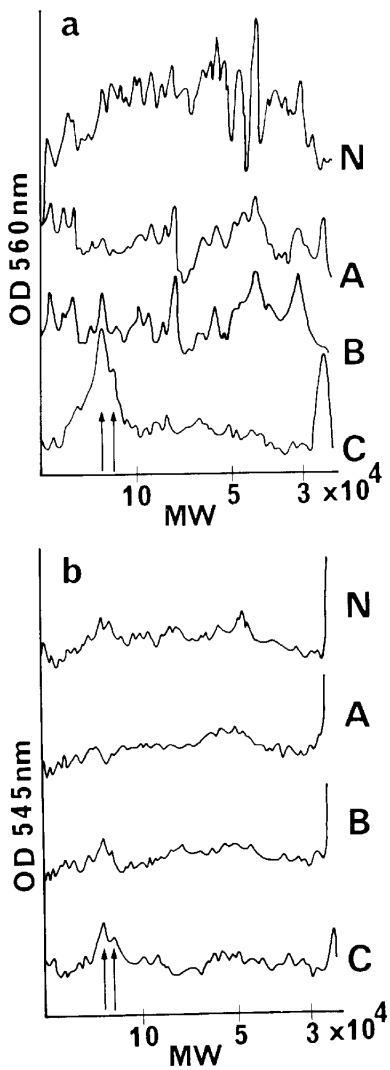

Fig. 6. (right) Scanning patterns of SDS polyacrylamide slab gel electrophoresis. a; Coomassie blue stain scanned at 560 nm. b; PAS stain at 545 nm. Arrows and abbreviations; refer to Fig. 5.

ly the same in G6Pase and ACP (Table 2).

CB added to the assay systems of ATPase, 5'ND, K-Pase and γ-GTP (10 μg / ml) had no ill effect on their activities of the NM fraction or untreated native EATCs. ConA added to these assay systems (50 μg / ml), however, induced a decrease in 5'ND activity of NM fraction by about one-half, but had no ill effect on the other enzyme activities of both the NM fraction and untreated native EATCs (Table 3.)

TABLE 2. ENZYME ACTIVITIES OF CONA-CB-BLEB FRACTION, CB-BLEB FRACTION, NM FRACTION AND NATIVE EATCS

	ATPase	K-Pase	5'ND	γ-GTP	G6Pase	ACP
ConA-CB-bleb fraction	5.25 (1.25)	1.41 (0.37)	0.07 (0.06)	0.105 (0.008)	0.076 (0.017)	0.40 (0.10)
CB-bleb fraction	5.05 (0.97)	2.41 (0.78)	0.05 (0.03)	0.096 (0.014)	0.082 (0.021)	0.39 (0.12)
NM fraction	7.09 (1.51)	1.34 (0.15)	0.05 (0.01)	0.094 (0.001)	0.094 (0.022)	0.26 (0.01)
Native EATCs	0.71 (0.37)	0.78 (0.22)		0.030 (0.005)	0.077 (0.008)	0.28 (0.08)

Values: μmol of products/mg protein /h.
Numerals in parentheses standard deviation

TABLE 3. EFFECT OF CONA AND CB ON THE ENZYME ACTIVITIES OF NM FRACTION AND NATIVE EATCS

	NM fraction			Native EATCs		
	Non	ConA	CB	Non	ConA	CB
ATPase	9.87 (1.38)	9.34 (0.90)	8.41 (0.91)	0.92 (0.48)	0.78 (0.41)	0.88 (0.49)
K-Pase	1.17	1.11	1.16	0.99	0.97	0.95
5'ND	0.095 (0.016)	0.040 (0.007)	0.081 (0.018)			
γ-GTP	0.096	0.101	0.099	0.029	0.029	0.026

Values: μmol of products/mg protein/h. Numerals in parentheses: standard deviation. Non: no reagent was added to each system. ConA: Con A was added (50μg/ml). CB: CB was added (10μg/ml).

DISCUSSION

As just demonstrated, the bleb fraction obtained from EATCs exposed to CB, CB-bleb fraction, was composed of small cytoplasmic particles surrounded by cytoplasmic membrane. This fraction was suitable material for studying plasma membrane components, because it had little contamination of mitochondria, lysosomes and microsomes as in NM fraction, though both fractions had many ribosomes. Its protein components were also nearly the same as the NM fraction as revealed by electrophoresis and the enzymatic analysis. The isolated blebs of EATCs were different from those of rat liver cells, which may include many mitochondria (30).

At the time when I was conducting this experiments, Henius and collaborators reported their studies on isolated blebs from the EATCs exposed to CB (10). Their method was similar to mine and their bleb fraction was also the same morphologically as that presented in this paper. They showed that their blebs retained nearly the same activities of active transport of Na^+, K^+ and α-aminoisobutyric acid as the living EATCs, indicating that the blebs had the function of plasma membrane.

CB may release fibronectin from a plasma membrane (31), reverably inhibit hexose transport across a plasma membrane (32) and reversably disrupt microfilaments (33-35), but CB had no effect on the activities of the enzymes assayed in this investigation.

The present observations also showed that ConA-CB-bleb fraction had plasma membranes solely from the non-capped areas which had membrane components not participaing in cap formation. The mechanism is that by CB-treatment, EATCs having caps form blebs solely on non-capped areas, probably by tight binding of membrane components in the caps mediated by ConA and certain cytoskeletal proteins. The ConA-caps of *Dictiosterium discoideum amoebae* are rigid and can be obtained as a purified fraction from cells having caps by treating with Triton-X-100 (9).

Cytochemical analysis showed ConA-BS as dense reactive products accumulating on the caps. Some reactive products were also found on the cytoplasmic membrane of the non-capped areas including the blebs. This may suggest the location of some remnant ConA-BS which did not participate in the ConA-cap formation being immobilized by the stable cytoskeletal system or by disconnection to the moving factors (1-6, 9), though it is possible that it may only be non-specific staining.

Electrophoresis revealed that CBS or ConA-BS was composed of two major glycoprotein components of about 140,000 and 120, 000 dalton and several minor ones, which is consistent with the observation of Nachbar and associates (16). Their ConA-BS were also composed of many glycoproteins including two major glucosamine-rich glycoproteins of about 135,000 and 95,000 dalton (16). Electrophoresis also revealed that the ConA-CB-bleb fraction decreased in the major

components of ConA-BS without distinct quantitative changes in the minor components of ConA-BS, indicating that the major components participate in the cap formation but the minor components do not. A quantity of ConA was found in the ConA-CB-bleb fraction. This indicates clearly that the ConA-BS remain in the non-capped areas without participating in the cap formation.

The K-Pase activity of the ConA-CB-bleb fraction was lower than that of the CB-bleb fraction by about 40%. This indicates that K-Pase moves to the capped area with the major components of ConA-BS, as ConA did not alter the K-Pase activity. The activities of ATPase and γ-GTP in the ConA-CB-bleb fraction were nearly the same as those of the CB-bleb fraction, indicating that these enzymes do not participate in the cap formation. ConA did not affect these enzyme activities, though some authors reported that it may alter ATPase activity (36, 37). ConA lowered the 5'ND activity by about one-half as previously reported by some anthors (38, 39), but the enzyme activity appeared nearly the same in intensity in both ConA-CB-bleb and CB-bleb fractions. This indicates that 5'ND does not move to the capped areas.

According to several authors, these marker enzymes of plasma membrane (21, 40-42) are a part of ConA-BS as supposed from their binding ability to ConA-columns (39, 43, 44). Therefore, the present results indicate that some enzymes having the characteristics of ConA-BS participate in the cap formation but others do not. That is, K-Pase participates in the cap formation but ATPase, γ-GTP and 5'ND do not, though these enzymes have the activity of ConA-BS. Enzymes not participating in the cap formation are thought to be fixed on the cell membrane, being inhibited from moving around as described above. Another possibility is that they may be cryptic in the living cells' plasma membrane and may not bind to ConA as described by Burger and associates (45, 46). It is likely that the plasma membrane-integrated ConA-BS are different from DOC-solubilized ConA-BS in the binding ability to ConA.

Raz and associates reported that, during capping of surface immunoglobulins of mouse splenic lymphocytes, Mg^{++}-ATPase and 5'ND accumulated in the capped area (47). This seems to indicate that the mobility of the enzymes during the cap formation differs with the cell source, though this needs to be clarified in the future.

Acknowledgment. I would like to thank Dr. S. Seno, Emeritus professor of Okayama University and the Director of Shigei Medical Research Institute, Dr. M. Awai, Professor of Pathology. Dr. M. Mori, Instructor in Pathology, Dr. K. Tomochika, Assistant in Bacteriology and Dr. J. Sasaki, Instructor in Anatomy in this medical school, for their valuable advice and the painstaking revision of the manuscript.

REFERENCES

1. Albertini, D. F. and Anderson, E.: Microtubule and microfilament rearrangements during capping of concanavalin A. Receptors on cultured ovarian granulosa cells. *J. Cell Biol.* **73,** 111-127, 1978.

2. Gabbiani, G., Chaponnier, C.,Zumbe, A. and Vassalli, P.: Actin and tubulin co-cap with surface immunoglobulins in mouse B lymphocytes. *Nature* **269,** 697-698, 1977.
3. Sasaki, J., Kanda, S., Otsuka, N., Nakamoto, S. and Mori, M.: Morphology of cap formation in Ehrlich ascites tumor cells induced by concanavalin A. *Cell Struct. Funct.* **4,** 1-10, 1979.
4. Williams, D. A., Boxer, L. A., Oliver, J. M. and Baehner, R. L.: Cytoskeletal regulation of concanavalin A capping in pulmonary alveolar macrophages. *Nature* **267,** 255-257, 1977.
5. Yahara, I. and Edelman G. M.: Modulation of lymphocyte receptor mobility by locally bound concanavalin A. *Proc. Natl. Acad. Sci. U.S.A.* **72,** 1579-1583, 1975.
6. Yahara, I. and Edelman, G. M.: The effect of concanavalin A on the mobility of lymphocyte surface receptors. *Exp. Cell Res.* **81,** 143-155, 1973.
7. Bourguignon, L. Y. W., Hyman, R., Trowbridge, I. and Singer, S. J.: Participation of histocompatibility antigens in capping of molecularly independent cell surface components by their specific antibodies. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 2406-2410, 1978.
8. Poulik, M. D., Bernoco, M., Bernoco, D. and Ceppellini, R.: Aggregation of HL-A antigens at the lymphocyte surface induced by antiserum to β_2-microglobulin. *Science* **182,** 1352-1355, 1973.
9. Condeelis, J.: Isolation of concanavalin A caps during various stages of formation and their association with actin and myosin. *J. Cell Biol.* **80,** 751-758, 1979.
10. Henius, G. V., Laris P. C. and Woodburn, J. D.: The preparation and properties of cytoplasts from Ehrlich ascites tumor cells. *Exp. Cell Res.* **121,** 337-345, 1979.
11. Kirizuka, K., Nakamoto, S., Mori, M., Seno, S. and Sasaki, J.: The mechanism of morphological change induced by cytochalasintB on Ehrlich ascites tumor cells. *Cell Struct. Funct.* **3,** 379, 1978.
12. Sasaki, J., Imanaka, M., Otsuka, N., Nakamoto, S. and Mori, M.: A scanning electron microscopic study of the two-step effect of cytochalasin B on Ehrlich ascites tumor cells. *Acta Med. Okayama* **35,** 1981 (in press).
13. Mori, M., Kirizuka, K., Nakamoto, S. and Sasaki, J.: (In preparation).
14. Dulbecco, R. and Vogt, M.: Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. Med.* **99,** 167-182, 1958.
15. Brunette, D. M. and Till J. E.: A rapid method for the isolation of L-cell surface membranes using an aqueous two-phase polymer system. *J. Membrane Biol.* **5,** 215-224, 1971.
16. Nachbar, M. S., Oppenheim, J. D. and Aull F.: Interaction of lectins with plasma membrane glycoproteins of the Ehrlich ascites carcinoma cell. *Biochim. Biophys. Acta.* **419,** 512-529, 1976.
17. Bernhard, W. and Avrameas, S.: Ultrastructural visualization of cellular carbohydrate components by means of concanavalin A. *Exp. Cell Res.* **64,** 232-236, 1971.
18. Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680-685. 1970.
19. Tonomura, Y., Watanabe, S. and Yagi, K.: Mechanism of muscular contraction. I. Interaction between actomyosin and adenosin triphosphate. *J. Biochem.* **40,** 27-54, 1953.
20. Masaki, T. and Takaiti, O.: Some properties of chichen α-actinin. *J. Biochem.* **66,** 637-643, 1969.
21. Nakao, T., Nagano, K. Adachi, K. and Nadao, M.: Separation of two adenosine triphosphatases from erythrocyte membrane. *Biochem. Biophys. Res. Commun.* **13,** 444-448, 1963.
22. Kent, C., Truesdale, N. J. and Constantine, L. A.: 5'-nucleotidase activity in developing chick pectoral muscle. *Exp. Cell Res.* **121,** 63-70, 1979.
23. Forte, J. G., Forte, T. M. and Heinz, E.: Isolation of plasma membranes from Ehrlich ascites

tumor cells. Influence of amino acids on (Na^+ + K^+)-ATPase and K^+-stimulated phosphatase. *Biochim. Biophys. Acta.* **298,** 827-841, 1973.

24. Baginski, E. S., Foa, P. P. and Zak, B.: Determination of rat liver microsomal Glucose-6-phosphatase activity: Study of citrate and G-6-P inhibition. *Anal. Biochem.* **21,** 201-207, 1967.
25. Fiske, C. H. and Subbarow, Y.: The colorimetric determination of phosphorus. *J. Biol. Chem.* **66,** 375-400, 1925.
26. Baginski, E. S., Foa, P. P. and Zak, B.: Microdetermination of inorganic phosphate, phospholipids and total phosphate in biologic materials. *Clin. Chem.* **13,** 326-332, 1967.
27. Orlowski, M. and Meister, A.: γ-glutamyl p-nitroanilide: A new convenient substrate for determination and study of L- and D-glutamyl transpeptidase activities. *Biochim. Biophys. Acta.* **73,** 679-681, 1963.
28. Kind, P. R. N. and King, E. J.: Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Pathol.* **7,** 322-326, 1954.
29. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193,** 265-275, 1951
30. Reith, A. and Seglen, P. O.: Morphometric electron microscopic and biochemical study of isolated and enucleated rat liver cells (LC). *J. Ultrastruct. Res.* **50,** 370, 1975.
31. Kurkinen, M., Wartiovaara, J. and Vaheri, A.: Cytochalasin B releases a major surface-associated glycoprotein, Fibronectin, from cultured fibroblasts. *Exp. Cell Res.* **111,** 127-137, 1978.
32. Lin, S., Lin, D. C. and Flanagan, M. D.: Specificity of the effects of cytochalasin B on transport and motile processes. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 329-333, 1978.
33. Hartwig, J. H. and Stossel, T. P.: Interactions of actin, myosin and an actin-binding protein of rabbit pulmonary macrophages. III. Effect of cytochalasin B. *J. Cell Biol.* **71,** 295-303, 1976.
34. Wever. K., Rathke, P. C., Osborn, M. and Franke, W. W.: Distribution of actin and tubulin in cells and in glycerinated cell models after treatment with cytochalasin B (CB). *Exp. Cell Res.* **102,** 285-297, 1976.
35. Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. and Yamada, K. M.: Microfilaments in cellular and developmental process. Contractile microfilament machinery of many cell types is reversibly inhibited by cytochalasin B. *Science* **171,** 135-143, 1971.
36. Novogrodsky, A.: Concanavalin A stimulation of rat lymphocyte ATPase. *Biochim. Biophys. Acta.* **266,** 343-349, 1972.
37. Swann, A., C., Daniel, A. Albers, R. W. and Koval, G. J.: Interactions of lectins with (Na^+ + K^+)-ATPase of eel electroric organ. *Biochim. Biophys. Acta.* **401,** 299-306, 1975.
38. Carraway, K. L., Doss, R. C., Huggins, J. W., Chesnut, R. W. and Carraway, C. A. C.: Effects of cytoskeletal perturbant drugs on ecto 5'-nucleotidase, a concanavalin A receptor. *J. Cell Biol.* **83,** 529-543. 1979.
39. Dornand, J., Bonnafous, J. C. and Mani, J. C.: Effects of ConA and othet lectins on pure 5'nucleotidase isolated from lymphocyte plasma membranes. *Biochem. Biophys. Res. Commun.* **82,** 685-692, 1978.
40. Cutler, L. S. and Rossomando, E. F.: Localization of adenylate cyclase in Dictyostelium Discoideum. II. Cytochemical studies on whole cells and isolated plasma membrane vesicles. *Exp. Cell Res.* **95,** 79-87, 1975.
41. Inoue, M., Horiuchi, S. and Morino, Y.: γ-Glutamyl transpeptidase in rat ascites tumor cell LY-5. Lack of functional correlation of its catalytic activity with the amino acid transport. *Eur.*

J. Biochem. **78,** 609-615, 1977.

42. Nakao, M.: Several topics concerning Na^+, K^+-ATPase. *Life Sci.* **15,** 1849-1859, 1974.
43. Horiuchi, S., Inoue, M. and Morino, Y.: γ-Glutamyl transpeptidase: Sidedness of its active site on renal brush-border membrane. *Eur. J. Biochem.* **87,** 429-437, 1978.
44. Marshall, M. O.: Studies on the glycoprotein component of (Na^+ + K^+)-ATPase from dog fish salt gland. Binding to concanavalin A and removal of sialic acid by neuraminidase. *Biochim. Biophys. Acta.* **445,** 837-848, 1976.
45. Burger, M. M.: A difference in the architecture of the surface membrane of normal and virally transformed cells. *Proc. Natl. Acad. Sci. U.S.A.* **62,** 994-1001, 1969.
46. Noonan, K. D., Renger, H. C., Basilico, C. and Burger, M. M.: Surface changes in temperature-sensitive Simian Virus 40-transformed cells. *Proc. Natl, Acad. Sci. U.S.A.* **70,** 347-349 1973.
47. Raz, A. and Bucana, C.: The redistribution of membrane surface immunoglobulin induces the rearrangement of some membrane integral proteins. *Biochim. Biophys. Acta.* **597,** 615-621, 1980.