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Surface Properties of Cell Membrane in Early Stage of Transformed Cell

I. Early Detection of Transformed Cell by Concanavalin A
II. Properties of Plasma Membrane of Transformed Rat Liver Cell Induced by 3'-Me DAB

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The present study was designed in order to investigate the lectin induced cytoagglutination properties of normal and transformed cells and surface alterations in the early stage of the transformed cells by characterizing the structural changes on the hepatoma surface membrane. Rat and rabbit erythrocytes and Sarcoma 180 ascites tumor cells were used for the lectin-induced cytoagglutination. Plotting % agglutination versus concanavalin A(Con A) concentration, sigmoid curves appeared in all cases. α-methyl-D-mannopyranoside(αMM) inhibited Con A induced cytoagglutination and the degrees of inhibition depended on the cell types and species. When rats were fed a diet containing 0.06% 3'-methyl-4-dimethylaminoazobenzene(3'-Me DAB) for 12 weeks, almost all of the rats had solid liver tumors. Polyacrylamide gel electrophoresis of surface membrane proteins of these rat livers and of transplanted tumor cells showed three distinct protein bands, of which two were absent in normal rat livers. The molecular weights of these proteins were 73,000, 66,000, and 57,000 daltons. Antiserum against primary hepatocarcinoma surface proteins precipitated with three membrane proteins obtained from primary hepatocarcinoma cells as well as transplanted hepatocarcinoma cells, suggesting the presence of specific tumor antigens in these cells.

Key Words: Concanavalin A, Transformed cell membrane.

Many aberrant propeties displayed by transformed cells are thought to arise from plasma membrane alterations which occurred during malignant transformation (Wallach, 1968; Emmelot, 1973). The first convincing biochemical changes observed in transformed cells are increased glycolysis (Warburg, 1930) and increased sugar transport (Hatanaka, 1974), suggesting the loss of a normal control device for glucose uptake into a cell. It is conceivable that tumor cells lack one or more of the proteins in the cell membrane that function in the active transport of glucose across the plasma membrane. It has been shown that there were decreased intercelluar communication (Loewenstein and Kanno, 1967; Haeysman, 1973) and the loss of contact inhibition of growth in tumor cells (Coman, 1953; Abercrombie and Ambrose, 1962). A strong evidence for the distinct difference in plasma membranes between cancer cells and normal cells comes from the comparison of their behavior after the contact of their ruffled edges. It has also been demonstrated that transformed cells lack normal cellular affinity (Loewenstein and Kanno, 1967) which is a specific cell-cell adhesion for a given type of cell. Although the nature of the sticky substances that attach normal cells to each other is still unclear, many cancer cells reveal much less organized arrangement of various muscle proteins compared to the normal cells (Pollack *et al.*, 1975; Goldman *et al.*, 1975). It is probable that the defect of the organized arrangement in cancer cells is due to the change in one or more components of the plasma membrane, which may hinder the attachment of individual actin filament to the membrane surface. Many investigators have reported that a number of lectins selectively agglutinate tumor cells (Nicolson, 1974; Burger, 1973) and suggested that cancer cell membranes have a uni-

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quely different quality for the precipitation by lectins from that of a normal cell membrane. Lectin from wheat seed, a glycoprotein, was thought to agglutinate only tumor cells, through a binding to sugar-containing receptor proteins unique to the cancer cells. However, it appears that both normal and cancer cells possess the same number and kind of agglutinin receptors but the receptors on the surface of cancer cells diffuse much more readily within the lipid bilayer than do the corresponding receptors in normal cells (Burger, 1973; Nicolson, 1974). Robinson and Bellett (1975) have reported the disappearance of surface glycoprotein of MW 46,000 in the early stage of transformed cells. In addition, the disapperance of 240,000 MW protein and 200,000 MW protein in the Rous sarcoma virus or various adenovirus induced transformed cells are another characteristic molecular change in cancer cells. The appearance of tumor specific antigen on the plasma membrane of hepatoma induced by azo dye was reported by Baldwin *et al.*, (1971) and the molecular weight of the antigen was estimated to be about 55,000, with minor proteins having larger molecular weights. Tumor specific antigen expressed in the methylcholanthrene induced fibrosarcoma cell surface has been reported to have a molecular weight of 70,000(Natori *et al.*, 1977) or 40,000 and 75,000 (Pellis *et al.*, 1976). The differences in the plasma membrane structure between normal and malignant cells may reflect the functional and morphological differences, but we are far from understanding exactly how the cell surface membrane changes modulate the growth control. The present experiment was designed in order to investigate the lectin-induced cytoagglutination properties of normal and transformed cells, and surface alterations in the early stage of the transformed cells by characterizing the structural changes on the hepatoma surface membrane.

METERIALS AND METHODS

Experiment I. Early detection of transformed cells by concanavalin A

Preparation of cells: Erythrocytes were obtained from rabbits and rats by cardiac or orbital fossa punctures. Sarcoma 180 mouse ascites cells were collected by syringe with a 17 gauge needle from the mouse peritoneum in which Sarcoma 180 ascites cells were inoculated for 2 weeks. The cells were washed 3 times in phosphate buffered saline(PBS, pH 7.4) by centrifugation (2,000 rpm, 10 minutes each). The upper 5% of the cell mass in each of three successive washings was discarded and the rest of cells were used for an agglutination test within 2 hours.

Preparation of Con A and α-methyl-D-mannopyranoside solutions: A stock solution of Con A (type III, Sigma Chem. Co., U.S.A.) containing 2 mg/ml of phosphate buffered saline (PBS, 0.05M, pH 7.4) was prepared and serial dilutions of this stock solution were made with PBS for working solutions. α-methyl-D-mannopyranoside (αMM, Grade III, Sigma Chem. Co.) stock (25 μmoles/ml) was prepared by dissolving the solid carbohydrate into PBS and serial dilutions were made with cold PBS for working solutions. A 1% bovine serum albumin (BSA, Sigma Chem. Co.) solution was freshly made with PBS on the day of assay.

Cytoagglutination assay: Cytoagglutination assay was performed by the method of Davis *et al.*, (1976) as described for Novikoff hepatoma cells, using a Coulter Counter (Model ZBI). The counter was equipped with a 100 μ aperture and a 0.5 ml manometer. Erythrocytes were counted at settings of 1/amplification=1.2, and 1/aperture current=1.0 with a window 14~100. Sarcoma 180 mouse ascites cells were counted at a setting of 1/aplification=32, 1/aperture current=1/8 with a window 24~100. To a 29 ml DiLu-Vial (Elkay products Inc., Mass, 01545), 10 μl of 1% BSA in PBS, 50 ul of each cell suspension containing (2.03~2.10)x10^7 rabbit erythrocytes /ml, 2.54 x10^7 rat erythrocytes/ml and (0.50~0.85)x10^7 mouse ascites cells/ml were added. The vial was capped, inverted and allowed to stand at 37°C in a water bath for 1 hour. After incubation, 10 ml of cold PBS (pH 7.4) was pipetted down the side of the vial and the vial was gently inverted three times, followed by counting single cells. The percent agglutination was defined as 100x[100-(a-b)/(c-b)]: where a, the number of single cells present at a serial dilution of lectin; b, the number of single cells present at maximal agglutination; and c, the number of single cells present without lectin.

Inhibition of Con A induced cytoagglutination by α MM: To a 20ml DiLu-Vial, 10ml of 1% BSA in PBS, 25μl of a serial dilution of inhibitor in PBS and 25μl of the diluted Con A solution, which gave a 95% agglutination in the absence of an inhibitor, were added. The vials were capped and incubated for 30 minutes in a 37°C water bath, then 50μl of cell suspensions were added and incubated for another 1 hour in a 37°C water bath. All countings were performed in the same way as in the cytoagglutination procedure.

Experiment II. Properties of plasma membrane of transformed rat liver induced by 3'-Me DAB

Induction of rat hepatocarcinoma and preparation of transplanted tumor cells: Male Albino rats (~80g BW) were fed a synthetic diet containing 0.06% 3'-mehtyl-4-dimethylaminoazobenzene (3'-Me DAB, Eastman Organic Chem.) for 12 weeks and a respective control diet thereafter (4 weeks). The composition of the control diet was described in the method of Lim et al. (1982). A liver tumor mass was removed and squashed into a single cell gruel (0.5gm/ml sterilized saline). A 1 ml portion was transplanted into a 5~10ml air pouch made on the back of the rat (~80g BW). The detailed techniques were described in the method of Chang et al. (1967).

Isolation of surface membrane proteins of liver cells: Rats were anesthetized with ether and a 0.5mM $CaCl_2$ containing saline solution was infused into liver through a portal vein to remove whole blood. Livers were removed, sqashed and 3M-KCl in PBS solution was added (2 ml/g tissue). The mixture was stirred for 18 hours at 4°C with the stirrer (Magnematic) and centrifuged at 20,000xg for 30 minutes. The supernate was centrifuged again at 110,000xg for 90 minutes and the supernate was dialyzed at 4°C for 18 hours against 500 volumes of PBS with 3 successive changes. The protein solution was freeze-dried and stored.

Preparation of rabbit antiserum against the surface protein of primary rat hepatocarcinoma: Five hundred micrograms of freeze-dried surface protein dissolved in 2ml of complete Freund adjuvant (Difco labortories, Detroit, Mich.) was injected into a male New Zealand White rabbit (~2kg BW) subcutaneously. The rabbit was then reinjected with 300 μg of the antigen dissolved in an incomplete adjuvant 2 weeks after the primary injection. Antiserum was prepared by centrifugation (5,000xg, 30minutes) of the clotted blood obtained from the rabbit immunized with the atigen.

Polyacrylamide gel electrophoresis and immunoelectrophoresis: An acrylamide gel column and slab were prepared according to the method described by Lim *et al.* (1982), in a previous report. Ten milligram of a freeze-dried surface protein was dissolved in 5ml of 0.062M Tris-HCl buffer (pH 6.8) containing 10% glycerol and 0.001% bromophenol blue. Fifty microliter of each sample was applied to the top of the gel and electrophoresed for 4 hours using 0.05M Tris-0.348M glycine buffer (pH 8.3) with a current of 2mA/gel and 12mA/slab (16.8cm×16.8cm×1mm) at 4°C. After the electrophoresis was finished, gels were fixed with a 50% TCA solution for 15 hours and stained with a 0.1% Coomassie brilliant blue R-250 (Sigma Chem. Co.) for 1 hour. Destaining of the gel was carried out using 7% acetic acid at 37°C until clear protein bands appeared.

Immunoelectrophoresis was carried out on an agarose gel plate (84mm×94mm) made by the spreading of 7ml of 1% agarose solution dissolved in hot 0.06M barbital buffer (pH 8.6). After the electrophoresis of surface protein (20~30μg) on a horizontal gel plate for 2 hours at 10 volts, troughs (2mm×52mm) were made along with sample wells. One hundred microliter of diluted antiserum (2×) was added to troughs and double diffusion was carried out in a humidity chamber for 18 hours. Gels were immersed into 0.1M NaCl solution to wash out the non-precipitated proteins. They were then dehydrated with filter paper under pressure (~1kg). By a hot blower, gels were completely dried and films were formed. Gel firms were stained with 0.5% Coomassie brilliant blue (90ml ethanol+20ml acetate+90ml water) for 5~10 minutes and destained with the same solution devoid of the dye.

RESULTS

Experiment I. Early detection of transformed cell by concanavalin A.

Con A induced agglutination of rabbit erythrocytes, rat erythrocytes and Sarcoma 180 mouse ascites cells: When % agglutinations versus the Con A concentrations were plotted, sigmoid curves were revealed in all cases. There were differences in the steepness and concentrations of Con A at which 50% agglutination is obtained, among cell types or species (Fig. 1). Final cell concentrations, to give an optimal agglutination in the assay were $(0.92\sim0.95)\times10^7$ cells/ml for rabbit erythrocytes, $(0.77\sim1.64)\times10^7$ cells/ml for rat erythrocytes and $(0.23\sim0.39)\times10^7$ cells/ml for Sarcoma 180 mouse ascites cells. Con A concentrations used to give a 95% agglutination of rabbit erythrocytes, rat erythrocytes and of Sarcoma 180 ascites cells were 22.73, 45.45 and 57.50 μg/ml, respectively. Con A concentrations which gave a 10% agglutination of rabbit erythrocytes, rat erythrocytes and Sarcoma 180 ascites cells were 1.56, 11.36 and 0.71 μg/ml, respectively.

Inhibition of Con A induced cytoagglutination by α MM: The inhibition of Con A induced cytoagglutination by α MM was varied with the concentration of the inhibitor. The degree of the inhibition also depended on the cell types and species (Fig. 2). The concentrations of α MM required for 50% inhibition of rabbit erythrocytes, rat erythrocytes and Sarcoma 180 ascites cells were 0.565, 0.072 and 3.677 μmoles per 1×10^7 cells, respectively. With a relatively low concentration of α MM (<mM), inhibition of Con A induced cytoagglutination of rat erythrocytes was remarkable.

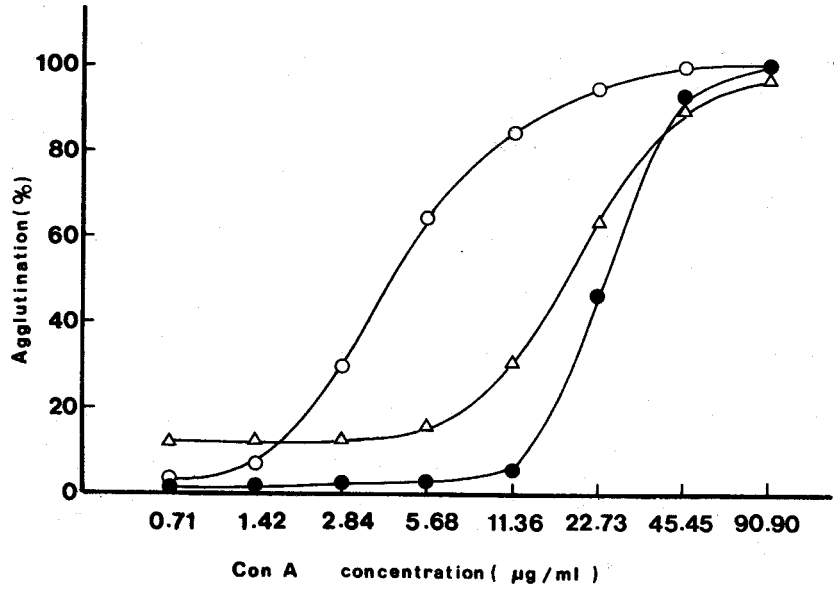

Fig. 1. Con A induced agglutination of rabbit erythrocytes, rat erythrocytes and Sarcoma 180 mouse ascites cells. Cell concentrations used in the assay were (0.92∼0.95)×10⁷ cells/ml for rabbit erythrocytes (○–○), (0.77∼1.64)×10⁷ cells/ml for rat erythrocytes (●–●) and (0.23∼0.39)×10⁷ cells/ml for Sarcoma 180 mouse ascites cells (△–△).

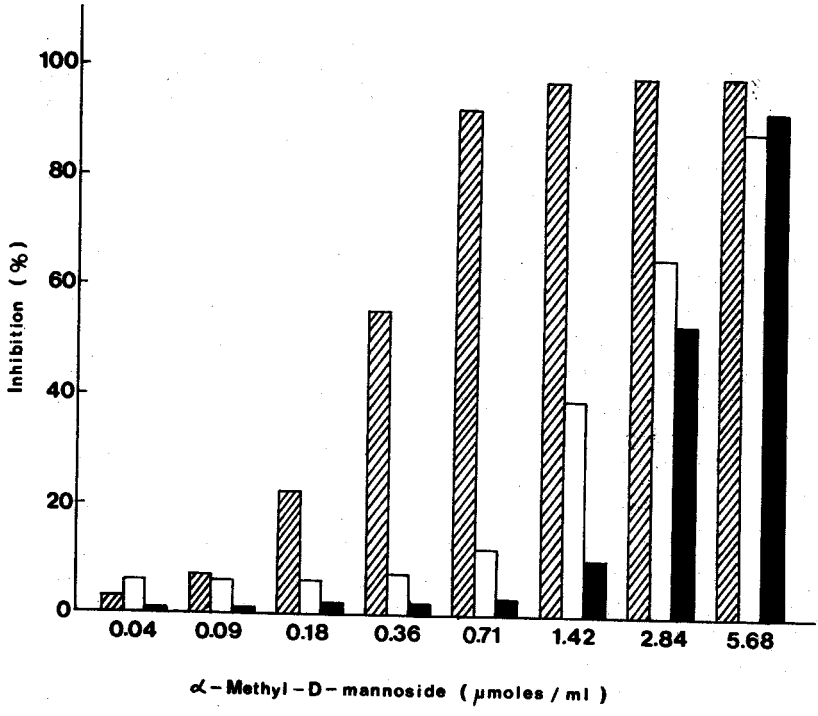

Fig. 2. Effect of inhibitor on Con A induced cytoagglutination. The Con A concentration used was the concentration required to give 95% agglutination as determined by a prior agglutination asaay.
Cell concentrations used in this assay were 2.2×10⁷ cells/ml for rabbit erythrocytes (■), 2.1×10⁷ cells/ml for rat erythrocytes (■) and 0.3×10⁷ cells/ml for Sarcoma 180 mouse ascites cells (□).

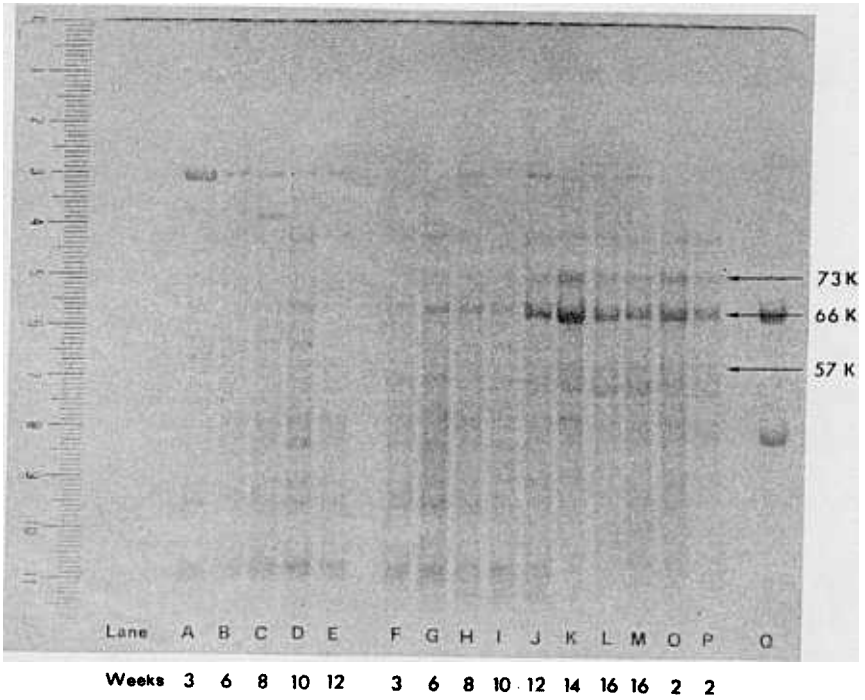

Fig. 3. SDS-gel electrophoretic analysis of the membrane surface proteins of normal hepatocytes, primary hepatocarcinoma cells induced by 3'-Me DAB and transplanted tumor cells.
Lane A∼E, control; lane F∼M, 3'-Me DAB treatment; lane O∼P, transplanted tumor; lane Q, standard proteins (ovalbumin, 45K; bovine serum albumin, 66K)

However, the inhibitory effects of αMM on the Con A induced agglutination of rabbit erythrocytes and Sarcoma 180 ascites cells were negligible.

Experiment II. Properties of plasma membrane of transformed rat liver cell induced by 3'-Me DAB

Appearance of tumor specific surface proteins in hepatocarcinoma induced by 3'-Me DAB: When rats were fed a diet containing 0.06% 3'-Me DAB for 12 weeks, followed by feeding of control diet for 4 weeks, almost all of the rats (>99%) had solid liver tumors. Polyacrylamide gel electrophoresis of surface membrane proteins of tumor bearing rat livers treated with 3'-Me DAB showed two abnormal protein bands (73K, 57K) which seem to appear newly in the hepatoma and one protein band (66K) which increased quantitatively compared to the normal rat liver (Fig. 3). The appearance of three distinct protein bands on slab gel electrophoretogram began at 12 weeks feeding of 3'-Me DAB and was pronounced at 14 weeks. These distinct proteins also appeared in the transplanted tumor cells (Fig. 3).

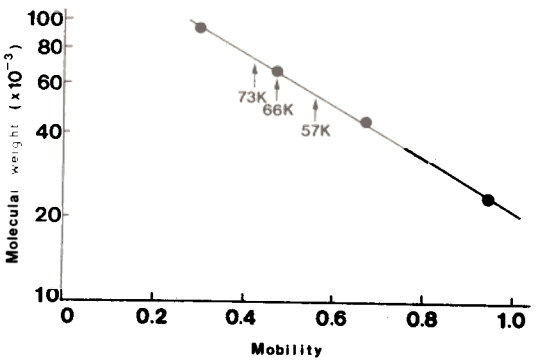

Fig. 4. Determination of the molecular weights of the polypeptide chains found in the transformed rat liver cells induced by 3'-Me DAB.

The arrows indicated the polypeptide chains appea red in the primary and transplanted hepatocarcino ma cell surface proteins.

The marker proteins used were phosphorylase b (94K), bovine serum albumin (66K), ovalbumin (45K) and trypsinogen (24K).

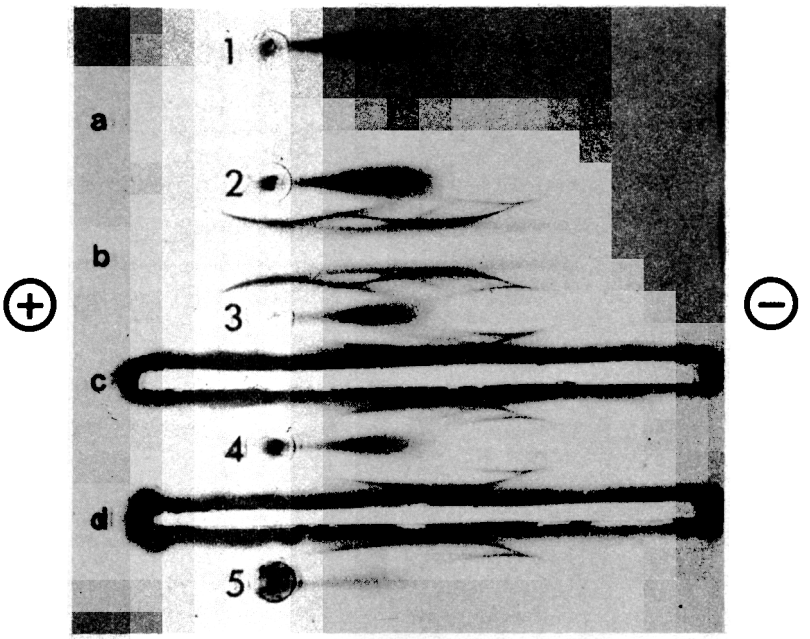

Fig. 5. Immunoelectrophoresis of surface membrane proteins of primary hepatocarcinoma cells induced by 3'-Me DAB and transplanted tumor cells:
a: control serum
b: antiserum against total surface proteins of hepatocarcinoma
c,d: antiserum against primary hepatocarcinoma surface protein, absorbed with surface membrane protein of normal rat liver
1,2,3,4: surface membrane protein (300 μg) prepared from primary hepatocarcinoma cells induced by 3'-Me DAB
5: surface membrane protein (300 μg) prepared from transplanted hepatocarcinoma cells

Determination of molecular weights of tumor specific surface membrane proteins of rat liver: There were about 20 protein bands in the control rat liver membrane fraction when separated by SDS-polyacrylamide gel electrophoresis (Fig. 3). A similar pattern with additional three distinct protein bands was observed in the transformed hepatoma cells. The molecular weights of three distinct surface proteins of the transformed rat liver cells were 73,000, 66,000 and 57,000 daltons, each corresponding to mobilities of 0.42, 0.47 and 0.56, respectively (Fig. 4).

Antigenicity of surface protein of rat hepatocarcinoma cells: Antiserum against primary hepatocarcinoma surface protein, absorbed with surface membrane proteins of normal rat liver, precipitated with two distinct membrane proteins obtained from the rat liver fed the diet containing 3'-Me DAB for 12 weeks, followed by feeding the control diet for 4 weeks (Fig. 5). Immunoelectrophoresis of surface membrane proteins of transplanted hepatocarcinoma cells revealed the presence of tumor specific antigens in these cells (Fig. 5).

DISCUSSION

Con A induced cytoagglutination of cells in the present study varied with the cell types and species. Due to the complication of the agglutination reaction between lectin and cell membranes and the limitation in the measurement of agglutination, the optimal conditions for the Con A induced cytoagglutination were different from cell to cell.

The optimal cell concentrations for each cell type were measured from the curve plotted by % agglutination versus the Con A concentration in the present study. At the optimal cell concentration, the curve will reach a plateau at a certain Con A concentration. Optimal cell concentrations for the cytoagglutination test of rabbit erythrocytes, rat erythrocytes and Sarcoma 180 ascites cells were $(0.92\sim0.95)\times10^7$ cells/ml, $(0.77\sim1.64)\times10^7$ cells/ml and $(0.23\sim0.39)\times10^7$ cells/ml, respectively. It has been reported that there are many factors affecting lectin-induced cytoagglutination. Among the factors, BSA concentration and

incubation time (David *et al.*, 1976), temperature (Vlodavsky *et al.*, 1972; Schnebli and Bachi, 1975), metabolic activity (Yahara and Edelman, 1972; Gunther *et al.*, 1973), receptor mobility (Yahara and Edelman, 1972) and mechanical shear (Schnebli and Bachi, 1975; Davis *et al.*, 1976) were considered to have an influence on the cytoagglutination. Con A concentrations required to give maximal and minimal agglutination of a single cell type at the optimal cell concentration were also variable. Con A concentrations required to give maximal cytoagglutination for rabbit erythrocytes, rat erythrocytes and Sarcoma 180 ascites cells were 1.43, 2.24 and 14.81 μg per 1×10^6 cells, respectively. For maximal cytoagglutination of Sarcoma 180 ascites cells about 10 times of Con A concentration were needed for maximal agglutination of rabbit erythrocytes and about 6 times of that of rat erythrocytes were required. This indicates the susceptibility to lectin induced cytoagglutination depended much on cell types as well as on the species of animals. The inhibitory effect of α MM on Con A induced cytoagglutination of rabbit erythrocytes and rat erythrocytes was comparable with the susceptibility of cell to the Con A induced agglutination. An inverse relationship between the susceptibility to the Con A induced cytoagglutination and the inhibition of agglutination by α MM was observed in both erythrocytes. However, Sarcoma 180 ascites cells were insensitive to the inhibition by α MM even though they have low susceptibility to the Con A induced cytoagglutination (Fig. 1, 2). It is assumed that Sarcoma 180 mouse ascites cells have a different topological distribution of agglutination sites without the change of the number of surface receptor sites which was demonstrated in transformed cells by a number of investigators (Cline and Livingston, 1971; Ozanne and Sambrook, 1971; Sela *et al.*, 1971; Singer and Nicolson, 1972).

Morphological changes of hepatocytes of rats fed a diet supplemented with 3'-Me DAB were very similar to the results reported by Cunningham *et al.* (1950) and Price *et al.* (1952) which showed occurrences of liver parenchymal tumor, bile duct tumor and the combination of these two. It took 15 days for the occurrence of a transplantable tumor in the first generation, and the rate of occurrence was 33% in this experiment. These results are well agreed on with the report of Chang *et al.* (1967).

Three distinct surface proteins appeared on SDS-gel electrophoretogram of the membrane protein fraction prepared from rat hepatocarcinoma, induced by 3'-Me DAB in the present study (Fig. 3). Among those three proteins, a protein corresponding to 66,000 daltons, could be separable from the similar protein present in normal rat liver by DEAE Sephadex (A-50) ion exchange chromatography (in press). Molecular weights of these three proteins were 73,000, 66,000 and 57,000 daltons, which were different from the 55,000 daltons of DAB induced hepatocyte tumor antigen reported by Baldwin and Glaves (1971). They used a limited papain digestion to solubilize the plasma membrane fraction. Meltzer *et al.* (1971) reported the molecular weight of diethylnitrosamine induced guinea pig hepatocyte tumor antigen to be 75,000-150,000 daltons, which were solubilized by hypertonic potassium chloride. Pellis *et al.* (1976) reported the methycholanthrene induced fibrosarcoma cell surface antigens, which have molecular weights of 40,000 and 75,000 daltons, solubilized with 0.25% trypsin and 3M KCl solution. Variability of the reported molecular weights of chemically induced specific hepatocyte tumor antigens could be a result of the difference in method of preparation of membrane proteins and separation or characterization. Different animal species used in this experiment may bring about different results with the same proteins. The appearance of new surface membrane proteins in 3'-Me DAB induced rat hepatocarcinoma was specific for the transformed solid tumor as evidenced by immunoelectrophoresis (Fig. 5).

REFERENCES

Abercrombie M, Ambrose EJ: *The surface properties of cancer cells. A review. Cancer Res 22:525, 1962*

Burger MM: *Surface changes in transformed cells detected by lectin. Fed Proc 32:91, 1973*

Baldwin RW, Glaves D: *Solubilization of tumor-specific antigen from plasma membrane of an aminoazo dye-induced rat hepatoma. Clin Exp Immunol 11:51, 1972*

Baldwin RW, Barker CR, Embleton MJ: *Demonstration of cell-surface antigens on chemically induced tumors. Ann NY Acad Sci 177:268, 1971*

Chang JP, Gibley CW Jr, Ichinoe K: *Establishment of transplantable hepatomas induced by 3'-methyl-4-dimethylaminoazobenzene with special reference to the histologic features of the transplants of early passages. Cancer Res 27:2065, 1967*

Cline MJ, Livingston DC: *Binding of ^3H-concanavalin A by normal and transformed cells. Nature 232:155, 1971*

Coman DR: *Mechanisms responsible for the origin and distribution of blood-borne tumor metastasis. A review. Cancer Res 13:397, 1953*

Cunningham L, Griffin AC, Luck JM: *Effect of a carcinogenic*

azo dye on liver cell structure. Isolation of nuclei and cytoplasmic granules. Cancer Res 10:194, 1950

Davis EM, Starling JJ, Walborg Jr. EF: *A microquantitative method to characterize lectin-induced cytoagglutination. Exp Cell Res 99:37, 1976*

Emmelot P: *Biochemical properties of normal and neoplastic cell surfaces. A review. Eur J Cancer 9:319, 1973*

Goldman RD, Chang C, Williams JF: *Properties and behavior of hamster embryo cells transformed by human adenovirus type 5. Cold Spring Harbor Symp Quant Biol 39 PT 1:106, 1975*

Gunther GR, Wang JL, Yahara I, Cunningham Ba, Edelman GM: *Concanavalin A derivatives with altered biological activities. Proc Natl Acad Sci USA 70:1012, 1973*

Haeysman J: *Locomotion of tissue cells. CIBA Foundation Symp 14 (new series):190, 1973*

Hatanaka M: *Transport of sugars in tumor cell membranes. Biochim Biophys Acta 355:77, 1974*

Lim IK, Hwang DS, Kim YS: *A study of plasma membrane surface of transformed rat liver cells induced by 3'-methyl-4-dimethyl-aminoazobenzene. Yonsei J Med Sci 15:308, 1982*

Loewenstein WRM, Kanno Y: *Intercellular communication and tissue growth. I. Cancerous growth. J Cell Biol 33:225, 1967*

Meltzer MS, Leonard EJ, Rapp HJ, Porsos T: *Tumor-specific antigen solubilized by hypertonic potassium chrolide. J Natl Cancer Inst 47:703, 1971*

Natori T, Law LW, Appella E: *Biological and biochemical properties of Nonidet P40-solubilized and partially purified tumor-specific antigens of the transplantation type from plasma membranes of a methylcholanthrene-induced sarcoma. Cancer Res 37:3406, 1977*

Nicolson GL: *The interactions of lectins with animal cell surfaces. Int Rev Cytol 39:89, 1974*

Ozanne B, Sambrook J: *Binding of radioactively labelled concanavalin A and wheat germ agglutinin to normal and virus transformed cells. Nature 232:156, 1971*

Pellis NR, Shulan DJ, Kahan BD: *Specific tumor immunity induced with soluble materials: Purification of antigens inducing tumor resistance. Biochem Biophys Res Commun 71:1251, 1976*

Pollack R, Osborn M, Weber K: *Patterns of organization of actin and mysoin in normal and transformed cultured cells. Proc Natl Acad Sci USA 72:994, 1975*

Price JM, Harman JW, Miller EC, Miller JA: *Progressive microscopic alterations in the livers of rats fed the hepatic carcinogens 3'-methyl-4-dimethylaminoazobenzene and 4'-fluoro-4-dimethylaminoazobenzene. Cancer Res 12:192, 1952*

Robinson AJ, Bellett JD: *A circular DNA-protein complex adenovirus and its possible role in DNA replication. Cold Spring Harbor Symp Quant Biol 39 PT 1:523, 1975*

Schnebli HP, Bachi T: *Reaction of lectins with human erhtyrocytes. I. Factors governing the agglutination reaction. Exp Cell Res 91:175, 1975*

Sela BA, Lis H, Sharon N, Sachs L: *Quantitation of N-append-acetyl-D-galactosamine-like sites on the surface membrane of normal and transformed mammalian cells. Biochim Biophys Acta 249:564, 1971*

Singer SJ, Nicolson GL: *The fluid mosaic model of the structure of cell membranes. Science 175:720, 1972*

Vlodavsky I, Inbar M, Sachs L: *Temperature-sensitive agglutinability of human erthrocytes by lectins. Biochim Biophys Acta 274:364, 1972*

Wallach DF: *Cellular membranes and tumor behavior. A new hypothesis. Proc Natl Acad Sci USA 61:868, 1968*

Warburg O: *The metabolism of tumor (translated in English by Dickens F). Constable London, 1930*

Yahara I, Edelman GM: *Restriction of the mobility of lymphocyte immunoglobulin receptors by concanavalin A. Proc Natl Acad Sci USA 69:608, 1972*