EFFECT OF METABOLIC INHIBITORS ON THE AGGLUTINATION OF TUMOR CELLS BY CONCANAVALIN A AND RICINUS COMMUNIS AGGLUTININ

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Summary: The agglutinations of rat ascites tumor cells by concanavalin A and by Ricinus communis agglutinin were inhibited by low temperature, 2,4-dinitrophenol and cytochalasin B but not by cycloheximide. These metabolic inhibitors, however, did not inhibit the binding of the agglutinins to the cells. These results suggest that the agglutination was dependent on an active process; probably on a microfilament system responsible for cell surface movement which requires ATP, but not on protein synthesis.

The plant agglutinin, concanavalin A (Con A) and Ricinus communis agglutinin (RC-PHA), can agglutinate malignant cells transformed by chemical carcinogens or viruses, but can not agglutinate normal cells or mouse cell line 3T3[1,2]. To explain this tumor specific agglutination, several theories have been proposed[3-7]. Nicolson has suggested[4] that the difference in the topological distributions of agglutinin binding sites between normal and tumor cell surfaces may be related with that of agglutinability. Another explanation was suggested by Inbar et al[5] that the agglutinability is related with a change in the location of binding sites and the activation of the Con A specific metabolic activity on the surface membrane.

In this communication, we report that the agglutinations of rat ascites tumor cells by Con A (specific for mannose-like residue) and RC-PHA (specific for galactose-like residue) may involve the movement of cell surface membrane

Methods and Materials

Concanavalin A (Con A) was obtained from jack bean meal (Sigma) by

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* Deceased on April 25, 1972

the method of Agrawal and Goldstein[8] and Ricinus communis agglutinin (RC-PHA) was purified by Sepharose affinity chromatography and subsequent Bio-Gel P-150 gel filtration[9]. These preparations were proved to be homogeneous by electrophoresis and sedimentation analysis. ^{3}H-Acetyl-concanavalin A (^{3}H-Con A) and ^{3}H-acetyl-Ricinus communis agglutinin (^{3}H-RC-PHA) were prepared as described previously[10]. Cytochalasin B was a product of Imperial Chemical Institutes, Alderley Park.

Rat ascites hepatoma free cells AH 13[11], AH 108 AF which is derived from AH 108 A[11], and Yoshida sarcoma (YS) cells were used 7 days after intraperitoneal inoculation of parent cells into female albino rats of Donryu strain. The cells were washed four times with Eagle's minimum essential medium before the binding and the agglutination test. The binding of agglutinin to the cells and agglutination of the cells were performed at the same time as follows; In siliconized glass tube, the cells were suspended at concentration of 1.0×10^{6} cells per ml in 0.4 ml Dulbecco Ca^{++} – Mg^{++} free phosphate buffered saline (PBS) in the presence of different amounts of ^{3}H-agglutinins. After appropriate time of incubation, the degree of agglutination of the cell suspensions with shaking (more than ten times) was scored on a qualitative scale from (–) to (++++) with a microscope: (–), no aggregate; (±), few small aggregates and free cell; (+), 3-10 cell aggregates; (++), 10-40 cell aggregates; (+++), 40-100 cell aggregates; (++++), 100-200 cell aggregates. The radioactive agglutinin bound to the cells was determined after washing three times with PBS by centrifugation as described previously[10].

Results and Discussion

Rat ascites hepatoma cells, AH 13 and AH 108 AF, and Yoshida sarcoma cells grown in vivo were tested for the binding of ^{3}H-Con A and ^{3}H-RC-PHA as well as for the agglutination. The binding of these ^{3}H-agglutinins to cells was completed within 1 hour and the amount of ^{3}H-agglutinins bound was directly proportional to the cell concentration over the range of $0.25 - 1.0 \times 10^{6}$ cells per ml and to the amount of ^{3}H-agglutinin added. Furthermore these bindings

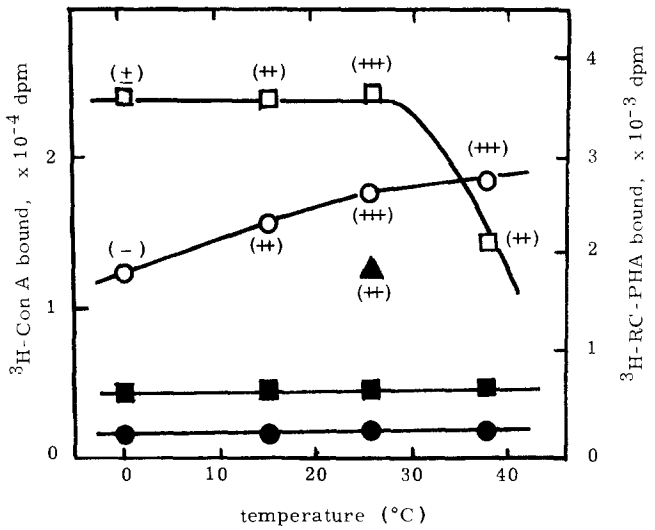

Fig. 1. Effect of temperature on the binding of ^{3}H-agglutinins and on the agglutination of AH 13 cells by ^{3}H-agglutinins

The cell suspension (0.4 ml) containing 12 μg of ^{3}H-agglutinins and 4.0×10^5 cells was stood for 1 hour at each temperature and the binding and the agglutination assays were performed as described in text. ^{3}H-Con A and ^{3}H-RC-PHA was 9×10^3 dpm/μg and 5.66×10^3 dpm/μg respectively, —○—○— , ^{3}H-Con A; —●—●— ^{3}H-Con A in the presence of 0.1 M α-methyl-D-glucoside; —□—□— , ^{3}H-RC-PHA; —■—■— , ^{3}H-RC-PHA in the presence of 0.1 M D-galactose; ▲ ^{3}H-Con A, 15 μg/ml. (-)~(+++) indicate agglutinability.

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reflect the specific ones because ^{3}H-Con A and ^{3}H-RC-PHA binding to cells was inhibited strongly by α-methyl-D-glucoside and D-galactose, respectively.

The effect of temperature on the binding of these agglutinins and on the agglutination of rat ascites hepatoma AH 13 cells was tested in the presence of excess agglutinins (Fig. 1). The results indicated that agglutination occurred strongly (+++) at 25° after 1 hour of incubation, but at 0° no (-) or a very weak (±) agglutination was observed in cases of both ^{3}H-Con A and ^{3}H-RC-PHA. On the other hand, the amount of ^{3}H-RC-PHA bound to the cells was constant between 0° and 25°, and that of ^{3}H-Con A was slightly increased with temperature rise. In a separate experiment in which lower concentration of ^{3}H-Con A was used, the agglutination was clearly detected at 25° even when the amount of ^{3}H-Con A bound was almost same as that at 0° shown in Fig. 1. Similar results were obtained in cases of AH 108 AF and YS cells. No parallelism between

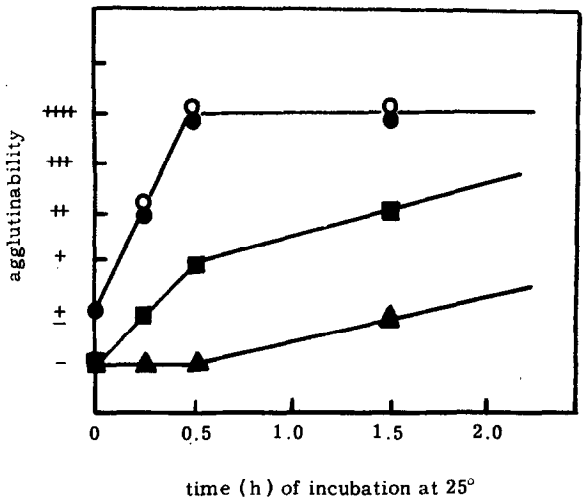

Fig. 2. Effects of 2,4-dinitrophenol, NaN_3 and cycloheximide on the rate of agglutination of AH 108 AF cells by ^{3}H-Con A

The cell suspension containing 4.0×10^5 cells and the inhibitor was preincubated at 37° for 10 min, then the suspension was cooled to 0° and ^{3}H-Con A (6 μg, 9×10^3 dpm/μg) was added to this suspension. After 1 hour at 0°, the cell suspension (0.4 ml) was incubated at 25° and the agglutinability was determined as described in text, —O—O— , no inhibitor; —●—●—, cycloheximide, 25 μg/ml; —■—■— , NaN_3, 10 mM; —▲—▲— , 2,4-dinitrophenol, 10 mM

agglutinability and binding of agglutinin indicates that the agglutination is not necessarily related to the amount of agglutinin bound to the cells. These results are in good agreement with observations[12] by Inbar et al that the tumor cells were not agglutinated by Con A at low temperature. When these rat ascites tumor cells which were pretreated with ^{3}H-agglutinins at 0° for 1 hour, were incubated at 25°, the cells became agglutinable rapidly. These results suggest that the agglutination is related with cell metabolism. Thus, the effects of various metabolic inhibitors on the agglutination were investigated. A typical experiment using AH 108 AF cells and ^{3}H-Con A is shown in Fig. 2. In the absence of inhibitors, the cells showed maximum agglutinability after 30 min at 25°. This recovery of agglutinability, however, was strongly inhibited by the presence of 2,4-dinitrophenol and NaN_3 which are inhibitors of ATP generation[13], but excess cycloheximide, inhibitor of protein synthesis[14], did not inhibit the agglutination. Similar results were obtained in cases of AH 13 and YS cells.

Table I Effect of metabolic inhibitors on the binding of ^{3}H-Con A and on the agglutination of AH 108 AF and AH 13 cells by ^{3}H-Con A

For the experiment with AH 108 AF cells, the cell suspension was treated with inhibitor described in the legend to Fig. 2, and preincubated at 0° with ^{3}H-Con A (7.2 μg, 1.28 x 10^4 dpm/μg) for 1 hour, then the suspension was incubated at 25°. The agglutinability and the radioactivity bound to the cells after 30 min of incubation at 25° were determined as described in text. For the experiment with AH 13 cells, the cell suspension was incubated at 25° under the same condition as above except no preincubation at 0° for 1 hour. After 2.0 hours at 25°, the agglutinability and the radioactivity bound were determined.

Inhibitor	concentration	AH 108 AF cells		AH 13 cells	
		agglutinability	bound	agglutinability	bound
none		+++	18,238 dpm	++	10,626 dpm
DMSO	1%	+++	15,134	++	10,636
α-methyl-D-glucoside	100 mM	−	989	−	1,220
2,4-dinitrophenol	10 mM	±	17,017	−	9,362
NaN_3	10 mM	+	17,426	±	10,710
cytochalasin B*	22.5 μg/ml	±	15,847	−	9,771
cytochalasin B*	45.0 μg/ml	±	16,517	−	11,915
cycloheximide	12.5 μg/ml	+++	13,262	++	8,206
colcemid	2.5 μg/ml	++	11,902	++	8,914

* Cytochalasin B was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was 1.0% in the experiments of Tables I and II.

Tables I and II show the effects of various metabolic inhibitors on the binding and on the agglutination of two cell lines by ^{3}H-Con A and ^{3}H-RC-PHA. Inhibitors such as 2,4-dinitrophenol, NaN_3 and cytochalasin B inhibited the agglutination, while they did not influence the binding. Cycloheximide inhibited neither the binding nor the agglutination. Under the conditions described above, these inhibitors had little effects on cell viability. Among these inhibitors, cytochalasin B is known to cause disorganization of the microfilaments which are thought to be involved in primitive contractile

Table II Effect of metabolic inhibitors on the binding of ^{3}H-RC-PHA and on the agglutination of AH 13 cells by ^{3}H-RC-PHA.

The cell suspension was treated as same as the case of AH 108 AF in the legend to Table I except using ^{3}H-RC-PHA (6.5 μg, 5.66 x 10^{3} dpm/μg). The agglutinability and the radioactivity bound to the cells were determined after 1.5 hours at 25°

Inhibitor	concentration	Agglutinability after 1.5 hour	^{3}H-RC-PHA bound after 1.5 hour
none		++	3,705 dpm
DMSO	1%	++	3,375
D-galactose	100 mM	−	654
2,4-dinitrophenol	10 mM	±	3,135
NaN_3	10 mM	±	3,919
cytochalasin B*	11.3 μg/ml	±	3,120
cytochalasin B*	22.5 μg/ml	±	3,031
cytochalasin B*	45.0 μg/ml	±	3,357
cycloheximide	2.5 μg/ml	++	3,120
cycloheximide	25 μg/ml	++	3,511

process[15-17] and to be similar to actin[18]. Based upon these results in our experiments, the agglutination of rat ascites tumor cells by Con A and RC-PHA seems to be dependent on cell metabolism; probably not on protein synthesis but on a microfilament system responsible for cell surface movement which requires ATP. Berlin and Ukena reported[7] that colcemid, which disrupts microtublar structures, inhibited the agglutination of polymorphonuclear leucocytes by Con A. In our experiments, however, colcemid (2.5 μg/ml) did not show significant effect on the agglutination of rat ascites tumor cells by Con A. The discrepancy remains to be clarified.

Taylor and Duffus[19] reported that cap formation of B lymphocyte induced by antiimmunoglobulin was inhibited by 2,4-dinitrophenol, NaN_3, cytochalasin B and low temperature but not by colcemid. Thus, they concluded

that cap-formation was an active, temperature dependent process involving contractile microfilament activity. Recently, Sundqvist[20] and Edidin[21] reported that the redistribution of surface antigen molecules induced by antibody was not only the case for B lymphocyte but a general property of animal cells. The effects of these inhibitors and temperature suggested the similarity between the agglutination and the patch- and cap formation. Thus, the agglutination of rat ascites tumor cells by Con A and RC-PHA may involve some redistribution of the agglutinin receptor sites, which is due to contractile microfilament activity. Alternatively it is possible that the agglutination requires some energy dependent movement of ruffles and microvilli[15] on the surface of the cells, which are observed in endocytosis[17, 22, 23] of macrophage and chang liver cells. The possible relation between agglutinability and ATP levels in the cell remains to be clarified.

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