RAPID COMMUNICATION ZIPGRAM

THE LOCALIZATION AND FATE OF CONCANAVALIN A IN AMOEBAE OF THE CELLULAR SLIME MOLD, DICTYOSTELIUM DISCOIDEUM (1)

MARTHA ULBRICK GILLETTE, R. E. DENGLER AND M. F. FILOSA
Departments of Zoology and Botany, Scarborough College,
University of Toronto, West Hill, Ontario, Canada

ABSTRACT Two different methods for the localization of Con A indicate that this lectin is bound to the surface of Dictyostelium discoideum amoebae. Redistribution of the Con A receptors occurs both on cells in suspension and cells moving on an agar surface. Internalization of the Con A does not occur.

Gillette and Filosa ('73) reported that when amoebae of the cellular slime mold Dictyostelium discoideum are put on agar containing 300 µg/ml of the plant lectin Concanavalin A (Con A), a four-fold increase in membrane-bound cyclic AMP phosphodiesterase activity occurs within the first 30 minutes. Activity continues to increase for at least an hour. Usually, the activity of this enzyme increases only at the time of aggregation, i.e., about 10-12 hours after the amoebae are placed on agar (Malchow et al., '72).

This paper is concerned primarily with the localization and fate of Con A in D. discoideum amoebae as a first step towards understanding how this lectin induces the premature appearance of phosphodiesterase. The extensive work on the effects of Con A on vertebrate cells has served as a guide throughout. Those studies, primarily on lymphocytes, have demonstrated that Con A binds to certain sites on glycoprotein receptors at the cell surface (Unanue et al., '72; de Petris et al., '73). They show that Con A binding can bring about the redistribution of these surface receptors, resulting in the formation of aggregates of the receptor molecules that can be visualized microscopically as caps or patches. Subsequent

to capping, the Con A-receptor complexes are found in intracytoplasmic vesicles, suggesting their internalization by endocytosis (Barat and Avrameas, '73; Unanue et al., '72). Barat and Avrameas ('73) reported that by 3 hours after initial treatment with Con A there is no longer any indication of the lectin on the cell surface. The present work gives evidence for the binding of Con A to the surface of D. discoideum amoebae and the subsequent redistribution of the Con A receptors

MATERIALS AND METHODS Amoebae of D. discoideum were grown to late log phase in shake cultures at 23°C using Escherichia coli as a food supply; the cells were harvested and washed as described previously (Gillette and Filosa, '73).

The localization of Con A was determined with fluorescein-labeled Con A (Fl-Con A) prepared by the method of Tkacz et al. ('71). Amoebae treated with Fl-Con A (100 µg/ml) at 21°C were examined with a Carl Zeiss fluorescence microscope. In addition, a cytochemical method, which enabled the ultrastructural localization of Con A, was modified from that of Barat and Avrameas ('73). For this method, amoebae were either suspended in saline containing Con A or spread on agar containing the lectin; in both cases the concentration of Con A used was 300 µg/ml, because it had been found to give maximum stimulation of phosphodiesterase activity (Gillette and Filosa, '73). After a specified period of time at 21°C, the cells were centrifuged out of suspension or scraped off the agar surface and 4×10^7 cells were then fixed in 4 ml of 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 for 20 minutes at 21°C. The cells were then washed in 0.1 M phosphate buffer and suspended in a 2 ml solution of horseradish peroxidase (100 µg/ml in 0.02 M phosphate buffer pH 6.0) for 30 minutes at 21°C. Following three washes in the 0.02 M phosphate buffer, the cells were incubated for 15 minutes in 4 ml of 3-3' diaminobenzidine hydrochloride (DAB) solution (500 µg/ml DAB in 0.1 TRIS pH 7.4 containing 0.01% H_2O_2), were washed three times with 0.1 M phosphate buffer pH 7.4 and were postfixed for one hour with

1% osmium tetroxide in the same buffer. (All chemicals were obtained from Sigma, St. Louis, U.S.A.).

Two types of controls for the cytochemical method were run. In one, horseradish peroxidase was omitted while in the other Con A treatment was omitted. In both cases there was no staining.

RESULTS AND DISCUSSION Within 15 minutes after amoebae were placed in Fl-Con A at 21^{o}C, capping occurred and the fluorescence was localized at one end of the cell (figs. 1, 2). Cells placed in Fl-Con A solution containing 0.05 M α-methyl-D-glucoside, a hapten inhibitor of Con A binding, exhibit no fluorescence. These results indicate that Con A binds to receptor sites on the surface of the slime mold amoebae and induces redistribution of these receptors.

The cytochemical localization of Con A leads to the same conclusions. When cells which have been washed at the end of the growth period are first fixed in 3% glutaraldehyde then suspended in Con A solution, the Con A is localized uniformly around the periphery of each cell (fig. 3). Since aldehyde fixation is thought to immobilize Con A receptor sites (Inbar et al., '73), this result indicates that normally the Con A receptors are randomly distributed over the surface of the amoebae. If the amoebae are first treated with Con A for 15 minutes, then fixed, staining appears at one end of each cell (fig. 4) indicating that a redistribution of the receptors has occurred.

In another series of experiments, instead of subjecting cells in suspension to Con A, the amoebae were placed on Con A-containing agar. When removed from the agar after 15 minutes as well as one hour later, the Con A was localized as a cap, apparently at the trailing end of these motile cells (fig. 5). Thus capping occurs both in cells in suspension and on agar where the cells are motile, suggesting that cell movement is not required for cap formation. This is anal-

ogous to the situation in polymorphonuclear leukocytes (Ryan et al., '74).

The fate of Con A bound to the cell surface was studied by incubating cells in Con A solution for 15 minutes, washing, and then suspending them in saline. At 15 minutes, 30 minutes, 1 hour, 3 hours, and 5 hours, samples were removed for cytochemical localization of Con A. Thin sections of the first 3 samples showed that the majority of cells had staining in the form of a cap. The 3 hour sample had fewer cells with caps, while in the 5 hour sample only an occasional cell stained for Con A. None of the samples showed evidence for the presence of Con A in intracytoplasmic vesicles. Likewise, cells kept on Con A agar and sampled at the same times as above showed no Con A in the cytoplasm. Thus the loss of Con A from the surface takes about 3 to 5 hours and is apparently not due to endocytosis This suggests the possibility that the Con A bound to the surface is lost by the shedding of Con A-receptor complexes, a process which occurs to some extent in lymphocytes (Ault et al., '73).

These data provide an interesting comparison with the results on vertebrate cells. They also show that the Con A-induced increase in phosphodiesterase activity could not result from internalization of the lectin but from some effect

---

FIGURE LEGENDS

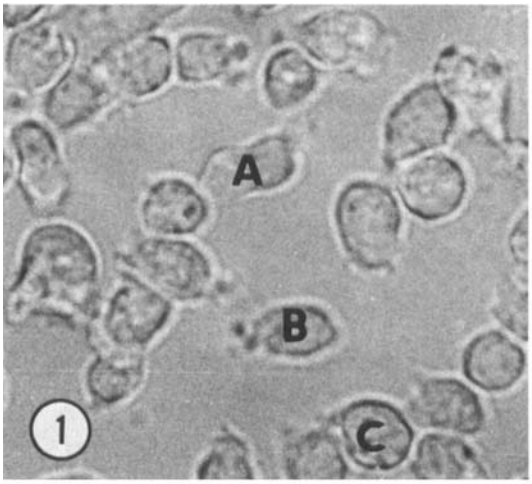
1 Late log phase amoebae treated with Fl-Con A and photographed under phase contrast. The letters indicate the same cells in figures 1 and 2. X 1,008.

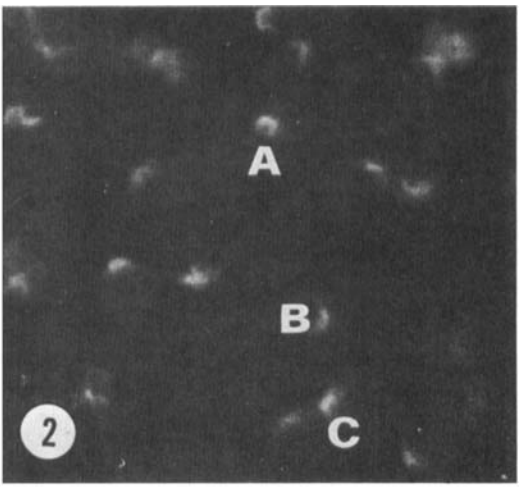
2 The same cells shown in figure 1 as seen by fluorescence microscopy about 15 minutes after treatment with Fl-Con A. The letters indicate the same cells in figures 1 and 2. Fluorescence is localized as a cap on the surface of each cell. X 1,008.

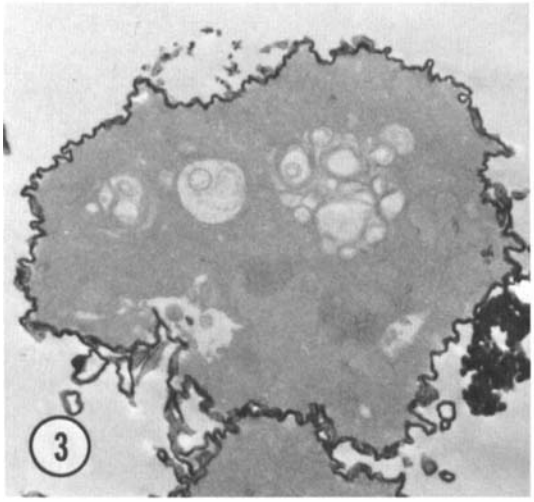
3 Late log phase amoebae pre-fixed in glutaraldehyde then treated with Con A. Cytochemical method shows Con A distributed uniformly on the surface. Unstained section. X 10,250.

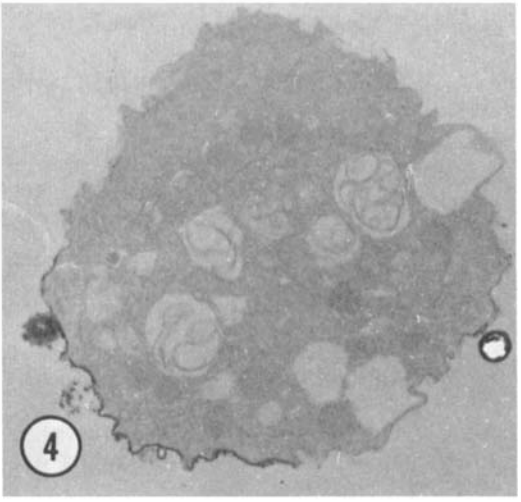
4 Late log phase amoebae treated in suspension with Con A for 15 minutes, then fixed and stained by the cytochemical method. Con A is localized as a polar cap. Unstained section. X 7,500.

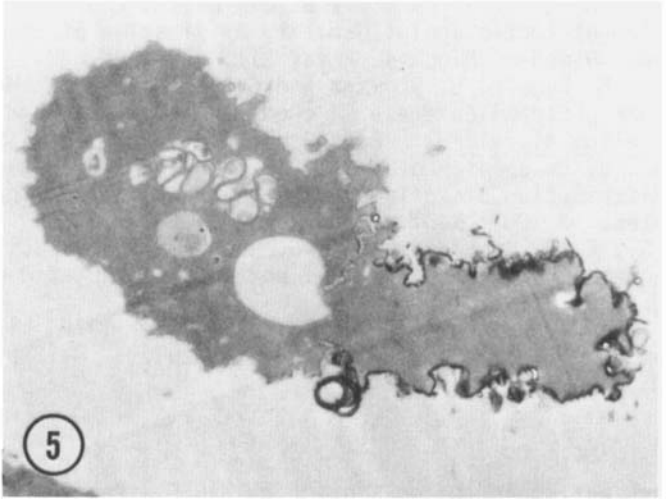
5 Late log phase amoebae exposed for 15 minutes to Con A in agar, then fixed and stained by the cytochemical method. Con A is localized as a polar cap at the posterior end of the cell. Unstained section. X 7,500.


A
B
C
1


A
B
C
2


3


4


5

on the cell surface itself. From these data it is not possible to determine if simple binding of the Con A to its receptors is sufficient for enzyme induction or whether capping of the receptors is also involved. We have previously suggested (Gillette and Filosa, '73) that Con A may be mimicking a factor, normally produced at aggregation, that is responsible for the increase in phosphodiesterase activity at that time. Now we can further suggest that this factor could act by binding to the cell surface and perhaps by causing the redistribution of its own receptor sites.

ACKNOWLEDGMENT Our thanks to Mrs. Monica Idler for the thin sections.

LITERATURE CITED

Ault, K. A., M. J. Karnovsky and E. Unanue 1973 Studies on the distribution of surface immunoglobulin on human B-lymphocytes. J. Clin. Invest., 52: 2507-2516.

Barat, N., and S. Avrameas 1973 Surface and intracellular localization of concanavalin A in human lymphocytes. Expt. Cell Res., 76: 451-455.

de Petris, S., M. C. Raff and L. Mallucci 1973 Ligand-induced redistribution of concanavalin A receptors on normal, trypsinized and transformed fibroblasts. Nature, New Biol., 244: 275-278.

Gillette, M. U., and M. F. Filosa 1973 Effect of concanavalin A on cellular slime mold development: premature appearance of membrane-bound cyclic AMP phosphodiesterase. Biochem. Biophys. Res. Comm., 53: 1159-1166.

Inbar, M., C. Huet, A. R. Oseroff, H. Ben-Bassat and L. Sachs 1973 Inhibition of lectin agglutinability by fixation of the cell surface membrane. Biochem. Biophys. Acta, 311: 594-599.

Malchow, D., B. Nagele, H. Schwarz and G. Gerisch 1972 Membrane-bound cyclic AMP phosphodiesterase in chemotactically responding cells of Dictyostelium discoideum. Eur. J. Biochem. 28: 136-142.

Ryan, G. B., J. Z. Borysenko and M. J. Karnovsky 1974 Factors affecting the redistribution of surface-bound concanavalin A on polymorphonuclear leukocytes. J. Cell Biol., 62: 351-365.

Tkacz, J. S., E. B. Cybulska and J. O. Lampen 1971 Specific staining of wall mannan in yeast cells with fluorescein-conjugated concanavalin A. J. Bact., 105: 1-5.

Unanue, E. R., W. D. Perkins and M. J. Karnovsky 1972 Ligand-induced movement of lymphocyte membrane macromolecules. J. Exp. Med., 136: 885-930.

REFERENCES

1 This work was supported by a grant to M.F.F. from the Health Sciences Committee, University of Toronto.