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CONCANAVALIN A PERTURBATION OF MEMBRANE ENZYMES OF MAMMARY GLAND*

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The plant lectin concanavalin A (Con A) specifically inactivates the 5′-nucleotidase of a plasma membrane-enriched fraction from lactating mammary gland. The lectin also causes an activation of the membrane Mg^{++}-ATPase, but does not affect galactosyltransferase or alkaline phosphatase. The enzyme perturbations are prevented by α-methylmannoside, an inhibitor of Con A binding, indicating that specific binding to carbohydrate structures rather than nonspecific protein-protein interaction is involved. Solubilization of the 5′-nucleotidase in detergents (0.2% Triton X-100 or 1% deoxycholate) does not prevent Con A inactivation, indicating that incorporation into the membrane structure is not a requirement for the Con A effect. The results suggest that Con A inactivates the 5′-nucleotidase by a direct interaction with the enzyme and that this enzyme is a Con A receptor site on the surface of mammary cells.

INTRODUCTION

The plant lectin concanavalin A (Con A) exhibits a variety of biological effects (1). Included among these are agglutination of certain cell types (2), induction of mitosis of lymphocytes (3) and inhibition of growth of some cells (4). Although the molecular bases for these phenomena are still uncertain, they appear to involve an initial binding of the lectin to oligosaccharide sequences present at cell surfaces. For this reason, considerable interest has developed in the identification of lectin receptor sites. Some of these have been isolated as glycoproteins (5) or glycopeptides (6) from cell membranes or proteolytic digests of cells. The biological functions of these receptor sites are still largely obscure.

Since a large number of enzymes, particularly those of plasma membranes, contain associated carbohydrate (7), it seems likely that lectins such as Con A might be able to alter cell surface enzyme activities, whether by direct interaction or via membrane alterations. Con A-induced alterations have been demonstrated for a galactosyltransferase (8) and Mg^{++}-ATPase (9). During the course of an investigation of the effects of Con A on mammary gland plasma membrane isolation, we discovered that the lectin was able to strongly inhibit 5′-nucleotidase from these membranes. The present report details studies on the mode of this inhibition.

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EXPERIMENTAL PROCEDURES

Materials

Con A (Type IV, highly purified), adenosine deaminase (Type I, calf intestinal mucosa), sodium dodecyl sulfate (SDS), sodium deoxycholate, Triton X-100, and all nonradioactive enzyme substrates and inhibitors were obtained from Sigma Chemical Co., St. Louis, Mo. Sodium N-lauroylsarcosinate (sarcosyl) was obtained from K and K Laboratories, and ^{14}C-UDP-galactose was from New England Nuclear, Boston, Mass.

Analytical Procedures

Protein was determined by the method of Lowry et al. (11). Inorganic phosphate released during enzyme assays was measured by the method of Lazarus (12), except in the final step phosphomolybdate was extracted by mixing the final reaction mixture with 1 vol of isoamyl alcohol. After centrifugation the absorbance of the alcohol phase was measured at 740 nm. 5′-Nucleotidase was assayed by phosphate release as described by Morré (13) or by coupled spectrophotometric assay (14) in the presence and absence of 0.1 mM β-glycerophosphate as an inhibitor of nonspecific alkaline phosphatase (15). Mg^{++} concentration for optimal nucleotidase activity was 1 mM, but Mg^{++} addition was not necessary to observe activity, possibly because of the presence of endogenous membrane Mg^{++}. Galactosyltransferase was assayed by the method of Fitzgerald et al. (16), using N-acetylglucosamine as the galactosyl acceptor. Alkaline phosphatase was measured by the release of p-nitrophenol from p-nitrophenylphosphate (17).

Membrane Preparation

A plasma membrane-enriched fraction was prepared from mammary glands of lactating rats, as previously described (10). The procedure involves homogenization of minced mammary gland with a Sorvall Omni-Mixer in 0.25 M sucrose-20 mM Tris (pH 7.4). Microsomes are isolated by differential centrifugation and fractionated by flotation through a discontinuous gradient. The lightest membrane fraction was found to contain the highest activities of plasma membrane markers 5′-nucleotidase and Na^+, K^+-ATPase and was the material used in all the experiments in this report.

RESULTS

Inhibition of 5′-Nucleotidase by Con A

Treatment of plasma membrane-enriched fractions isolated from lactating rat mammary gland with Con A causes a marked inhibition of the 5′-nucleotidase, as measured by a phosphate release assay (Fig. 1). The amount of Con A required for 50% inhibition in this system is about 120 μg Con A per mg membrane protein. Inclusion of α-methylmannoside, an inhibitor of Con A binding to oligosaccharides, prevents the inhibition effect of Con A on the nucleotidase. Since the nucleotidase activity assayed by phosphate release may contain contributions from nonspecific phosphatases (15), we have also used a coupled spectrophotometric assay which measures the liberation of adenosine from 5′-AMP by nucleotidase by converting it to inosine in the presence of excess adenosine deaminase. In this assay the contribution of nonspecific phosphatases can be essentially eliminated by adding β-glycerophosphate as an alkaline phosphatase inhibitor (15). The results in Table I show that 89–97% of the enzyme activity measured by the spectrophotometric assay in either membrane-bound or detergent-solubilized form is not

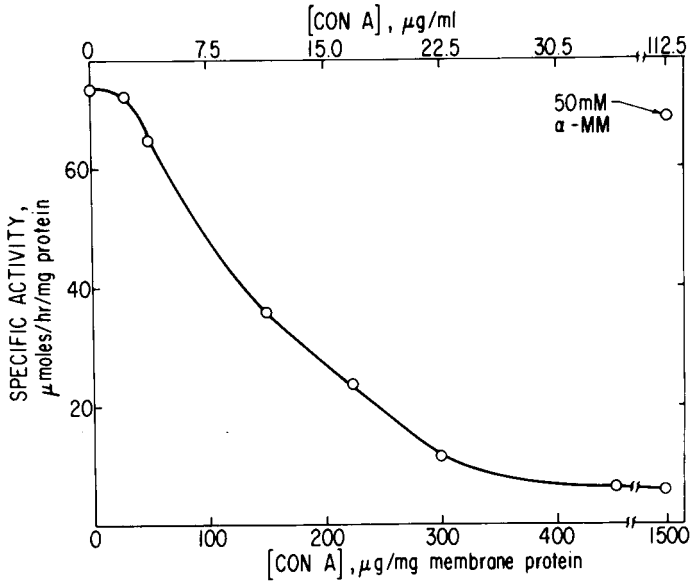

Fig. 1. Con A inhibition of 5′-nucleotidase. Membranes (0.75 mg/ml) were incubated with Con A at 37°C for 30 min before assaying by the phosphate release assay. α-MM, α-methylmannoside.

TABLE I. Specificity of 5′-Nucleotidase Assay and Con A Inhibition

Preparation	$\frac{5'\text{-Nucleotidase}^1}{\text{Phosphohydrolase}} \times 100$	Con A inhibition[2] of phosphohydrolase (%)
Membrane	96.6	94.0
SDS solubilized before centrifugation[3]	88.9	89.3
SDS solubilized after centrifugation[3]	90.4	90.4

[1] Activity was assayed by spectrophotometric method in presence and absence of β-glycerophosphate as described in Methods. 5′-Nucleotidase (%) is expressed as ratio of activity in presence of the inhibitor to activity in its absence × 100.
[2] Activity in the absence of β-glycerophosphate was assayed in the presence and the absence of 1,500 μg Con A/mg protein and is expressed as percent inhibition of total activity by Con A.
[3] Membrane samples (160 μg/ml protein) were solubilized by incubating in 0.05% SDS in 10 mM Tris, pH 7.6, at 37°C for 20 min. Samples were centrifuged at 40,000 × g for 1.75 hr.

affected by the β-glycerophosphate. In addition, a similar 89–94% of the total activity is inhibited by Con A. The results indicate that about 90% of the phosphohydrolase in these preparations is due to 5′-nucleotidase and that the nucelotidase is the predominant, if not the exclusive, enzyme being inhibited by Con A. These conclusions are supported by data (Table II) which show little effect of Con A on nonspecific alkaline phosphatase. This lack of effect on nonspecific phosphatase probably explains the failure of Con A to cause complete inhibition of the enzyme activity as shown in Fig. 1.

TABLE II. Effect of Con A on Other Enzymes

Enzyme	Con A (μg/mg membrane protein)	α-Methyl-mannoside (mM)	Specific Activity (μmoles/hr/mg protein)
Mg^{++}-ATPase	0	0	13.2
	0	50	12.8
	660	0	23.1
	660	50	13.4
Galactosyl transferase	0	0	1.5
	1,920	0	1.4
	1,920	50	1.3
Alkaline phosphatase	0	0	2.5
	1,500	0	2.2

Effects of Con A on Other Mammary Membrane Enzymes

In order to determine if the inhibition of 5′-nucleotidase by Con A was the result of nonspecific membrane-Con A interactions, the effects of Con A on several other membrane enzymes were examined (Table II). The Mg^{++}-ATPase activity is stimulated by Con A by almost 100%, an effect which is reversed by α-methylmannoside. A similar stimulation of the Mg^{++}-ATPase of adipocyte plasma membranes has been noted (9). No effect of Con A was observed on the galactosyltransferase activity of these membranes. This is contrary to the findings of Podolsky et al. (8), who reported an inhibition by Con A of galactosyl-transferase activity from several cell types. Since different assay methodology was used in evaluating the enzyme activity and inhibition, it is possible that two different enzymes are being observed. The collected data on the four different enzymes show a diversity of responses, indicating that the effects of Con A are not due to a single nonspecific mechanism, such as membrane aggregation. Characterization of the kinetics of the enzyme and Con A inhibition (unpublished observations) indicate that the inhibition cannot be due to a direct interaction of 5′-AMP with Con A.

Effect of Detergent Solubilization on 5′-Nucleotidase and Its Inhibition by Con A

An important consideration in understanding the effects of Con A on membrane enzymes is whether the Con A is acting directly on the enzyme or acting at another site to produce a membrane structural change which is transmitted to the enzyme. In order to distinguish between these possibilities we have examined the effects of detergents on the Con A inhibition of 5′-nucleotidase. If the effects of Con A are transmitted through the membrane, the inhibition should be eliminated by detergent treatments which destroy the membrane structure and solubilize the enzyme. Mammary membranes have been treated with three different detergents which disaggregate membrane structure in different ways and affect the nucleotidase differently. Triton X-100 has no effect (or a slight activation) at concentrations up to 0.2%. Sarcosyl causes activations up to 15% between 0 and 1% detergent, but decreases activity at higher concentrations. Deoxycholate causes activations as high as 100% over the range of 0–2% detergent. The effects of the detergents on 5′-nucleotidase solubilization and Con A inhibition of the enzyme are shown in Table III. Deoxycholate and Triton X-100 are most effective at solubilizing the enzyme, which is almost completely sedimented in the absence of detergent. Inhibition can be demonstrated on all of the detergent-solubilized preparations. The failure to demonstrate complete

TABLE III. Detergent Effects on 5′-Nucleotidase Inhibition by Con A

Detergent (concentration %)	Specific activity (μmoles/hr/mg protein)	
	−Con A	+ Con A[1]
None, uncentrifuged[2]	34.9	4.4
None, centrifuged[2]	1.1	0.8
Triton (0.2)[2]	39.3	6.8
Sarcosyl (1.0)[2]	7.9	0.55
Deoxycholate (1.0)[3]	157	51

[1] All Con A incubations were performed at 37°C for 30 min with 1,500 μg Con A/mg membrane protein.
[2] The membranes were solubilized by treating with detergent for 20 min at 37°C. These samples were centrifuged for 1.75 hr at 40,000 × g.
[3] The deoxycholate experiment was performed with a different membrane preparation, which had specific activities of 86 and 143 μmoles/hr/mg protein, respectively, for uncentrifuged samples in the presence and absence of 1% deoxycholate. The membranes were incubated with deoxycholate for 45 min at 37°C and centrifuged at 100,000 × g for 1 hr.

inhibition may be due to an effect of the detergents on the Con A interaction with its receptor.

DISCUSSION

The interaction of Con A with cell surface receptors appears to involve very specific and complex interactions, which can ultimately lead to a variety of biological effects (1). Investigation of Con A receptors has been primarily limited to efforts to solubilize and isolate their molecular constituents because of an absence of identifiable activity other than Con A binding. In the present work we have demonstrated the ability of Con A to inhibit the membrane enzyme 5′-nucleotidase specifically. The effect presumably occurs via an interaction with a carbohydrate moiety, since inhibition can be prevented by α-methylmannoside. The inhibition appears to occur by direct interaction with the enzyme rather than through a membrane-mediated process, since detergent solubilization of the enzyme does not prevent inhibition. The 5′-nucleotidase is known to be a surface constituent of several cells (18, 19) and purifies with a putative plasma membrane fraction from mammary tissue (10). These facts lead us to propose that the 5′-nucleotidase is a Con A receptor on the surface of the mammary epithelial cell. At present we are unable to determine what percentage of the total Con A binding sites are represented by 5′-nucleotidase. However, preliminary affinity chromatography results (D. Snow, unpublished observations) with solubilized bovine milk fat globule membranes, a mammary plasma membrane derivative, suggests that they may represent a fairly small proportion. A recent report of Con A inactivation of 5′-nucleotidase of liver plasma membranes (20) indicates a similar role in liver cells. The biological significance of the lectin receptor activity of this enzyme is unclear, but it may present opportunities for investigations of the role of the enzyme and the effects of lectins that have hitherto been impossible.

The apparent specificity of the reaction for 5′-nucleotidase, as opposed to other phosphohydrolases, suggests that the Con A inhibition might be a useful adjunct to the utilization of the nucleotidase as a plasma membrane marker. The specificity of the Con A toward surface-located nucleotidases needs to be evaluated in whole cells, membranes, and homogenates from a number of cell types. If the lectin proves to be specific for surface

nucleotidase, this will significantly enhance the usefulness of the enzyme as a plasma membrane marker.

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REFERENCES

1. Lis, H., and Sharon, N., Annu. Rev. Biochem. 42:541 (1973).
2. Burger, M. M., Fed. Proc. 32:91 (1973).
3. Novogrodsky, A., and Katchalski, E., Biochim. Biophys. Acta 228:579 (1971).
4. Shoham, J., Inbar, M., and Sachs, L., Nature 227:1244 (1970).
5. Findlay, J. B. C., J. Biol. Chem. 249:4398 (1974).
6. Wray, V. P., and Walborg, E. F., Jr., Cancer Res. 31:2072 (1971).
7. Evans, W. H., Hood, D. O., and Gurd, J. W., Biochem. J. 135:819 (1973).
8. Podolsky, D. K., Weiser, M. M., LaMont, J. T., and Isselbacher, K. J., Proc. Natl. Acad. Sci. U.S.A. 71:904 (1974).
9. Jarett, L., and Smith, R. M., J. Biol. Chem. 249:5195 (1974).
10. Shin, B. C., Ebner, K. E., Hudson, B. G., and Carraway, K. L., Cancer Res. 35:1135 (1975).
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193:265 (1951).
12. Lazarus, L. H., and Chou, S. -C., Anal. Biochem. 45:557 (1972).
13. Morré, D. J., Methods Enzymol. 22:130 (1971).
14. Ipata, P. L., Anal. Biochem. 20:30 (1967).
15. Belfield, A., and Goldberg, D. M., Nature 219:73 (1968).
16. Fitzgerald, D. K., Colvin, B., Mawal, R., and Ebner, K. E., Anal. Biochem. 36:43 (1970).
17. Garen, A., and Levinthal, C., Biochim. Biophys. Acta 38:470 (1960).
18. DePierre, J. W., and Karnovsky, M. L., J. Biol. Chem. 249:7111 (1974).
19. DePierre, J. W., and Karnovksy, M. L., Science 183:1096 (1974).
20. Riordan, J. R., and Slavik, M., Biochim. Biophys. Acta 373:356 (1974).