Proc. Nat. Acad. Sci. USA
Vol. 70, No. 12, Part I, pp. 3650–3654, December 1973

Mitogenic Properties of Pea Lectin and Its Chemical Derivatives

(mitogenesis/chemical modification/binding affinity)

IAN S. TROWBRIDGE

The Armand Hammer Center for Cancer Biology, The Salk Institute, Post Office Box 1809, San Diego, California 92112

Communicated by Robert W. Holley, August 6, 1973

ABSTRACT **A mitogenic lectin that has a carbohydrate-binding specificity similar to that of concanavalin A (Con A) can be isolated from pea seeds. Chemical modification (succinylation, acetylation, or treatment with the diazonium salt of sulfanilic acid), changes its biological properties. In mitogenic stimulation of mouse spleen cells and in hemagglutination, the differences between the chemically modified pea lectin and the native molecule are similar to those observed between succinyl-Con A and native Con A. However, whereas chemical modification converts tetrameric Con A to a dimeric molecule, similar treatments of the pea lectin do not change its quaternary structure. The results of binding studies of the pea lectin and its derivatives to mouse spleen cells suggest that the differences in biological activities may be explained by a reduction in binding affinity of the pea lectin for glycoproteins on the spleen cell surface after chemical modification.**

The stimulation of lymphoid cells to initiate DNA synthesis and to divide after the binding of certain plant lectins to cell surface receptors (1, 2), affords an excellent opportunity to study the transfer of an external mitogenic signal from the cell membrane into the cytoplasm. The availability of well-characterized plant lectins has enabled a molecular approach to this problem to be attempted (3). Studies using chemical derivatives of concanavalin A (Con A) with altered biological properties and quaternary structure (4) have prompted the construction of a hypothesis concerning the initial events that lead to the transfer of the mitogenic stimulus into the cytoplasm after lectin binding (5).

I report here analogous studies with another mitogenic lectin, isolated from pea seeds (6), and its chemical derivatives, which also differ from the native molecule in their biological properties in a manner similar to the chemical derivatives of Con A.

MATERIALS AND METHODS

Pea lectin was isolated from the 0–60% ammonium sulfate fraction of a water extract of pea flour by affinity chromatography on Sephadex G-100, with 0.2 M glucose used to elute the purified lectin. Con A twice crystallized was purchased from Miles-Yeda Ltd., Miles Laboratories, Inc., Kankakee, Ill., and for most experiments was used without further purification. Succinyl and acetyl derivatives of Con A and pea lectin were prepared as described (4). Using [^{14}C]-succinic anhydride and [^{14}C]acetic anhydride (New England Nuclear Corp.) it was estimated that succinyl pea lectin contained an average of 16 succinyl residues per lectin molecule and acetyl pea lectin about 19 acetyl groups per lectin molecule. The sulfanilic-azo derivatives of pea lectin and Con A were prepared by treatment of the lectins (5 mg/ml) with the diazonium salt of sulfanilic acid at a concentration of 4 mM in pH 8.5 borate buffer at 0–4° for 60 min. The reaction was terminated by the addition of 3 volumes of saturated ammonium sulfate. The precipitated lectin was dissolved in water, dialyzed, and lyophilized. Both sulf-pea lectin and sulf-Con A dissolved in pH 7.2 phosphate-buffered saline (PBS) had similar absorption spectra, exhibiting a broad peak between 300 and 400 nm with a λ max at 330 nm and a shoulder at 370 nm. Under the same conditions the A_{280}/A_{330} of both derivatives was 0.64. [^{125}I]-Pea lectin (specific activity 1.7×10^5 cpm/μg) was prepared by the method of McConahey and Dixon (7) and purified by affinity chromatography on Sephadex. [^{14}C]Succinyl pea lectin used below had a specific activity of 4.2×10^3 dpm/μg.

Polyacrylamide gel electrophoresis was performed using a high-pH discontinuous system (8) in the absence or presence of sodium dodecyl sulfate (SDS). Isoelectric focussing was in polyacrylamide gels (9). Low-speed sedimentation equilibrium was studied with a Spinco model E analytical ultracentrifuge equipped with Schlieren optics. Results were calculated as described by Lamm (10). The partial specific volume of pea lectin was calculated from its amino-acid composition (11), and the same value was used for calculations involving the derivatized pea lectins.

Specific binding to Sephadex was determined by chromatography of 4-mg samples on a 1×60-cm Sephadex G-100 column equilibrated with PBS. The specifically bound lectin was eluted with 0.1 M glucose in PBS.

Mitogenic activity of the lectins and their derivatives was assayed in mouse spleen cell suspensions, prepared from spleens of $B_6D_2F_1$ hybrid mice 2–8 months old (12), and incubated in Roswell Park Memorial Institute 1640 medium supplemented with 2 mM glutamine, 10% Sephadex G-50 filtered fetal-calf serum (13), and penicillin and streptomycin. Cells (1×10^6/ml) in 1.0 ml of medium were incubated in 16-mm multi-well tissue culture plates (Linbro Chemical Co., Inc., Los Angeles, Calif.). DNA synthesis in triplicate cultures was estimated after 42 hr incubation by pulsing cells for 6 hr with 2 μCi of [^{3}H]thymidine (5 μM) and measuring the incorporation of radioactivity into trichloroacetic acid-precipitable material.

The binding of lectins to mouse spleen cells was assayed by

Abbreviations: Con A, concanavalin A; sulf-pea lectin, sulfanilic acid-azo-pea lectin; PBS, phosphate-buffered saline (pH 7.2); PBS–BSA, phosphate-buffered saline (pH 7.2) containing 2% bovine-serum albumin; SDS, sodium dodecyl sulfate; BSA, bovine-serum albumin.

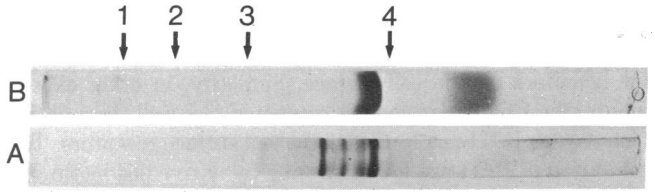

FIG. 1. (*A*) Isoelectric focussing of 20 μg of pea lectin on a pH 3–10 Ampholine gradient in 6% acrylamide gel. The *left* of the gel is the acidic region. (*B*) SDS electrophoresis of 50 μg of pea lectin on 13% acrylamide gel. Protein was stained with Coomassie blue. The position of protein molecular weight markers are indicated: (*1*) BSA; (*2*) ovalbumin; (*3*) Con A; (*4*) cytochrome *c*.

incubation of the lectins with spleen cells freed of erythrocytes by incubation at room temperature with Tris-buffered ammonium chloride (14). Experimental conditions were selected to minimize endocytosis of lectin molecules after binding to the cell surface (15). After incubation for 50 min at 4° with 3×10^6 spleen cells in 0.3 ml of PBS–BSA containing 10 mM sodium azide, the reaction was terminated by the addition of 2.0 ml of cold PBS–BSA and the cells were collected on Whatman 2.4-cm GF/C glass fiber filters, presoaked in PBS–BSA, by filtration at a flow rate of 60 ml/min, and washed with two 5-ml portions of PBS–BSA. The results of the binding experiments presented below are corrected for nonspecific binding of the lectins, which was determined as the radioactivity bound to filters when the lectins were incubated with cells in the presence of 100 mM methyl α-D-mannoside. The nonspecific binding of pea lectin did not exceed 5% of the total radioactivity bound, even at a lectin concentration of 100 μg/ml. Nonspecific binding of [^{14}C]succinyl pea lectin accounted for approximately 15–20% of the radioactivity bound in the absence of methyl α-D-mannoside. Without cells in the incubation mixture, virtually no radioactivity was retained by the filters. In competition studies, unlabeled lectins were added to [^{125}I]-pea lectin in a total volume of 0.2 ml of PBS–BSA. The binding reaction was initiated by the addition of 3×10^6 spleen cells in 0.1 ml of PBS–BSA.

Hemagglutination assays using human erythrocytes were carried out in a final volume of 50 μl of PBS containing 100 μg/ml of BSA at a cell concentration of 1×10^8 cells per ml. Agglutination of spleen cells was estimated under the same conditions as used in the mitogenic assays, after 42 hr of culture.

RESULTS

Pea lectin prepared by affinity chromatography showed two major components together with a number of minor bands on isoelectric gels (Fig. 1A). The two major components could be separated by chromatography on DEAE–cellulose in 0.01 M Tris·HCl–0.05 M NaCl buffer (pH 8.8) at 4°, and had identical biological properties and similar amino-acid compositions. On SDS electrophoresis in the absence or presence of mercaptoethanol they gave an identical gel pattern, a major component, molecular weight approximately 13,000, and a minor diffuse band, molecular weight less than 10,000 (Fig. 1B). Sedimentation equilibrium studies carried out in PBS gave a molecular weight of 43,000 for the native lectin, reduced to 13,100 in 6 M guanidine hydrochloride.

Native pea lectin was mitogenic. Under the conditions of assay maximum stimulation of mouse spleen cells occurred at a

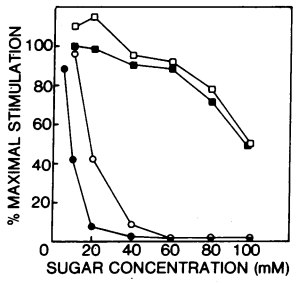

FIG. 2. Inhibition of the mitogenic activity of pea lectin by various sugars added at the beginning of culture. (●), methyl α-D-mannoside; (○), methyl α-D-glucoside; (□), methyl β-D-glucoside; (□), methyl α-D-galactoside.

lectin concentration of 2 μg/ml. The mitogenic activity of pea lectin could be specifically inhibited by methyl α-D-mannoside and methyl α-D-glucoside (Fig. 2), suggesting pea lectin has a sugar-binding specificity similar to that of Con A. However, the specificities of the two lectins are not identical, since about 10% of [^{125}I]-pea lectin bound to spleen cells and displaceable by unlabeled pea lectin could not be displaced by Con A.

The mitogenic activities of native pea lectin and its chemically modified derivatives are shown in Fig. 3A. Those of native Con A and its derivatives are shown in Fig. 3B. The dose–response curve of native pea lectin was similar to that of Con A, displaying maximum mitogenic activity at low concentrations of lectin and inhibition of the response at high concentrations of lectin. However, whereas Con A exhibited a relatively sharp peak of mitogenic activity at a lectin concentration of 1 μg/ml, pea lectin showed substantial activity over a concentration range of 1–10 μg/ml. Succinyl-pea lectin, acetyl-pea lectin, and sulf-pea lectin are not mitogenic at concentrations at which the native lectin is maximally active, but exhibit a broad plateau of activity at high concentrations. The maximal mitogenic stimulation obtained with chemically modified pea lectins, particularly succinyl-pea lectin, was less than the maximal stimulation that could be achieved with the native lectin.

Confirming the observations of Gunther *et al.* (4), succinyl-Con A exhibits a broad plateau of mitogenic activity at concentrations at which the native lectin is inhibitory. The dose–

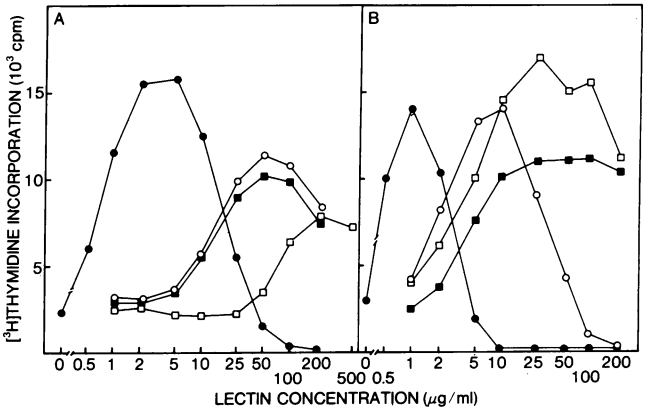

FIG. 3. (*A*) Mitogenic activity of pea lectin and its chemically modified derivatives. (●), pea lectin; (○), acetyl-pea lectin; (□), succinyl-pea lectin; (■), sulf-pea lectin. (*B*) Mitogenic activity of Con A and its chemically modified derivatives. (●), Con A; (○), acetyl-Con A; (□), succinyl-Con A; (■), sulf-Con A.

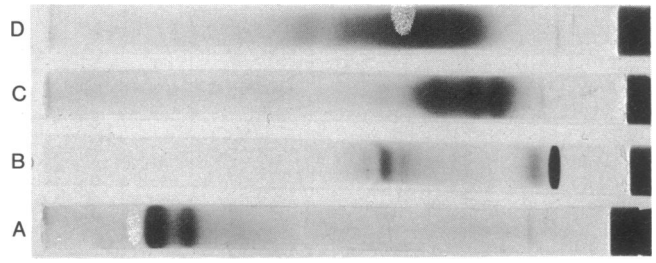

FIG. 4. Gel electrophoresis of pea lectin and its chemically modified derivatives, performed as described in *Materials and Methods* on 7% acrylamide gels. (*A*) 20 μg of native pea lectin; (*B*) 80 μg of succinyl-pea lectin; (*C*) 20 μg of acetyl-pea lectin; (*D*) 40 μg of sulf-pea lectin.

response curve of sulf-Con A parallels that of succinyl-Con A, while that of acetyl Con A resembles the native lectin, but is displaced to slightly higher concentrations (Fig. 3B).

The properties of the chemical derivatives of pea lectin are summarized in Table 1 and are similar to those of the Con A derivatives in a number of respects. They bind specifically to Sephadex, do not agglutinate erythrocytes, and show a reduced capacity to agglutinate mouse spleen cells. However, in contrast to derivatization of Con A, succinylation, acetylation, or treatment with the diazonium salt of sulfanilic acid does not change the extent of subunit aggregation of pea lectin.

Since the derivatized pea lectins bind almost quantitatively to Sephadex, their failure to agglutinate erythrocytes cannot be explained by contamination of inactive derivatives with small amounts of native lectin. Also, gel electrophoresis fails to reveal any indication of such contamination (Fig. 4). Under the conditions used, succinyl-pea lectin migrated as a single major band of high mobility, acetyl-pea lectin gave three slower moving components, while sulf-pea migrated as a diffuse band.

To discover the cause of the differences in the mitogenic properties of pea lectin and its derivatives, their binding to mouse spleen cells was compared. In competition studies illustrated in Fig. 5 it was found that the pea lectin derivatives were much less efficient than the native lectin in displacing

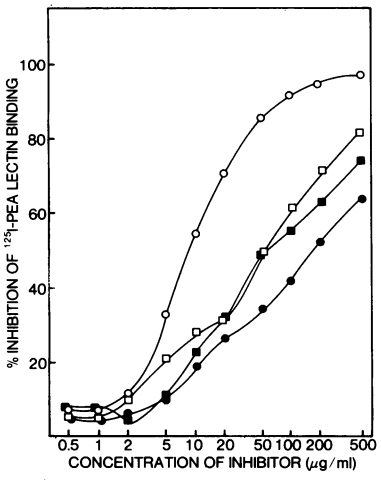

FIG. 5. The inhibition of the binding of [^{125}I]-pea lectin (2 μg/ml) to mouse spleen cells by unlabeled native pea lectin and its chemically modified derivatives. (○), native pea lectin; (●), succinyl-pea lectin; (□), acetyl-pea lectin; (■), sulf-pea lectin.

[^{125}I]-pea lectin from the surface of spleen cells. This implies that chemically derivatized pea lectins have a reduced affinity for receptors on the cell surface. Similarly, in other experiments, the Con A derivatives competed less well than native Con A with [^{125}I]-pea lectin for the cell surface receptors. The inhibition of [^{125}I]-pea lectin binding by native pea lectin was less effective than predicted by a simple competition model, and probably reflects cooperative binding. This was suggested by Cuatrecasas to explain similar results observed in binding experiments of Con A to isolated fat cells (16).

The reduced affinity of succinyl-pea lectin for receptors on the spleen cell surface compared with the native lectin was confirmed by direct binding studies using [^{14}C]succinyl pea lectin and [^{125}I]-pea lectin (Fig. 6). The concentration of succinyl-pea lectin required to saturate the cell receptors was about 10-fold higher than that of the native lectin. Roughly 2-fold less succinyl-pea lectin than native lectin was bound at the highest concentrations of lectin tested. The maximum mitogenic activity of the native pea lectin occurred at a lectin concentration at which, under the conditions of the binding assay, about 20% of the available binding sites on the cell surface are occupied. The mitogenic activity of succinyl-pea lectin was observed at concentrations of lectin at which the maximum number of succinyl-pea lectin molecules are bound to the cell surface.

DISCUSSION

The chemical derivatives of pea lectin described above differ from the native molecule in their biological properties. These differences are like those between similarly prepared derivatives of Con A and native Con A recently reported (4a), and confirmed in this paper. However, whereas derivatization of Con A causes the molecule to change from a tetramer to a dimer under physiological conditions, the quaternary structure of pea lectin under the same conditions is unchanged by chemical modification. The results of the sedimentation

TABLE 1. *Properties of pea lectin and its derivatives*

Properties	Pea lectin	Acetyl-pea lectin	Succinyl-pea lectin	Sulf-pea lectin
1. Concentration having maximal mitogenic activity (μg/ml)	2–5	25–100	100–500	25–100
2. Molecular weight in PBS	43,000	45,000	41,000	56,000*
Molecular weight in 6M guanidine·HCl	13,100	—	—	—
3. Minimum concentration for agglutination of erythrocytes (μg/ml)	8	>500	>500	>500
4. Minimum concentration for agglutination of mouse spleen cells (μg/ml)	1–2	10–25	50–100	10–25
5. Percent binding to Sephadex	>95	90	90	75

* The presence of small amounts of aggregated material indicated by the deviation from linearity of the Lamm plot accounts for the high molecular-weight value observed.

equilibrium studies and SDS electrophoresis suggest native pea lectin is a tetrameric molecule. The minor band observed on SDS electrophoresis may result from proteolytic cleavage of pea lectin before or during its isolation. Naturally occurring fragments of Con A have been described (17). Further investigations are required to confirm the detailed molecular structure of pea lectin and to determine the number of carbohydrate-binding sites per molecule of native lectin and its derivatives. Until these studies are completed it is not possible to formally exclude the possibility that chemical modification inactivates a proportion of the carbohydrate-binding sites of pea lectin, leading to a change in valency without a concomitant change in the degree of subunit aggregation.

The differences in mitogenic and agglutination properties of pea lectin and its derivatives can largely be accounted for by a reduced affinity of the derivatives for receptors on the cell surface. A similar explanation can account for the differences between the Con A derivatives and the native molecule in mitogenic and agglutination properties. Preliminary results of Gunther *et al.* (4) indicate succinyl-Con A has the same affinity as the native molecule for methyl α-D-glucoside. Since the competition studies reported here suggest succinyl-Con A binds to spleen cells with lower affinity than the native molecule, it is probable that, following succinylation, there is a differential change in the affinity of Con A for the cell surface receptor compared to the simple sugar. The increased susceptibility of the mitogenic activity of acetyl-Con A to inhibition of methyl α-D-mannoside reported by Reichert *et al.* (18) is consistent with this possibility. The differential effect could result from multivalent binding of Con A to the cell surface compared with univalent binding to the monosaccharide. It is probable that the reduced valence of the succinyl-Con A molecule would lead to a reduced apparent affinity in the former case, but not the latter. In addition, succinylation may modify the carbohydrate-binding site in such a way as to differentially change its affinity for the cell-surface receptor, the precise structure of which is unknown, with little effect on the binding of methyl α-D-glucoside.

The close relationship between valency and the apparent affinity of ligand binding to the cell surface is an important consideration with regard to the question of whether crosslinking of cell-surface receptors is essential for the mitogenic response. The probability that a reduction in valence of a ligand binding multivalently to the cell surface will lower the apparent affinity of the interaction means that, in the absence of direct binding studies, the failure of monovalent antibody fragments directed against lymphoid cell-surface determinants to stimulate mitogenesis under conditions which divalent fragments are active (19, 20), must be interpreted cautiously. Such results do not *per se* demonstrate that crosslinkage of cell receptors is necessary for the mitogenic response, other than to increase the number of ligand molecules bound at any given concentration of free ligand. A recent study of the specific binding of dinitrophenyl conjugates to the cell surface of murine myeloma cells clearly indicates the influence of valency on the apparent affinity of ligand binding (21). If crosslinkage of receptors is not an essential requirement for mitogenesis, it may be possible to reconcile the conflict between the observations by Sell (22), that univalent antibody fragments are mitogenic, with the contrary finding of others (19, 20), in terms of the affinities of the antibody preparations used in the separate investigations. Crosslinking of cell-surface

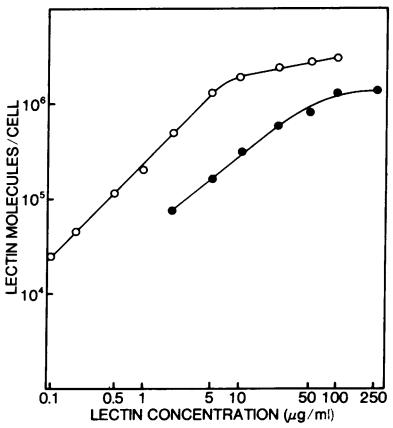

FIG. 6. The binding of native [^{125}I]-pea lectin (○)and [^{14}C]-succinyl-pea lectin (●) to mouse spleen cells.

receptors seems to be required for clustering of receptors ("capping"), but capping itself does not seem to be required for mitogenesis. This has been demonstrated most elegantly by Gunther *et al.* (4), who showed that succinyl-Con A, although active in the mitogenic assay, neither induces capping of its own receptors nor inhibits capping of the receptors of other ligands. There is no evidence contrary to the view that capping is simply a reflection of membrane fluidity and cell mobility (23), unrelated to the transfer of information across the cell membrane. It is therefore reasonable to consider other mechanisms for transferring the mitogenic stimulus from the exterior of the cell membrane into the cytoplasm, such as the direct binding of mitogenic lectins to membrane-bound enzymes (24, 25) or hormone receptors (26, 27).

The dose–response curves of succinyl-Con A and sulf-Con A exhibit a broad plateau of mitogenic activity, in contrast to the relatively sharp peak of mitogenic stimulation of the native lectin. Similar differences between native pea lectin and its derivatives are much less marked. The broad plateau of mitogenic activity of succinyl-Con A and sulf-Con A compared to the relatively sharp peak of mitogenic activity of the native lectin may not be attributable to the binding affinity differences. An alternative possibility relates to the physical properties of the native lectin. Con A will aggregate under physiological conditions (28). Cell toxicity could result from an analogous event occurring on the cell surface, which could presumably magnify changes in membrane structure caused by extensive capping. The introduction, by succinylination, of a large number of negatively charged residues increases the solubility of some proteins at neutral pH (29). Whatever the precise cause of cell death, the toxicity of Con A towards many cells in culture has been established (30), and consequently raises questions concerning the use of the high-dose inhibition of mitogenesis as a model for specific immunological tolerance (31).

Maximal mitogenic activity of pea lectin, in common with that of other lectins (32, 33), occurs only when a fraction of the cell-surface receptor sites are occupied. It is possible that at this concentration of free ligand, the binding sites that participate in the mitogenic response are saturated, by virtue of their high affinity in comparison with that of the bulk of the receptors that bind lectin. An alternative explanation is that only a proportion of the total available receptors need be occupied for maximal stimulation. While the biological activ-

ity of most polypeptide hormones is proportional to the number of hormone receptor sites occupied (34–36), a biological precedent for the latter possibility does exist. The biological activity of thyrotropin-releasing factor, a tripeptide that stimulates secretion of thyrotropin from pituitary cells, is maximal at concentrations at which only 5–10% of the available hormone receptor sites are occupied (27).

Succinyl-pea lectin at optimal concentrations apparently induces DNA synthesis in a lower proportion of spleen cells than the native lectin does (Fig. 3A). A possible explanation is that the spleen cell population stimulated by the native lectin is heterogeneous with respect to the number and affinity of glycoprotein mitogenic receptors on the surface of individual cells. The affinity of succinyl-pea lectin may be too low for it to bind to the critical number of receptors required for mitogenic stimulation in a fraction of this cell population.

The heterogeneity of both the spleen cell population and the glycoprotein receptors on individual cells imposes the most serious limitation in comparing the results of lectin-binding experiments with those of the mitogenic assay. The binding assay measures the binding of lectin to all the classes of glycoprotein receptors of the entire spleen cell population, whereas the mitogenic stimulus involves only a subpopulation of spleen cells and a fraction of the receptors on these cells. This is a problem intrinsic to all studies of this nature and can only be partially resolved by using purified cell preparations.

In conclusion, the results reported here suggest that the changes in mitogenic activity of pea lectin induced by chemical modification result from a reduction in binding affinity of the derivatized lectin for the glycoprotein receptors on the surface of spleen cell. It has been suggested that similar changes in the mitogenic and agglutinating activity of Con A after chemical modification are attributable to their reduced valence (4). However, since, in contrast to that of Con A, the quaternary structure of pea lectin is unchanged by chemical derivatization, an alternative explanation is that the essential difference between Con A and its derivatives, which results in changed mitogenic and agglutination activities, is a reduced affinity of the derivatives for glycoprotein receptors on the cell surface.

I thank Dr. R. W. Holley for advice throughout this work and Dr. W. T. Shier for criticism of the draft manuscript. This work was supported in part by Grant CB 32391X from the National Science Foundation. I am a recipient of a postdoctoral fellowship from the Jane Coffin Childs Memorial Fund for Medical Research, no. 61–318.

1. Andersson, J., Edelman, G. M., Möller, G. & Sjöberg, O. (1972) *Eur. J. Immunol.* **2**, 233–235.
2. Greaves, M. F. & Bauminger, S. (1972) *Nature New Biol.* **235**, 67–70.
3. Edelman, G. M. (1973) *Science* **180**, 830–840.
4. Gunther, G. R., Wang, J. L., Yahara, I., Cunningham, B. A. & Edelman, G. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1012–1016.
5. Edelman, G. M., Yahara, I. & Wang, J. L. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1442–1446.
6. Entlicher, G., Kostir, J. V. & Kocourek, J. (1970) *Biochim. Biophys. Acta* **221**, 272–281.
7. McConahey, P. G. & Dixon, F. J. (1966) *Int. Arch. Allergy Appl. Immunol.* **29**, 185–189.
8. Maziel, J. V. (1971) in *Methods in Virology*, eds. Maramorosch, K. & Koprowski, H. (Academic Press, New York), Vol. V, pp. 179–246.
9. Wrigley, C. (1968) *Sci. Tools* **15**, 17–22.
10. Lamm, O. (1929) *Ark. Mat. Astron. Fys.* **21B**, No. 2.
11. McMeekin, T. L., Groves, M. L. & Hipp, N. J. (1949) *J. Amer. Chem. Soc.* **71**, 3298–3300.
12. Mishell, R. I. & Dutton, R. W. (1967) *J. Exp. Med.* **126**, 423–442.
13. Piez, K. A., Oyama, V. I., Levintow, L. & Eagle, H. (1960) *Nature* **188**, 59–60.
14. Boyle, W. (1968) *Transplantation* **6**, 761–764.
15. Taylor, R. B., Duffus, W. P. H., Raff, M. C. & de Petris, S. (1971) *Nature New Biol.* **233**, 225–229.
16. Cuatrecasas, P. (1973) *Biochemistry* **12**, 1312–1323.
17. Wang, J. L., Cunningham, B. A. & Edelman, G. M. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1130–1134.
18. Reichert, C. F., Pan, P. M., Mathews, K. P. & Goldstein, I. J. *Nature New Biol.* **242**, 146–147.
19. Woodruff, M. F. A., Reid, B. & James, K. (1967) *Nature* **215**, 591–594.
20. Fanger, M. W., Hart, D. A., Wells, J. V. & Nisonoff, A. (1970) *J. Immunol.* **105**, 1484–1492.
21. Bystryn, J.-C., Siskind, G. W. & Uhr, J. W. (1973) *J. Exp. Med.* **137**, 301–316.
22. Sell, S. (1967) *J. Immunol.* **98**, 786–791.
23. Edidin, M. & Weiss, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2456–2459.
24. Resch, K., Ferber, E., Odenthal, J. & Fischer, H. (1971) *Eur. J. Immunol.* **1**, 162–165.
25. Hadden, J. W., Hadden, E. M., Haddox, M. K. & Goldberg, N. D. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3024–3027.
26. Cuatrecasas, P. & Tell, G. P. E. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 485–489.
27. Krug, U., Krug, F. & Cuatrecasas, P. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2604–2608.
28. Cunningham, B. A., Wang, J. L., Pflumm, M. N. & Edelman, G. M. (1972) *Biochemistry* **11**, 3233–3239.
29. Klotz, I. M. (1957) in *Methods in Enzymology*, ed. Hirs, D. H. W. (Academic Press, New York), Vol. XI, pp. 576–580.
30. Shoham, J., Inbar, M. & Sachs, L. (1970) *Nature* **227**, 1244–1246.
31. Andersson, J., Sjöberg, O. & Möller, G. (1972) *Immunology* **23**, 637–645.
32. Allan, D. & Crumpton, M. J. (1973) *Exp. Cell Res.* **78**, 271–278.
33. Andersson, J., Sjöberg, O. & Möller, G. (1972) *Transplant. Rev.* **11**, 131–177.
34. Cuatrecasas, P. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1264–1268.
35. Lefkowitz, R. J., Rother, J., Pricer, W. & Pastan, I. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 745–752.
36. Rodbell, M., Krans, M. J., Pohl, S. L. & Birnbaumer, L. (1971) *J. Biol. Chem.* **246**, 1861–1871.
37. Grant, G., Vale, W. & Guillemin, R. (1972) *Biochem. Biophys. Res. Commun.* **46**, 28–33.