The Potential Significance of Potato Hemagglutinins (Lectins) in Serodiagnosis

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The technical assistance of G. Secor and J. Morton is gratefully acknowledged.
Montana Agricultural Experiment Station Journal Series Paper No. 151.
Accepted for publication 11 June 1970.

ABSTRACT

Extracts from both virus-free and potato virus X-infected potato tubers were found to contain hemagglutinating compounds referred to as lectins. The precipitating activity of the potato lectin was demonstrable in single-radial-immunodiffusion systems containing agar impregnated with either normal or immune sera. In radial-diffusion, the lectin reaction with blood group antigen was identical in appearance with that of potato virus X-degraded protein and its homologous antibody. To eliminate possible confusion in serological test systems, means were devised to control lectin reactions. No lectin-blood group antigen precipitation occurred in radial-diffusion systems which contained dialyzed or ammonium sulfate fractionated sera if a final concentration of 0.3% ammonium sulfate was present in the buffering medium. Phytopathology 60:1623-1625.

Nonspecific precipitation in plant saps is a well-recognized problem in serological testing. The causes for spontaneous agglutination or precipitation of cellular components, either in the presence or absence of serum, are varied. Tannins or tanninlike compounds, for example, must be controlled for reliable serodiagnosis. Nondescript flocculations in plant saps apparently free from tannins may also be problematical. Immunodiffusion techniques are useful for minimizing some of the sources of spurious reactivity, but cannot be considered free of them. Defining and controlling the causes of undesirable reactions in any type of serological test can significantly enhance the reliability of the procedure.

One class of compounds present in several plant species which can produce "nonspecific" reactions with either normal or immune sera are the plant hemagglutinins or lectins. Lectins are normally capable of both the agglutination of erythrocytes and the precipitation of certain red blood cell antigens. The seeds, and to a lesser extent other plant parts, of numerous plant species, particularly the legumes, possess significant hemagglutinin concn. The nature and behavior of lectins from these plants has been extensively reviewed elsewhere (1, 2, 4).

Recently (5), a lectin was characterized from potato tubers. This compound was described as a low mol-wt glycoprotein with a low degree of solubility in dilute salt solutions. Furthermore, it was classified as nonspecific with respect to the blood group antigen with which it reacted. Few, if any, lectins are truly nonspecific, however. Rather, they react only with erythrocyte receptor or chemically similar polysaccharide determinants but not with protein determinants. Lectins and blood group antigens exhibit precipitation curves similar to those characteristic of antigen-antibody reactions.

The report of significant lectin concn in potato tubers may be of special significance in serodiagnosis. Although tuber tissue is rarely indexed serologically for viral infection, tubers have been used in other instances (7). Furthermore, lectin concn may vary depending upon environmental conditions (4), perhaps to the point where potato tuber sprouts or foliage may also contain the hemagglutinin. Recent experience in our laboratory with field-grown potatoes has indicated that approx 1 leaf extract/1,000 tested will produce a precipitation ring on normal serum radial-diffusion plates. Such reactions invalidate corresponding tests with the same extract in radial-diffusion systems containing immune serum, and necessitate either the testing of additional leaves from the same plant or the testing of the same leaf by other techniques.

In this study, the activity of the potato lectin was verified in tuber extracts. Also investigated were means of eliminating the potato lectin as a possible cause of spurious reactions in single-radial-diffusion systems.

MATERIALS AND METHODS.—Potato (*Solanum tuberosum* L.) lectin was prepared from virus-free and potato virus X (PVX)-infected tubers of two potato cultivars Russet Burbank and Norgold Russet. Virus-free tubers were collected from increased virus-free stock, and subsequently indexed for possible reinfection. Juice was expressed from tuber sprouts and mechanically inoculated onto *Gomphrena globosa* L. PVX-infected potato stocks (which also contained potato virus S) were increased and tubers indexed for PVX in similar manner.

Juice was extracted with a blender from virus-free and PVX-infected tubers. The crude homogenate was pressed through cheesecloth and centrifuged at 10,000 *g* for 10 min. The low-speed supernatant which contained the potato lectin was used without further purification. Comparative extracts were made in a similar way from tuber sprouts and greenhouse-grown foliage of the same varieties. Field-grown potato foliage was not available for testing.

Hemagglutination tests were conducted with rabbit and human red blood cells in the manner described by Campbell et al. (3). To assay for precipitating activity, potato extracts were placed in single-radial-diffusion depots. Diffusion media consisted of 3% purified agar (Difco) mixed with either normal serum or antiserum specific for PVX-degraded protein (D-protein). Unless stated otherwise, the agar was dissolved in 0.05 M Tris[tris(hydroxymethyl) amino methane]-HCl buffer,

pH 7.2, containing 0.85% NaCl. Basic procedures for PVX D-protein antiserum production and for conducting single-radial-diffusion tests were as previously described (6).

RESULTS AND DISCUSSION.—When extracts from all tubers investigated were mixed 1:1 with human or rabbit red blood cell suspensions, a rapid agglutination of erythrocytes occurred. Tuber sprout extracts, in contrast, produced either faint hemagglutination or none. The intense hemagglutination as produced by tuber extracts was never observed with sprout sap. Foliar extracts consistently failed to induce detectable agglutination of red blood cells. In radial-diffusion systems, tuber extracts invariably produced stable precipitation rings when tested against either normal or PVX D-protein immune serum. The precipitation rings were identical in appearance to those characteristic of specific reactions (Fig. 1), and varied in intensity and diam with serum concn. Extracts from tuber sprouts sometimes produced a faint diffuse ring of precipitation close to the periphery of the radial-diffusion depot when tested against normal or immune serum (Fig. 1). In at least 90% of the cases, however, such diffuse rings dissolved after a 16-hr incubation period. Foliar extracts failed to exhibit precipitating activity in normal serum-radial-diffusion tests. In D-protein antiserum-radial-diffusion systems, a faint precipitin ring was observed in a few instances with PVX-infected leaf homogenates.

Unlike several plant hemagglutinins, the potato lectin is not inhibited by simple sugars (5). Thus, other means were investigated to eliminate the potato lectin reaction. The addition of one part normal serum to one part tuber extract prior to placement in radial-diffusion depots was nominally effective. A reduction in the intensity of the lectin-blood group antigen reaction was consistently observed, and frequently the reaction was entirely eliminated; however, to achieve a reliable control of lectin reactions from all tubers, the proportions of normal serum and tuber extract had to be varied according to the lectin concn in the extract. This necessitated a laborious series of tests for each tuber.

The most effectual means of preventing potato lectin reactions in either normal or immune serum radial-diffusion plates was through the elimination of the blood group antigen from the serum prior to its incorporation into the agar. This could be done by dialysis of sera overnight against a large volume of 0.05 M Tris-HCl buffer pH 7.2 containing 0.85% NaCl. That the blood group antigen was in fact dialyzable was verified by dialyzing a serum aliquot against an equal volume of buffer. Following dialysis, this buffer and a control buffer were separately combined with agar and poured into plastic petri dishes. Strong precipitation rings developed when tuber extracts were tested against the dialyzing buffer-radial-diffusion system. Similar reactions did not occur in control plates. Another method for separating the blood group antigen from the antibody portion of sera was through the precipitation of antibody with ammonium sulfate.

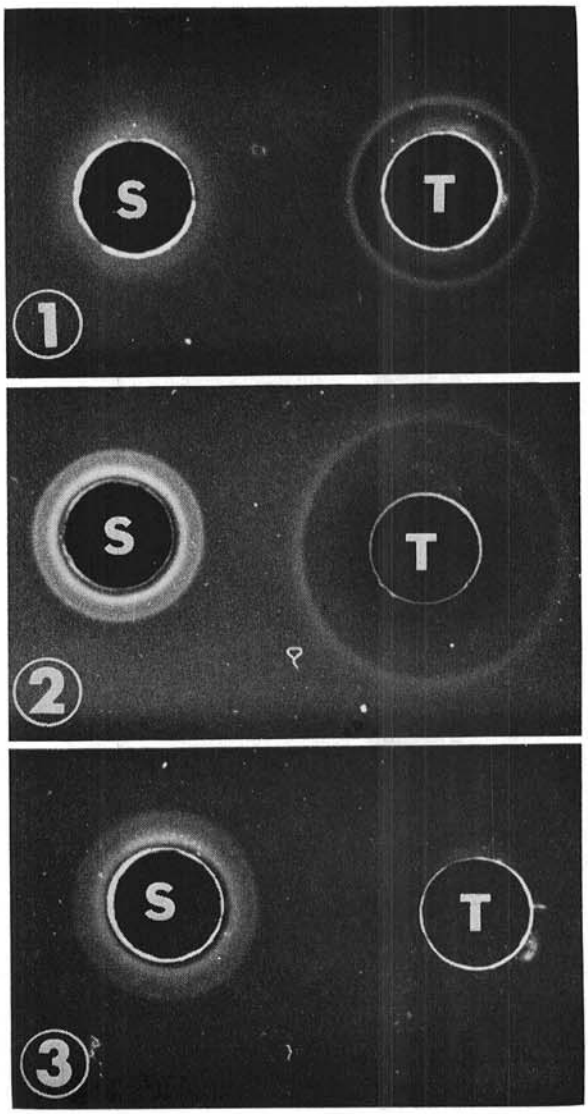

Fig. 1-3. **1)** A comparison between the reactions of PVX-infected Norgold Russet potato tuber (T) and tuber sprout (S) extracts in a radial-diffusion system containing normal serum. The faint reaction near the (S) depot periphery dissolved after an overnight incubation. **2)** Pyridine-treated extracts from infected tubers (T) and sprouts (S) in a radial-diffusion plate containing dialyzed PVX-degraded protein (D-protein) antiserum. The precipitin ring surrounding the (S) depot is PVX D-protein specific, whereas the ring encircling the (T) depot illustrates the insolubility of the potato lectin at low salt concn. **3)** The control of specific and nonspecific potato lectin precipitation in radial-diffusion systems. The same test extracts as illustrated in Fig. 2 against dialyzed PVX D-protein antiserum-agar; 0.3% ammonium sulfate was added to the standard Tris-saline buffering medium.

Normal and immune gamma globulin were precipitated in the cold by the addition of 12 g of ammonium sulfate/50 ml of crude serum. Precipitated gamma globulin was sedimented by centrifugation at 5,000 *g* for 10 min, after which the supernatant was discarded. The gamma globulin fraction was resuspended in the

same volume as the original serum, using 0.05 M Tris buffer plus saline, and then dialyzed for 16 hr against the same buffer. Complete control of the specific lectin reaction, i.e., lectin combination with blood group antigen from tuber extracts, was achieved with this procedure.

Control of the specific lectin reaction in radial-diffusion systems created yet another potential problem. If the lectin present in tuber extracts was not specifically precipitated near the periphery of radial-diffusion depots, as when plates contained dialyzed or precipitated serum or when they contained no serum, the lectin diffused out to greater distances into the agar and then spontaneously became insoluble (Fig. 2). That this unusual manner of precipitation was most probably correlated with the sparing solubility of the potato hemagglutinin in dilute salt solutions (5) was suggested from experiments with ammonium sulfate. The addition of a final concn of 0.3% ammonium sulfate to the radial-diffusion medium prevented spontaneous lectin precipitation in all combinations tested (Fig. 3). Thus, potato tuber hemagglutinin and similar compounds should be completely controlled in radial-diffusion systems if antisera are fractionated with ammonium sulfate or freed of antigen by dialysis and supplemental ammonium sulfate is added to the buffering medium.

It is of interest to note that PVX D-protein reactions were not observed when pyridine-treated extracts from infected tubers were assayed in radial-diffusion against D-protein antibody. This indicates that PVX is in too low a concn in potato tuber tissue for reliable serological detection with the single-radial diffusion procedure. Such a conclusion could not have been reached, however, if the tuber lectins were left uncontrolled. Lectin precipitation would have taken place with both virus-free and infected tuber extracts, and if PVX D-protein specific reactions did occur in some of these systems, their presence would have been undetectable. Thus, tuber testing is one instance where the control of plant hemagglutinins is an important facet of serodiagnosis.

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