# Cell Wall Protein in Plants: Autoradiographic Evidence

Abstract. *Autoradiography of phloem-parenchyma tissue from carrots, which was allowed to incorporate radioactive proline and then plasmolyzed, indicates that a stable protein moiety is associated with the cell wall.*

A protein component rich in hydroxyproline is associated with plant cell walls rigorously purified by differential centrifugation (*1*). Experiments with labeled proline show that protein-bound hydroxyproline in the cytoplasm and in the cell wall have entirely different kinetics, suggesting that the hydroxyproline-rich protein component of the wall is not a cytoplasmic contaminant (*2*). However, electron-microscope autoradiography of rapidly proliferating carrot explants which had incorporated radioactive proline led to the conclusion that there is no cell wall protein (*3*). Using a system in which cells were plasmolyzed after incorporation of proline, but before they were coated with emulsion, we obtained evidence that a protein moiety accumulates in the plant cell wall.

Our experiments were performed on disks (1 cm in diameter and 1 mm thick) of phloem parenchyma from the storage roots of carrots (*Daucus carota* cv.). When such disks are incubated, they undergo a large number of physiological and metabolic changes collectively termed "aging" (*4*). Disks were incubated at 30°C for 24 hours in 2 m*M* phosphate buffer (*p*H 6.5) and 50 μg of chloramphenicol per milliliter (*5*). Tritiated proline (*6*) (5 μc per disk) was added, and the disks were incubated for another 24 hours. The disks were washed once with 1 m*M* proline, then five times with water, and subsequently plasmolyzed for 1 hour in 1.75*M* polyethylene glycol (average molecular weight, 190 to 210) to separate the cytoplasm from the wall. After fixation in acetic acid and alcohol, and embedding in paraplast, mounted sections (6 μ) of disks were coated with Ilford K-5 emulsion and exposed for 3 days at 4°C.

In a section through a plasmolyzed cell (Fig. 1A) the cytoplasm forms a ring around the central vacuole and is distinctly separated from the cell wall. Silver grains appear over both the cell wall and the cytoplasm. The wall label can be accentuated by changing the level of focus. In Fig. 1B a large amount of label is associated with the cell wall. When grain counts were made of various cellular areas, we found 32 percent of the cellular label associated with the wall. Disks from the same incubation were homogenized, and we isolated and purified the cell walls. In this case, 26 percent of the protein-bound cellular radioactivity was associated with the cell wall. Whereas quantitation of autoradiographs with the light microscope is hazardous (*7*), the results are compatible with our biochemical observations (*8*). Cytoplasmic proteins and cell walls from labeled disks were precipitated with trichloroacetic acid and subjected to acid hydrolysis (6*N* HCl for 18 hours at 107°C). Amino acids were separated by paper chromatography, and the distribution of radioactivity was determined. In cytoplasmic proteins, 85 percent of the radioactivity was in proline and 15 percent in hydroxyproline, whereas in the cell wall 48 percent of the label was in proline and 52 percent in hydroxyproline. This suggests that the silver grains found over the wall represent a hydroxyproline-rich protein component.

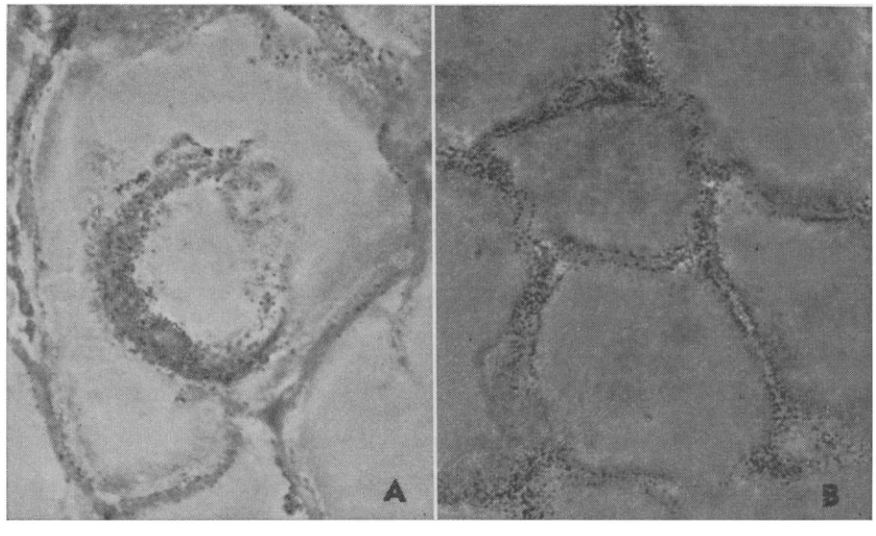


Fig. 1. Plasmolyzed carrot phloem-parenchyma cell labeled with tritiated proline for 24 hours. (A) Focused on cell structures (× 850). (B) Focused on cell wall (× 340).

Several control experiments confirm our interpretation of these results. When disks were placed in plasmolyzing solution throughout the period of incubation in radioactive proline, both cytoplasmic and cell wall labeling were drastically reduced (more than 95 percent) as measured on purified walls and by autoradiography; this makes adsorption of nonincorporated proline onto the wall an unlikely explanation of our results. Incubation of the disks for 72 hours in buffer after incorporation of labeled proline led to a substantial decrease in cytoplasmic label, and an increase in wall label; this supports the suggestion that the cell wall protein is comparatively stable. When tissue sections were incubated in pronase (*9*) before being coated with emulsion, no significant amount of label appeared over the cell wall or cytoplasm. This parallels our observation that most of the protein label associated with purified carrot cell walls and cytoplasm can be removed by pronase.

Whether plasmolysis completely separated cytoplasm from the wall is unknown. It can be argued that pieces of cell membrane were broken off and adhered to the wall, possibly with some cytoplasmic contaminants. If this were the case, however, localized areas of wall label would appear; instead, our cells consistently show label over the entire cell wall. The contribution of plasmodesmata to the wall label is not known. The predominance of silver grains over the inside area of the wall indicates that protein may be deposited by apposition, a situation similar to carbohydrate deposition in the cell walls of some plants (*10*). This makes plasmolysis a necessary prerequisite to resolving whether the radioactive label observed is in the wall or in the nearby cytoplasm.

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**References and Notes**

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## Human Leukocyte Antigenic Specificity HL-A3: Frequency of Occurrence


Abstract. *Reactivity of antiserum against HL-A3, a human leukocyte and tissue antigenic specificity, depends upon a property of the lymphocyte as well as on the potency of the serum. Many reactions of HL-A3 antiserums can only be recognized through absorption or by a two-stage test not in general use. Interpretations of donor-recipient compatibility and of the constitution of HL-A alleles are affected by these findings.*


Formal designations have been given to six specificities of the HL-A system of human leukocyte and tissue antigens (*1*). These factors are recognized by serums which have been shown, through absorption, to be oligo- or monospecific (*2*). Other serums which give comparable but not identical reactions to these "type specific" serums are quite commonly found. Additional reactions are frequently due to the presence of a second antibody. Where two antibodies are present in a serum, either component can be removed, leaving the other intact. With certain serums, selective absorption has not been possible, and cross-reactivity between antigens has been invoked as an explanation (*3*). We have had occasion to study a subject, BC, whose serum reacts with cells that carry HL-A3 specificity (positive) and also with cells generally considered to be HL-A3–negative. The reaction with HL-A3–negative cells does not appear to be a simple cross-reaction, and the various samples of BC serum subjected to absorption appear to be monospecific. This suggests that the HL-A3 specificity is more widely distributed than supposed. We now suggest that there are wide variations, genetically determined, in the amount of HL-A3 antigen available on the lymphocyte surface.

Subject BC was immunized against lymphocyte donor MF on four separate occasions over an 18-month period by intradermal injection of $5 \times 10^6$ lymphocytes at each of ten sites. Two series of injections spaced 7 weeks apart were needed to initiate the production of cytotoxic antibody; thereafter one injection induced a rapid rise in antibody titer (Fig. 1). Samples obtained at intervals were titrated against lymphocytes from a panel of unrelated donors in a simple cytotoxity test (*4*).

Cells from seven subjects were distinguished by their greater reactivity with all serum samples. These were grouped as class 1. Cells from a second group (class 2) of subjects consistently reacted at a lower titer and failed completely to react with several of the serum samples. Cells from a third donor class (class 3) reacted only with an undiluted serum. Most members of this class failed to exhibit any reaction with weaker serums. A few other reactions appeared among class 3 cells in a more sensitive assay system in which the cells were washed after incubation with antibody and before exposure to complement (*4*). A fourth class of cell could be distinguished in that they reacted only in this two-stage test. The four major categories were clearly distinguishable from each other, but within each category there was a certain amount of variability. This is indicated by the ranges of reactivity represented in Fig. 1, and by the frequency of reaction of the most sensitive cells within a class with certain serums (Table 1). The same reaction groups were found when cells from the panel were tested with antiserums to HL-A3 (*5*). Various samples of these serums covered a range of reactivity comparable to that obtained with different samples of BC serum (Table 1). The frequency of reactivity

Table 1. Cytotoxicity reactions of antiserums to HL-A3 including 12 bleedings of a single subject (BC). Samples from BC were obtained during four immunizations with the lymphocytes of another human subject (MF).

| Serum identification* | Class 1† | Class 2 | Class 3 | | Class 4 | | Frequency‡ (%) |
|---|---|---|---|---|---|---|---|
| | | | One-stage | Two-stage | One-stage | Two-stage | |
| *Antiserums to HL-A3 from serum bank* | | | | | | | |
| 2506-03-01-03 | 7/7 | 5/5 | 6/10 | 6/10 | 0/4 | 2/4 | 58 |
| 2506-02-22-01 | 7/7 | 1/5 | 0/10 | 1/10 | 0/4 | 0/4 | 22 |
| 2506-02-17-01 | 6/7 | 0/5 | 0/10 | 0/10 | 0/4 | 0/4 | 17 |
| 2506-03-09-02 | 7/7 | 5/5 | 1/10 | 1/10 | 0/4 | 0/4 | 36 |
| 2506-03-22-02 | 7/7 | 5/5 | 0/10 | 4/10 | 0/4 | 2/4 | 50 |
| 1017-02-07-03 | 7/7 | 5/5 | 2/10 | 6/10 | 0/4 | 3/4 | 58 |
| 2506-06-22-01 | 7/7 | 5/5 | 0/10 | 0/10 | 0/4 | 0/4 | 33 |
| *BC serum* | | | | | | | |
| 6-16-65 | 7/7 | 0/5 | 0/10 | 1/10 | 0/4 | 0/4 | 22 |
| 7-1-65 | 7/7 | 1/5 | 0/10 | 1/10 | 0/4 | 0/4 | 25 |
| 10-15-65 | 7/7 | 5/5 | 0/10 | 3/10 | 0/4 | 1/4 | 44 |
| 11-11-65 | 7/7 | 3/5 | 0/10 | 0/10 | 0/4 | 0/4 | 28 |
| 3-9-66 | 7/7 | 5/5 | 9/10 | 10/10 | 0/4 | 4/4 | 72 |
| 3-15-66 | 7/7 | 5/5 | 5/10 | 8/10 | 0/4 | 0/4 | 58 |
| 3-24-66 | 7/7 | 5/5 | 1/10 | 1/10 | 0/4 | 0/4 | 36 |
| 6-14-66 | 7/7 | 0/5 | 0/10 | 0/10 | 0/4 | 0/4 | 19 |
| 10-5-66 | 7/7 | 0/5 | 0/10 | 0/10 | 0/4 | 1/4 | 22 |
| 11-22-66 | 7/7 | 5/5 | 10/10 | 10/10 | 0/4 | 4/4 | 72 |
| 11-27-66 | 7/7 | 5/5 | 10/10 | 10/10 | 0/4 | 0/4 | 61 |
| 2-2-67 | 7/7 | 5/5 | 0/10 | 0/10 | 0/4 | 0/4 | 33 |

* NIH catalog number or bleeding date. † Proportion of positively reacting samples that were obtained from donors from classes 1 to 4 (see text). ‡ Including positive reactions in the two-stage test.