growth phases. These bands might be part of the α-helix amide III bands or might arise from vibrations of protein other than those involving the amide groups.

The triple helical region that comprises the bulk of the native collagen molecule has a structure distinctly different from the α-helix or β-sheet structures of other proteins and polypeptides. Thus, the spectral band assignments mentioned previously cannot be used without a great deal of caution.

The point of this report is not to explain the spectral changes observed during collagen fibrillogenesis, but rather to point out the wealth of detail that can be observed by FTIR in this clean system and the ability to dynamically detect subtle changes which reflect both intramolecular configurational and intermolecular packing alterations. It is nevertheless tempting to speculate that the appearance of the 1640 cm^{-1} shoulder on the amide I and the broadening seen in the lag phase are indicative of conformational changes in the telopeptides, while the increase in the amide III band at 1242 cm^{-1} with fibril growth may indicate the perfection of the triple helix. This was suggested by Helseth and Veis (*6*) as the consequence of telopeptide-collagen helix packing. Identification of the structural origins of these spectral changes and the use of this technique to elucidate the mechanisms of fibrillogenesis will require significantly more study, but FTIR techniques are directly applicable to protein interaction studies in aqueous solution.

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Localization of Wheat Germ Agglutinin–Like Lectins in Various Species of the Gramineae

Abstract. *Antigenically similar chitin-binding lectins are present in the embryos of wheat, barley, and rye, members of the Triticeae tribe of the grass family (Gramineae). However, the lectins display different localization patterns in these embryos. Lectin is absent from the coleoptile of barley but is present in the outer surface cells of this organ in wheat and in both inner and outer surface cells of rye coleoptiles. All three cereals contain lectin at the periphery of embryonic roots. Similar lectins were not detected in oats and pearl millet, members of other tribes of the Gramineae. Rice, a species only distantly related to wheat, contains a lectin that is antigenically similar to the other cereal lectins and located at the periphery of embryonic roots and throughout the coleoptile.*

Barley and rye embryos contain *N*-acetylglucosamine (GlcNAc)-binding lectins that are virtually indistinguishable by biochemical and immunological criteria from the well-characterized wheat lectin, wheat germ agglutinin (*1*). Although their function is not known, evolutionary conservatism such as this suggests that these lectins perform adaptationally significant roles in the plants in which they are found. We reported earlier that the wheat lectin is localized in peripheral portions of wheat embryos, that is, in the root cap, coleorhiza, and the outer layers of the radicle, coleoptile, and scutellum (*2*). We have sought to determine whether the barley and rye lectins are similarly localized.

Antiserum to wheat germ agglutinin (WGA) was prepared as described (*3*). In order to determine whether this antiserum was able to cross-react with lectins from other grasses, we prepared extracts of wheat, barley, and rye embryos as described (legend to Fig. 1). Results obtained with our antiserum to WGA were similar to those reported by Peumans *et al.* (*1*). After Ouchterlony double diffusion of these extracts against antiserum to WGA, precipitin arcs of identity formed between the wheat, rye, and barley extracts (Fig. 1).

We identified WGA-like lectins in sections of these grass species with the peroxidase-antiperoxidase procedure (Fig. 2)

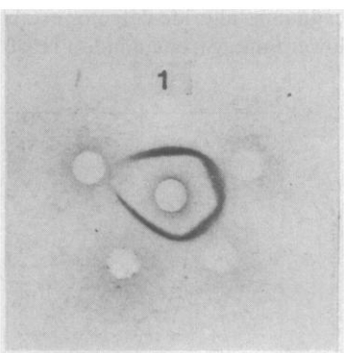

Fig. 1. Diffusion of grain extracts against antiserum to WGA. Wheat, barley, rye, and oat grains were soaked in distilled water overnight at 4°C. Fifty embryos of each species were excised, ground in a mortar with 6 ml of 50 m*M* HCl, and shaken for 4 hours at 4°C. The mixtures were centrifuged at 10,000*g* for 15 minutes, after which the supernatants were collected and brought to 50 percent saturation with ammonium sulfate. After standing overnight at 4°C, the precipitates were pelleted by centrifugation, suspended in 1 ml of distilled water, and dialyzed against distilled water. A hemagglutination assay, performed as described (*3*), revealed titers of 64, 64, and 32 for the wheat, barley, and rye extracts, respectively. These activities were totally inhibited by oligomers of GlcNAc. The oat embryo extracts displayed no hemagglutinating activity. Double diffusion was performed in 1 percent agarose gels prepared in 50 m*M* barbital buffer (*p*H 8.6), with 100 m*M* GlcNAc included to inhibit precipitation caused by lectin-sugar interactions. The gels were dried and stained with Coomassie blue before being photographed. Monospecific immunoglobulins to WGA (10 μl of a solution containing 1 mg/ml) were loaded into the center well and (counterclockwise beginning at well 1) purified WGA, or extracts of wheat, barley, rye, and oat embryos were added to the peripheral wells. Note the fusion of the precipitin arcs of the wheat, barley, and rye extracts, and the absence of a precipitation reaction between antiserum to WGA and the oat extract.

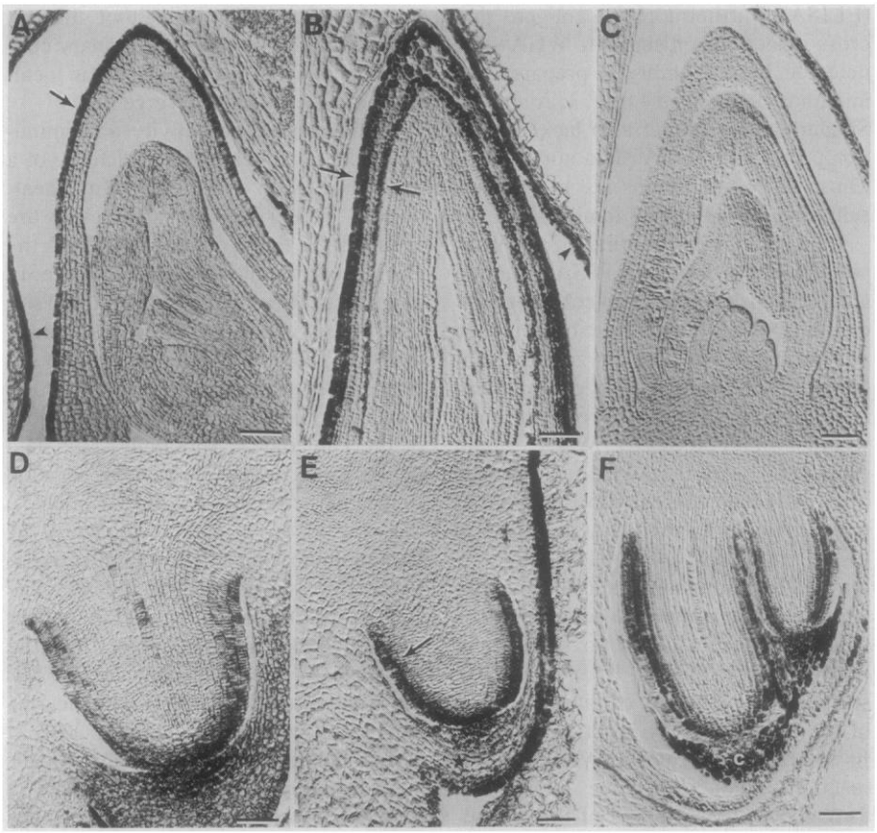

Fig. 2. Immunocytochemical localization of WGA-like proteins in wheat, rye, and barley embryos. Immunocytochemistry was performed as described (*2*). Briefly, embryos excised from imbibed grain were fixed in 4 percent formaldehyde, frozen, and sectioned in a cryostat. Sections 6 to 8 μm thick were suspended in phosphate-buffered saline (PBS) containing 3 percent formaldehyde and 0.3 percent glutaraldehyde, washed in PBS, and incubated with antiserum to WGA. Antibodies bound to the tissue were then made visible by the peroxidase-antiperoxidase procedure (*4*). Reaction product indicative of WGA is localized in the outer cell layer of the wheat coleoptile (arrow in A), in both inner and outer cell layers of the rye coleoptile (arrows in B), but is absent from the barley coleoptile (C). (D to F) WGA-specific reaction product is visible at the periphery of wheat, rye, and barley radicles (arrow in E) and in the root cap covering the tip of the barley radicle (*C* in F). The internal layer of the grain coat (arrowheads in A and B) and cells throughout the coleorhiza (for example, star in E) also display reaction product. Reaction product was not detected in sections treated with nonimmune immunoglobulin G in place of antiserum to WGA (data not shown). Grain varieties were Era (wheat), Vita-graze (rye), and McNair 601 (barley). Scale bars, 100 μm.

[see legend to Fig. 2 and (*2*, *4*) for a description of the technique]. For comparison, the localization pattern of WGA in wheat is also shown in Fig. 2. Both barley and rye embryos, like wheat embryos, displayed reaction product indicative of a WGA-like lectin at the periphery of the radicle (primary root) and in the coleorhiza (Fig. 2, D to F). Sections incubated with nonimmune rabbit serum in place of antiserum to WGA did not exhibit reaction product (data not shown).

The localization patterns in the coleoptile, in contrast to the radicle, varies between species. Whereas reaction product in the wheat coleoptile is limited to the outer surface of this organ, rye coleoptiles contain lectin in both the inner and external cell layers (Fig. 2B). Barley differs markedly from wheat and rye in that it lacks a discernible reaction product in the coleoptile (Fig. 2C) despite the abundance of lectin in the radicle (Fig. 2F).

Comparative studies of cereal lectins have so far been limited to the species found in the Triticeae tribe of the Gramineae family, the tribe that includes wheat, barley, and rye. We thus sought to determine whether WGA-like lectins are also found in three non-Triticeae species. Oats, a member of the Aveneae tribe, lacks both extractable hemagglutinating activity and antigens that cross-react with antiserum to WGA discernible by Ouchterlony double diffusion (Fig. 1) or enzyme-linked immunosorbent assay

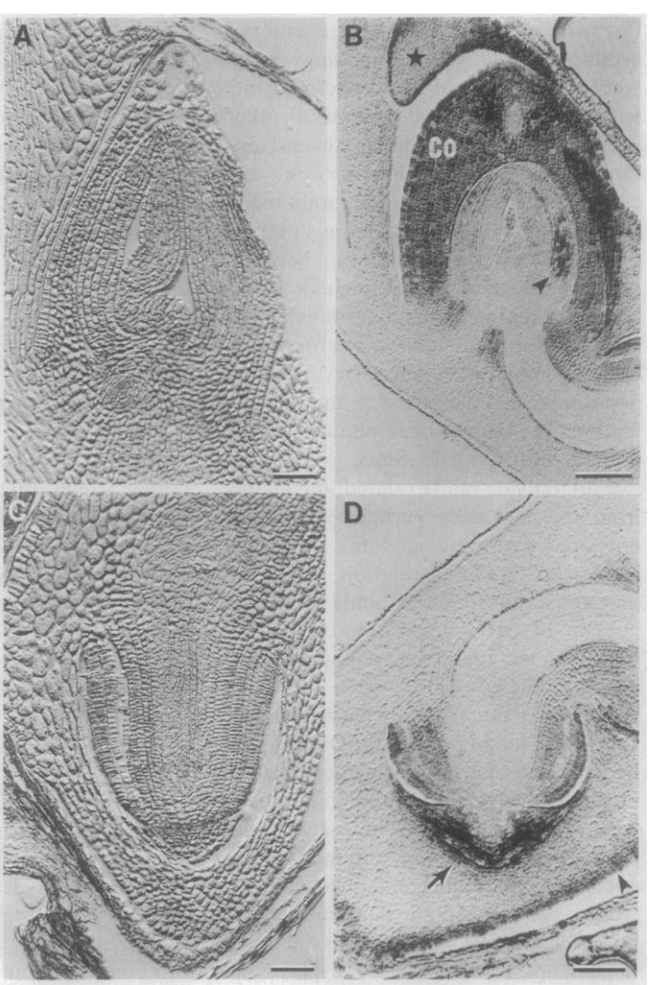

Fig. 3. Immunocytochemistry of oat and rice embryos incubated with antiserum to WGA. Sections of oat (A and C) and rice (B and D) embryos were obtained as described [Fig. 2 and (*2*)] and incubated with antiserum to WGA followed by the peroxidase-antiperoxidase procedure for visualization of antibodies bound to the section. The radicle (D) is in a section parallel to that shown in (B). Reaction product is absent in the oat embryo. In the rice embryo, reaction product is present throughout the coleoptile (*Co* in B), at the periphery of the scutellum (star in B), and in certain cells of the embryonic leaf (arrowhead in B). Reaction product is visible as well in the peripheral tissue of the radicle, the root cap (arrow in D), and the edge of the coleorhiza (arrowhead in D). Grain varieties were Cayuse (oats) and Nato (rice). Scale bars, 100 μm.

(ELISA). Furthermore, no antigens that cross-react with antiserum to WGA were detected in oat embryos prepared for immunocytochemistry (Fig. 3, A and C). Similarly, pearl millet also lacked reaction product for WGA (data not shown). Thus, WGA-like lectins are not universally found in the grass family.

Tsuda (*5*) reported that a GlcNAc-binding lectin similar to WGA in amino acid composition and molecular weight can be purified from rice bran. Since rice, a member of the Oryzoideae subfamily of the Gramineae, is only distantly related to wheat (*6*), we questioned whether antibodies to WGA would cross-react with a component of rice bran. Although a precipitin reaction between a rice bran extract and antiserum to WGA was not observed on Ouchterlony double diffusion (data not shown), we found that antiserum to WGA does bind immunospecifically to sections of rice embyros (Fig. 3, B and D). The surface and a number of internal layers of the radicle display reaction product. In contrast to wheat and rye, however, reaction product in rice is located throughout all cell layers of the coleoptile (Fig. 3B). A few cells in the embryonic leaves also display reaction product (arrowhead in Fig. 3B). Rice embryo sections treated with nonimmune serum were not stained. Moreover, our results with rice, as with those from all other species, were consistent in several different experiments. The apparent discrepancy between the immunodiffusion and immunocytochemical assays may be a function of the distant relation between rice and wheat. Whereas single or weakly recognized antigenic determinants are sufficient for the detection of cross-reactivity by immunocytochemistry, multiple determinants are necessary for the formation of immunoprecipitates during double diffusion (*7*). Since wheat and rice are only distantly related, the lectins from these grasses perhaps share few cross-reactive antigenic sites. In agreement with this interpretation, we found that antiserum to WGA binds to a component of rice bran extracts in an ELISA, a test that detects nonprecipitating antibodies.

A general localization pattern of the WGA-like lectins emerges. Organs that become externalized during germination of the embryo tend to contain lectin, at least at the periphery. The radicle, root cap, and coleorhiza were found without exception to contain lectin. The extent to which the coleoptile contains lectin, however, varies; barley lacks lectin in the coleoptile, whereas rice displays lectin-containing cells throughout this organ. Thus, despite the evolutionary conservatism of these proteins, their localization patterns have diverged.

Hemagglutinating activity and immunological cross-reactivity indicative of a WGA-like lectin were detected in wheat, barley, and rye. These grasses are grouped in the Triticeae tribe of the Pooideae subfamily of the Gramineae. In addition to these cereal grains, the Triticeae tribe includes forage and range grasses. WGA-like lectins were not detected in the Aveneae tribe of the Pooideae (oats) nor in pearl millet, a member of the Panicoideae subfamily. Our data demonstrate, however, that, in addition to the characteristics noted by Tsuda (*5*), the rice lectin is similar to WGA by immunological and histological criteria. Since rice is classified in the Oryzeae tribe of the Oryzoideae, a subfamily considered to be more primitive than the Pooideae by morphological criteria (*8*), our results suggest that WGA-like lectins may be a primitive character lost in certain lineages during the adaptive radiation of the grass family but retained in species of the Triticeae and Oryzeae tribes.

A close evolutionary relation exists among the lectins of various plant families including the Leguminosae (*9*) and the Solanaceae (*10*), as well as the Gramineae (*1*). In the latter, the capacity to bind chitin has been conserved despite the variations in patterns of localization that we observed. Thus, chitin-binding may be integrally associated with lectin function. Whether the differences in patterns of localization reflect the optimization of this function in the particular environments to which these species have adapted merits further investigation.

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Light-Enhanced Free Radical Formation and Trypanocidal Action of Gentian Violet (Crystal Violet)

Abstract. *Transmission of Chagas' disease by transfusion of blood containing* Trypanosoma cruzi *has often been reported, and gentian violet, a triarylmethane dye, is widely used by blood banks in attempts to eliminate such transmission. In a study of intact trypanosomes, gentian violet was found to undergo a one-electron reduction to produce a carbon-centered free radical as demonstrated by electron spin resonance spectroscopy. Either reduced nicotinamide adenine dinucleotide or the reduced dinucleotide phosphate could serve as a source of reducing equivalents for the production of this free radical by homogenates of* Trypanosoma cruzi. *The formation of this free radical, and the trypanocidal action of gentian violet, were enhanced by light. The enhanced free radical formation may be the basic cause of the selective toxicity of gentian violet to* Trypanosoma cruzi.

Transmission of Chagas' disease by blood transfusion in endemic and nonendemic areas has often been reported (*1*). The epidemiological importance of this mechanism of transmission is increasing in endemic areas, and it may be taken into consideration as a possible cause of transmission of the disease in cases encountered in nonendemic countries such as Canada (*2*).

Ever since Nussensweig *et al.* (*3*) demonstrated the activity of gentian violet against the trypomastigote forms of *Trypanosoma cruzi* in vitro, this compound