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INTERMEDIATE FILAMENTS: DETECTION OF LECTIN-LIKE ACTIVITY IN PURIFIED ALCOHOLIC HYALINE

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SUMMARY: Alcoholic hyaline, an intracellular, filamentous (10 nm) aggregate isolated from human alcoholic livers,bound the glycoprotein enzyme, horseradish peroxidase, in a specific and reproducible manner. Using a solid-phase assay system consisting of adsorbed alcoholic hyaline, we have shown that this binding is thermolabile, relatively insensitive to both pH extremes and high ionic strength, and highly sensitive to the presence of neutral and amino sugars. The results suggest that the binding of horseradish peroxidase is not a passive adsorption but rather an "active" phenomenon involving carbohydrate groups on the enzyme. The presence of an intracellular, filament-associated lectin is strongly indicated.

Intermediate filaments (10 nm) are a class of subcellular structures with widespread occurrence. IFs have been described in a number of tissues including nerve (1), glial (2), smooth muscle (3), and liver (4), as well as in several tissue culture systems (5-7). Recent studies have indicated that these filaments possess considerable immunological polymorphism which is both species and cell specific (8).

IFs have also been reported in a number of pathological systems (9, 10). One such system involves an accumulation of branched 10 nm filaments in human alcoholic liver disease (11-13). This material, AH, is thought to arise through an antimicrotubular action of ethanol (14) and appears to be immunologically related to prekeratin, the major component of IFs present in epithelial cells (5, 15).

Although considerable analytical work has been carried out on IFs, little functional information concerning these structures has been obtained.

Abbreviations: AH, alcoholic hyaline; IF, intermediate filament; HRP, horseradish peroxidase.

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In a general sense, IFs are assumed to perform a variety of cytoskeletal functions, including the maintenance of cell shape and elasticity. However, to date, specific enzymatic functions have not been assigned to these structures. Recently, during an investigation of AH glycoprotein localization, we observed that AH possessed the unique property of binding the glycoprotein enzyme, HRP (16). The present report describes some of the properties of this binding activity. The results suggest that AH contains lectin-like activity.

METHODS

Isolation of AH. AH was isolated from human autopsy livers by the method of Tinberg et al. (17). The preparations were routinely 95-99% pure by both light and electron microscopy (16). AH (1-2 mg protein/ml) was stored in deionized water at 4°C and used within 12 hours.

Determination of HRP Binding. The binding of HRP was quantified using a solid-phase colorimetric assay described previously (16). Purified AH (20-40 µg protein) was adsorbed to the bottom of 13x100 mm pyrex glass tubes. Following formaldehyde-methanol fixation (16), the samples were incubated with HRP (0.05 to 0.2 mg/ml, 0.2 ml in 0.01 M sodium phosphate-0.15 M sodium chloride buffer (pH 7.2)) for 30 min at room temperature. Excess HRP was removed by buffer washings (3X) and the HRP activity was determined using the antipyrine-phenol-H_2O_2 reaction system (18). The absorbance at 530 nm was determined at 30 min. To ascertain whether elution of AH material occurred during the HRP incubation, the protein remaining in the tubes after treatment was determined (19).

Affinity Labeling of AH. HRP (5 mg/ml) was treated with glutaraldehyde (distilled, 2%) in phosphate-buffered saline for 60 min at room temperature to produce an "activated" derivative of HRP (HRP*). Excess glutaraldehyde was removed by gel filtration through Sephadex G-25 using phosphate-buffered saline as the eluting buffer. The protein concentration of the HRP* was determined and the material used immediately. Binding of HRP* to AH was carried out in the standard manner except that formaldehyde was omitted from the fixation step. Following incubation, the extent of binding of HRP* was determined as described above. In some tubes, the AH-HRP* complex was solubilized by the addition of a sodium dodecyl sulfate-mercaptoethanol mixture (17). The samples were heated to 100°C and subjected to electrophoresis in the discontinuous Laemmli (20) system containing 10% acrylamide.

RESULTS

The Ficoll isolation procedure affords excellent preservation of AH filament morphology. The isolated preparation were composed primarily of branched filaments, approximately 10 nm in diameter (Fig. 1)

Effect of Incubation Conditions on HRP Binding. HRP binding was essentially unaffected by incubation at either pH 5 or pH 9 (Table 1)

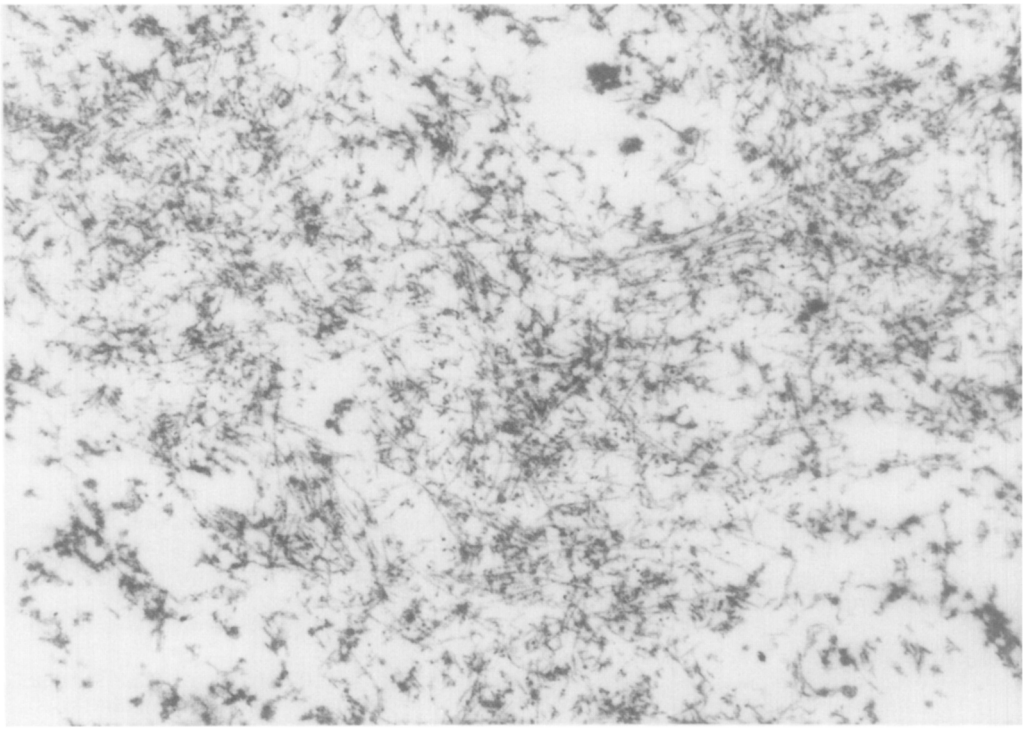


Fig. 1. Electron micrograph of isolated AH. The filamentous substructure of AH is clearly evident. The section was stained with lead citrate and uranyl acetate. X31,360.

suggesting that the binding was not due primarily to simple electrostatic adsorption. Furthermore, high ionic strength, which should also interfere with electrostatic interactions, only partially prevented HRP binding (36% inhibition). Control experiments indicated that these treatments did not affect HRP activity directly or induce the elution of HRP material.

HRP binding was heat labile. Treatment of AH at 80°C for 60 min resulted in a marked inhibition of HRP binding (Fig. 2). Inactivation occurred when AH was heated in the presence ("wet") or absence of deionized water ("dry"). However, wet heat appeared to be more effective than dry heat in inactivating the binding activity. Apparently, the presence of solvent accelerates or amplifies the thermally induced structural modifications leading to inactivation.

Effect of Carbohydrate on HRP Binding. Since HRP is a glycoprotein (approximately 18% carbohydrate), experiments were carried out to determine

TABLE 1

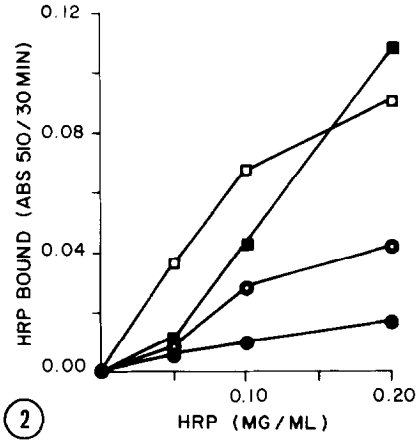
Effect of Incubation Conditions on HRP Binding

CONDITION	HRP BOUND (% Control)	HRP ACTIVITY (% Control)
PBS	100	100
pH 5	79	97
pH 9	93	112
KCl (1 M)	64	100

HRP binding was carried out as described in the text except that additional experimental conditions, as listed, were used. HRP activity refers to the enzyme activity of HRP exposed to these conditions. After 30 min of incubation (absence of AH) aliquots were taken and assayed for activity.

whether binding involved the sugar residues on the enzyme. Inclusion of increasing amounts of glucose into the incubation medium resulted in a corresponding decrease in the amount of HRP bound (Fig. 3). However, once bound to AH, HRP could not be readily displaced by added carbohydrate (Fig. 3). Apparently, the AH-HRP interaction, once established, is quite stable suggesting the possible participation of other non-carbohydrate components. Several other neutral hexoses as well as an amino sugar, galactosamine, were also effective in competing with HRP for AH binding sites (Table 2). It should be noted the inhibitory effect of the sugars did not appear to result from simple tonicity since glycerol at the same concentration (1-2 M) had little effect on HRP binding.

Binding of Activated HRP. In an attempt to localize the molecular binding site of HRP, AH was treated with activated HRP. In the concentrations used (0.1-0.5 mg/ml), HRP* bound to AH at levels similar to that noted for the unmodified enzyme. Moreover, the derivatized HRP was also sensitive to glucose competition. To determine which AH polypeptides bound HRP*, the labeled samples were solubilized and electrophoresed. Components binding HRP would exhibit increased molecular weight and would be detectable on the gels. The results are shown in Fig. 4. Treatment with low levels of HRP* resulted in a decreased staining intensity of all major AH polypeptides



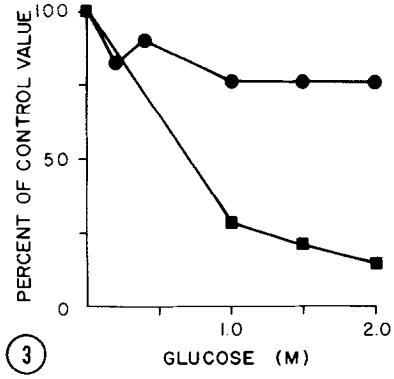


Fig. 2. Effect of heat on binding of HRP to purified AH. AH was isolated as described in the text. The solid-phase colorimetric assay for HRP binding is also described in the text. After adsorption to glass tubes, the samples were heated at 80°C for 60 min in a water bath. Deionized water (0.2 ml) was added to samples exposed to "wet heat". Control samples were treated identically but held at 20°C. ●, "wet heat" (80°C); ◉, "dry heat" (80°C); ■, "wet" control; □, "dry" control.

Fig. 3. Effect of carbohydrate on HRP binding (competition vs. displacement). Binding of HRP was carried out as described in the text. In the competition experiments, glucose was added during HRP binding. Prior to this step, the samples were preincubated for 30 min with glucose at the same concentration used in the competition phase. Displacement was carried out as follows. After binding HRP, glucose in PBS (0.2 ml) was added to the tubes and an additional incubation of 30 min was carried out. Following this incubation, the tubes were aspirated, washed, and assayed for HRP content. ■, competition; ●, displacement.

TABLE 2

Effect of Carbohydrate on HRP Binding

ADDITIONS	HRP BOUND (% Control)
PBS	100
Glucose	35
Mannose	32
Galactose	28
Fructose	37
Galactosamine	30
Glycerol	85

HRP binding was carried out as described in the text except that the carbohydrates were included in the incubation medium at a concentration of 1 M.

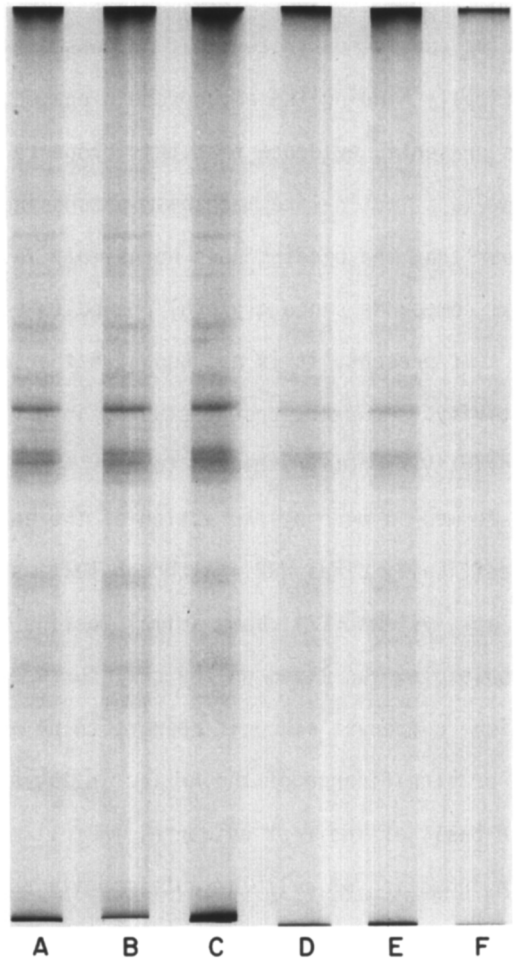


Fig. 4. SDS-polyacrylamide gel electrophoresis of affinity-labeled AH. Isolated AH was treated with various concentrations of glutaraldehyde-activated HRP (HRP*) as described in the text. Following treatment, the samples were solubilized and subjected to electrophoresis in a discontinuous 10% gel system. A. 0.1 mg HRP/ml; B. 0.2 mg HRP/ml; C. 0.5 mg HRP/ml; D. 0.1 mg HRP*/ml; E. 0.2 mg HRP*/ml; F. 0.5 mg HRP*/ml.

Extensive crosslinking occurred at higher HRP* concentrations resulting in the disappearance of all bands. Since little free glutaraldehyde was present in the incubation system, the crosslinking must be due to the HRP*. These results suggest that the major AH polypeptides are arranged in a compact structure within the filament and that the HRP binding site is localized within this supramolecular complex. Once bound, HRP*, by virtue of both size and multireactive aldehyde groups, is able to covalently stabilize the entire complex.

DISCUSSION

Previous studies have demonstrated that AH possesses the interesting property of specifically binding the glycoprotein enzyme, HRP (16). The present report has presented evidence that this property is thermolabile and appears to proceed via a lectin-like mechanism possessing broad specificity. However, it is clear that the binding does not simply involve reactivity toward polyhydroxyl compounds since glycerol proved to be a relatively poor binding inhibitor. At present, it is not known whether other IFs also possess lectin activity. However, recent studies in our laboratory indicate that the lectin activity is not restricted to AH. A similar filamentous hyaline aggregate formed in mice by the action of the antibiotic, griseofulvin (21), also specifically binds HRP. Although lectins are most often associated with plant systems (22), some animal lectins have been described (23-25) and when possible, localized on the cell surface. The AH lectin is a particularly unique component since it appears to be an intracellular lectin

The function or significance of the AH lectin is, at present, unknown. We suggest, on the basis of indirect evidence, that it may be involved to some degree in the induction of cellular autoimmunity and therefore in the pathogenesis of alcoholic liver disease. It has been demonstrated that incubation of purified AH with lymphocytes produced a substance which inhibited the migration of polymorphonuclear leucocytes (26). We are currently investigating whether the production of this lymphokine involves an interaction of AH lectin with lymphocyte surface glycoproteins.

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