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Cellulose Adherence Factors in *Ruminococcus albus*

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Summary

*The cell wall of the bacterium **Ruminococcus albus** was mixed with cellulose particles, allowing any molecules with an affinity for cellulose to bind. The cellulose particles and any bound molecules can be retrieved by centrifugation, providing a relatively simple procedure to enrich for molecules involved with attachment of the bacterium to plant fiber. Four proteins have been identified using this procedure, suggesting that adherence involves a protein-carbohydrate interaction. The quantity of these proteins appears to be affected by the nutrient composition of the medium used to grow the bacterium. It seems likely that nutrients which stimulate cellulose degradation also positively affect the amount of the adherence factors present on the bacterial cell surface.*

Introduction

Grazing and forage-fed animals depend upon the rumen microorganisms' ability to breakdown polysaccharides present in the leaf and stems of plants. The rate and extent of this breakdown has a major impact on animal nutrition, therefore understanding the mechanics of this process offers the potential to optimize and further improve animal performance. The limited published studies to-date suggest that: 1) colonization and adherence by some rumen bacteria are specific in nature and; 2) adherence can be modified by nutrients and growth conditions, such as ruminal pH. Better understanding and future improvements of fiber digestion in the rumen will be afforded by the identification and isolation of the molecules controlling bacterial colonization and adherence.

Procedure

Detection of proteins with an affinity for cellulose

The rumen bacterium *R. albus* strain 8 was used for study, because it is a very active degrader of plant material. The

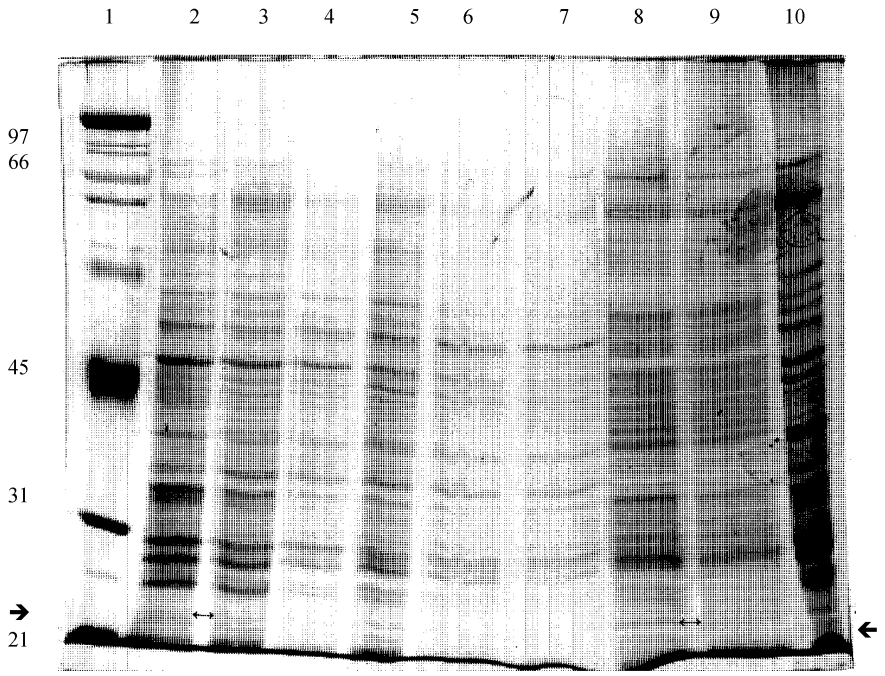

Figure 1. Identification of a 22 kDa protein from *R. albus* 8 membrane fractions, on the basis of its affinity for cellulose. Membrane fragments with or without cellulose added were incubated at room temperature for 1 hour, then subjected to centrifugation. Note the disappearance of a 22 kDa protein in lane 2 (arrowed, + cellulose), compared with the control (- cellulose, lane 3). Lanes 4 through 7 represent the wash fractions. The 22 kDa protein is virtually absent in these fractions but is readily visible in lane 8 (arrowed), following the boiling of the cellulose pellet in SDS-PAGE running buffer. Lane 10 is a sample of the crude membrane fragments.

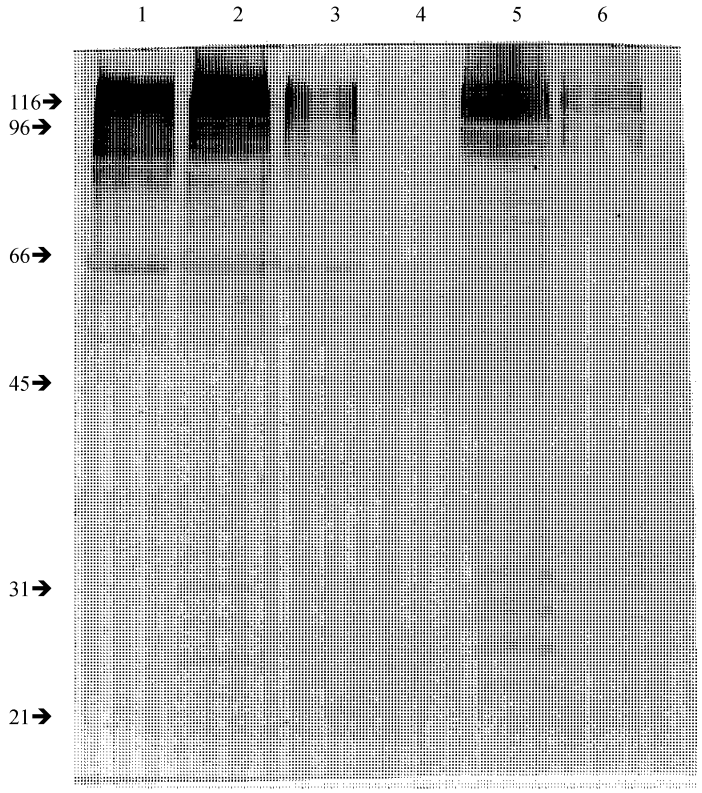

Figure 2. Identification of glycosylated proteins from *R. albus* 8 membrane fractions, on the basis of their affinity for cellulose. Membrane fragments, with or without cellulose added, were incubated at room temperature for 1 hour, then subjected to centrifugation. Note the disappearance of the glycosylated proteins between the 21 and 32 kDa molecular weight range in lane 1 (+ cellulose), compared to the control (lane 2, - cellulose). Lanes 3 and 4 represent the supernatant fractions obtained after washing the control and test reactions with phosphate buffer, containing 0.05% (w/v) Triton X-100. The proteins are virtually absent in these wash fractions, but are readily visible in lane 5 (arrowed) following the boiling of the cellulose in SDS-PAGE sample running buffer.

bacterium was grown using media with or without added rumen fluid. Rumen fluid is known to contain nutrients which can affect cellulose degradation, and potentially then, the ability of the bacterium to attach and degrade the substrate. The bacterial cells were harvested by centrifugation and the cell wall fragmented by passage through a French pressure cell. The membrane fragments contain proteins and other molecules that may be involved with adherence, and these molecules were released from the cell wall by treatment with a detergent. The suspension was then mixed with cellulose, and incubated with occasional agitation at room temperature for one hour. The cellulose particles were harvested by centrifugation and washed, first with phosphate buffered saline, then with detergent. The washes and a sample of the cellulose were boiled with a protein running buffer, then subjected to denaturing polyacrylamide gel electrophoresis (SDS-PAGE). Membrane fragments without added cellulose were subjected to the same procedures, and also subjected to SDS-PAGE.

*Reaction of **R. albus** whole cells with lectins and erythrocytes*

We anticipate that the binding process between the bacteria and the plant surface involves either a protein-carbohydrate, or a carbohydrate-carbohydrate interaction. One approach to identify such interactions is to incubate the bacteria with lectins (proteins that recognize a specific carbohydrate on the bacterium's surface). Another is to incubate the bacteria with erythrocytes from different species of animals. The erythrocytes possess different carbohydrates on their outer surface and will bind (hemagglutinate) with bacteria that possess a protein which can bind that particular carbohydrate. Agglutination of the bacteria upon incubation with lectins and(or) erythrocytes will provide new information, which will be useful for the isolation of the adherence molecules.

Cultures of *R. albus* 8 and *Prevotella ruminicola* strains D31d, 23, and $B_1$4 were harvested at mid-log phase of growth by centrifugation, washed, and resuspended in buffer to give a consistent cell density. Fifty microliter a liquots of the cell suspensions were then mixed in microtiter dishes with lectin suspensions (50 µg total) derived from jack bean, peanut, castor bean, winged pea, wheat germ, and lentil. Cell-lectin mixtures were shaken for 15 minutes, then left stationary at room temperature for two hours.

Erythrocytes obtained from rabbit, ox, calf, guinea pig, horse, sheep, and goat were washed and resuspended in phosphate buffered saline. Cell-erythrocyte mixtures were treated in the same manner as described above. With both assays, agglutination can be easily distinguished by macro- and microscopic observations. A positive reaction results in the dispersion of the mixture, rather than the formation of a tight "button" in the bottom of the assay well.

Results

The results of the cellulose-binding assays are shown in Figure 1. Despite the presence of some background protein bands, a protein of approximately 22 kDa molecular weight is clearly absent following incubation with cellulose (compare lanes 2 and 3 of Figure 1), but is readily visible once the cellulose particles are washed and boiled in protein running buffer to remove bound protein(s) (lane 8). This type of assay has since been combined with staining procedures to identify glycosylated proteins. No less than four protein bands, all ranging in size between 21 and 31 kDa possess affinity for cellulose with

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the assay conditions used (Figure 2). Moreover, the presence of these glycoproteins in the membrane fragments of *R. albus* requires growth of the bacterium in the presence of rumen fluid. These glycosylated proteins seem to be excellent candidates for further investigation by a variety of molecular-based approaches.

Of the seven lectins tested so far, only the winged pea lectin caused agglutination of *P. ruminicola* D31d cells. This lectin has affinity for terminal L-fucose (deoxygalactose) residues. None of the lectins tested so far agglutinate *R. albus* 8 whole cells, indicating some difference(s) from previous studies with another type of *R. albus.*

Both *P. ruminicola* D31d and *R. albus* 8 cell preparations can agglutinate rabbit erythrocytes. However, hemagglutination appears to be affected by the age of the erythrocytes, suggesting some removal of the terminal sugars recognized by these putative "adhesins". The results with *R. albus* 8 to date have been the most variable. So far, all assays have been conducted under aerobic conditions, and this may have some impact upon the results.

Although these studies are still preliminary, the findings support the contention that glycosylated proteins present in the bacterial membrane will bind specifically with cellulose. Further studies are underway to better characterize these proteins. The potential impact from these studies could be far-reaching. It may be possible to identify the "rate limiting" binding/receptor sites, in either plant tissue or ruminal bacteria, that affect adherence. Factors affecting the expression and(or) chemical "viability" of binding/receptor sites (e.g. ruminal pH), and the relationship between these specific interactions and cellulose-degrading enzymes may be identified. Finally, the information gained may ultimately be utilized to model the impact of ruminal conditions, plant quality, and the adherence mechanism(s) upon the kinetics of ruminal fiber digestion.

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