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α-L-Arabinofuranosidase from *Ruminococcus albus* 8: Purification and Possible Role in Hydrolysis of Alfalfa Cell Wall

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An α-L-arabinofuranosidase has been purified from the extracellular broth of cultures of *Ruminococcus albus* 8. The purification procedure utilized gel filtration, $(NH_4)_2SO_4$ precipitation, and isoelectric focusing. The purified enzyme appeared to be homogeneous when chromatographed on disc and analytical isoelectric focusing gels. The estimated molecular weight of the native protein was 305,000 to 310,000. Sodium dodecyl sulfate-gel electrophoresis analysis suggested that the native protein is a tetramer composed of 75,000-molecular-weight subunits. The enzyme appeared to have no metal cofactor requirement but was sensitive to several sulfhydryl reagents. The pH optimum with *p*-nitrophenyl-α-L-arabinofuranoside as the substrate was 6.9 and the K_m was 1.3 mM. Several lines of evidence indicated that the enzyme is a glycoprotein. When assayed against alfalfa cell wall material, the enzyme hydrolyzed only small amounts of arabinose from the substrate. When assayed together with hemicellulolytic or pectinolytic enzymes against the same substrate, the arabinosidase significantly enhanced the hydrolytic action of the glycanases.

The various plant materials consumed by ruminants contain considerable quantities of carbohydrate polymers. The largest proportion of this material is generally cellulose, with the remainder made up of hemicellulose and pectin (6). Together the hemicellulosic and pectic components can account for as much as 50% of the total carbohydrate in the cell wall and so constitute a potentially important energy source for the ruminant.

Whereas the digestibility of the in situ hemicellulose diminishes as the plant matures, hemicellulose extracted from rye grass becomes more susceptible to digestion by xylanases with maturation (18). This increased digestibility is well correlated with the decrease in the degree of substitution of the hemicellulose polymers with arabinosyl residues. Morrison (18) postulates that the rumen hemicellulase catalyzes cleavage of bonds between adjacent unsubstituted hemicellulosic xylosyl residues. The maturation-dependent increase in susceptibility of extracted hemicelluloses by hydrolysis may result from a decrease in hemicellulose arabinose content, which creates a larger number of unsubstituted hydrolysis sites.

Accordingly, any mechanism removing the arabinosyl side chains from hemicellulose should increase its digestibility. In this paper we describe the purification and partial characterization of an α-L-arabinofuranosidase (α-AF) from *Ruminococcus albus* 8. This enzyme removes arabinosyl residues from alfalfa cell wall (ACW) pectic and hemicellulosic polysaccharides, making them more susceptible to attack by other glycanases.

MATERIALS AND METHODS

Culture procedures. Extracellular broth from cultures of *R. albus* 8 was the enzyme source. The stock culture was maintained by daily transfers of a 1% inoculum into 6 ml of 0.15% (wt/vol) pebble-milled cellulose broth, as described previously (14), except that 3-phenyl propanoic, isobutyric, and 2-methylbutyric acids were included at a concentration of 2.5×10^{-5} M and *n*-valeric and isovaleric acids were deleted.

One-liter cultures for the production of enzyme were similar to the stock cultures except that pebble-milled cellulose was replaced with 0.3% (wt/vol) pebble-milled ACW and grown for 96 h at 39°C.

Purification of the α-AF. One-liter cultures of *R. albus* 8 were filtered by suction through an 11-cm GF/A glass-fiber disk. The filtrate was centrifuged at 25,000 × *g* for 20 min to remove cells and debris. This and all subsequent steps in the purification were performed at 25°C unless otherwise specified. The pellet was discarded, and the supernatant was filtered through a polycarbonate membrane (0.4-μm pore size; Nuclepore Corp., Pleasonton, Calif.) The extracellular enzymes were concentrated to 25 ml by ultrafiltration through a PM-10 membrane (nominal molecular weight cutoff of 10,000; Amicon Corp., Danvers, Mass.). The enzyme concentrate was then stored under N_2 at 4°C until used.

A 3-ml portion of the enzyme concentrate (containing ca. 15 mg of protein) was applied to a column (53 by 3 cm) of S-300 Sephacryl (Pharmacia Corp., Uppsala, Sweden) and eluted with 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer, pH 6.8 (Sigma Chemical Co., St. Louis, Mo.). The absorbance profile at 280 nm was recorded with a Gilson model HM holochrome column monitor (Gilson Medical Electronics, Inc., Middletown, Wis.). Fractions containing the α-AF (see assay procedure below) were pooled, and solid ammonium sulfate was added to 30% of saturation. This solution was stirred for 4 h at 4°C and then centrifuged for 20 min at 25,000 × *g*. The pellet was discarded, and ammonium sulfate was added to the supernatant to 50% of saturation. The precipitate from this treatment was collected by centrifugation and resuspended in a minimal volume of water. Residual ammonium sulfate was removed, and the buffer was changed to 1 mM glycine (pH 7.0) by ultrafiltration through a PM-10 membrane.

For preparative isoelectric focusing the technique of Radola (20) was followed with some minor modifications. A flatbed containing 100 ml of prewashed Biolyte electrofocusing gel (Bio-Rad Laboratories, Richmond, Calif.) containing

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0.5 to 1.0 mg of protein and 5% (wt/vol) ampholytes, pH 3 to 7 (Bio-Rad Laboratories), was prepared in a glass tray (11 by 30 cm). The protein-containing gel was then dehydrated with an air stream to 73% of its original weight. Equipment utilized included a Bio-Rad model 1415 electrofocusing unit, an MRA model 158 power supply (MRA Corp., Clearwater, Fla.), and a Haake model E4391 cooling apparatus (Haake Corp., Berlin, Germany).

The protein was focused at 2°C for 16 to 18 h at a constant power of 15 W. The gel bed was then fractionated with a Bio-Rad gel divider. Each gel fraction was transferred to a 12-ml plastic syringe barrel containing a glass-wool plug and eluted with 3.0 ml of 0.1 M HEPES (pH 6.8). Fractions were assayed for α-AF activity. The purified enzyme was stable for at least 8 weeks when held at 4°C under N_2.

Analytical isoelectric focusing. Analytical isoelectric focusing was performed as described by Wrigley (28) to assess both the purity of the enzyme and the possibility that proteins present might contain covalently linked carbohydrate. Gels containing 6.2% (wt/vol) acrylamide, 2% (wt/vol) ampholytes (pH 3 to 7), 100 to 150 μg of protein (in 1 mM glycine, pH 7.0), and 0.01% (wt/vol) ammonium persulfate were cast in glass tubes (5 by 120 mm). The gels were focused for 3 to 5 h at 2 mA/gel, using an Isco model 490 power supply (Isco Co., Lincoln, Neb.). Gels were stained in 0.1% Coomassie brilliant blue R-250 in isopropanol-acetic acid-water (27:10:63) containing 0.5% cupric sulfate (21). Gels were destained by rinsing overnight in a mixture of methanol-acetic acid-water (5:2:12). Periodate-Schiff staining for carbohydrate followed previously described methods (9).

Molecular weight determination. Molecular weight of the isolated enzyme was estimated by both gel filtration (2) and polyacrylamide electrophoresis (12, 26). Gel filtration analysis used the S-300 Sephacryl column described previously. The column was calibrated with a standard sample containing myoglobin, horseradish peroxidase, catalase, ferritin, and thyroglobulin (Sigma Chemical Co.).

Electrophoretic estimations of molecular weight were done with both native and sodium dodecyl sulfate (SDS) gels. For the native analysis the method of Hedrick and Smith (12) was used. Standards were the same as those listed for gel filtration, minus catalase. Gels of 4, 6, and 8% (wt/vol) acrylamide were prepared in glass tubes (5 by 120 mm) and electrophoresed at 1.5 mA/gel for 3 h, using an Isco model 490 power supply. SDS electrophoresis was run in 5.6% acrylamide gels following the method of Weber and Osborne (26), using the gel system described by Fairbanks et al. (9). Protein samples (25 to 50 μg) were electrophoresed at 2 mA/gel until the tracking dye (pyronin Y) was 5.0 mm from the bottom of the gel. All gels for molecular weight estimation were stained as described above (21).

Evaluation of divalent cation requirements. Purified α-AF was dialyzed against 4 liters of 0.05 M potassium phosphate, pH 6.8, containing 0.01 M EDTA for 16 h and then against 0.05 M potassium phosphate, pH 6.8, for 48 h (with three changes of 4 liters each) to remove the EDTA. After this treatment aliquots of the enzyme were assayed for α-AF activity.

Inhibition by sulfhydryl reagents. The sensitivity of the α-AF to sulfhydryl reagents was examined by preincubation of the pure enzyme for 1 h at 30°C with $HgCl_2$, *p*-mercuribenzoate, or iodoacetamide at concentrations ranging from 0.01 mM to 0.15 M. Aliquots of the treated enzyme were then assayed to determine the effectiveness of the treatment.

Enzyme assays. α-AF activity was determined with *p*-nitrophenyl-α-L-arabinofuranoside (Sigma) as substrate. The typical assay medium consisted of 0.5 ml of 0.1 M HEPES buffer, pH 6.8, containing 0.01 M *p*-nitrophenyl-α-L-arabinofuranoside. The reaction was initiated by the addition of enzyme (0.01 to 0.02 ml), incubated for 30 min at 37°C, and terminated by the addition of 0.5 ml of 1 M NH_4OH. The absorbance at 400 nm of the resulting solution was measured with a Beckman spectrophotometer (model DU) and converted to micromoles of *p*-nitrophenol by comparison to a standard curve. One unit of activity was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol per min.

Arabinosidase assays that used polysaccharide substrates were quantitated by monitoring the enzyme-dependent increase in reducing sugar equivalents. Reducing sugar content was measured by the method of Nelson (19), as modified by Somogyi (22). Substrates used in these assays were ACW and polygalacturonase-soluble and base-soluble carbohydrates (PGSC and BSC, respectively; see below) from ACW.

ACW was prepared by homogenization of 100 g of fresh alfalfa (apical 10 cm of shoots from growing plants) in 500 ml of water with a Waring blender. The insoluble material in the slurry was collected by filtration through Miracloth (Calbiochem-Behring, La Jolla, Calif.). The residue was extracted three times with 1 liter of chloroform-methanol (3:1), or until no further green pigment could be removed. This treatment was followed by a single extraction with 500 ml of acetone. Residual acetone was removed under vacuum, and the dried ACW was stored at 25°C.

PGSC and BSC are two distinct polysaccharide fractions derived by sequential extraction of ACW. The extraction process was initiated by pebble milling 3.0% (wt/vol) ACW at 75 rpm for 24 h in distilled water (3.0%, wt/vol). Aliquots of milled material (250 to 300 mg) were washed by stirring for 24 h with 0.1 M sodium acetate, pH 5.0, at 37°C to remove materials solubilized by the milling process. The washed residue was filtered on a Büchner funnel and rinsed thoroughly with distilled water. These wall preparations were then treated with a polygalacturonase from *Fusarium oxysporum cubense* (FPG) which had been purified free of all other glycanase and glycosidase activities (25). The incubation mixture contained 250 to 300 mg of cell wall, 20 ml of 0.1 M sodium acetate (pH 5.0), and 2.0 ml of FPG. This mixture was stirred at 37°C for 12 h, and the PGSC was collected by filtering the slurry on a sintered-glass funnel. The filtrate and a subsequent wash (20.0 ml of 0.1 M sodium acetate, pH 5.0) were combined and stored at 4°C. This treatment was repeated twice for a total FPG hydrolysis period of 36 h. The cell wall material remaining after this treatment was saved for further extraction, whereas the solutions resulting from the FPG treatments were pooled and made 80% (vol/vol) with ethanol. The resulting precipitate (PGSC) was collected by centrifugation (2,000 × *g*, 15 min) and stored dry at 4°C.

The FPG-treated ACW was suspended in 20 ml of 1 N NaOH containing 1 mg of $NaBH_4$ per ml and stirred overnight at 25°C. The slurry was centrifuged at 10,000 × *g* for 30 min to remove cell wall debris. Ethanol was added to the supernatant to a final concentration of 90% (vol/vol). The resulting solution was kept at 4°C for 24 h and then centrifuged at 2,000 × *g* for 15 min. The supernatant was discarded, and the pellet (BSC) was washed three times in 90% (wt/vol) ethanol, dried at reduced pressure, and stored at 4°C until needed.

Xylanase and polygalacturanase activities were also measured by following the generation of reducing sugar (19, 22) when larch xylan and polygalacturonic acid (Sigma) were

used as substrates. Before use the xylan was purified as described by Taiz and Honigman (23). Before its use in assays the polygalacturonic acid was precipitated from aqueous solution with HCl at pH 3.0 to remove low-molecular-weight oligosaccharides and contaminating neutral polysaccharides. Reducing sugar assays were run at 37°C for 30 min in 0.1 M HEPES (pH 6.8) containing 0.001 M $CaCl_2$ and 0.2% (wt/vol) substrate. One unit of activity is defined as that amount of enzyme generating 1 μmol of reducing equivalents per min.

Carbohydrate analysis. The neutral sugar components of PGSC and BSC were identified by gas chromatography after hydrolysis with 2 N trifluoroacetic acid as described by Albersheim et al. (1). The same procedures were used in the analysis of carbohydrate products of enzymatic hydrolysis of PGSC and BSC. Determination of the neutral sugar components of the α-AF also used this method except that hydrolysis was carried out in degassed trifluoroacetic acid under N_2 to preserve amino sugars.

Hexosamine content of the pure α-AF was measured by the Elson-Morgan method as described by Kabat (15). Analysis of the amino sugar was performed on a Sigma 3 gas chromatograph (Perkin-Elmer Corp., Norwalk, Conn.), using nickel columns (3 ft by 1/8 in. [ca 91 by .32 cm]; Alltech Co., Deerfield, Ill.) containing 3% SP-2340 on 100/200 Supelcoport (Supelco Inc., Bellafonte, Pa.). Temperature programming after a 6-min post injection hold ran from 180 to 240°C at 2°C/min. Carrier gas (N_2) flow rate was 25 ml/min and detection was by flame ionization.

Additional assays. Protein was measured by the method of Bradford (4). Total sugar concentration was estimated by the orcinol assay (7). Uronic acid was measured by the method of Blumenkrantz and Asboe-Hansen (3).

RESULTS

Enzyme purification. Table 1 presents a summary of the purification scheme. The Sephacryl S-300 chromatography resulted in a 49-fold increase in specific activity (Fig. 1). The ammonium sulfate precipitation after gel filtration provided a significant additional 3.4-fold increase in specific activity and, more importantly, decreased the variability in the results of the terminal isoelectric focusing step. Without the precipitation step electrofocusing gave either marginal recoveries of enzyme activity or incomplete resolution of α-AF from other activities in the ammonium sulfate-precipitated protein.

The results of the preparative isoelectric focusing are shown in Fig. 2. The purity of the α-AF recovered from the preparative focusing was examined by disc gel electrophoresis at gel concentrations of 4, 6, and 8% acrylamide, by analytical isoelectric focusing, and by SDS-gel electrophoresis. All of these methods demonstrate the presence of a single Coomassie blue-staining band. Figure 3, which shows an analytical isoelectric focusing gel, illustrates this point.

TABLE 1. Summary of the purification of α-AF from *R. albus* 8

Purification step	Total activity (U)	Total protein (mg)	Sp act (U/mg of protein)	% Recovery
Crude culture filtrate	52.26	67.00	0.78	100
PM-10 concentrate	48.60	60.80	0.80	93
S-300 isolate	40.21	1.02	39.46	77
$(NH_4)_2SO_4$ precipitate	39.92	0.30	133.06	76
Isoelectrically focused protein	27.53	0.04	655.47	52.6

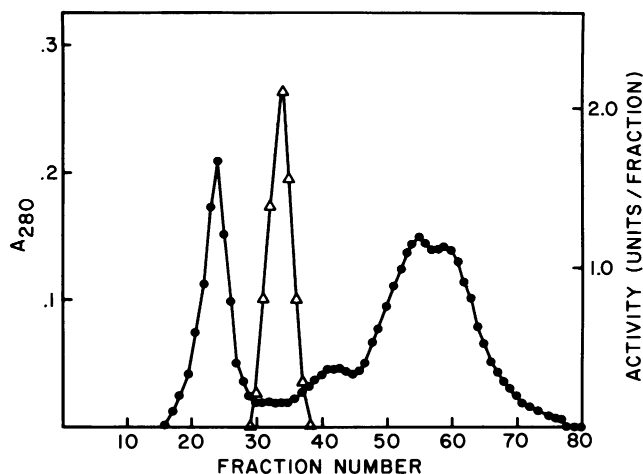

FIG. 1. Sephacryl S-300 chromatography of the α-AF from *R. albus* 8. Chromatographic conditions are described in the text. Fraction volume is 5.2 ml. Aliquots (100 μl) of each fraction were assayed to determine the distribution of the α-AF. Symbols: △, enzyme activity; ●, absorbance at 280 nm (A_{280}).

Coincidence of enzyme activity with the Coomassie blue-staining band (as shown in Fig. 3) was tested in both the disc gel and isoelectric focusing systems by slicing duplicate gels into 2-mm sections and then assaying eluates from those sections for α-AF activity. In all cases the enzyme activity was coincident with the protein band.

Molecular weight determinations. The molecular weight of the purified α-AF was estimated by S-300 gel filtration to be 310,000 and by disc gel electrophoresis to be 305,000. SDS analysis of the purified enzyme showed a single protein band with a molecular weight estimated to be 75,000.

The effect of pH on the rate of hydrolysis of *p*-nitrophenyl-α-L-arabinofuranoside and of arabinose-containing PGSC was examined. The activity maximum for action on both substrates is pH 6.9.

The rate of hydrolysis with respect to substrate concentration (*p*-nitrophenyl-α-L-arabinofuranoside) was examined. The enzyme displayed typical Michaelis-Mentien kinetics,

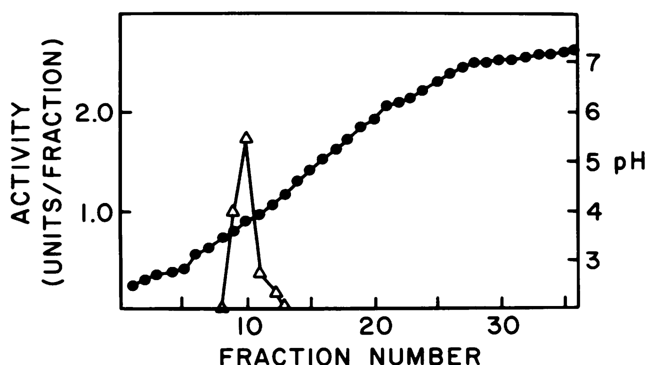

FIG. 2. Preparative isoelectric focusing of the α-AF from *R. albus* 8. The gel was divided into 36 sections which were eluted as described in the text. The pH of each fraction was determined with a surface pH electrode. Symbols: △, enzyme activity; ●, pH.

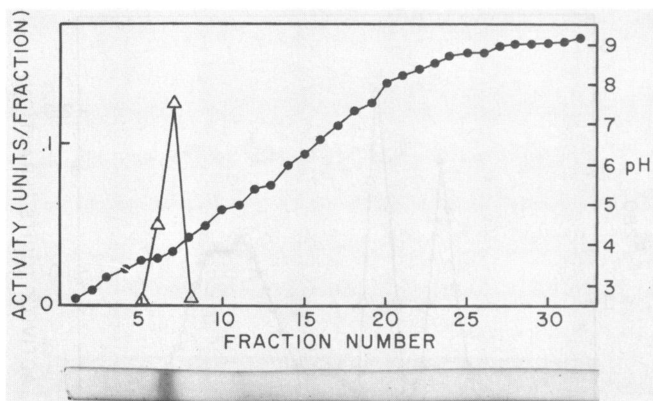

FIG. 3. Analytical isoelectric focusing of the α-AF from *R. albus* 8. Duplicate gels containing 100 μg of the purified α-AF protein were cast as described in the text. After focusing for 4 h, one gel was stained for protein and another was cut into 3-mm segments. Protein was eluted from these sections into 1.0 ml of 0.1 M HEPES, pH 6.8, and each eluate was assayed for α-AF activity. A third gel, containing no protein, was prepared in the same manner and focused simultaneously. This gel was sectioned and the sections were eluted into 1 ml of water. The pH of each of these water eluates was measured. Symbols: △, enzyme activity; ●, pH.

yielding a K_m of 1.6 mM as determined by a Lineweaver-Burke plot.

The isoelectric point of the enzyme was 3.8 both on the preparative apparatus and in the analytical isoelectric focusing gels (Fig. 2 and 3).

No evidence for metal ion cofactors was obtained; preparations dialyzed against EDTA had activities identical to those of untreated control enzymes even when assays were performed in the presence of EDTA.

The α-AF was extremely sensitive to a variety of sulfhydryl reagents. Concentrations as low as 0.05 mM (as for iodoacetamide) completely inhibited activity, and 50% inhibition was effected by 0.05 mM $HgCl_2$ or *p*-mercuribenzoate and 0.01 mM iodoacetamide.

Colorimetric analysis of the enzyme indicated that it contained approximately 6% carbohydrate by weight. The composition of the carbohydrate was determined by gas-liquid chromatography as described above. The sugars detected were mannose, galactose, glucosamine, and fucose (42, 32, 17, and 8% of the measured carbohydrate, respectively). Periodate-Schiff-stained isoelectric focusing gels were also positive for carbohydrate at the location of the α-AF.

Enzymatic activity of polysaccharide substrates. The enzyme was assayed with ACW, PGSC, and BSC as substrates. The carbohydrate composition of these materials is shown in Table 2. When incubated with ACW, the pure α-AF generated monosaccharide arabinose that accounted for only a small quantity of the arabinose available in ACW. However, the α-AF generated significantly larger proportions of monosaccharide arabinose when incubated with the soluble PGSC and BSC substrates (Table 3). Other than arabinose, no other carbohydrate moieties were detected after α-AF treatment of the three substrates, indicating that this enzyme probably functions as a specific exoenzyme.

The α-AF was incubated with ACW, PGSC, and BSC, respectively, either alone (as above) or in combination with a β-1,4-xylanase (purified from *R. albus* 8; L. C. Greve, J. M. Labavitch, and R. E. Hungate; unpublished data) or FPG.

TABLE 2. Neutral sugar composition of BSC, PGSC, and ACW

Sugar	μg of residue/100 μg of total carbohydrate		
	BSC	PGSC	ACW
Rhamnose	0	6.0	0.2
Fucose	0	0.4	0
Arabinose	17.0	27.5	6.9
Xylose	41.0	2.2	7.8
Mannose	6.4	0.8	1.3
Galactose	8.9	9.0	4.1
Noncellulosic glucose	26.0	2.6	4.8
Uronic acids	0	51.0	26.0
Cellulose	0	0	48.9

Both the xylanase and FPG were assayed against a variety of substrates (including cellulose) and found to be without contaminating activities (data not shown). The effectiveness of these enzyme treatments in catalyzing hydrolysis was judged by comparing the generation of reducing sugar equivalents from identical amounts of substrate. The results (Table 4) clearly indicate that there is synergism when the α-AF acts in conjunction with either the FPG or the xylanase (compare, for example, the generation of reducing sugars from ACW by FPG and α-AF, in combination, with the sum of the reducing equivalents generated by the two enzymes acting separately).

DISCUSSION

The α-AF was first detected during a screening of *R. albus* 8 culture filtrate for enzymes capable of degrading cell wall polysaccharide. In terms of total activity it is the most abundant extracellular glycosidase produced by *R. albus* 8 when grown on ACW (11). Its presence as a major hydrolytic activity in the culture medium was relatively unexpected since we have shown α-AF to be marginally effective in hydrolyzing ACW-bound arabinose (Table 3). To determine whether the enzyme had different activities, or other unusual characteristics, which might explain why *R. albus* 8 would make such an apparently large investment in its production, we undertook a study of its physical and kinetic properties.

Our analysis shows that the α-AF purified from *R. albus* 8 differs in several ways from α-AFs isolated from a variety of plant, bacterial, and fungal sources (10, 13, 16, 17, 24, 27).

The enzyme from *R. albus* 8 has a pH optimum of 6.8, noticeably higher than the optima of 4.5 to 6.0 reported for the α-AFs of other organisms. However, pH 6.8 is near the upper range of the normal pH of the rumen.

TABLE 3. Percent arabinose (as monosaccharide) hydrolyzed from polysaccharide substrates by the purified α-AF

Substrate	% Hydrolysis[a]
ACW	1.2
BSC	47.2
PGSC	21.8

[a] Percent hydrolysis calculated as monosaccharide arabinose hydrolyzed from substrate compared with total arabinose available in substrate aliquot (see Table 2 for arabinose content of substrates). After incubation with α-AF, monosaccharide arabinose was measured in the soluble fraction after removal of undigested substrate by centrifugation (ACW) or ethanol precipitation (BSC and PGSC) of the relatively undigested polysaccharide substrate. All values for arabinose released were determined by subsequent gas-liquid chromatographic analysis of unhydrolyzed aliquots of the cleared incubation media.

TABLE 4. Effects of assaying the α-AF alone and in conjunction with β-1,4-xylanase and polygalacturonase against BSC, PGSC, and ACW[a]

Enzyme(s)	Substrate	μg of reducing sugar per h
A. Xylanase	BSC	2,281
	ACW	196
B. FPG	PGSC	3,400
	ACW	314
C. α-AF	BSC	516
	PGSC	312
	ACW	20
D. Xylanase + α-AF	BSC	7,600
	ACW	1,130
E. FPG + α-AF	PGSC	9,676
	ACW	1,732

[a] A 20-mg portion of substrate (ACW, BSC, or PGSC) was incubated with a fixed amount of a single enzyme in 5-ml total volume and the reducing equivalents generated in 60 min were recorded (A, B, and C). In addition, both the FPG and the xylanase were assayed together with the α-AF in the same manner (D and E).

Weinstein and Albersheim reported that an α-AF from *Bacillus sublitis* (27) was unaffected by sulfhydryl reagents such as $HgCl_2$. Our data indicate that the enzyme from *R. albus* is extremely sensitive to sulfhydryl modifiers, even though no loss of activity resulted from oxidation during purification. Inclusion of β-mercaptoethanol or dithiothreitol did not affect the yield of enzyme activity. The reduction potential of the rumen would probably ensure that the sulfhydryl groups essential for enzyme activity remain reduced.

Higashi and Yasui (13) have reported that carbohydrate is associated with the α-AF from *Streptomyces* sp. However, they concluded that their enzyme was not a glycoprotein since the quantity of carbohydrate bound was highly variable and could be removed with detergent (13). Our investigations show the quantity and composition of the carbohydrate associated with the α-AF from *R. albus* 8 to be very consistent. Also, the presence of glucosamine in the carbohydrate and the observation that, during electrophoresis in SDS, the carbohydrate remains with the α-AF (shown by periodate-Schiff staining) suggest that the *R. albus* 8 enzyme is a glycoprotein.

The molecular weight of the enzyme (305,000 to 310,000) also differs considerably from those of most isolated α-AFs (63,000 to 92,000) (16, 24, 27). SDS-gel analysis yields a single band migrating at 75,000, indicating that the enzyme as isolated from *R. albus* 8 culture broth may be a tetramer. It is not known whether the monomer or some other oligomer is the active species in vivo.

The foregoing physical characterization of the α-AF provides no explanation for the abundance of the enzyme in the culture medium. However, the assays in which the enzyme was combined with either the xylanase or the FPG do provide information which allows us to speculate on the role of α-AF in the rumen environment. Morrison (18) has reported that isolated "hemicellulases" (ovine rumen) will not efficiently solubilize untreated cell walls from rye grass. However, if the hemicellulose carbohydrate was first solubilized, it was then readily degraded by hemicellulases. The extent to which this hydrolysis proceeds seems to be correlated with the degree of substitution of the hemicellulosic backbone with arabinosyl residues. We have shown that the *R. albus* 8 α-AF removes arabinosyl residues from various substrates (Table 3) and actively promotes the hydrolysis of isolated ACW and solubilized cell wall polymers by both *R. albus* 8 xylanase and FPG (Table 4). By removing arabinosyl groups from otherwise undigestible pectic and hemicellulosic (i.e., arabinose-bearing) polymers, the α-AF may increase the quantity of fermentable substrate available to the rumen flora.

An α-AF has been purified from *R. albus* 8 and shown to be homogeneous by several criteria. We have provided data which are compatible with the hypothesis that the enzyme functions biochemically to provide other enzymes (and, hence, other rumen microbes) with suitable substrates.

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