Development of a 96-Well Enzyme-Linked Solid-Phase Assay for β-Glucanase and Xylanase†

Guojie Wang,‡,§ Ronald R. Marquardt,*,‡ Hao Xiao,‡,|| and Zhiqun Zhang‡

Department of Animal Science, Faculty of Agriculture and Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

An enzyme-linked sorbent assay (ELSA) for estimating β-glucanase activity was developed on the basis of the use of biotinylated β-glucan as a solid-phase substrate. The assay involves the coating of titer plate wells with biotinylated β-glucan, the partial hydrolysis of this substrate with β-glucanase, the reaction of the biotin from the unhydrolyzed substrate with an alkaline phosphatase−streptavidin complex, and quantitation of the remaining β-glucan using alkaline phosphatase. The activity of the bound indicator enzyme, alkaline phosphatase, is proportionally related to the β-glucanase activity in the sample. The ELSA is simple, can be readily adapted to the routine assay of a large number of samples (as many as 200 per person/day), and has good precision (CV = 4.0−6.4%) and high sensitivity (detects as low as 0.001 mU of β-glucanase/assay). A similar assay was developed for xylanase using biotinylated arabinoxylan. The ELSA provides a simple and sensitive estimate of β-glucanase and xylanase activity.

Keywords: *β-Glucanase; xylanase; enzyme assay; biotinylated glucan*

INTRODUCTION

There has been a continuing interest in the development of simple and reliable assay procedures for β-glucanase, as this enzyme plays an important role in the depolymerization of barley β-glucan in both the brewing and the poultry production industries. Several methods have been reported for this assay, including viscometry (Bourne and Pierce, 1970), reducing sugar production (Denalt et al., 1978), radial gels diffusion (Edney et al., 1986; Martin and Bamforth, 1983), and the use of *azo*-barley glucan (McCleary and Shameer, 1987). The detection and the quantitation of enzyme activity in finished feeds by any method developed to date are technically challenging due to the requirement for high sensitivity and the complex nature of the feed itself. The development of a highly sensitive photometric method will be welcomed, particularly if this could lead to a high degree of assay automation. Microtitration using microtiter plates and a microtiter plate reader would greatly facilitate such an assay. There have been two approaches in this direction; one was the studies of Wirth and Wolf (1992) using a microplate colorimetric assay. The principle of this assay is the same as the *azo*-barley glucan method except the absorbance is read in microtiter plate wells. This procedure, as well as the original *azo*-barley glucan procedure, has the disadvantage of requiring precipitation and centrifuging steps. It also does not have a high degree of sensitivity, as the lowest detection limit is approximately 3 mU/assay. Another approach has been to quantitate the amount of enzyme using the immunological properties of enzymes (Bühler, 1991). The main drawback of this technique is its inability to assess the biological activity of a particular enzyme, as the immunoassay will estimate the amount of enzyme protein but not its biological activity. Also this assay would only be useful for enzymes from closely related species as antibodies tend to have high specificity. In a recent review, Headon (1993) concluded that no suitable method has been reported that facilitates detection and quantitation of enzymes added to feed. This may in part be attributed to the lack of an assay that is able to detect the very low levels of enzymes that are usually added to feed.

The purpose of this study was to develop a simple, sensitive avidin−biotin enzyme-linked sorbent assay (ELSA) for β-glucanase using aminated and biotinylated glucan as substrate. In this assay, the substrate is incubated with β-glucanase and the amount of β-glucan−biotin remaining in the titer plate wells is quantitated enzymatically following the binding of an alkaline phosphatase−streptavidin complex to the unreacted substrate complex. The color produced by the activity of the bound alkaline phosphatase in turn can be indirectly related to the β-glucanase activity. The use of a second enzyme not only greatly amplifies the signal but provides the basis for the development of a simple method for monitoring β-glucanase activity.

MATERIALS AND METHODS

Materials. The following materials were obtained from Megazyme International (Bray, Ireland): lichenase (*endo*-1,3−1,4-β-D-glucan-4-glucanohydrolase, EC 3.2.1.73) from *Bacillus subtilis* (batch MLI 82001), *exo*-1,3-β-glucanase (EC 3.2.1.58) from *Trichoderma* sp. (EBG 00703), barley β-glucan (lot BBG 30108), rye flour arabinoxylan (pentosan, batch MRP 90801), xylanase (*endo*-1,4-β-D-xylan xylanohydrolase, EC 3.2.1.32) from *Trichoderma viride* (batch MXY 80202), and *azo*-barley glucan. Cellulase (1,4-β-D-glucan-4-glucanohydrolase, EC 3.2.1.4) from *Aspergillus niger* (Type 2), Pullulanase (limited Dextranase, amylopectin 6-glycohydrolase, EC 3.2.1.41) from *Entero-*

* To whom correspondence should be addressed. Fax: (204) 474 7268. Phone: (204) 474 8188. E-mail: rr_marquardt@umanitoba.ca.

† Patent pending on method.

‡ University of Manitoba.

§ Current address: Laboratory of Physiology and Biochemistry, Nanjing Agricultural University, Nanjing, China 210095.

|| Present address: ImmuneChem Pharmaceutical Inc., 204-8678 Greenall Ave., Burnaby, BC, Canada V5T 3M6.

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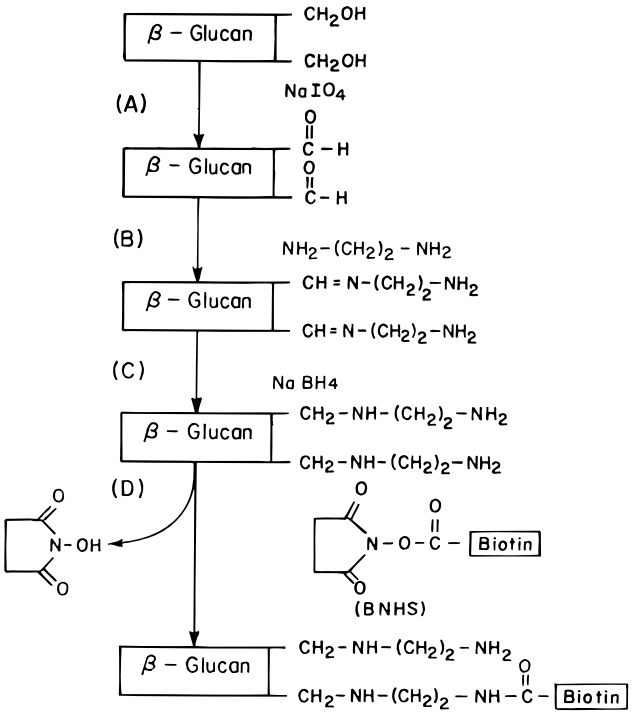

Figure 1. Synthesis of biotinylated β-glucan. The hydroxyl groups of β-glucan are partially oxidized to an aldehyde with $NaIO_4$ (A), followed by reaction with ethylenediamine [NH_2-$(CH_2)_2NH_2$] (B). The Schiff bases that are formed are reduced with $NaBH_4$ (C), followed by reaction of the free amide groups of the β-glucan−ethylenedimine complex with *N*-hydroxysuccinimide ester of biotin (BNHS) to form a biotinylated β-glucan (D).

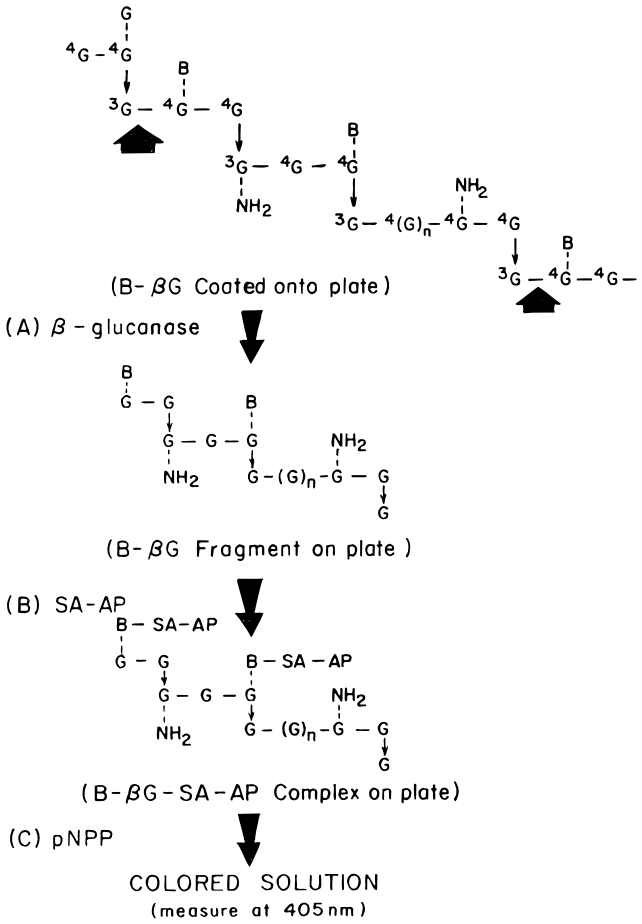

Figure 2. Steps in the ELSA for β-glucanase. The substrate, biotinylated β-glucan (B-βG), is incubated with β-glucanase (lichenase) and the hydrolyzed substrate is removed by washing (A). Excess alkaline phosphatase−streptavidin complex (SA−AP) is then incubated with hydrolyzed B-βG followed by washing to remove unreacted SA-AP (B). The amount of SA-AP bound to the unhydrolyzed substrate (B-βG) is quantitated by incubating with pNPP (C). Arrows indicate bonds hydrolyzed by the β-glucanase.

bacter aerogenes, α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) from porcine pancreas (Type 1-A), biotinyl-*N*-hydroxysuccinimide esters (BNHS), ethylenediamine, *p*-nitrophenyl phosphate (pNPP), diethanolamine, and Tween-20 were from Sigma Chemical Co. (St. Louis, MO). Sodium periodate and sodium borohydride were from Fisher Scientific Co. (Fairlawn, NJ); alkaline phosphate−streptavidin from Zymed Laboratories, Inc. (San Francisco, CA); microtiter plates (Falcon 3911, Microtest III) from Becton Dickinson Labware (Oxnard, CA); dimethyl sulfoxide (DMSO) from J. T. Baker Chemical Co. (Phillipsburg, NJ); and instant skim milk powder was from Nestle (Don Mills, ON). RM-1 was a crude enzyme preparation from Finnfeeds International Ltd. (Wiltshire, U.K.) and contained high β-glucanase, xylanase, and other enzyme activities. All solvents and reagents were of analytical grade.

Preparation of Biotin−Glucan and Biotin−Arabinoxylan Conjugates. The technique is based on the principle that an active aldehyde group which is generated after sodium periodate oxidation of the hydroxyl group of the polysaccharide moiety reacts with ethylenediamine to form an aminated polysaccharide. The Schiff base that is formed is stabilized by reaction with sodium borohydride (Figure 1; Wong, 1991). The amine groups can then be conjugated to biotin using BNHS. In brief, 75 mg of β-glucan was dissolved in 2 mL of distilled water, and 0.1 mL of 100 mM $NaIO_4$ was then added. The reaction was protected from light and mixed for 0.5 h at room temperature. The reaction mixture was treated with 1 mL of ethylenediamine for 2 h, and unreacted reagents were removed by ethanol precipitation. This involved the addition of 8 mL of 95% (v/v) ethanol to the reaction mixture (3 mL), followed by mixing of the sample, centrifugation at 10000*g* for 10 min at 0 °C, and dissolving the pellet in 2 mL of distilled water. The preparation was washed three times. The washed precipitate was then dissolved in 2 mL of distilled water, 5 mg of sodium borohydride were added, and the reaction was allowed to proceed for 4 h at 4 °C. The washing steps as indicated above were repeated three times. The biotin ester (BNHS, 15 or 0.15 mg) was dissolved in 0.2 mL of DMSO; the mixture was allowed to react at room temperature for 3 h followed by three precipitations with ethanol as described above. The final precipitate was dissolved in 3 mL of distilled water, divided into aliquots, and each aliquot of the BNHS−β-glucan complex was stored at −20 °C in sealed polypropylene containers. The ratios of BNHS to β-glucan were 0.2 and 0.002 for the complexes that were synthesized from 15 or 0.15 mg BNHS and 75 mg β-glucan, respectively.

The method for preparing the arabinoxylan substrate was the same as that used for β-glucan except the substrate was arabinoxylan rather than β-glucan. The BNHS to arabinoxylan ratio was 0.2 with the stock substrate, being diluted 10 000-fold prior to being coated on the plate.

Buffers and Coating of Plates. Phosphate-buffered saline [PBS, 4.39 g/L NaCl, 8.19 g/L Na_2HPO_4, and 2.45 g/L NaH_2PO_4; pH 7.2] containing 0.3% (w/v) skim milk was used for dilution of the alkaline phosphate−streptavdin complex. The wash buffer for the titer plates was PBS (pH 7.2) containing 0.05% (v/v) Tween 20 (PBS-T). Sodium phosphate buffer (20 mM, pH 6.5) was used for the dilution of all enzyme preparations, except for xylanase which was diluted in 25 mM acetate buffer (pH 4.7). Alkaline phosphate substrate solution contained 1 mg/mL of *p*-nitrophenyl phosphate in 1 M diethanolamine buffer (pH 9.8).

The microtiter plates were directly coated with 0.1 mL/well of the biotin−glucan complex diluted in 0.05 M carbonate−bicarbonate buffer (pH = 9.6) and then left at room temperature for 1 h and overnight at 4 °C. The usual dilutions of the

Table 1. Relative Activity of Different Enzymes[a]

enzyme	origin	relative activity compared to lichenase (mean ± SD, $n = 12$)
pure enzymes		
lichenase (*endo*-1,3-1,4-glucanase)	Megazyme, *Bacillus subtilis* (EC 3.2.1.73)	100 ± 4
endo-β-xylanase	Megazyme, *Trichoderma viride* (EC 3.2.1.32)	2.1 ± 0.1
exo-1,3-β-D-glucanase	Megazyme, *Trichoderma sp.* (EC 3.2.1.58)	6.2 ± 0.3
partially purified enzymes		
cellulase (1,4-β-D-glycohydrolase)	(EC 3.2.1.4)	101 ± 7
pullulanase (limit dextranase)	(EC 3.2.1.41)	1.8 ± 0.1
α-amylase	(EC 3.2.1.1)	4.3 ± 0.2
crude enzyme		
RM-1[b]	(Finnfeeds)	108 ± 4

[a] Assay procedures were as described in Figure 3. All enzyme concentrations were diluted to 5 U of enzyme activity/mL according to the activity values given by the manufacture. The enzymes were then assayed according to procedures in Materials and Methods and Figure 5. The substrate in all wells was 0.2 biotin−glucan (diluted 1 to 50 000) with the hydrolysis time being 15 min and the color development time being 30 min at 22 °C. The mean CV was less than 5%. [b] RM1 contained 900 U/(g of β-glucanase) (pH 5.0) and a mixture of other enzymes.

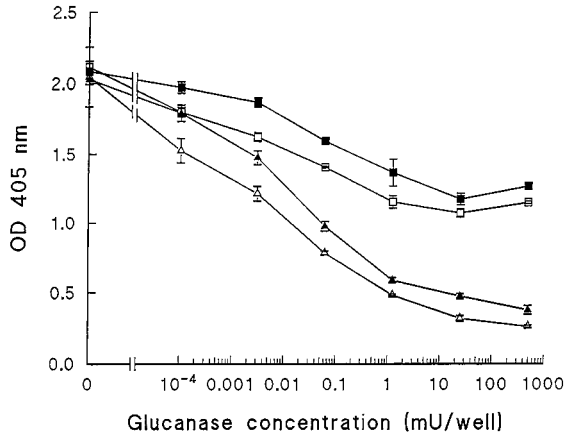

Figure 3. Hydrolysis of biotin−glucan substrates by β-glucanase (lichenase) followed by the quantitation of unhydrolyzed β-glucan using a second enzyme. Two concentrations each of the 0.002 biotin−glucan complex [1−50 (■) and 1−100 (□) dilutions] and of the 0.2 biotin−glucan complex [1−50 000 (▲) and 1−100 000 (△) dilutions] were coated onto titer plates and prepared as described in Materials and Methods. β-Glucanase (100 μL, 20 mM sodium phosphate buffer, pH 6.5) was added to the wells at the concentrations indicated (10^{-4}−10^{3} mU/100 μL) and the mixtures were incubated at 22 °C for 15 min. The reaction was stopped by washing with PBS-T, and phosphatase−streptavidin was added to each well followed by washing to remove the unbounded complex. The amount of bounded phosphatase was quantitated enzymatically following incubation with *p*-nitrophenyl phosphate in 1 M diethanolamine buffer for 30 min at 22 °C. Values represent the mean ± SD of triplicate analysis.

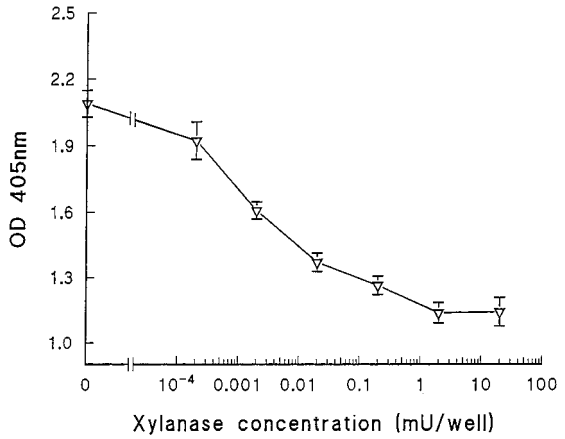

Figure 4. Standard curve for the xylanase assay. The biotin (BNHS) to arabinoxylan ratio was 0.2 with the dilution of the substrate being 1 to 10 000. The amount of enzyme added to each well is indicated on the abscissa. Other conditions were as described in Figure 3 and in Materials and Methods. Values represent the mean ± SD of triplicate analysis.

0.2 stock biotin−glucan complex was 50 000. The plates were washed three times with PBS-T buffer, and the emptied plates were stored in sealed containers at 0 °C for up to several months.

Test Procedures. A summary of the procedure is outlined in Figure 2. β-Glucanase (lichenase) or xylanase were, respectively, diluted with 20 mM sodium phosphate buffer (pH 6.5) or 25mM acetate buffer (pH 4.7) to the desired concentration. The enzyme (100 μL) was added to each well in the biotin−glucan coated microtiter plate, the plate was sealed with a low evaporation lid, and the mixture was incubated for the desired period of time (from 1−30 min) and at the desired temperature (usually 22−24 °C). The reaction was stopped by emptying the plates followed by washing of the wells three times with pH 7.2 PBS-T. Blanks contained the incubation buffer without enzyme. Alkaline phosphatase−streptavidin (100 μg) diluted 1:1000 in pH 7.2 PBS was added to each well and incubated for 30 min at room temperature. The plates were washed six times with pH 7.2 PBS-T and dried at ambient temperature for approximately 10−20 min. Alkaline phosphatase substrate solution was then added to each well (100 μL), and the microtiter plates were incubated at 22 °C for 30 min or until absorbency of the well with no β-glucanase yielded a value of from 1.5 to 2.0 optical density units. The plates were read at 405 nm using a microtiter plate reader (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada; Model 450). Further details of individual studies are given in Figures 3−5 and Table 1. The individual activity values for the enzymes are also given in the legends of the figures or tables. The activities of stock preparation of β-glucanase and xylanase were as given by the manufacture.

Other Procedures. The precision of the ELSA was studied using three concentrations of enzyme (0.076, 2.1, and 160 mU)/well. They were added (100 μL) to wells containing the 0.2 biotin−β-glucan diluted 1 to 50 000, and the mixture was incubated for 15 min at 22 °C. The assay within the same titer plate was replicated eight times. The entire assay was replicated six times. Other procedures were as described above. Another study (Figure 6) determined the effects that β-glucan had on enzyme activity. In this study, β-glucan (5.12 mg/mL) in 20 mM sodium phosphate buffer (pH 6.5) was mixed with an equal volume of 0.2 U/mL of β-glucanase and hydrolyzed at 40 °C for 60 min. The reaction was stopped by putting the enzyme solution in a boiling water bath for 15 min. The control sample contained β-glucan but was not incubated with enzyme and was not subjected to boiling. The hydrolyzed β-glucan and nonhydrolyzed solutions were added to wells (50 μL) containing 0.2 biotin−glucan (1 to 50 000 dilution), and the solution was incubated with 50 μL of β-glucanase (0.5 mU). In a final study (Figure 7), the *azo*-barley glucan method for the assay of

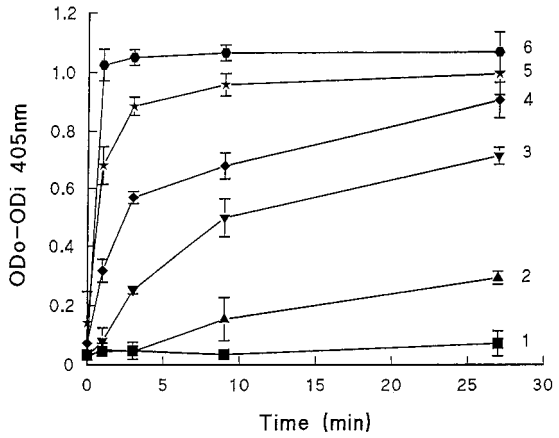

Figure 5. Time course for the hydrolysis of biotin−β-glucanase (lichenase). The enzyme was incubated for 0, 1, 3, 9, and 27 min at 22 °C with the dilution of the 0.2 biotin−glucan being 1 to 50 000. The six concentrations of enzymes were as follows: 0.032 (1), 0.16 (2), 0.8 (3), 4 (4), 20 (5), and 100 (6) mU/well. The color development time was 30 min at 22 °C. Other procedures were as described in Materials and Methods and Figure 3. The net β-glucanase activity was obtained by subtracting the absorbency values obtained in the absence of enzyme (OD_o) from those containing enzymes (OD_i).

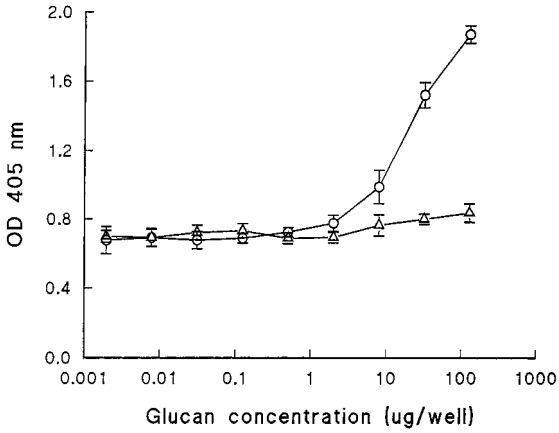

Figure 6. Influence of prehydrolyzed (△) and unhydrolyzed (○) β-glucan on β-glucanase (lichenase) activity. Different amounts of the two forms of β-glucan as shown in the figure were added to wells containing bound biotin−β-glucan followed by the addition of 0.5 mU of β-glucanase/well (50 μL). The reaction was carried out for 15 min at 22 °C. Other conditions were as described in Materials and Methods or Figure 5.

β-glucanase was compared to the method developed in this paper. The *azo*-barley glucan procedure was as described by McCleary and Shameer (1987).

RESULTS AND DISCUSSION

Hydrolysis of β-Glucan. The optical density changes obtained by the hydrolysis of different biotin−glucan substrates in the presence of different concentrations of β-glucanase followed by the detection of the unhydrolyzed substrate using an alkaline phosphatase−streptavidin complex is shown in Figure 3. The absorbency values provide a measure of the amount of substrate that was not hydrolyzed. The net amount of substrate hydrolyzed can therefore be estimated by subtracting the absorbance value in the absence of enzyme from those obtained in the presence of the enzyme.

The labeling of glucans at a biotin/glucan ratio of 0.2 (w/w) gave a steeper standard curve with larger absorbency changes than the values obtained with the complex that had a 0.002 ratio. Also the higher dilution of substrate with either degree of biotinylation yielded assays that were more sensitive. These results suggest that increased sensitivity of the assay with lower background values can be achieved by increasing the biotin/glucan ratio of the substrate and by coating the plate with a more dilute solution of the substrate. There are limitations, however, to the degree that this can be carried out as excessive biotinylation of the substrate may reduce its accessibility to the enzyme. The 0.2 biotin/glucan substrate at a dilution of 50 000 or 100 000 was able to detect β-glucanase activity in the range from 10^{-4} to 1 mU/well when the hydrolysis time was 15 min. A dilution of 50 000 was selected for routine analysis as the interplate variation was lower than that of the higher dilution. These data suggest that the assay is able to detect very low enzyme activity values.

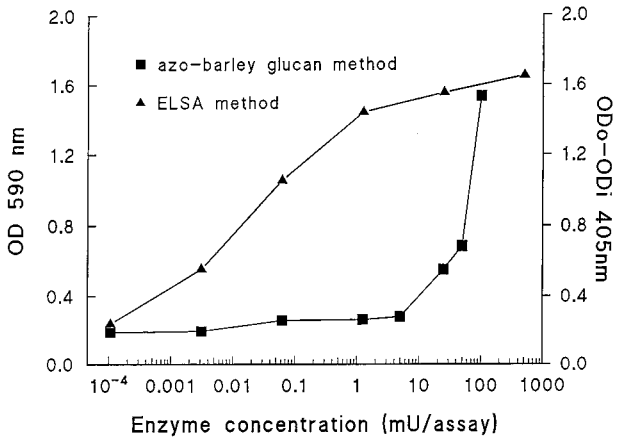

Figure 7. Standard curves comparing the *azo*-barley glucan and ELSA methods for β-glucanase (lichenase): ELSA (▲); *azo*-barley glucan (■). The ELSA reaction was carried out for 15 min at 22 °C. See Materials and Methods and Figure 5 for details of the two assays.

A curve similar to that obtained with β-glucanase was obtained with xylanase when the substrate was a biotin−arabinoxylan complex (Figure 4). No activity was obtained with this assay when β-glucanase was added to the incubation mixture.

Time Course Response at Different Enzyme Concentrations. The biotin−glucan complex was hydrolyzed by 0.032−100 mU/well of β-glucanase over a time period of from 1 to 27 min (Figure 5). In this and subsequent assays the net absorbency values due to enzyme activity were plotted (OD_o-OD_i at 450 nm). The results demonstrated that at the high concentration of enzyme (100 mU/well) the reaction was completed within 1 min, whereas at a low concentration (0.16 mU/well) the reaction rate was low and remained near linear over a period of 27 min. Similar overall trends, however, were also obtained at all time periods, indicating that the time of assay is not critical provided a standard curve is used. However, a better proportionality is obtained when short assay times are used (i.e., less than 5 min), especially when the enzyme concentration is high [i.e., above 20 mU per assay (100 μL)]. Also when the concentration of enzyme is very low (less than 0.16 mU/100 μL), incubation periods of longer then 30 min should be used. Assay periods of 15 min would be convenient for routine assays as this would provide sufficient time to add the enzyme and color developing reagents to the titer plate wells. Under such conditions,

errors caused by differences in the time that reagents are added to the wells in a 96 titer plate assay would be minimal compared to that obtained when assay times of less than 5 min are used. These data, therefore, indicate that the assay is highly flexible and can be readily adapted to meet the needs of the user. An advantage of the assay in addition to being highly sensitive and flexible is that more than 200 assays can be completed by one person in a single day. A disadvantage of this assay is that it provides a measure of the relative rather than the absolute units of enzyme activity. The procedure, however, can be calibrated against an enzyme of known activity in a manner similar to that used with the *azo*-barley glucan assay (McCleary and Shameer, 1987). Also the assay can only estimate the enzyme activity and therefore does not represent a fully optimized procedure.

Influence of Exogenous β-Glucan on Assay Values. It is well-known that β-glucanase as well as other carbohydrate hydrolyzing enzymes are tightly bound to their substrate (Headon, 1993; Yu et al., 1995). Therefore, the presence of high concentrations of exogenous substrate in a sample can interfer with the assay of these enzymes. The current study demonstrated that the presence of prehydrolyzed β-glucan did not affect the results, whereas there was a reduced activity of the enzyme, as seen by the absorbency changes, when the amount of intact β-glucan in the well exceeded 1 μg/well (Figure 6); this would be equivalent to 10 μg/mL of β-glucan in an extract containing the enzyme. Barley that contains a high concentration of β-glucan (i.e., 5%) when extracted with a near minimum amount of buffer (assume a 1 to 10 weight-to-volume ratio) would therefore contain 5 μg of β-glucan/mL. Under such conditions, the β-glucans in the extract would have only a minimal effect on β-glucanase activity. However, if there is a suppression of β-glucanase activity by exogenous β-glucans, its effects can be reduced by prior enzymatic hydrolysis of the extracted β-glucans in a manner analogous to that shown for Figure 6. Interference caused by endogenous substrates with other β-glucanase assays, as discussed below, would be greater as the dilution of the enzyme extract, due to reduced sensitivity of the assay, would be much less than that used in the current study.

The data from this study, also, suggest that a modified form of the assay can be used to quantitate the amount of β-glucan in an extract. Under such conditions a competitive ELSA could be developed with the two forms of substrate; the unknown amount in the extract and the reference amount, the biotinylated β-glucan, which would be bound to the surface of the well. Such an assay would be analogous in principle to an ELISA, which is widely used for the detection of low molecular weight analytes (Kemeny and Challacombe, 1988). Further research is required to explore this possibility.

Precision of Assay. The within-assay means ±SD for the ELSA, as assessed by eight repeated analyses of three concentrations of enzyme (160, 2.1, and 0.076 mU/mL), yielded absorbency values of 0.33 ± 0.016 (CV = 6.4%), 1.25 ± 0.06 (CV = 4.9%), and 1.92 ± 0.078 (CV = 4.0%), respectively. There were greater variations in absorbency values between runs (average CV = 12.9%) than within runs (average CV = 5.1%). Differences in incubating and color development times, ambient temperature, or other variables could have contributed to the between-run variation. This can be reduced by the use of appropriate reference standards and by the more rigid control of assay conditions.

Relationship between the *azo*-Barley Glucan Method and the ELSA for the Assay of β-Glucanase Activity. The objective of this study was to compare the sensitivity of the assay as developed in this study with another indirect and very popular assay, the *azo*-barley β-glucan method (McCleary and Shameer, 1987). In this comparison the concentration of enzyme was plotted on a logarithmic scale so as to compare the sensitivity of the two assays over a wide range of activities. As indicated in Figure 7 the azo-dye procedure gave a steep response at a relatively high concentration of the enzyme whereas the ELSA produced a more gradual response but was much more sensitive. The detection range for the ELSA was from 0.001 to 1 mU/assay, whereas that of the *azo*-barley glucan procedure was from 10 to 100 mU/assay, a difference in sensitivity of from 10-fold to 100 000-fold. The limitations of the ELSA were discussed above. The azo-blue dye also has limitations as the method, which is considerably more simple than other standard methods, requires a precipitation step followed by a centrifugation step to separate the hydrolyzed and unhydrolyzed substrate. These steps preclude the adaptation of the procedure to the formate that is used for the ELSA. The azo-dye method must also be standardized since it suffers from changes in parameters such as the solubility of the dyed polysacharide fragments as influenced by factors such as the ionic strength and temperature of the precipitant, and the centrifugation conditions. These data suggest that, for the assay of β-glucanase activity, using the ELSA is much easier to carry out and is considerably more sensitive than the corresponding *azo*-barley glucan method.

Hydrolysis of Biotinylated-β-Glucan by Other Enzymes. Results shown in Table 1 compare the degree of hydrolyses of biotinylated β-glucan by different enzyme preparations. In all of these assays the enzyme was diluted to a common activity on the basis of assay values provided by the producer of the enzyme; as a result, the comparisons are not precise but only approximate. The results, nevertheless, demonstrate that those enzymes that are capable of hydrolyzing β-glucan such as lichenase (the reference enzyme) and cellulase (McCleary and Glennie-Holmes, 1985) are able to hydrolyze biotinylated β-glucan. RM-1, a crude enzyme preparation high in β-glucanase activity, was also able to hydrolyze the biotinylated β-glucan. Other enzymes with a low ability to hydrolyze β-glucan such as *exo*-1, 3-β-D-glucanase (Wood and Bhat, 1988) or no ability to hydrolyze the substrate (i.e., pullulanase, β-xylanase, and α-amylase) also yielded low values relative to those obtained with lichenase. The activity associated with α-amylase may be due to the presence of some contaminating starch in the β-glucan preparation or possibly to residual β-glucanase activity in the enzyme preparation. The ability of cellulase to hydrolyze the substrate also suggests that a modification of the substrate (i.e., use of cellulose rather than β-glucan) would provide a basis for its assay.

CONCLUSIONS

The ELSA assay developed in this study provides a new and alternate procedure for estimating β-glucanase activity. The study indicated that a 50 000-fold dilution of substrate having a BNHS to β-glucan ratio of 0.2

yielded near optimal activity values. The optimal assay times and amount of enzyme that could be used were from 3 to 30 min and from 0.16 to approximately 100 mU of β-glucanase per assay (100 μL). Conditions within this range can be selected for maximum sensitivity. The method is simple, highly sensitive, and inexpensive and can be carried out in any laboratory that has ELISA equipment. It is not greatly affected by endogenous substrate, and only small amounts of reagents are needed. Several hundred assays may be carried out during a working day by one person. Also the assay can probably be adapted to the assay of any enzyme that is able to hydrolyze a polymeric structure as suggested by the results obtained with cellulase using biotinylated β-glucan and xylanase using biotinylated arbinoxylan. As indicated above, the type of response to the enzyme can also be varied by altering the degree of biotinylation and dilution of the substrate. Further research will need to be carried out, not only to improve the assay for β-glucanase but also to determine if the method can be utilized for the assay of other enzymes such as cellulases, xylanase, other polysaccharide hydrolyzing enzymes, proteases, DNAases and RNAase, etc. The procedure for the assay of other hydrolytic enzymes would be the same as for β-glucanase or xylanase. The only requirement is that the appropriate substrate be selected. This assay should be particularly useful for the assay of enzymes in animal feeds as it is highly sensitive and is not greatly affected by the presence of endogenous substrates, two factors that limit the use of the currently available assays (Headon, 1993).

Finally the results from the inhibition studies with β-glucan suggest that the assay can be modified so that the amount of soluble β-glucan in a sample can be quantitated. If this procedure is successful, it should also be possible to extend the current assay to the quantitation of other macromolecules in a competitive ELSA. The results of this study have outlined the basis of a new type of assay for the estimation of the activity of enzymes that are able to hydrolyze polymeric substrates and suggest that it can also be utilized in the quantitation of the same polymeric substrates when present in a soluble form.

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