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A Sandwich Inhibition Enzyme-Linked Immunosorbent Assay of Water-Soluble Pentosans in Rye Grain

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ABSTRACT

A sandwich ELISA for determination of fraction II from a DEAE ion-exchange column fractionation of water-soluble rye pentosans is described. A modification of this method, called a sandwich inhibition ELISA, is also described to determine pentosans in whole-rye grains. This procedure includes the following steps: (1) antibody is bound to a polystyrene solid phase, (2) the immunogen to be assayed is reacted with the bound antibody, (3) a second antigen is reacted with the unreacted antibody, (4) the enzyme-labelled antibody is reacted with the second antigen, (5) the enzyme activity of the bound enzyme-labelled antibody is then measured. This activity decreases with increasing immunogen content. The assay can be used in the range of concentration between 2 and 100 μg for water-soluble rye pentosans. Procedures for preparation of the sample, production and separation of the antibodies and the conjugate are described.

INTRODUCTION

An important fraction of the non-starch polysaccharides in cell walls of cereal grains consists mainly of polymers containing xylose and arabinose and called *pentosans*. Small amounts of glucose, galactose, ferulic acid and protein are linked to these pentose chains (Fausch *et al.*, 1963). The dry

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substance of whole-rye grains usually contains about 8–10% pentosans, and in wheat it is around 6–7% (Henry, 1987).

Generally, pentosans are classified into water-soluble and water-insoluble fractions. The chemical composition of the water-soluble pentosans and some of their physical properties have been described earlier by Girhammar *et al.* (1986). Water-soluble pentosans, which form 16–23% of the total pentosan content of rye (Drews & Seibel, 1976), and 20–25% of wheat (Neukom *et al.*, 1967), are of technological importance mainly due to their ability to bind large quantities of water. In wheat dough, 23% of the water is found associated with these water-soluble pentosans (Bushuk, 1966), even though they constitute only 1·4–2·1% of the total dry substance of wheat.

Another important physical property of the water-soluble pentosans from the point of view of the baking process is their ability to form gels, especially in the presence of oxidising agents (Baker *et al.*, 1943). Kündig *et al.* (1961*b*) separated the wheat pentosans on an ion-exchange (DEAE-cellulose) column and studied the gelation properties of various fractions. They found that the fraction (fraction II) which was eluted with 0·01M sodium borate buffer was the only one which showed ability to form gels in the presence of oxidising agents. According to Morita *et al.* (1974) this fraction was a polysaccharide (xylose 48·8%, arabinose 41·4% and galactose 1·8%) containing ferulic acid (0·2%) free of proteins.

In the absence of gluten-forming proteins, pentosans contribute to the structure of the bread made from rye flour. Pentosans hold the water that is available in the bread efficiently in competition with starch, and as a consequence influence the rate of the staling process (Jankiewicz & Michniewicz, 1987). Endogenous enzymes, present in the grains, degrade pentosans under wet storage conditions, causing decreased dough viscosity. The amount of water-soluble pentosans in the grains can vary widely depending on weather conditions. Crops grown during a wet summer season produce grains with a higher content of degraded pentosans, which give doughs of low viscosity (Drews & Seibel, 1976).

The baking quality of rye depends to a great extent on its pentosan content. Too high amounts of water-soluble pentosans yield doughs that can be mixed easily, but which have low stability, thus producing bread with low loaf volume. Too low amounts of pentosans in the flour produce a low viscosity dough and on baking a bread with a low loaf volume. It is therefore important that rye used for baking purposes contains an optimal quantity of soluble pentosans. In crop-breeding projects, an accurate and sensitive method for determining pentosans in small samples of rye grains is therefore necessary for the selection of rye varieties with suitable baking qualities.

Most of the methods available for the determination of pentosans in cereals have two main disadvantages in common. One is lack of sensitivity

and the other is interference of other compounds. According to one method, analysis is performed by first hydrolysing pentoses to furfural. Furfural is then separated from the acidic mixture by distillation (Cerning & Guilbot, 1973) or is protected from decomposition by extraction with an immiscible solvent (AACC, 1961) before it is quantified. Distillation is not considered a method of choice, because some amount of furfural is found to decompose during distillation. Hexoses and uronic acids interfere due to formation of compounds similar to furfural on hydrolysis.

The present paper reports the development of an immunochemical method for determination of water-soluble pentosans in rye grains. Immunochemical methods have been in use for analysis of food components during the last 20 years (Daussant & Bureau, 1984). The specific nature of the reaction between antigen and antibody is very useful for detecting a single component in complex mixtures like food products. The enzyme-linked immunosorbent assay (ELISA) (Engvall & Perlmann, 1971) is one of the most common immunochemical methods used in food analysis. The enzyme immunosorbent assay uses either enzyme-labelled antigen or enzyme-labelled antibodies. Polystyrene microtitre plates are used for the immobilised phase. The specificity and sensitivity of ELISA is normally very high, making it suitable for measurement of small amounts in complex samples. Immunosorbent assays are also very fast and well suited for screening purposes.

MATERIALS AND METHODS

Isolation of antigen

Water-soluble pentosans were isolated from whole-rye grains (var. Petkus obtained from Svalöf AB, Svalöf, Sweden) as described by Antoniou *et al.* (1981). Milled whole-grain rye flour (1 mm screen) was boiled with 3·5 volumes (w/v) of 80% ethanol for 1 h under reflux. The residue obtained by filtration was washed with 95% ethanol and dried at room temperature. The dried rye was then extracted with four volumes of distilled water for 15 min followed by centrifugation at 4000 rpm for 20 min. The pH of the supernatant was adjusted to 7·5 with NaOH before porcine pancreatin (Purum, Fluka AG, Buchs, Switzerland) was added to digest the protein and the starch. The suspension was incubated at 34°C for 24 h with 0·05% NaN_3 under continuous stirring. The solution was then centrifuged at 4000 rpm for 20 min and filtered (pore size 3 μm, Millipore, Bedford, Massachusetts). From the clear supernatant, pentosans were precipitated with ethanol, adjusting the final concentration to 80%. The pentosan precipitate was

filtered (Munktell No. 0, Grycksbo pappersbruk AB, Grycksbo, Sweden), washed with 95% ethanol and dried at room temperature under a nitrogen atmosphere. This product is referred to as the *original pentosan preparation.* Part of the original pentosan preparation was then dissolved in water and fractionated on a diethylaminoethyl (DEAE) A-25 Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden) ion-exchange column (52 cm × 5·3 cm) according to Kündig *et al.* (1961). Five fractions (Fig. 1) were collected by eluting first with H_2O, and then with solutions of 0·002 5M, 0·025M and 0·125M disodium tetraborate ($Na_2B_4O_7$) and 0·4M sodium hydroxide. The pentose concentrations of the fractions were determined by measuring the absorbance of the colour developed with an orcinol reagent (Volkin & Cohn, 1954). The fraction eluted with 0·002 5M $Na_2B_4O_7$, called *fraction II* hereafter, was concentrated using a Diaflo ultrafiltration apparatus (Amicon Corporation, Lexington, Massachusetts) with membranes having cut-off 30 000 daltons under nitrogen pressure, dialysed to remove the salts and then evaporated to dryness under reduced pressure. Small aliquots of this fraction were stored frozen in 0·9% NaCl solution (400 μg/500 μl) for use in immunisation of rabbit or in sample dilution buffer (200 μg/200 μl) for use in the ELISA method.

Determination of the monosaccharide content of fraction II was done according to Nyman & Asp (1988), and determination of the amino acid content was carried out using an ion-exchange chromatographic method after acid hydrolysis as described by Nair (1977).

Production of antisera and separation of antibodies

A rabbit (New Zealand, white male, 4 kg, bred at the Department of Biochemistry, University of Lund, Sweden) was injected intramuscularly at two sites under the shoulder blade with 400 μg of fraction II in 1 ml of an emulsion of Freund's complete adjuvant oil and 0·9% NaCl solution (1:1 by volume, Harboe & Ingild, 1983). The rabbit was boosted with the same dose at intervals of approximately 3–4 weeks and blood (40–45 ml) was collected from the marginal ear vein 10–21 days after each booster injection. The serum was separated from red blood cells and immunoglobulin G (IgG) was isolated from the serum by precipitation with ammonium sulphate (41% saturation), followed by ion-exchange chromatography on a DEAE Sephadex A-50 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (15·5 cm × 1·6 cm), equilibrated with 0·05M Tris-HCl buffer of pH 7·2 (Harboe & Ingild, 1983) (Fig. 2).

A small fraction containing contaminating proteins was eluted with the void volume. Bound IgG was eluted by raising the salt concentration to

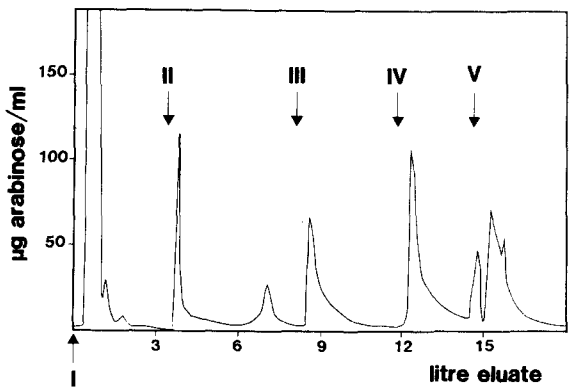

Fig. 1. Fractionation of water-soluble pentosans from rye grain on a diethylaminoethyl (DEAE) Sephadex A-25 ion-exchange column (52 cm × 5·3 cm). Pentose concentrations were measured with an orcinol reagent. Second peak contains fraction II. Elution with: (I) water, (II) 0·002 5M $Na_2B_4O_7$, (III) 0·025M $Na_2B_4O_7$, (IV) 0·125M $Na_2B_4O_7$ and (V) 0·4M NaOH.

0·075M NaCl in the same elution buffer. The eluate was concentrated in the Diaflo using a membrane with cut-off 30000 daltons under nitrogen pressure. The immunoglobulin solution was concentrated to 7–8 ml, giving a final protein concentration of 4·4–7·0 mg/ml, determined spectrophotometrically at 280 nm, where a protein concentration of 1 mg/ml gives the absorbance 1·43 (Hudson & Hay, 1980).

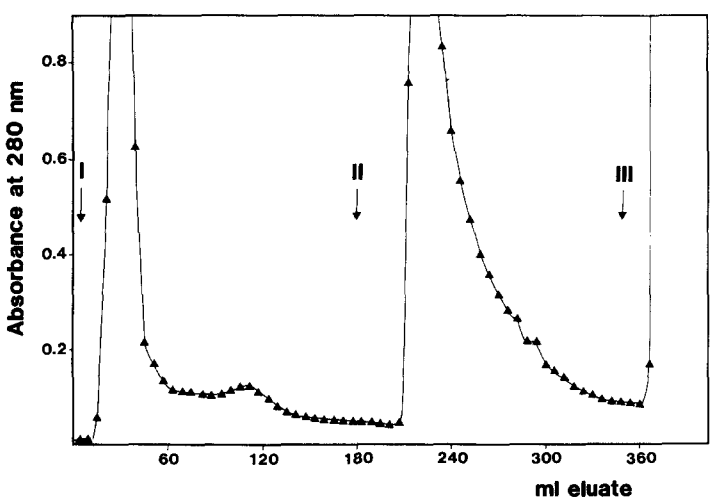

Fig. 2. Separation of immunoglobulin G (IgG) from other serum proteins on a DEAE Sephadex A-50 ion-exchange column (15·5 cm × 1·6 cm). Second peak contains IgG molecules. Elution with: (I) 0·05M Tris-HCl pH 7·2, (II) 0·05M Tris-HCl with 0·075M NaCl pH 7·2 and (III) 0·05M Tris-HCl with 0·5M NaCl pH 7·2.

Production of enzyme-labelled conjugate

The IgG was conjugated to horseradish peroxidase (HRPO, Type VI, Sigma Chemicals Co., St. Louis, Missouri) according to Nakane & Kawaoi (1974). Horseradish peroxidase (4 mg) dissolved in distilled water (1 ml) was oxidised with 0·2 ml freshly prepared 0·1M sodium meta-periodate solution for 20 min at room temperature. The HRPO-aldehyde solution thus obtained was dialysed against 1 mM sodium acetate buffer, pH 4·4, overnight at 4°C. At the same time, 8 mg IgG was dissolved in 1 ml 0·01M sodium carbonate buffer, pH 9·5, and dialysed overnight at 4°C against the same buffer. The pH of the HRPO-aldehyde solution was raised to 9–9·5 by adding 40 μl freshly made 0·2M sodium carbonate buffer, pH 9·5; IgG was added immediately and the mixture was allowed to react under constant stirring for 2 h at room temperature. The conjugate mixture was dialysed against phosphate-buffered saline (PBS) of pH 7·2 containing sodium borohydride (1 mg/ml) for 1 h at 4°C and then 1 h against PBS alone. The mixture was separated on a Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (40 cm × 2·5 cm), which was equilibrated with PBS (Fig. 3). Absorbance of each fraction was measured at 403 and 280 nm. Those fractions that showed a relation between absorbance at 403 nm and 280 nm from 0·3 and 0·6 were pooled and frozen in small aliquots (Nakane & Kawaoi, 1974).

Sandwich ELISA procedure

To analyse fraction II (antigen) a sandwich ELISA procedure was followed. Wells of a polystyrene microtitration plate (Greiner, Nürtingen, West Germany) were coated by incubation of 100 μl IgG in coating buffer, 0·01M sodium carbonate buffer, pH 9·6, with 0·02% sodium azide for 18 h at room temperature. They were then washed eight times with a washing solution containing 0·15M sodium chloride and 0·05% Tween 20 (polyoxyethylene sorbitan monolaurate, Kebo Lab., Stockholm, Sweden). After each fill the washing solution was emptied out of the wells by tapping the plate on a bed of tissue paper. Samples with different antigen concentrations diluted in sample dilution buffer (SDB) containing 10 mM phosphate buffer, pH 8·0; 0·5M NaCl and 0·1% Tween 20 were added to each well in 100 μl portions. A few wells were filled with SDB to be used as blanks. After incubation at 37°C and washing as before, 100 μl enzyme-labelled conjugate, diluted 1:67 in SDB, was added to all the wells and incubated at 37°C. To measure enzymatic activity, 200 μl newly made substrate containing 20 ml 0·05M citrate buffer, pH 4·0, 80 μl 40 mM ABTS (2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid), Sigma Chemicals Co., St. Louis,

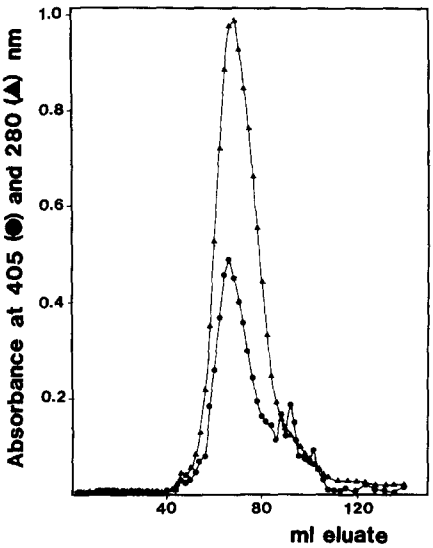

Fig. 3. Fractionation of bound and unbound horseradish peroxidase on a Sephacryl S-200 column (40 cm × 2·5 cm) equilibrated with PBS. Absorbance was measured at 280 nm and 403 nm.

Missouri) and 20 μl 0·5M H_2O_2, were added to each well after washing as above and allowed to react at room temperature before the absorbance at 405 nm was measured with a microplate photometer which had the capacity to read the plate within a period of 20 s (Titertek Multiscan Plus, Flow Laboratories, Herts, England) (Fig. 4a).

Sandwich inhibition ELISA

Since the original pentosan preparation did not react in the sandwich ELISA system, a sandwich inhibition ELISA procedure was also developed. The sandwich ELISA procedure was modified to include one additional step as shown in Fig. 4b. The microtitration plates were first coated with antibodies against fraction II. Then, the plates were incubated with a suitable number of dilutions of each unknown sample containing pentosan. After washing, 100 μl of a constant dilution of fraction II was incubated in all the wells. After this step the plates were washed and incubated with 100 μl enzyme-labelled conjugate that was diluted 1:67 in SDB. After washing unbound conjugate, the colour was developed with substrate before absorbance was measured as described above.

Preparation of supernatant from rye

Milled whole grains of rye (10 g) were mixed with distilled water (40 ml) for 15 min at room temperature to extract the soluble substances. After centrifugation and filtration, this supernatant (SW-1) was analysed in both

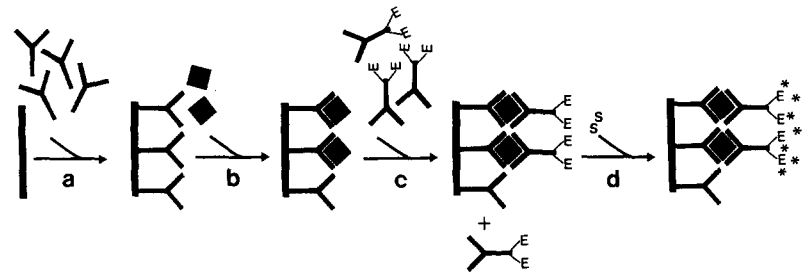

Fig. 4a. Principle of the sandwich ELISA demonstrating four incubations, (a) antibody adsorbed to plate surface, (b) sample containing fraction II (antigen) bound to the immobilised antibody, (c) conjugate bound to the bound antigen and (d) measurement of enzymatic activity after substrate addition. ——, Plate surface; —≺, antibody; ■, fraction II; ≻—≺ E/E, conjugate; S^S, substrate; **, enzymatic activity.

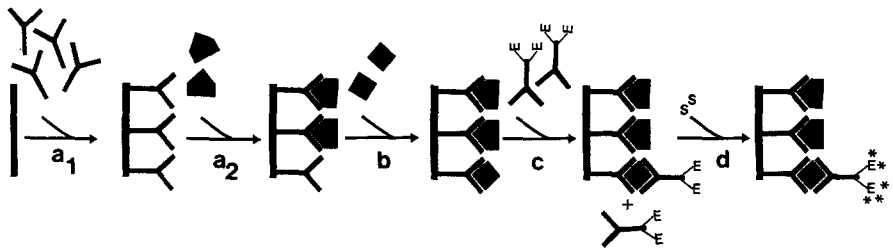

Fig. 4b. Principle of the sandwich inhibition ELISA demonstrating five incubations. (a_1) antibody adsorbed to plate surface, (a_2) sample containing pentosan bound to the immobilised antibody, (b) (c), and (d) same as (b), (c) and (d) in Fig. 4a. ■, pentosan; other symbols as Fig. 4a.

the sandwich ELISA and the sandwich inhibition ELISA. The supernatant obtained above was also analysed in both the sandwich ELISA and the sandwich inhibition ELISA after boiling (SW-2) for 15 min. A precipitate (SW-3) produced from the supernatant (SW-2) by adding ethanol to a final concentration of 80% was separated, dried and redissolved in water before it was analysed in both the sandwich ELISA and the sandwich inhibition ELISA.

Another batch of milled whole grains of rye (50 g) was boiled with 80% ethanol as described by Antoniou *et al.* (1981) for inactivation of enzymes, denaturation of protein, gelatinisation of starch and removal of ethanol-soluble substances. After filtration the residue was dispersed in water and the water-soluble substances were extracted and separated by filtration. The water extract (SE) was analysed in both the sandwich ELISA and the sandwich inhibition ELISA. The water extract (SE) was incubated with

pancreatin for 24 h at 34°C to digest starch and protein so that the pentosans could be released from the matrix. This supernatant (SD) was filtered and analysed in both the sandwich ELISA and the sandwich inhibition ELISA. The pentosans which were present in the extract were precipitated with 80% ethanol. The dried precipitate, designated *new pentosan preparation* hereafter, was redissolved in water and analysed in both the sandwich ELISA and the sandwich inhibition ELISA.

RESULTS AND DISCUSSION

Chemical composition of fraction II used for immunisation of the rabbit

Table 1 shows the amino acid composition of fraction II, fractionated from the original pentosan preparation on the DEAE ion-exchange column, which was used as immunogen for immunisation of the rabbit. Most of the earlier work has shown that pentosans are found in the cell walls bound to other constituents, especially to protein matrix. The binding mechanism has not been completely elucidated yet. However, it has been proposed that most

TABLE 1
Amino Acid Composition

Amino acid	*nmol/mg*
Aspartic acid	5·6
Threonine	2·7
Serine	4·2
Glutamic acid	5·4
Proline	2·4
Glycine	5·5
Alanine	3·9
Valine	5·7
Isoleucine	1·5
Leucine	4·7
Tyrosine	2·1
Phenylalanine	2·4
Lysine	1·3
Arginine	1·5

Determination of the amino acid content (Nair, 1977) from a single protein (0·6%) sample of fraction II, fractionated from water-soluble pentosans in rye grain on a DEAE ion-exchange column (Kündig *et al.*, 1961*a*).

of the pentosans exist in relation to proteins through covalent ester bonds between carboxyl groups of the amino acids and hydroxyl groups of the xylose (Fincher & Stone, 1974). Thus, on separation and isolation, the pentosans carry a small amount of protein with them, unless very drastic treatments with proteolytic enzymes are included in the isolation procedure. The fraction II used in this study for immunisation of rabbit contains 0·6% protein as determined by amino acid analysis. One of the main objectives of separating pentosans on the DEAE ion-exchange column is to obtain well-defined fractions that contain less protein and, as expected, the second fraction contains a low amount of protein.

Table 2 shows the results of monosaccharide analysis of fraction II. Holas *et al.* (1971) have also fractionated water-soluble pentosans from rye on a DEAE ion-exchange column. Their fraction II, eluted with 0·01M sodium borate, contained more protein (2%) and higher proportions of glucose and galactose, as compared with the fraction II isolated for the present study. An explanation of this difference could be that the enzymatic digestion with pancreatin included in the procedure for isolation of pentosans in the present study might have caused more extensive degradation of protein attached to the pentose polymers. On the other hand, Antoniou *et al.* (1981) isolated pentosans in the same way, and the pentosans prepared by them were more similar to our fraction II, whereas galactose and glucose were only found in small quantities in relation to the total dry substances. The main difference, however, is the larger xylose/arabinose ratio, 2·3 in our

TABLE 2
Monosaccharide Content

	Fraction II (%) weight		*Pentosan (%) weight*
Monosaccharide	*Present research*	*Holas* et al. *(1971)*	*Antoniou* et al. *(1981)*
Arabinose	28	25	35
Xylose	64	34	47
Mannose	3	—	6
Galactose	trace	10	4
Glucose	5	31	8
Total	100	100	100

Determined (Nyman & Asp, 1988) on a single sample of fraction II, fractionated on a DEAE ion-exchange column (Kündig *et al.*, 1961*a*) from water-soluble pentosans in rye grain, compared to fraction II from Holas *et al.* (1971) and water-soluble pentosan from Antoniou *et al.* (1981).

fraction II, compared with 1·3 for the water-soluble pentosans of Antoniou et al. (1981) and 1·4 for the fraction II reported by Holas *et al.* (1971). Pentosans contain xylose chains with arabinose units bound to them (Geissmann & Neukom, 1973). Our fraction II contained a higher proportion of xylose than was reported by Antoniou *et al.* (1981) for rye water-soluble pentosans, probably because our preparation is a pentosan subfraction with a xylose backbone containing fewer bound arabinose molecules.

Optimisation of the sandwich ELISA procedure

The sensitivity of the enzyme-immunosorbent assay depends on the efficiency of binding in the various steps, described below.

Coating

Antigen binding was studied for various coating concentrations of IgG. Concentrations below 0·2 μg/well gave lower antigen binding, whereas 0·3–0·5 μg/well gave similar results. Coating with 0·5 μg/well was chosen for all the experiments (Fig. 5).

Antigen binding

All incubations were carried out at 37°C when the incubation time was optimised. The binding was studied for six different incubation periods.

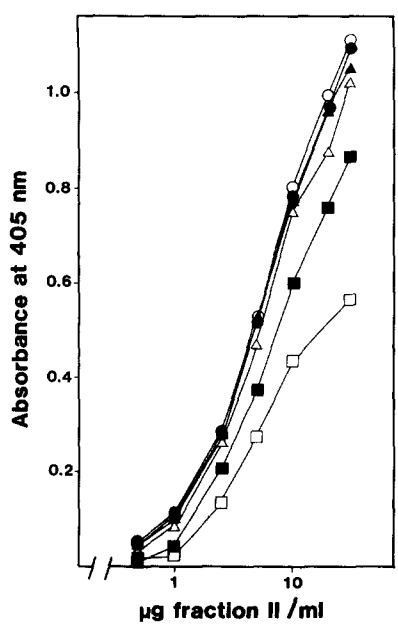

Fig. 5. Effect of different antibody (IgG) coating concentrations on the standard curve. (□) 0·5, (■) 1·0, (△) 2·0, (▲) 3·0, (○) 4·0 and (●) 5·0 μg/well IgG.

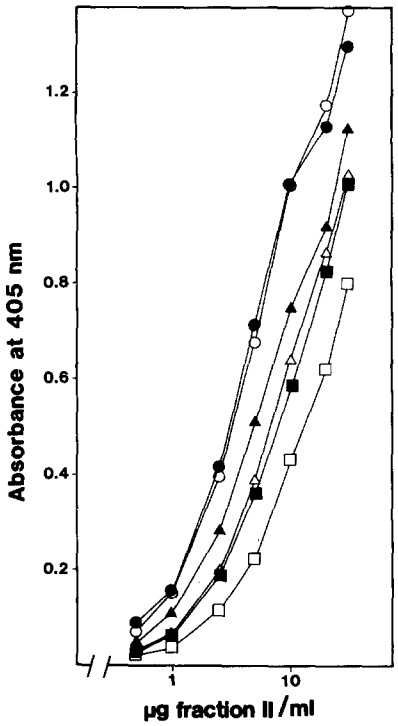

Fig. 6. Effect of various incubation periods for antigen (fraction II) on the standard curve. (□) 15, (■) 30, (△) 45, (▲) 60, (○) 75 and (●) 90 min.

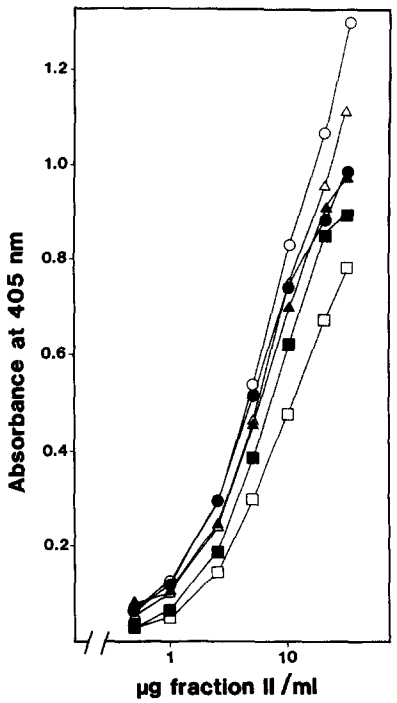

Fig. 7. Effect of various incubation peroids for enzyme-linked conjugate on the standard curve. (□) 15, (■) 30, (△) 45, (▲) 60, (○) 75 and (●) 90 min.

When increasing the incubation time, a steeper standard curve was obtained. An incubation of 60 min was chosen from these experiments (Fig. 6).

Enzyme-labelled conjugate binding

The incubation of enzyme-labelled conjugate was varied between 15 and 90 min. The binding increased with increasing incubation time, but levelled out between 60 and 90 min of incubation. An incubation time of 60 min was chosen (Fig. 7).

Substrate incubation

The absorbance became easily measured after 25 min. Therefore, a substrate incubation time of 30 min was chosen (Fig. 8).

Sandwich ELISA

Varying dilutions of fraction II were incubated in the sandwich ELISA procedure. A standard curve was constructed for the concentration range

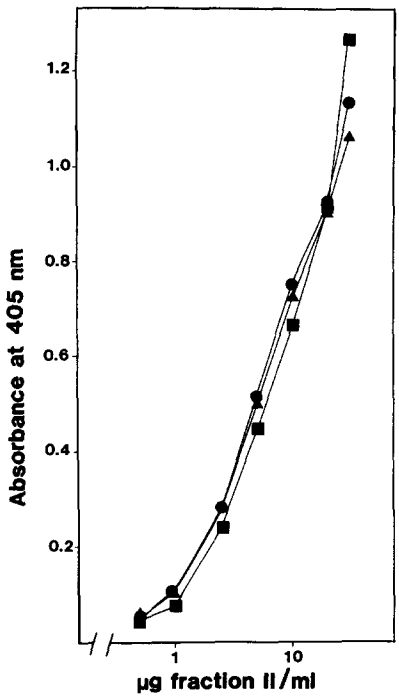

Fig. 8. Effect of different incubation periods with substrate on the standard curve. (▲) 25, (●) 30 and (■) 35 min.

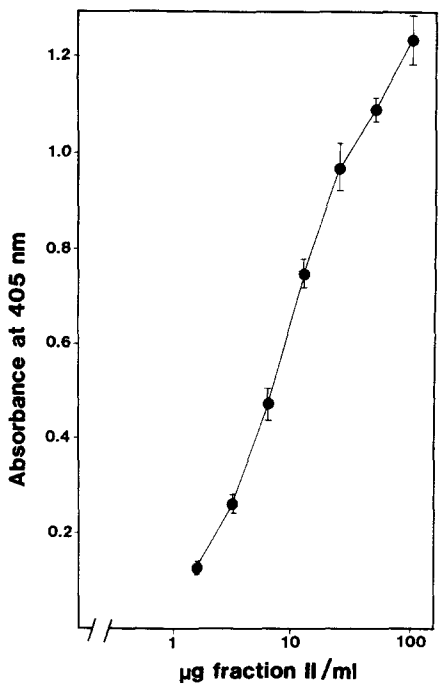

Fig. 9. A standard curve for determining dilutions of fraction II in relation to absorbance readings at 405 nm with sandwich ELISA. The vertical bars represent the coefficient of variation for each value.

1·5–100 µg/ml of fraction II (Fig. 9). The methodological design shows that fraction II has at least two antigenic determinants that can bind the antibodies. The standard curve obtained for fraction II was used to analyse the total pentosan content of preparations SW-1, SW-2, SW-3, SE, SD, original and new pentosan preparations. But the sandwich ELISA method could not detect any pentosans in the preparations.

Fraction II originated from these preparations. It makes up approximately 2·25% of the original and new pentosan preparations and 0·04% of the rye supernatant preparations (SW-1, SW-2, SW-3, SE and SD). However, not even when the above preparations were diluted to give a fraction II concentration that would fall within the standard curve could any antigen be detected with the sandwich ELISA.

Samples of soluble wheat starch (Merck, Darmstadt, West Germany), monopol wheat protein (Lindahl, 1987) and the monosaccharides xylose, arabinose and galactose gave no absorbance when analysed in the ELISA. Wheat starch was chosen as the chemical composition, and properties of wheat starch are very similar to rye starch (Hoseney, 1986).

Sandwich inhibition ELISA

To determine if the analysed samples contained any pentosans that reacted with the antibodies and thus could inhibit the binding of fraction II in a following incubation step, the ELISA procedure was modified (Fig. 4b). Samples of the original pentosan preparation, the new pentosan preparation and the supernatants (SW-1, SW-2, SW-3, SE and SD) from rye were incubated in microtitration plates for 60 min at 37°C. All the other incubation steps were identical to the steps in the sandwich ELISA procedure. Samples of the original pentosan preparation showed increased inhibition of binding by fraction II as the concentration of pentosans increased (Fig. 10). The inhibition curve obtained could thus be used as a standard curve for determination of pentosans in unknown samples in the concentration range 20–1000 μg/ml. No inhibition was obtained either in the supernatants SW-1, SW-2 and SW-3, or in the rye boiled in ethanol and

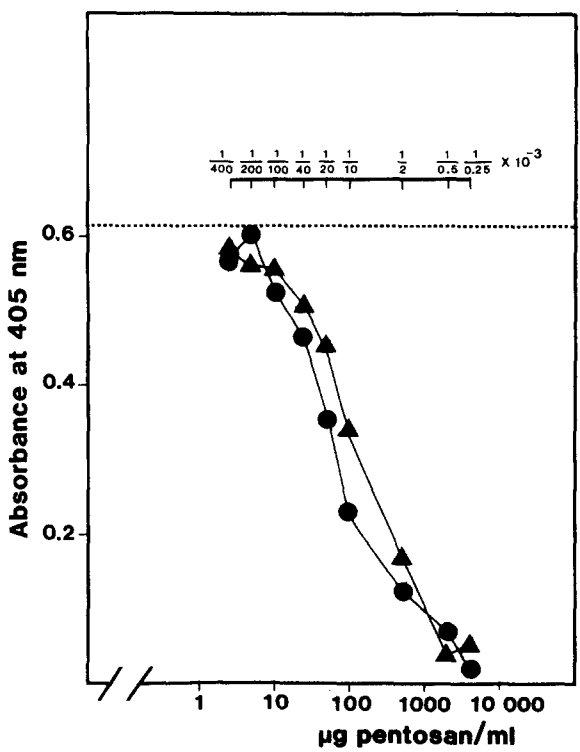

Fig. 10. Standard curves for determining dilutions of pentosans in relation to absorbance readings at 405 nm with sandwich inhibition ELISA. ▲, Original pentosan preparation; ●, new pentosan preparation; -------, represents 5 μg/ml of fraction II, without inhibiting substances. The bar shows the dilutions for the pentosan preparations.

extracted in water (SE). However, after pancreatin digestion of this extract (SD) certain inhibition of fraction II was measured. This inhibition, at least partially, could be due to digestion of the adsorbed antibodies by active pancreatin still present in the extract. Consequently, the new pentosan preparation, precipitated with 80% ethanol from the extract (SD), was produced to eliminate the pancreatin activity. This preparation also demonstrated inhibition of fraction II. As can be seen from Fig. 10, the slopes of the curves were very similar for the original pentosan and the new pentosan preparations, indicating that the same molecule was measured in both cases. Control experiments with ethanol fractionation of pancreatin solutions showed no inhibitory activity in the sample prepared from dissolved filter deposits, which proves that the observed inhibitions are due to pentosan binding.

This means that, when sandwich inhibition ELISA was used, the original pentosan preparation was capable of binding to the solid-phase antibodies, thus inhibiting the fraction II introduced later from binding to any immobilised antibodies except the remaining vacant ones. The conjugate, however, could bind only to fraction II. Thus, only the amount of bound fraction II was detected, and this amount decreased as the concentration of the original pentosan preparation increased. The supernatant preparations (SW-1, SW-2, SW-3 and SE) were all non-inhibiting; and only when starch and protein were digested by pancreatin, was a preparation (SD) obtained that demonstrated inhibition of fraction II binding. The antigenic determinants in the undigested (SW-1, SW-2, SW-3 and SE) appear to be hidden and become reactive for binding at one site in sandwich inhibition ELISA only after degradation of protein and starch with pancreatin as is the case in digested (SD) rye extract, original and new pentosan preparations. It is possible that pancreatin is able to digest the starch and/or protein that interfere with the antibody/antigen and antigen/conjugate binding. Further fractionation of the original pentosan preparation shows that a small fraction, fraction II, exposes at least two determinants that simultaneously can bind to the antibodies and consequently can be assayed in a sandwich ELISA.

For wheat protein, wheat starch and the monosaccharides arabinose, xylose and galactose, no inhibition was demonstrated.

CONCLUSIONS

The above results show that a sandwich ELISA method has been successfully developed for fraction II. To measure water-soluble pentosans with sandwich inhibition ELISA, a standard curve was constructed

from a preparation of known amounts of pentosans. It is shown that, by this procedure, pentosan concentrations in the range 20–1000 μg/ml could be determined. However, it is necessary to prepare the sample from rye flour with pancreatin digestion and ethanol precipitation before determining pentosans. This extra step lengthens the time required for analysis by 1 day. The digestion time with pancreatin has, however, not been optimised.

In conclusion, the sandwich inhibition ELISA method described above seems to be a sensitive method that may be useful for determining the pentosans in cereals; for instance, in plant breeding programmes.

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