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ALLERGENIC GLUCANS FROM DERMATOPHYTES

PART II. ENZYMIC DEGRADATION

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ABSTRACT

A glycogen-like structure is proposed for an allergenic glucan isolated from the mycelium of *Trychophyton rubrum* on the basis of the identification of oligosaccharides obtained by hydrolyses with alpha- and beta-amylase and pullulanase. This glucan has a completely different structure from glucans hitherto isolated from dermatophytes.

INTRODUCTION

Allergenic α-D-glucans were recently identified[1] as components of the mycelia of the dermatophytes *Trichophyton rubrum*, *T. mentagrophytes*, and *Microsporum canis* grown in surface culture. Preliminary structural studies indicated that these polysaccharides contain (1→4)-linked residues of α-D-glucose. Further structural details of the glucan from *T. rubrum*, as revealed by hydrolysis with alpha- and beta-amylase and pullulanase, are the subject of the present paper.

MATERIALS AND METHODS

The glucan component of the mycelium of *T. rubrum* (surface culture) was isolated and purified as described in the previous paper[1]. alpha-Amylase solution [α-1,4-glucan 4-glucanohydrolase] *ex Bacillus subtilis* and pullulanase [α-D-1,4;1,6-glucan 6-glucanohydrolase] *ex Aerobacter aerogenes* were generous gifts from Ranks, Hovis McDougall. beta-Amylase [α-1,4-glucan maltohydrolase] *ex* sweet potato was obtained from Sigma, glucoamylase [α-1,4-glucan glucohydrolase] *ex Aspergillus niger* from Glaxo Laboratories, and β-D-glucosidase [β-D-glucoside glucohydrolase] *ex* sweet almonds from Koch–Light Laboratories Ltd.

Fractionation of hydrolysates of T. rubrum *glucan: general methods.* — Product mixtures were concentrated to dryness at ambient temperature under reduced pressure and were fractionated by elution with water from Dowex-AG50W x2(Li^+) resin[2].

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Carbohydrate components in the eluates were detected by reaction with cysteine and sulphuric acid[3]. In all cases, preliminary experiments were carried out to determine the conditions for maximal hydrolysis of the glucan.

Treatment of T. rubrum *glucan with pullulanase.* — Aliquots (0.5 ml) of a solution of pullulanase[4] (100 mg) in 0.02M phosphate buffer (pH 6.8, 3 ml) were added to a solution of the glucan (5.8 mg) in 0.02M phosphate–citrate buffer (pH 5.0, 4.5 ml) and to buffer (4.5 ml) alone. The solutions were dispensed through Millipore filters (pore size, 0.45 μm) into sterile, screw-capped bottles and were maintained at 35° for 14 h. The solutions were heated at 100° for 5 min and then concentrated to dryness. Solutions of the products in water (0.5 ml) were separately eluted from a column (95 × 1.3 cm) of Dowex-AG50W x2(Li^+) resin.

Sequential treatment of T. rubrum *glucan with pullulanase and alpha-amylase.* — Pullanase solution (0.5 ml) was incubated with the glucan (6.1 mg) and with buffer alone under sterile conditions, as described above. The solutions were heated at 100° for 10 min, the pH of the solutions was adjusted to pH 6.5 with sodium hydroxide solution, and an aliquot (1.0 ml) of a solution (10 ml) of alpha-amylase [previously dialysed at 4° against 0.02M phosphate buffer (pH 6.5)] was added to each. The solutions were sterilised by filtration and were maintained at 37° for 15 h, prior to heating at 100° for 5 min. The solutions were centrifuged and concentrated to dryness, and solutions of the products in water (0.5 ml) were separately eluted from a column (97 × 1.3 cm) of Dowex-AG50W x2(Li^+) resin.

Isolation and characterisation of oligosaccharides from T. rubrum *glucan following reaction with alpha-amylase.* — A solution of the glucan (50 mg) in 0.02M phosphate buffer (pH 6.5, 40 ml) was incubated for 15 h at 37° with a solution (10 ml) of alpha-amylase under sterile conditions as described above. The product mixture was heated as described and, after concentration, was eluted from a column (57.5 × 5 cm) of Dowex-AG50W x2(Li^+) resin. Four components (**1**–**4**) were isolated. Components **2** and **3** were further purified by elution from a column (94 × 1.3 cm) of Dowex-AG50W x2(Li^+) resin.

Component **1**. — Aliquots of a solution of Component **1** were analysed for glucose by using the cysteine–sulphuric acid reaction[3], and for D-glucose by using D-glucose oxidase[5].

Component **2**. — (*a*) *Oxidation with alkaline hypoiodite.* An aliquot (1 ml) of a solution of purified **2** (627 μg of glucose/ml) was diluted with water (1.5 ml) and was reacted with a solution (0.5 ml) of 0.1M iodine in aqueous potassium iodide (40 g/l) and 0.2M sodium hydrogen carbonate (1 ml) buffered to pH 10.6 with sodium hydroxide. After 3 h at 25°, silver carbonate was added and the solution was centrifuged and treated with Dowex-AG50W x8(H^+) resin (5 g) at 4° for 1 h. The filtered solution was concentrated to dryness. A solution of the product in water (1 ml) was titrated to pH 8 with sodium hydroxide and, after 15 min, was eluted from a column (105 × 0.5 cm) of Dowex-AG1 x8 resin (acetate form) with an approximately linear gradient (0→0.2M) of ammonium acetate. Aliquots of the fractions (2 ml) of the eluate were analysed for carbohydrate[3]. The product aldonic acid was recovered by sequential

treatment of the solution with Dowex-50W x8(H^+) resin, evaporation to dryness, and removal of acetic acid by repeated evaporation of water from the residue. Aliquots of a solution of the sodium salt of the aldonic acid were analysed for glucose[3], and for formaldehyde[6] and glyoxylic acid[7] liberated on periodate oxidation[7].

(*b*) *Treatment with glucoamylase and β-D-glucosidase.* Solutions of glucoamylase (1 mg/ml) in M ammonium acetate buffer (pH 5) and of β-D-glucosidase (2 mg/ml) in 0.02M potassium phosphate buffer (pH 6.5) were clarified by centrifugation. Aliquots (0.1 ml) of solutions of maltose (400 μg/ml), cellobiose (400 μg/ml), and Component **2** (4.62 mg of glucose/ml) were diluted to 0.5 ml with the appropriate buffer and were incubated at 50° with solutions (0.5 ml) of each of the enzymes. Solutions of the enzymes without substrates were treated similarly as controls. At intervals during the period of incubation (5 h), the D-glucose contents of the test and control solutions were determined[5] after heating at 100° for 5 min. The total glucose content of the solutions of the substrates was determined[3].

Component 3. — (*a*) *Preparation of the derived aldonic acid.* The aldonic acid derivative of 3 was prepared and fractionated by elution from Dowex-AG1 x8 resin (acetate form), essentially as described for the corresponding derivative of **2**. The sodium salt of the product aldonic acid was analysed for glucose, and for formaldehyde and glyoxylic acid liberated on periodate oxidation.

(*b*) *Oxidation with periodic acid.* Aliquots (0.05 ml) of a solution (448 μg/ml) of 3 were reacted with 25mM periodic acid (0.1 ml) for 26 h in the dark at ambient temperature. Excess periodic acid was destroyed with M sodium metabisulphite (0.05 ml), and the solutions were analysed for glucose[8].

(*c*) *Smith degradation of the derived aldonic acid.* A solution (4.8 ml) of the aldonic acid (171 μg of glucose/ml) was reacted with 10mM periodic acid (4.8 ml) in the dark at ambient temperature and the reduction of periodic acid was measured spectrophotometrically[9]. Aliquots (0.1 ml) of the solution were analysed for formaldehyde[6] and glyoxylic acid[7] at intervals during 45 h, after which time the solution was neutralised by the addition of barium carbonate, centrifuged, and reacted with sodium borohydride (20 mg) which was added during 3 h at room temperature. Dowex-50W x8(H^+) resin (4 g) was added, and after 1 h at 4° the solution was filtered and concentrated to dryness by evaporation under diminished pressure at 40°. Boric acid was removed by repeated distillation of methanol from the residue. The product was hydrolysed with 0.5M sulphuric acid (7 ml) for 4 h at 100° in a sealed tube, and the hydrolysate was neutralised ($BaCO_3$), deionised with Dowex-50W x8(H^+), and diluted to 10 ml with water. An aliquot (0.5 ml) of this solution was fractionated by elution with water from a calibrated column[10] of Dowex-AG1 x8 resin (molybdate form, 53 × 0.6 cm), and the eluate was monitored continuously by a spectrofluorimetric analysis for formaldehyde[11] liberated on periodate oxidation. The remainder of the solution (9.5 ml) was concentrated nearly to dryness at ambient temperature, and a solution of the product in 86% ethanol (1 ml) was eluted with that solvent from a calibrated column[10] of Dowex-AG1 x8 resin (sulphate form, 64 × 0.6 cm). Part of the eluate was monitored for formaldehyde liberated on periodate oxidation and the

remainder was collected automatically in fractions. Components with the same elution characteristics as glycerol and erythritol were isolated and separately converted into *O*-trimethylsilyl (Me_3Si) derivatives[12] which were analysed by g.l.c., using a Pye 104 instrument with a column packing of 10% SE-30 on siliconised Celite (100–200 mesh, Pye-Unicam Ltd.) at 151° (erythritol) and 123° (glycerol), respectively.

(*d*) *Alkaline degradation.* An aliquot (1.2 ml) of a solution of **3** (433 μg of glucose/ml) was concentrated to dryness at ambient temperature. The product was dissolved in 20mM barium hydroxide (2.7 ml) and the solution was maintained at 25° for 96 h in a sealed tube. Dowex-50W x8(H^+) resin (5 g) was added and the solution was filtered and concentrated to dryness. The product was dissolved in water (1 ml), and the solution was titrated to pH 8 with sodium hydroxide and after 15 min was eluted from a column (19 × 0.5 cm) of Dowex-AG1 x8 resin (acetate form), using an approximately linear gradient of ammonium acetate. Fractions (0.96 ml) were analysed for glucose[3] and for formaldehyde[6] liberated on periodate oxidation. Appropriate fractions were analysed for 2-*C*-(hydroxymethyl)-3-deoxypentonic acid (isosaccharinic acid) and for 3-deoxyhexonic acid (metasaccharinic acid)[13].

Fractions that gave positive colour reactions for isosaccharinic acid were combined and treated with Dowex-50W x8(H^+) resin, followed by removal of acetic acid by evaporation. Hydrochloric acid (1 drop) was added to a solution of the residue in the minimal amount of water and the solution was concentrated to dryness by heating at 50° for 30 min under diminished pressure. The product and a sample of authentic isosaccharino-1,4-lactone were separately converted into Me_3Si derivatives[12] which were analysed by g.l.c. at 177° as described above. The absence of metasaccharino-1,4-lactone in the test sample was confirmed by subsequently increasing the temperature of the column to 200° and maintaining that temperature for 15 min.

(*e*) *Treatment with glucoamylase and β-D-glucosidase.* Component **3** was reacted with the two enzymes essentially as described for **2**.

Reaction of the T. rubrum *glucan with beta-amylase: characterisation of maltose and reaction of the beta-limit dextrin with pullulanase.* — A solution of the glucan (8 mg) in water (4 ml) was mixed with 0.4M acetate buffer (pH 4.8, 4 ml) and a suspension of beta-amylase (10 μl, 200 μg of enzyme as supplied). The solution was maintained at 37° and aliquots (0.1 ml) were analysed for reducing sugars[14] at intervals during 4 h. The solution was heated at 100° for 5 min, cooled, and centrifuged prior to elution with water from a column (96 × 1.3 cm) of Dowex-AG50W x2(Li^+) resin and analysis of the eluate for glucose[3]. The contents of appropriate tubes were combined and concentrated to dryness by rotary evaporation or freeze-drying. The oligosaccharide component thus obtained was characterised as maltose by using the methods described above for the characterisation of **2** obtained on treatment of the glucan with alpha-amylase. The experiment was repeated on a larger scale to obtain the beta-limit dextrin for further structural studies.

Purification and assay of pullulanase. — Pullulanase was assayed by using pullulan as substrate and determining the amount of maltotriose formed[4].

Crude pullulanase (200 mg), which contained a carbohydrate component of

high molecular weight, was dissolved in 20mM phosphate buffer (pH 6.8) and dialysed against that buffer prior to concentration to 3 ml by ultrafiltration. Pullulanase was purified by sequential elution of the solution from columns of Bio-Gel P-150 (75 × 2.4 cm) and DEAE-Sephadex A-50 (chloride form, 79 × 2.4 cm). Fractions that showed pullulanase activity were combined and dialysed against 20mM phosphate buffer (pH 6.8). The solution (50 ml) of purified pullulanase thus obtained[4] had an activity of 0.66 unit/ml and contained a relatively small proportion of carbohydrate.

Reaction of the beta-limit dextrin with purified pullulanase. — An aliquot (24 ml) of a solution of the dextrin (27 mg) in 20mM phosphate–citrate buffer (pH 5, 25 ml) was added to the solution of purified pullulanase (20 ml) and buffer (6 ml). The solution was sterilised by filtration and was maintained at 37° for 20 h. Pullulanase solution (1 ml) and buffer (1.5 ml) were treated similarly as a control. The solutions were heated at 100° for 5 min and the test solution was concentrated to 5 ml prior to elution with water from a column (93 × 4.5 cm) of Dowex-AG50W x2(Li^+) resin. Aliquots of fractions (10 ml) were analysed for glucose[3] and three fractions (*P1*, *P2*, *P3*) were obtained by combining the contents of appropriate tubes. The control solution was fractionated similarly.

Characterisation of P1 *and* P2. — Fraction *P1* was analysed by paper chromatography (Whatman No. 1 paper), using 1-butanol–pyridine–water[15] (6:4:3), and for hexose (as glucose[3]) and D-glucose[5]. The components of *P1* were converted into aldonic acid derivatives which were separated and isolated by elution of the product mixture from Dowex-AG1 x8 resin (acetate form) and were analysed for glucose, and for formaldehyde and glyoxylic acid released on periodate oxidation. Fraction *P1* was reacted with glucoamylase and β-D-glucosidase, as previously described. Fraction *P2* was oxidised with 10mM periodic acid and was analysed for residual glucose[8] after 24 and 120 h. It was also treated with beta-amylase, essentially as described above for the glucan, and the product mixture was eluted with water from Dowex-AG50W x8(Li^+) resin. Aliquots of the eluate were analysed for hexose (as glucose) and for D-glucose. Oligosaccharide components in the eluate were characterised in the same way as described for the components from *P1*.

Characterisation of P3. — A solution of *P3* in 20mM Tris–hydrochloric acid buffer (pH 7.6) was eluted from a column of DEAE-Sephadex A-50 (chloride form, 56 × 2.4 cm) with that buffer, and the eluate was monitored for carbohydrate[3]. Two fractions (*P3a*, *P3b*) thus obtained were treated with beta-amylase as described for the glucan, and the product mixtures were eluted with water from Dowex-AG50W x2 (Li^+) resin. The oligosaccharides of lower d.p. thus obtained were converted into their aldonic acid derivatives which were fractionated and analysed, as described above for *P1*, and were also reacted with glucoamylase and β-D-glucosidase. The oligosaccharides of higher d.p. that were obtained on treatment of *P3a* with beta-amylase were reacted with 20mM periodic acid, and the reduction of periodate was measured[16] at intervals during 25 h. Aliquots of the solution were then analysed for glucose[8], and the solution was neutralised ($BaCO_3$), centrifuged, and then reacted with excess of sodium borohydride added during 3 h. The product, isolated as

described for **3**, was hydrolysed with 0.5M sulphuric acid at 100° for 4 h. Aliquots of the neutralised ($BaCO_3$) hydrolysate were analysed for erythritol and glycerol by chromatography on Dowex-AG1 x8 resins (molybdate and sulphate forms[10]). The identities of the components in the hydrolysate were confirmed by g.l.c. analysis of their Me_3Si derivatives.

RESULTS

Oligosaccharides that were produced by enzymic degradation of the glucan from *T. rubrum* were conveniently isolated by elution of the product mixtures with water from Dowex-AG50W x2(Li^+) resin. Fractionation of the enzymes alone by this method showed that the oligosaccharide components of such mixtures were not derived from the enzymes. A small proportion of the glucan was degraded by pullulanase to oligosaccharides of relatively small d.p., thus indicating the presence in the glucan of α-(1→6)-linked residues of D-glucose flanked by α-(1→4)-linked residues but excluding a pullulan-type structure for the glucan. Treatment of the glucan with alpha-amylase resulted in more-extensive degradation to at least four oligosaccharide components, one (**4**) of which was absent from the product mixture obtained by sequential treatment of the glucan with pullulanase and alpha-amylase. Component **4** is thus an oligosaccharide containing one or more α-(1→6)-D-glucoside linkage susceptible to hydrolysis by pullulanase. Components **1**, **2**, and **3** represented 29.5% of the glucan. Component **1** was identified as D-glucose from its reactions with cysteine and sulphuric acid and with D-glucose oxidase.

The aldonic acid derivative of **2** was purified by elution from Dowex-AG1 x8 resin (acetate form). The ratio of glucose in the aldonic acid derivative of **2** to formaldehyde and glyoxylic acid liberated on periodate oxidation was 1.0:0.88:0.89. This is consistent with a 4-*O*-D-glucosyl-D-glucose structure for **2**. Component **2** was hydrolysed quantitatively to D-glucose by glucoamylase, but was not hydrolysed by β-D-glucosidase. Since the glucoamylase preparation contained some β-D-glucosidase activity, it was necessary to incubate **2** with both enzymes to confirm that **2** was maltose (4-*O*-α-D-glucopyranosyl-D-glucose).

Similar methods were used to characterise **3**. The ratio of glucose in the aldonic acid derivative to glyoxylic acid and formaldehyde released on periodate oxidation was 1.0:0.43:0.48. This is consistent with a linear trisaccharide structure for **3**, in which the reducing glucose unit is linked through O-4. All the glucose residues in **3** were oxidised by periodate, thus precluding the presence of (1→3)-linked residues. The results of periodate oxidation of the aldonic acid derivative of **3** are summarised in Table I. Acid hydrolysis of the alcohol obtained on treatment of the oxidised aldonic acid derivative with borohydride liberated erythritol and glycerol in the molar ratio 1.0:0.97. These data are consistent with a trisaccharide structure of the type *O*-D-glucopyranosyl-(1→4)-*O*-D-glucopyranosyl-(1→4)-D-glucose for **3**. The trisaccharide was hydrolysed quantitatively to D-glucose by glucoamylase, but was not hydrolysed by β-D-glucosidase, thus indicating that it was maltotriose. Reduction

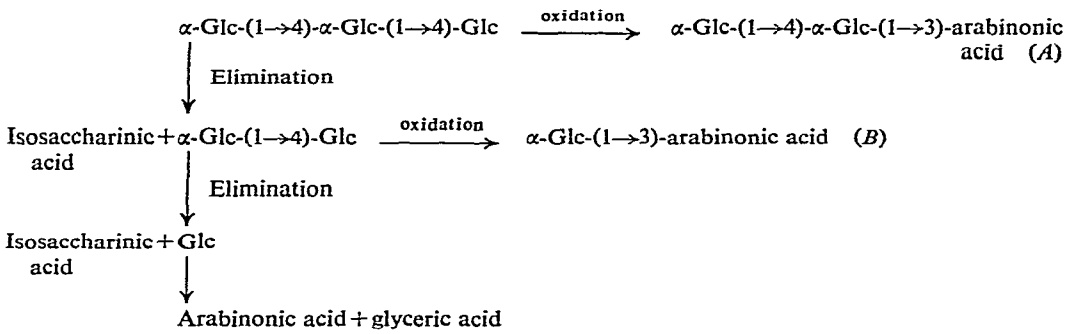
of periodate by the aldotriouronic acid derivative of 3, in excess of the theoretical amount, is due in part to oxidation of the product glyoxylic acid (see Table I).

TABLE I

PERIODATE OXIDATION OF THE ALDONIC ACID DERIVATIVE OF COMPONENT 3

Periodate consumed (moles/mole of aldotriouronic acid)	*Products*	*Moles/mole of aldotriouronic acid, after reaction for*		
		0.5 h	*8 h*	*45 h*
5 (after 8 h)	Formaldehyde	0.97	1.00	1.03
6 (after 45 h)	Glyoxylic acid	0.95	0.95	0.74

Alkaline degradation of the trisaccharide in the presence of air gave a mixture of acidic products including two *O*-glucosylaldonic acids, both of which liberated formaldehyde on periodate oxidation. Two other components of the product mixture were tentatively identified as arabinonic and glyceric acids, and a third component was identified as isosaccharinic acid by reaction with 2-thiobarbituric acid after periodate oxidation and by g.l.c. of the Me_3Si derivative of the corresponding lactone. Metasaccharinic acid was not identified in the products of alkaline degradation of 3. The identification of these five acidic products is consistent with the proposed linkage sequence in 3 (see Fig. 1).



Product	*Reaction in*	
	L-Cysteine–sulphuric acid assay	*Periodate–formaldehyde assay*
A	++	+
B	+	+
Isosaccharinic acid	−	++
Arabinonic acid	−	+
Glyceric acid	−	+

Fig. 1. Acidic products obtained by alkaline degradation of 3 (maltotriose) in the presence of air.

Incubation of the glucan with beta-amylase liberated a limit dextrin and maltose (equivalent to 31.9% of the glucan) which was characterised from the ratio (1.0:1.05: 0.95) of D-glucose in the derived *O*-glycosylaldonic acid to glyoxylic acid and formaldehyde liberated on periodate oxidation, and by quantitative hydrolysis by glucoamylase to D-glucose. Further degradation of the limit dextrin was not achieved by repeated incubation with beta-amylase, but incubation with pullulanase caused significantly more fragmentation than was observed on similar treatment of the parent glucan. The product mixture obtained on treatment of the limit dextrin with purified pullulanase was separated into three fractions (*P1*, *P2*, and *P3*) by elution from Dowex-50W x2(Li^+) resin. The components of *P1* were identified as maltose and maltotriose by paper chromatography and enzymic hydrolysis of the parent mixture, and by analysis of the products obtained by periodate oxidation of their isolated aldonic acid derivatives. The amounts of these derivatives indicated that *P1* contained maltose and maltotriose in the molar ratio 1:1.05. Treatment of *P2* with beta-amylase gave maltose and maltotriose (8.1:1) as the sole products.

Residual pullulanase was removed from *P3* by chromatography on DEAE-Sephadex. Two oligosaccharide-containing components (*P3a* and *P3b*) thus obtained were hydrolysed by beta-amylase with the production of maltose (25.5 and 34.5%, respectively) and oligosaccharides. Structural features of the latter type of product obtained from *P3a*, as revealed by oxidation with periodic acid and Smith degradation, are summarised in Fig. 2.

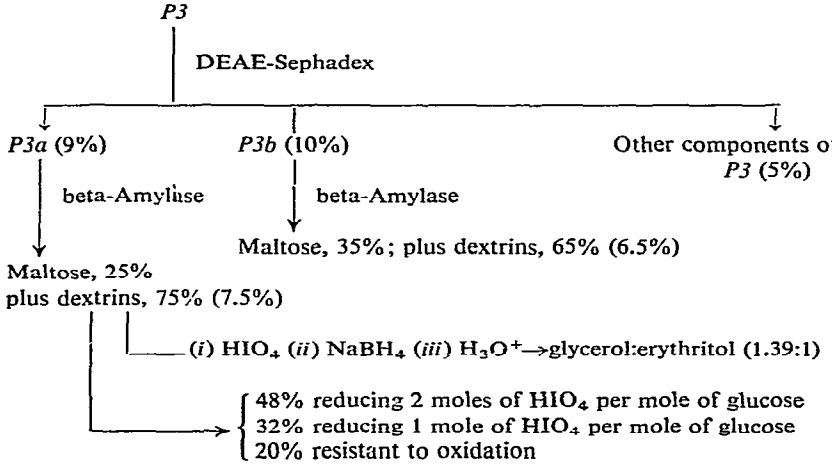


Fig. 2. Characterisation of some of the components of Fraction *P3* obtained from *T. rubrum* beta-limit dextrin by hydrolysis with pullulanase.

DISCUSSION

Hydrolysis with alpha-amylase and pullulanase has shown the presence of α-(1→4)- and α-(1→6)-linked D-glucose residues in the parent glucan from *T. rubrum*. Oligosaccharides obtained by enzyme-catalysed hydrolyses of the glucan were

characterised conveniently by using techniques which we consider are of general application in structural studies of oligosaccharides.

The quantitative identification of D-glucose, maltose, and maltotriose as the products of lower d.p. obtained on treatment of the glucan with alpha-amylase indicates that at least 29.5% of the D-glucose residues in the glucan are linked through C-4. Furthermore, the glucan probably contains sequences of more than seven α-(1→4)-linked residues, since maltosaccharides smaller than maltohexaose are markedly resistant to hydrolysis by alpha-amylase from *B. subtilis*, and there was no accumulation of maltopentaose, the preponderant product obtained on hydrolysis of maltohexaose and maltoheptaose[17]. The close similarity between the amount of maltose (31.9% of the glucan) released on treatment of the glucan with beta-amylase and the total amount of D-glucose, maltose, and maltotriose (29.5%) obtained on treatment with alpha-amylase suggests that the latter products were derived from peripheral chains of α-(1→4)-linked D-glucose residues.

The anionic nature of pullulanase[18] was exploited in the removal of non-diffusible, carbohydrate-containing contaminants from the crude enzyme. Sequential treatment of the glucan with pullulanase and alpha-amylase hydrolysed a dextrin, present in the products obtained on reaction of the glucan with alpha-amylase alone, which therefore contains one or more α-(1→6)-linkages which are susceptible to hydrolysis by pullulanase. The limited hydrolysis of the glucan by pullulanase alone precludes a pullulan-type structure for the glucan. The removal of exterior chains of α-(1→4)-linked D-glucose residues from the glucan by prior treatment with beta-amylase, however, increased the susceptibility of the interior structure of the glucan to hydrolysis by pullulanase. Other workers have shown that, for steric reasons, pullulanase is unable to hydrolyse all the α-(1→6)-linkages in glycogen[4]. Two classes of branch links have also been defined in waxy-maize amylopectin[19]. The first type is directly accessible to the debranching action of R-enzyme; the second becomes accessible only after initial beta-amylolysis. The broad size-distribution of the oligosaccharides obtained on hydrolysis of the beta-limit dextrin with pullulanase suggests that α-(1→6)-linked D-glucose residues are distributed randomly in the beta-limit dextrin. Identification of these oligosaccharides gave further information about the structure of the glucan. Thus, the recovery of equimolar (1:1.05) amounts of maltose and maltotriose (Fraction *P1*) suggests that these were obtained from "stubs" which remain after beta-amylolysis of unbranched, peripheral chains of α-(1→4)-linked D-glucose residues in the glucan. Such chains correspond to the *A* chains in amylopectin and glycogen[19], which are degraded to two- or three-unit "stubs" by beta-amylase according to whether the original chains contain an even or an odd number of D-glucose residues, respectively. If all the maltose and maltotriose released by the sequential actions of beta-amylase and pullulanase on the *T. rubrum* glucan derived from *A* chains, a value for the average length of an *A* chain can be calculated. Thus, if the average number of units in such chains is p, then 3 in every $2p$ D-glucose residues are released as maltotriose, and the ratio of the amount of maltotriose released to the combined amounts of maltose and maltotriose is $3:2p$. The value of p

thus calculated is 6.5. The validity of the assumption that maltose and maltotriose originated only from *A* chains is established by the relatively high molecular weight of oligosaccharides in *P2* and *P3* obtained by treatment of the beta-limit dextrin with pullulanase. Significant proportions of maltotetraose were not obtained. Such products would be expected if the maltose and maltotriose was obtained from *B* chains in an amylopectin-type structure[19].

The identification of maltose and maltotriose as the sole products on reaction of *P2* with beta-amylase shows that this fraction contains maltosaccharides derived from sequences of α-(1→4)-linked D-glucose residues in the interior of the glucan. If it is assumed that the numbers of maltosaccharides containing odd and even numbers of D-glucose residues are equal, the ratio of maltose to maltotriose liberated from *P2* with beta-amylase corresponds to a mean chain-length of 9.5 α-(1→4)-linked D-glucose residues. This value indicates that extensive fragmentation of such chains in the glucan by reaction with alpha-amylase is prevented by steric reasons.

Fractions *P3a* and *P3b*, obtained from *P3* following the removal of residual pullulanase, both contained peripheral sequences of α-(1→4)-linked D-glucose residues, as shown by degradation by beta-amylase. The absence of maltotriose in such product mixtures indicates that it is unlikely that either fraction contains linear, as well as branched, dextrins. The dextrins in *P3a* are probably more highly branched than those in *P3b*, as shown by the greater conversion into maltose in the latter fraction and by the higher proportion of residues in *P3a* which reduce 2 moles of periodate per mole. The molar ratio of glycerol to erythritol (1.39:1) in the Smith-degradation products from *P3a* was in good agreement with the ratio of D-glucose residues reducing 2 moles of periodate per mole to those consuming 1 mole/mole (1.48:1).

These investigations show that at least 80% of the *T. rubrum* glucan can be accounted for as a linear sequence of α-(1→4)-linked D-glucose residues joined by α-(1→6)-linkages such as are present in glycogen. Glycogen-like polysaccharides have not been reported previously as components of dermatophytes, although glycogen is present as a storage material in many other yeasts and fungi[20]. This allergenic glucan from *T. rubrum* has a completely different structure from the glucans isolated from dermatophytes, including *T. rubrum*, by Bishop *et al.*[21,22] who used more-drastic methods of extraction. Thus, those glucans either had low, positive, specific optical rotations or were laevorotatory, and contained (1→6)- and (1→3)-linked D-glucose residues with 1,3,6-branch points. These differences in structural features were further emphasised by studies of the *T. rubrum* glucan using the techniques of periodate oxidation and methylation which will be reported elsewhere.

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