activity was collected and rotary evaporated to dryness. The residue was extracted (∗5) with 10 ml of methanol, redissolving the dried residue in water and drying before each extraction. The pooled methanol extracts were dried under vacuum, redissolved in 1 ml of methanol/chloroform/2.5 M ammonium hydroxide (40:60:8, by vol.) and run onto a silica gel column (50 cm × 1 cm) equilibrated with the same solvent at 0.5 ml/min. Fractions (10 ml) were collected and inhibitor was shown to be eluted between fractions 3 and 10. Fractions 4-7 inclusive were pooled, rotary evaporated to dryness then redissolved in ammonium bicarbonate (10 mm). The sample was finally purified by h.p.l.c. using a C18 (Spherisorb ODS, 25 cm × 4.6 mm) column equilibrated in 10 mm-ammonium bicarbonate and run at 1.5 ml/min. After loading of the sample, a linear gradient from 10% to 50% methanol was applied over a 25-min period. The inhibitor eluted between 11 and 13 min and gave a single spot (Rf = 0.22) t.l.c. silica plates in methanol/chloroform/2.5 M-ammonium bicarbonate (40:60:8, by vol.), when developed with 50% (v/v) sulphuric acid. Development with phenol sulphuric acid gave a brown spot and carbazole sulphuric acid gave a violet spot, indicating the presence of a sugar. Positive fast atom bombardment mass spectroscopy on the purified material gave an M, of 323. Further study using chemical ionization of the trimethylsilylated derivative and analysis by nuclear magnetic resonance has provided the proposed structure shown in Fig. 1.

Methanolic extracts of the algae Mesotaenium calcicantorum and Mougeotia sp. have been shown to inhibit α-glucosidase. For both extracts the active components have been extensively purified by chromatography on Sephadex LH 20 (41 cm × 1.6 cm) in 95% (v/v) methanol/5% (w/v) ammonium bicarbonate/100 mM β-mercaptoethanol, followed by chromatography on silica in water containing 100 mM-β-mercaptoethanol for Mesotaenium calcicantorum and 80% (v/v) water/20% (v/v) methanol/100 mM-β-mercaptoethanol for Mougeotia sp. The active fractions were collected and each showed a single spot on silica (t.l.c. (butanol/acetic acid/water, 2.8: 1:1, by vol.) when developed with 50% (v/v) sulphuric acid. However, in both cases, attempts at further analysis by h.p.l.c. and mass spectroscopy were unsuccessful due to the consistent loss of activity on concentration or drying of the sample. Concurrent with the loss of activity was the appearance of a reddish brown precipitate in each sample. Such changes are symptomatic of the polymerization of tannins, which can be caused by air oxidation [4], a not uncommon problem associated with the purification of tannins. Use of excess β-mercaptoethanol did not prevent this problem. Treatment of the initial extracts with polyvinylpyrrolidone resulted in the loss of enzyme inhibitory activity in both cases and, with t.l.c., the active components gave a positive reaction with K3Fe(CN)6/FeCl3 spray, which supported the hypothesis that the active compounds were tannins. The non-specific precipitation of proteins by tannins have been well documented [5], but, since in both cases methanol extracts of these algae did not inhibit other enzymes tested it seems that these tannins inhibit α-glucosidase specifically. Indeed, we have previously shown that the tannin pentagalloylglucose from Sphiregira varians specifically inhibits α-glucosidase [2] and others have suggested a role for tannins as specific enzyme inhibitors [6].


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The N-linked oligosaccharides of bovine skin proteodermatan sulphate

PAUL G. SCOTT and CAROLE M. DODD
Department of Oral Biology, University of Alberta, Edmonton, Alberta T6G 2N8, Canada

A small proteoglycan, referred to as proteodermatan sulphate (PDS) or dermatan sulphate proteoglycan, consisting of a protein core of approximate M, 40,000 carrying one dermatan sulphate chain [1], is found in many fibrous connective tissues [2]. It has been shown to be regularly arrayed on the surfaces of collagen fibrils [3], giving rise to suggestions that it may regulate fibril diameter or prevent calcification of normally non-calciﬁed connective tissues. Complete deglycosylation of PDS puriﬁed from mature bovine skin with hydrogen ﬂuoride yields a smaller protein core than that produced by digestion of the glycosaminoglycan alone with chondroitinase ABC [4], indicating the presence of oligosaccharides. Nakamura et al. [5] reported that PDS from newborn calf skin carried three O-linked oligosaccharides and probably one N-linked oligosaccharide. Fibroblasts from human skin synthesize a PDS which appears to carry two or three N-linked oligosaccharides but no O-linked oligosaccharides [6]. The present investigation was undertaken to establish the number, locations and chemical nature of the oligosaccharides on PDS from mature bovine skin.

The protein core of PDS was prepared [4], and peptides made from it by incubation at a concentration of 1 mg/ml in 70% (w/v) formic acid with CNBr (12 mg/ml), under nitrogen, for 4 h at room temperature. The peptides were separated on a 1 cm × 102 cm column of Sephadex G-100 Superfine (Pharmacia) eluted with 0.01% (v/v) trifluoroacetic

Abbreviation used: PDS, proteodermatan sulphate.

Fig. 1. Proposed structure for the α-amylase inhibitor produced by Anabaena flo-taquae
forms, juvenile (JCL), late-infantile (LICL) and infantile (ICL), can be distinguished on the basis of clinical course. The ceroid-lipofuscinoses (CL) (Batten’s disease) are a group of diseases characterized by the accumulation of tertiary lysosomes containing a storage material insoluble in water. Neurophysiological findings and ultrastructural appearance suggest that the storage material consists of high-mannose type oligosaccharides. The primary biochemical abnormalities underlying these disorders have yet to be discovered, but recent studies have shown that concentrations of phosphorylated dolichol are much higher in brains from each form of the disease than in age-matched controls. The solubility properties of the accumulating phosphorylated dolichol in JCL and LICL brain are consistent with their being present largely in the form of dolichyl pyrophosphoryl phosphorylated oligosaccharides (Dol-PP-OS). [2]. The structures of the oligosaccharide chains from JCL and LICL suggest that they are derivatives of dolichol-linked glycosylation intermediates [5, 6]. After liberation from Dol-PP-OS, by mild-acid hydrolysis, at least 12 different oligosaccharides were identified by their reactivity on nitrocellulose paper with concanavalin A. The compositions preclude the presence of significant proportions of high-mannose type oligosaccharides, suggested as possible components of PDS synthesized by human skin fibroblasts [6]. Sialic acid was not detected on chemical analysis. The primary biochemical abnormalities underlying these disorders have yet to be discovered, but recent studies have shown that concentrations of phosphorylated dolichol are much higher in brains from each form of the disease than in age-matched controls. The solubility properties of the accumulating phosphorylated dolichol in JCL and LICL brain are consistent with their being present largely in the form of dolichyl pyrophosphoryl phosphorylated oligosaccharides (Dol-PP-OS). [2]. The structures of the oligosaccharide chains from JCL and LICL suggest that they are derivatives of dolichol-linked glycosylation intermediates [5, 6]. After liberation from Dol-PP-OS, by mild-acid hydrolysis, at least 12 different oligosaccharides were identified by their reactivity on nitrocellulose paper with concanavalin A. The compositions preclude the presence of significant proportions of high-mannose type oligosaccharides, suggested as possible components of PDS synthesized by human skin fibroblasts [6]. Sialic acid was not detected on chemical analysis.