Glycosidase inhibitors from algae

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Previous studies in our laboratory have shown microalgae and cyanobacteria to be a novel source of glycosidase inhibitors. A screen of microalgae and cyanobacteria identified 38 different strains producing inhibitors of α-amylase, α-glucosidase or β-galactosidase [1]. We have previously shown one of the α-glucosidase inhibitors to be pentagalloylgucose [2]. Three further inhibitors are currently under investigation and are described here.

Anabaena flos-aquae produces an extracellular α-amylase inhibitor. The inhibitor was purified using the following procedure. Algae were grown in media BGM 11 [3] for 12–14 days. The culture supernatants were recovered by centrifugation (3900 g), filtered through a Whatman no. 1 filter and rotary evaporated to dryness. The residue from 2.0 litres of culture supernatant was dissolved in 8 ml of ammonium bicarbonate solution (10 mM) and passed through a Sephadex G 15 column (90 cm X 2.4 cm) equilibrated in the same buffer. The peak of enzyme inhibitory

Table 1. Specific activity (nmol/min per mg of protein) of marker enzymes in mouse cerebellum explants maintained in the absence (control) or presence of castanospermine (10 μg/ml)

Results are means of duplicate experiments that agreed within 10%.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Localization</th>
<th>Control</th>
<th>+ Castanospermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate dehydrogenase</td>
<td>Cytosol</td>
<td>2565</td>
<td>2650 (103%)*</td>
</tr>
<tr>
<td>Na⁺,K⁺-ATPase</td>
<td>Plasma membrane</td>
<td>119</td>
<td>111 (93%)</td>
</tr>
<tr>
<td>α-Mannosidase I</td>
<td>Golgi complex</td>
<td>1.29</td>
<td>1.06 (88%)</td>
</tr>
<tr>
<td>Hexosaminidase</td>
<td>Lysosomes</td>
<td>23.4</td>
<td>18.3 (78%)</td>
</tr>
<tr>
<td>α-Glucosidase (pH 6.5)</td>
<td>Rough endoplasmic reticulum</td>
<td>5.00</td>
<td>0.91 (18%)</td>
</tr>
<tr>
<td>α-Glucosidase (pH 4.0)</td>
<td>Lysosomes</td>
<td>1.27</td>
<td>0.36 (28%)</td>
</tr>
</tbody>
</table>

*Compared to corresponding control.
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 mM-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 eluted 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 elution. 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 mM-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 carbazol/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 caldarium and Maugeotia 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 caldarium and 80% (v/v) water/20% (v/v) methanol/100 mM-β-mercaptoethanol for Maugeotia 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 has 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 Spirogyra 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

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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 only 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.