Note

Primary Structure of an *O*-Linked Sugar Chain Derived from Glucose Oxidase of *Aspergillus niger*

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Glucose oxidase (EC 1.1.3.4) catalyzes the oxidation of glucose to produce gluconic acid and hydrogen peroxide, and has been purified from extracts of *Aspergillus niger*.^{1,2)} The enzyme contains 10–16% carbohydrates,^{2–5)} but the arrangement and structures of the carbohydrate moieties of the enzyme have not been identified.

We have investigated the action of *Flavobacterium* sp. endo- β -*N*-acetylglucosaminidase on glucose oxidase from *A. niger*, and found that the enzyme has not only *N*-linked but also *O*-linked sugar chains.⁶⁾ For this paper, we examined the structures of the *O*-linked sugar chains derived from glucose oxidase of *A. niger*.

Glucose oxidase was purchased from Sigma Chemicals Co. (Type VII-S) and further purified to homogeneity by DEAE-Toyopearl 650M column chromatography. For the preparation of *O*-linked carbohydrates, glucose oxidase (30 mg) was treated with 0.3 M NaBH₄ in 0.1 M NaOH at 40°C for 24 hr as described previously.⁶⁾ The carbohydrate released by β -elimination was separated from the protein moiety and desalted by Dowex 50W X-2 (1.8 $\times$ 10 cm) and Dowex 1-X2 (1.8 $\times$ 10 cm) column chromatographies. The neutral sugars were analyzed by high-performance liquid chromatography (HPLC). HPLC for saccharide analysis was done with a Hitachi model L6000 chromatograph equipped with a Shodex RI SE-31. For the separation of oligosaccharides, a column of Shim-Pack ION S-802 (eluted with deionized water at the flow rate of 1 ml/min at 60°C) was used. Analysis of the isolated carbohydrate by HPLC found only one major peak at a position corresponding to monosaccharide (Fig. 1), and this peak

Fig. 1. Separation of the Oligosaccharides Isolated from Alkali-treated Glucose Oxidase by HPLC.

The isomaltooligosaccharide standards of glucose units were prepared from an acid hydrolysate of dextran.⁷⁾ Mono-, monosaccharide; Di-, disaccharide; and Tri-, trisaccharide.

Fig. 2. HPLC Pattern of the Alditol Obtained from Alkali-treated Glucose Oxidase.

The sample was eluted with deionized water at the flow rate of 1 ml/min at 60°C. GlcNAc-ol, *N*-acetylglucosaminitol; Man-ol, mannitol; Glc-ol, glucitol; and Gal-ol, galactitol.

was collected.

Further characterization of the main peak was done by HPLC analysis with a Hitachi GL-C 611 column. Only one peak was obtained at the elution position of mannitol (Fig. 2), and other sugars such as glucitol, galactitol, and *N*-acetylglucosaminitol were not detected. Mannitol was also identified by gas-liquid chromatographic analysis. From these results, the *O*-linked sugar chains of glucose oxidase were mainly present as single mannose residues.

There are only a few reports concerning the presence of *O*-linked neutral sugar chains in fungal glycoenzymes,^{8–10)} and these enzymes were glycosidases such as cellulase and

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amylase. These short *O*-glycosidically linked oligosaccharide units are thought to be essential for the digestion of insoluble substrates.¹¹⁾ We found them in the glucose oxidase from *A. niger*, which is an oxidoreductase contained mannosyl-serine and mannosyl-threonine linkages, but the role of the *O*-linked mannosyl residues in glucose oxidase is still obscure. However, the *O*-linked sugar chains may be widely distributed in fungal glycoproteins. Svensson and co-workers had reported that glucoamylase from *A. niger* also contains *O*-linked sugar chains¹²⁾ and these sugar chains are located in a cluster in the threonine and serine-rich region near the C-terminal end.¹³⁾ Recently the amino acid sequence of glucose oxidase from *A. niger* has been identified from the DNA sequence of this gene.¹⁴⁾ The enzyme contains 8 potential sites for *N*-linked glycosylation. However, the glycosylation sites for *O*-linked sugar chains is still unknown. Studies are now in progress to find the glycosylation sites for *N*- and *O*-linked sugar chains in glucose oxidase from *A. niger*.

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