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# Purification and Characterization of Glucose Oxidase from Ligninolytic Cultures of *Phanerochaete chrysosporium*†

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**Glucose oxidase, an important source of hydrogen peroxide in lignin-degrading cultures of *Phanerochaete chrysosporium*, was purified to electrophoretic homogeneity by a combination of ion-exchange and molecular sieve chromatography. The enzyme is a flavoprotein with an apparent native molecular weight of 180,000 and a denatured molecular weight of 80,000. This enzyme does not appear to be a glycoprotein. It gives optimal activity with D-glucose, which is stoichiometrically oxidized to D-gluconate. The enzyme has a relatively broad pH optimum of 4 to 5. It is inhibited by $Ag^+$ (10 mM) and *o*-phthalate (100 mM), but not by $Cu^{2+}$, NaF, or KCN (each 10 mM).**

Hydrogen peroxide ($H_2O_2$) plays an important role in the ligninolytic system of *Phanerochaete chrysosporium*, a basidiomycete extensively used in studies on lignin biodegradation (16, 21). Forney et al. (9, 10) showed a temporal correlation between ligninolytic activity and $H_2O_2$ production. Both $H_2O_2$ production and ligninolytic activity increased when cultures were incubated under 100% $O_2$, and lignin degradation was inhibited by catalase, which metabolizes $H_2O_2$ to yield $O_2$ and $H_2O$ (7). Nutritional parameters which are known to affect ligninolytic activity, such as nitrogen and carbohydrate concentration, were shown to have a similar effect on $H_2O_2$ production (12, 14, 18; C. A. Reddy and R. L. Kelley, *in* C. O'Rear and G. C. Llewellyn, ed., *Biodeterioration 6*, in press). $H_2O_2$-producing periplasmic microbodies were seen only in lignin-degrading cultures, not in nonligninolytic cultures (10). $H_2O_2$-dependent, extracellular, lignin-degrading oxygenases (ligninases) and a demethylase have been demonstrated in ligninolytic cultures of *P. chrysosporium* (2, 11, 15, 24).

Glucose oxidase (EC 1.1.3.4) appears to be an important source of $H_2O_2$ in ligninolytic cultures of *P. chrysosporium* (Reddy and Kelley, in press), as evidenced by the following observations. Glucose supported the highest level of $H_2O_2$ production in cell extracts. Polyacrylamide gel electrophoresis of these extracts showed the presence of a single protein band that supported glucose-dependent $H_2O_2$ production; this protein band was missing in extracts of nonligninolytic cultures. An $H_2O_2$-producing protein band with similar electrophoretic mobility was observed in cell extracts regardless of whether glucose, cellobiose, xylose, or succinate was employed as the growth substrate. Both glucose oxidase and ligninolytic activities were shown to be secondary metabolic events, and both were triggered in response to nitrogen or carbohydrate starvation (Reddy and Kelley, in press). In this report, we describe the characteristics of a glucose oxidase purified to electrophoretic homogeneity from ligninolytic cultures of *P. chrysosporium*.

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## MATERIALS AND METHODS

**Organisms and culture conditions.** *P. chrysosporium* ATCC 34541 was maintained and conidial inoculum was prepared as previously described (17). Sterile media in foam-stoppered flasks were inoculated with conidial suspensions in water ($1.25 \times 10^6$ conidia per ml, 0.5 ml of inoculum per 10 ml of medium) as previously described (17). The flasks were incubated at 37°C without agitation for 6 days.

**Assay for glucose oxidase.** We determined glucose oxidase activity at 37°C by monitoring the change in $A_{460}$ due to oxidation of *o*-dianisidine by horseradish peroxidase and using a molar extinction coefficient of 8.3 (5). The reaction mixture consisted of 1.5 ml of citrate-sodium phosphate buffer (0.1 M, pH 4.5), 1.0 ml of *o*-dianisidine (0.31 mM), 0.3 ml of a 1 M solution of the substrate (such as D-glucose, L-sorbose, D-xylose, or D-maltose) in water, 0.1 ml of horseradish peroxidase (60 U/ml; EC 1.11.1.7; Sigma Chemical Co., St. Louis, Mo.), and 0.1 ml of glucose oxidase solution. The reaction mixture was bubbled with 100% $O_2$ for 10 min before addition of glucose oxidase.

**Electrophoresis.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of different glucose oxidase preparations was done as described by Laemmli (19) with gel slabs (14 by 18 cm by 1.5 mm) with a 4% stacking gel and a 10% running gel. Samples (50 μl) were placed on the slab gel in 0.05 M Tris hydrochloride buffer (pH 6.7) containing 20% (wt/vol) glycerol, 4% sodium dodecyl sulfate, and 10% 2-mercaptoethanol. Electrophoresis was performed at 30 mA for 3.5 h with a model 3-1014A power supply (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N.J.) set at constant amperage. Gels were stained for protein with Coomassie brilliant blue R250 as previously described (27). Gels were stained for protein-bound carbohydrate by the dansyl hydrazine staining procedure as described by Eckhardt et al. (6).

**Protein and flavin assays.** Protein content was determined by the procedure of Lowry et al. (20) with bovine serum albumin (IV; Sigma) as the standard. Flavin content was determined by the method of Cerletti et al. (4).

**Purification of glucose oxidase.** All purification steps were carried out at 4°C. The pH of the phosphate buffer used was 6.8. Protein solutions were concentrated as needed by ultra-

filtration with an ultrafiltration unit (Amicon Corp., Lexington, Mass.) equipped with a PM-10 filter (10,000-dalton pore size).

**(i) Preparation of cell extracts.** Cultures of *P. chrysosporium* grown in low-nitrogen medium for 6 days at 37°C were collected by filtration on six layers of gauze in a Büchner funnel. The mycelium from 10 liters of culture (~15 g [dry weight]) was washed 3 times with 200 ml of 0.1 M sodium phosphate buffer ($PO_4$ buffer). The washed mycelium was resuspended in 100 ml of the same buffer, mixed with glass beads (0.1 mm in diameter) in a 1:1 ratio (glass beads/mycelium [wet weight]), and blended at 4°C with an Omni-mixer (Ivan Sorvall, Inc., Norwalk, Conn.) for 15 min. The glass beads and unbroken mycelium were removed by centrifugation at 4,080 × *g* for 10 min. The supernatant was saved, and the pellet was suspended in 100 ml of $PO_4$ buffer and blended for an additional 15 min. The supernatants were combined and frozen until needed.

**(ii) DEAE-Sephadex chromatography.** The frozen cell extracts were thawed, clarified by centrifugation at 27,000 × *g* for 15 min at 4°C, and diluted fivefold with distilled water. This protein solution was applied to a DEAE-Sephadex (A50; Pharmacia Fine Chemicals, Piscataway, N.J.) column (50 ml of gel, 2.4- by 16-cm gel bed) previously equilibrated with 0.01 M $PO_4$ buffer. The column was washed with 50 ml of the same buffer, and the protein was eluted stepwise from the column with 100-ml volumes of 10 mM $PO_4$ buffer containing 0.05, 0.10, and 0.25 M NaCl. Fractions (2.25 ml) were collected and tested for glucose oxidase activity as described above. Fractions with the highest activity were pooled and concentrated by ultrafiltration.

**(iii) Sephacryl chromatography.** The concentrated protein from the DEAE-Sephadex step was loaded onto a Sephacryl S-300 column (Pharmacia; 170 ml of gel, 2.4- by 46-cm gel bed) equilibrated with 0.1 M $PO_4$ buffer. The column was eluted with the same buffer at a flow rate of 0.25 ml/min. Fractions (1.5 ml) were collected, and those with the highest activity were pooled.

**(iv) DEAE-Sepharose chromatography.** The pooled fractions were applied to a DEAE-Sepharose CL-6B column (Pharmacia; 20 ml of gel, 1.6- by 20-cm gel bed) previously equilibrated with 0.01 M $PO_4$ buffer. Protein was eluted from the column with a linear salt gradient (440 ml total volume; 0 to 100 mM NaCl) in 0.01 M $PO_4$ buffer. The NaCl concentration in each fraction was calculated by comparing conductivity values with those of NaCl standards. The flow was 0.25 ml/min, and fractions (1.5 ml) were collected and tested for activity.

**Molecular weight determination.** The molecular weight of purified glucose oxidase was determined by gel filtration chromatography with a Sephacryl S-300 column. The column was calibrated with a gel filtration standard containing (molecular weight) thyroglobulin (670,000), gamma globulin (158,000), ovalbumin (44,000), myoglobin (17,000), and vitamin $B_{12}$ (1,350) obtained from Bio-Rad Laboratories, Richmond, Calif. A molecular weight marker kit for sodium dodecyl sulfate gel electrophoresis (MW-SDS-200) was purchased from Sigma.

**Effect of pH.** Citrate-sodium phosphate buffer adjusted to pHs from 3 to 6 was used to determine the optimal pH for glucose oxidase activity. Because pH could affect glucose oxidase activity as well as the peroxidatic oxidation of *o*-dianisidine, glucose oxidase activity in this experiment was determined by monitoring production of $H_2O_2$ directly by the change in $A_{240}$ at 37°C. The reaction mixture consisted of 0.5 ml of citrate-$PO_4$ buffer (0.1 M, pH 3 to 6), 0.1 ml of glucose solution (1 M), and 0.4 ml of glucose oxidase (0.1 mg of protein per ml).

**Enzyme inhibition.** Different metal ions or *o*-phthalate in citrate-$PO_4$ buffer (0.1 M, pH 4.5) was added to the glucose oxidase assay mixture described above. Since KCN is known to inhibit horseradish peroxidase, activity of purified glucose oxidase in the presence of KCN was determined by measuring anaerobic reduction of 2,6-dichlorophenol-indophenol (25).

**Determination of apparent $K_m$ and $V_{max}$.** The apparent $K_m$ for glucose of the purified glucose oxidase was determined by measuring initial velocities over a range of glucose concentrations (2 to 125 mM) at an $O_2$ concentration of 1.6 mM. For determining the $K_m$ for oxygen, reaction mixtures containing 0.1 M glucose in stoppered cuvettes were bubbled with 100, 80, 60, 40, 20, or 10% $O_2$ in $N_2$, which was obtained by mixing the gases through a pair of calibrated flow meters (model 7322; Matheson Scientific, Inc., East Rutherford, N.J.). After bubbling for 10 min at 37°C, the reaction mixtures were allowed to equilibrate for 15 min at 37°C. The initial oxygen concentration in each reaction mixture was determined by measuring the amount of dissolved $O_2$ present in an identically treated parallel cuvette with a biological oxygen monitor (model 5331; Yellow Springs Instrument Co., Yellow Springs, Ohio) equipped with a Clark-type electrode (22). Apparent $K_m$ values were calculated from Lineweaver-Burk plots.

**Quantification of glucose and gluconate.** D-Glucose and D-gluconate were identified and quantified by gas-liquid chromatography. Purified enzyme (0.5 ml containing 0.35 U/ml) was added to a reaction mixture containing 200 μl of catalase solution (1 mg/ml) and 0.5 ml of glucose solution (0.1 M in 0.1 M citrate-phosphate buffer) and incubated for 16 h at 37°C. An internal standard of L-erythritol was added to the reaction mixture prior to sialylation. The reaction mixture was evaporated to dryness and dissolved in methanol (acidified with 50 μl of trifluoroacetic acid per ml). Undissolved material was removed by centrifugation, and the supernatant was evaporated to dryness, dissolved in 0.5 ml of acetonitrile plus 0.5 ml of *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (Pierce Chemical Co., Rockford, Ill.), and heated for 30 min at 70°C. Samples were analyzed with a Varian 3700 gas chromatograph equipped with a flame ionization detector, a Hewlett-Packard 3390A digital integrator, and a glass column (2 m by 0.3 cm) packed with 3% SE-30 on 80/100 mesh Chromosorb W(HP). The carrier gas was He at 25 ml/min, and the chromatograph oven was temperature programmed to hold at 140°C for 10 min and then to increase at 2°C/min to 220°C. D-Glucose and D-gluconate were quantified from peak areas compared with those of the standards.

TABLE 1. Purification data for glucose oxidase from ligninolytic cultures of *P. chrysosporium*

| Fraction | Sp act (U/mg)[a] | Total protein (mg) | Total activity (U) | Yield (%) | Purification (fold) |
|---|---|---|---|---|---|
| Crude cell extract | 0.17 | 285 | 48.4 | 100 | 1.0 |
| DEAE-Sephadex | 0.91 | 32 | 29.1 | 60 | 5.4 |
| Sephacryl S-300 | 6.39 | 3.1 | 19.8 | 41 | 37.8 |
| DEAE-Sepharose | 15.1 | 0.28 | 4.2 | 9 | 89.3 |

[a] Specific activity is defined in units per milligram of protein. One unit of activity represents the oxidation of 1 μmol of *o*-dianisidine per min at 37°C and pH 4.5.

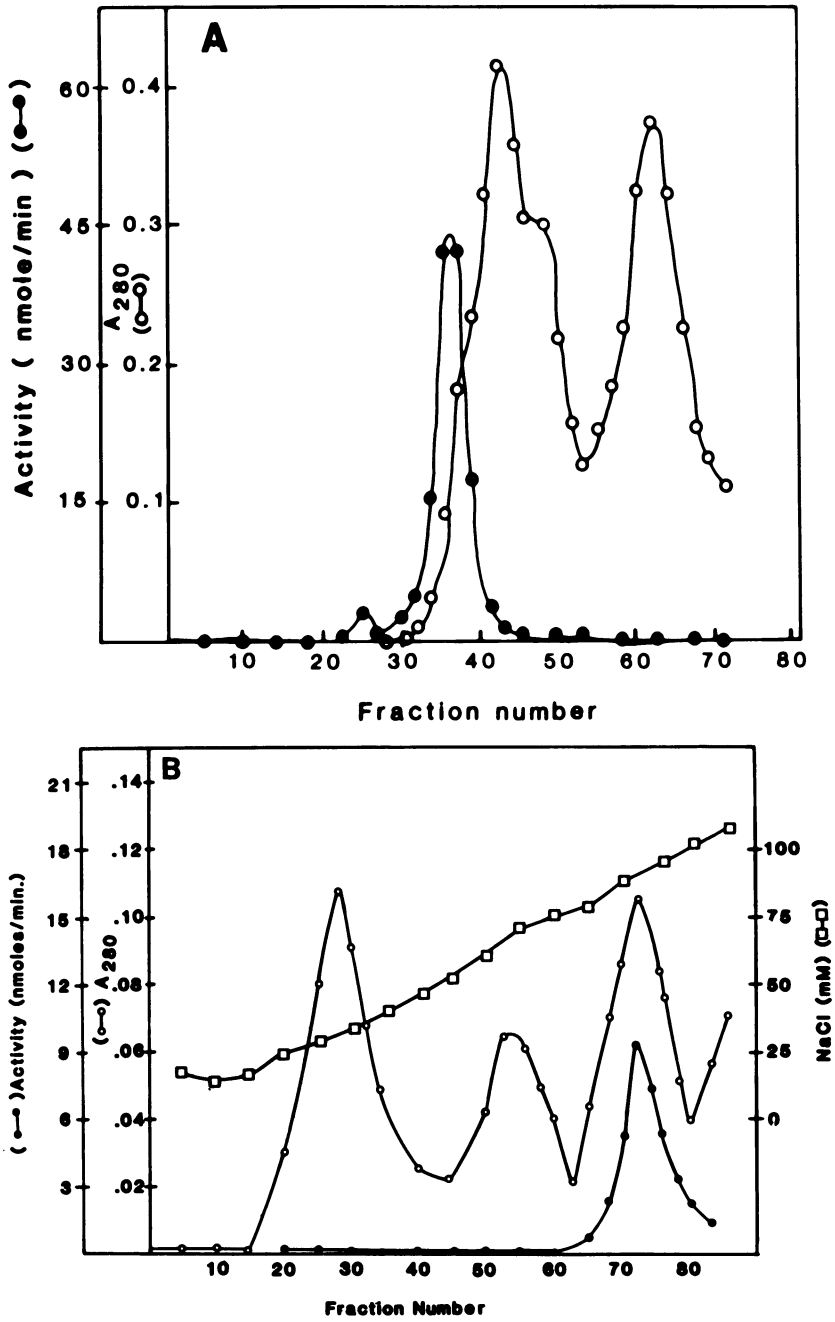


FIG. 1. (A) Elution profile of protein and glucose oxidase activity (nanomoles per minute per milligram of protein) on a Sephacryl S-300 column. Fractions of 1.5 ml were collected and assayed for enzyme activity and protein. (B) Elution profile of protein and glucose oxidase activity from a column of DEAE-Sepharose. Protein was eluted from the column with a linear salt gradient (see Materials and Methods). Each datum point for salt concentration was determined by conductivity measurements. Fractions (1.5 ml) were assayed for glucose oxidase activity and protein content.

## RESULTS

**Purification of glucose oxidase.** The purification of glucose oxidase from *P. chrysosporium* is summarized in Table 1. Purification of about 90-fold was routinely achieved. In the DEAE-Sephadex step, about 60% of the total glucose oxidase activity present in crude cell extracts was recovered in the 0.25 M NaCl eluate, giving about a fivefold increase in specific activity. The subsequent Sephacryl S-300 step (Fig. 1A) produced 38-fold enrichment in specific activity and 41% recovery of total activity. The elution profile from the DEAE-Sepharose column (Fig. 1B) showed that a single protein peak had all the glucose oxidase activity, with approximately 90-fold enrichment in specific activity and enzyme recovery of 9%. The DEAE-Sepharose protein fraction was found to be homogeneous based on SDS-PAGE analysis (Fig. 2).

**Molecular weight.** Based on gel filtration chromatography on a Sephacryl S-300 column, the apparent molecular weight of purified glucose oxidase was estimated to be 180,000 (Fig.

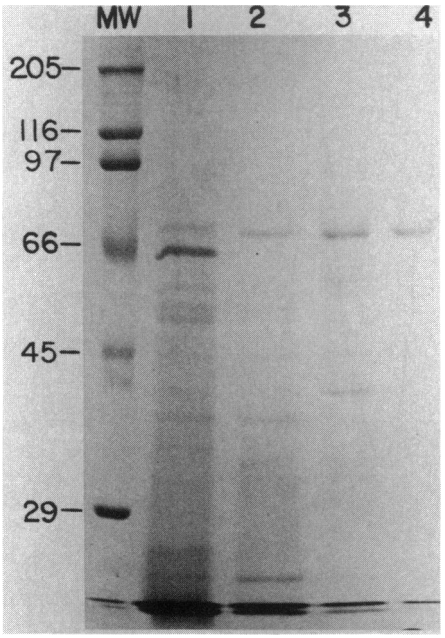


FIG. 2. SDS-PAGE of glucose oxidase preparation obtained from different purification steps (Table 1). Molecular weight standards (lane MW; numbers on the left in thousands), the crude cell extract (lane 1; 0.14 mg of protein), the DEAE-Sephadex fraction (lane 2; 20 μg of protein), the Sephacryl S-300 fraction (lane 3; 9.5 μg of protein), and the DEAE-Sepharose fraction (lane 4; 1 μg of protein) were stained with Coomassie blue as described in the text.

3). The denatured molecular weight, determined by SDS-PAGE, was estimated to be 80,000 (Fig. 4A).

**Carbohydrate and flavin content.** Staining of purified glucose oxidase from *P. chrysosporium* for protein-bound carbohydrate by the dansyl hydrazine method showed no detectable carbohydrate (Fig. 4B, lane 2), whereas an equal amount of commercially prepared glucose oxidase from *Aspergillus niger*, which is known to be a glycoprotein (5, 23), stained positive (Fig. 4B, lane 1). Flavin analysis indicated that the purified enzyme contained 1.5 mol of flavin per mol of protein.

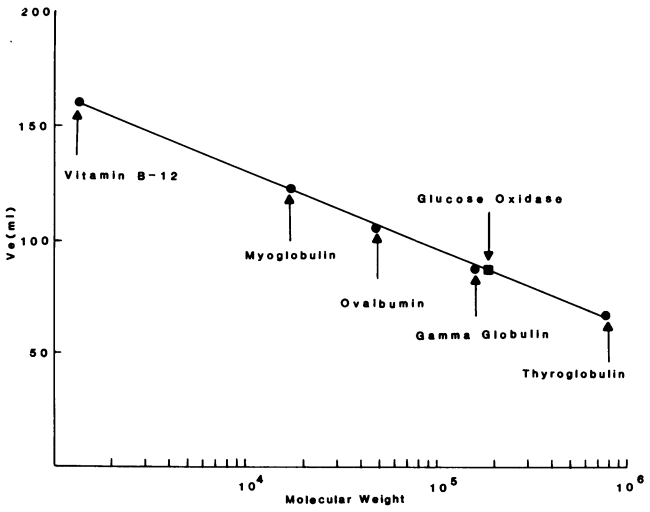


FIG. 3. Molecular weight determination of purified glucose oxidase by gel filtration chromatography with a Sephacryl S-300 column. See the text for details. $V_e$, Elution volume.

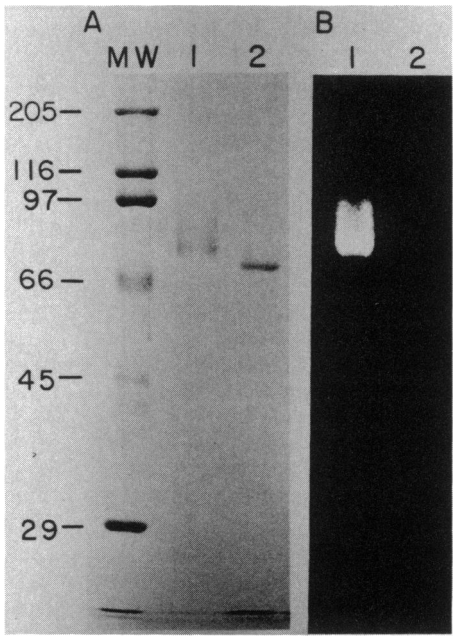


FIG. 4. Molecular weight determination and staining for carbohydrate and protein after SDS-PAGE of commercial glucose oxidase from *A. niger* and that from *P. chrysosporium*. In panel A, molecular weight standards (lane MW; numbers on the left in thousands), *A. niger* glucose oxidase (lane 1) and glucose oxidase from *P. chrysosporium* (lane 2) were stained with Coomassie blue. In panel B, *A. niger* glucose oxidase (lane 1) and glucose oxidase from *P. chrysosporium* (lane 2) were stained for carbohydrate by the dansyl hydrazine method as described in the text. In each panel, 1 μg of the respective enzyme was used.

**pH optimum.** The purified enzyme had a pH optimum between 4.6 and 5.0. In comparison, glucose oxidase from *Penicillium notatum* and *A. niger* were reported to have pH optima of 5.5 and 5.6, respectively (1, 3).

**Enzyme inhibition.** Glucose oxidase from *P. chrysosporium*, like that from *A. niger*, was inhibited by $Ag^+$ but was not inhibited by $CU^{2+}$, KCN, or NaF; in fact, there was substantial stimulation of activity in the presence of the latter (Table 2). Also, glucose oxidase from *P. chrysosporium* was severely inhibited by *o*-phthalate, whereas commercial glucose oxidase from *A. niger* showed only limited inhibition of activity.

TABLE 2. Comparison of the effects of *o*-phthalate, KCN, NaF, and different metal ions on glucose oxidase from *P. chrysosporium* and commercially prepared *A. niger* glucose oxidase

| Addition | Final concn (mM) | % Activity of glucose oxidase from: | |
|---|---|---|---|
| | | *A. niger*[a] | *P. chrysosporium* |
| None | | 100 | 100 |
| KCN | 10 | 128 | 135 |
| NaF | 10 | 101 | 111 |
| $CuCl_2$ | 10 | 133 | 123 |
| $AgSO_4$ | 10 | 36 | 25 |
| *o*-Phthalate | 50 | 86 | 12 |

[a] Commercial *A. niger* glucose oxidase was obtained from Sigma. The concentration of each enzyme preparation was adjusted to give 0.015 U of activity per ml.

**Kinetic properties and substrate specificity.** The apparent $K_m$ values for glucose and $O_2$ for this enzyme were 38 and 0.95 mM, respectively. The $V_{max}/K_m$ data (Table 3) show that glucose is the primary substrate for the enzyme, whereas the others are relatively minor substrates at best. Furthermore, compared with a specific activity of 12.27 μmol/min per mg with glucose as the substrate, the following substrates had ≤1% activity: cellobiose, glycolate, mannose, gluconate, ethanol, acetate, lactate succinate, pyruvate, D-galactose, and β-D-gluconolactone.

D-Glucose was stoichiometrically oxidized to D-gluconate; from 28.6 μmol of D-glucose oxidized, we obtained 26.1 μmol of gluconate, which amounts to 91.2% recovery. These results are in agreement with the results obtained with glucose oxidases from other fungi (1, 3, 5).

## DISCUSSION

Fungal glucose oxidase (β-D-glucose:oxygen oxidoreductase, EC 1.1.3.4) catalyzes the oxidation of D-glucose to δ-D-gluconolactone and $H_2O_2$ in the presence of molecular oxygen (1, 3, 4, 25; Fig. 5). In a subsequent step, δ-D-gluconolactone is nonenzymatically hydrolyzed to D-gluconic acid. This enzyme has been demonstrated in various *Aspergillus* and *Penicillium* species (3, 23, 28). Certain enzymes in animal tissues also catalyze oxidation of D-glucose (or derivatives) to δ-D-gluconolactone, but these are readily differentiated from glucose oxidase because they do not require molecular oxygen and $H_2O_2$ is not a product (28). Glucose oxidase from different fungi has a molecular weight range from 150,000 to 186,000 and normally consists of two identical polypeptide chain subunits covalently linked by disulfide bonds (3, 5, 28).

The glucose oxidase that we isolated from *P. chrysosporium* is a flavoprotein with a native molecular weight of 180,000 and a denatured molecular weight of 80,000. Presumably, this enzyme, like other glucose oxidases, consists of two identical polypeptides (a molecular weight of 80,000 each). Overestimation of the native molecular weight may perhaps be due to hydrodynamic properties of this enzyme that are different from those of other glucose oxidases. Our flavin analysis data revealed 1.5 mol of flavin per mol of purified glucose oxidase from *P. chrysosporium*. Using identical procedures, we showed that *A. niger* glucose oxidase has 1.6 mol of flavin per mol of protein. Since *A. niger* enzyme has been shown to have two flavins per mol of protein by a number of earlier investigators (3, 23, 25), we believe that both *P. chrysosporium* and *A. niger* glucose oxidases actually contain 2 mol of flavin per mol of protein, and the lower value of 1.5 to 1.6 that we obtained experimentally is apparently due to a limitation of the analytical procedure we used.

No carbohydrate was detectable in glucose oxidase from *P. chrysosporium* based on the dansyl hydrazine method (6) used in this study (Fig. 4B). Under identical conditions, an equal amount of the enzyme from *A. niger*, which has been reported to contain approximately 18% sugar residues (5), stained strongly positive. These results suggest either that *P. chrysosporium* glucose oxidase is not a glycoprotein or that it contains a very low level of carbohydrate which is not detectable by the procedure used (6). It would be of interest to see whether *P. chrysosporium* glucose oxidase proves to be a nonglycoprotein in light of previous observations that a majority of peroxisomal proteins are not glycosylated (25, 26) and that $H_2O_2$ production in ligninolytic cultures of *P. chrysosporium*, presumed to be due to glucose oxidase activity, has been shown to be localized in periplasmic, peroxisomelike structures (10).

TABLE 3. Substrate specificity of purified glucose oxidase[a]

| Substrate | $V_{max}$ (μmol/min per ml) | $K_m$ (mM) | $V_{max}/K_m$ | % Sp act |
|---|---|---|---|---|
| D-Glucose | 15 | 38.0 | 0.395 | 100 |
| L-Sorbose | 5 | 217.4 | 0.023 | 5.8 |
| D-Xylose | 2 | 105.2 | 0.019 | 4.8 |
| D-Maltose | 1 | 55.5 | 0.018 | 4.5 |

[a] Initial velocity was determined by the *o*-dianisidine–horseradish peroxidase assay described in Materials and Methods.

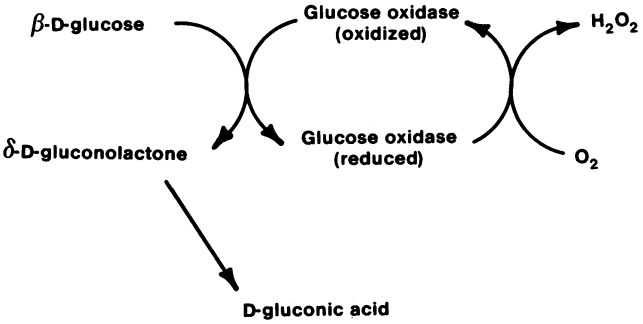


FIG. 5. A schematic illustration of the glucose oxidase reaction.

Glucose oxidases from other fungal sources have been shown to possess relatively low affinity for glucose, with $K_m$ values ranging from 0.11 to 33 mM and slightly higher affinity for $O_2$, with $K_m$ values from 0.2 to 0.83 mM (3, 24). The $K_m$ values for glucose and $O_2$ (38 and 0.95 mM, respectively) for the enzyme isolated from *P. chrysosporium* fall within the range of the values reported for previously described glucose oxidases.

Glucose oxidase from *Aspergillus* and *Penicillium* spp. was shown to be highly specific for β-D-glucose. Although D-mannose, D-galactose, 2-deoxy-D-glucose, and D-xylose have been shown to exhibit low activities as substrates, no greater than 2% of the activity found with glucose was found with these or 50 other carbohydrates tested (1, 3). The enzyme from *P. chrysosporium* had 33, 13, and 7% specific activity, respectively, with sorbose, xylose, and maltose compared with that seen with glucose as the substrate. However, comparison of the $V_{max}/K_m$ ratios for the different substrates clearly shows that glucose is the primary substrate for this enzyme. Another $H_2O_2$-producing enzyme, designated carbohydrate oxidase, has been partially purified from extracts of a white-rot fungus, *Polyporus obtusus* (13). This enzyme exhibited 59 and 38% activity, respectively, with L-sorbose and D-xylose compared with that observed with glucose. *Polyporus obtusus* enzyme was, however, different from *P. chrysosporium* glucose oxidase in that it could utilize D-gluconate as a substrate (14% of the activity observed with glucose) and showed no activity with D-maltose. A third type of $H_2O_2$-generating oxidase, L-sorbose oxidase from *Trametes sanguinea*, which catalyzed the oxidation of L-sorbose, D-glucose, D-galactose, D-xylose, and D-maltose, has been described (29). It has been suggested that this enzyme is similar to *Polyporus obtusus* carbohydrate oxidase (13). The ability of *P. chrysosporium* glucose oxidase to utilize xylose as a substrate may allow the organism to utilize sugars derived not only from cellulose but also from hemicelluloses found in woody material, its natural

habitat, to produce $H_2O_2$, which is known to be important to the ligninolytic system.

Inhibition studies showed that glucose oxidase from *P. chrysosporium*, similar to glucose oxidase from *A. niger*, is inhibited by $Ag^+$ but not by $Cu^{2+}$, NaF, or KCN (Table 2). Earlier results showed inhibition of lignin degradation when *o*-phthalate was used as a buffer in the growth medium (8). The results of this study show that glucose oxidase from *P. chrysosporium* is severely inhibited by *o*-phthalate, suggesting that inhibition of lignin degradation by this compound may at least partially be due to its effect on $H_2O_2$ production by glucose oxidase.

In this report we have described the purification, characterization, and kinetic properties of glucose oxidase from *P. chrysosporium*. This enzyme is similar in its physical and kinetic properties to glucose oxidases isolated from other fungal sources, except that we were unable to demonstrate the presence of carbohydrate in this protein. The enzyme is severely inhibited by *o*-phthalate and $Ag^+$.

## ACKNOWLEDGMENTS

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