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PURIFICATION AND CHARACTERIZATION OF HEXOSE OXIDASE FROM THE RED ALGA *CHONDRUS CRISPUS**

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SUMMARY

Hexose oxidase (D-hexose:O_2 oxidoreductase, EC 1.1.3.5) from the red alga *Chondrus crispus* has been purified by a procedure involving extraction with 0.1 M sodium phosphate (pH 6.8), *n*-butanol treatment, $(NH_4)_2SO_4$ precipitation, DEAE-cellulose chromatography, pepsin–trypsin digestion, and gel filtration on Sephadex G-200. The purified enzyme shows only a single band on disc gel electrophoresis. The enzyme has a mol. wt of 130 000 as determined by gel filtration and contains approximately 12 gram-atoms of copper per mole. FAD was not detected in the enzyme. The enzyme is a glycoprotein containing about 70% of a carbohydrate moiety consisting mainly of galactose and xylose. Optimum temperature and pH of the enzyme are 25 °C and 6.3, respectively. The enzyme oxidizes D-glucose, D-galactose, maltose, cellobiose, and lactose. The products of hexose oxidation are H_2O_2 and hexonolactone. The enzyme is strongly inhibited by diethyldithiocarbamate and to a less extent by cyanide, azide, hydroxylamine, and acetate. The production of H_2O_2 by the enzyme is responsible for the toxicity of *Chondrus crispus* to *Chlorella*. The red alga *Euthora cristata* also appears to contain this enzyme.

INTRODUCTION

It was previously found that two red algae, *Chondrus crispus* and *Euthora cristata* which occur off the New England Coast, inhibit the growth of the green alga *Chlorella pyrenoidosa*[1]. This inhibition of growth has been found to be due to H_2O_2 produced by the action of a hexose oxidase in the red algae on glucose in the *Chlorella* growth medium. The properties of this oxidase appear to be very similar to the D-hexose:O_2 oxidoreductase (EC 1.1.3.5) reported in the red alga *Iridophycus flaccidum*[2]. Both oxidases can utilize a variety of substrates which include D-glucose, D-galactose, maltose, lactose and cellobiose. This wide range of substrate specificity clearly distinguishes these algal enzymes from glucose oxidase (EC 1.1.3.4). Preliminary studies indicate that *Euthora cristata* also contains a hexose oxidase. *Chondrus* and *Iridophycus* are closely related taxonomically and both genera are classified in

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the family *Gigartinaceae* of the order *Gigartinales*. The presence of similar enzymes would, therefore, not be surprising. *Euthora*, however, is classified into a different order, *Cryptonemiales*, of the red algae. *Gigartina stellata* does not inhibit *Chlorella* and would therefore appear not to contain this enzyme.

In this paper we wish to report on the purification of the hexose oxidase from *Chondrus crispus* and some of the characteristics of the enzyme.

METHODS AND MATERIALS

Materials

The following were obtained from commercial sources: Sephadex G-200 (Pharmacia Fine Chemicals); Whatman DE 52 DEAE-Cellulose (Reeve Angel); pepsin and trypsin (Nutritional Biochemicals); *Aspergillus niger* glucose oxidase (1100 units per ml), *o*-dianisidine·2HCl, and peroxidase (Sigma Chemical). Standards for gel filtration included ribonuclease (Nutritional Biochemicals), and myoglobin, chymotrypsinogen, ovalbumin, albumin, γ-globulin, apoferritin (Schwarz/Mann). The other chemicals used were of reagent grade.

Methods

Protein was determined by the method of Lowry *et al.*[3] using bovine serum albumin as the standard. Carbohydrate was determined by the anthrone method[4,5] using D-galactose as the standard.

Copper was determined by atomic absorption spectroscopy and the dithizone method[6,7]. The determinations were made on lyophilized samples (approx. 10 mg) which had been wet ashed with a 3.5-ml mixture containing 3 ml HNO_3 and 0.5 ml 35% $HClO_4$ and then neutralized with NH_4OH[6].

Disc gel electrophoreses were run on standard gels (7%) at 5 °C and 2 mA per tube, using a Tris–barbital buffer (pH 7.0) with a running pH of 8.0 as described by Williams and Reisfeld[8]. Gels were stained with Coomassie blue (0.25% in methanol–water–acetic acid; 5:5:1, by vol.) and destained electrophoretically with a Canalco gel destainer using 7% acetic acid.

Assay of hexose oxidase

The procedure used for the assay of the *Chondrus* enzyme was based on methods given for the assay of glucose oxidase[9,10]. The assay mixture consisted of the following: 1.5 ml glucose (0.1 M in 0.1 M sodium phosphate, pH 6.3), 1.2 ml sodium phosphate buffer (pH 6.3), 0.1 ml *o*-dianisidine·2HCl (3.0 mg/ml in water), 0.1 ml peroxidase (0.1 mg/ml in sodium phosphate buffer), and 0.1 ml enzyme solution. The mixture was incubated at 25 °C for 15 min, the reaction stopped by adding 1 drop of conc. HCl, and the absorbance read at 402 nm. A standard curve was constructed using varying concentrations of H_2O_2 (0–3.0 μg/ml) in place of the enzyme solution. One enzyme unit was defined as that amount of enzyme which catalyzes the production of 10^{-3} μmole H_2O_2 per min at 25 °C, pH 6.3, and at a substrate concentration of 0.05 M.

Chlorella assay

Chlorella assays were carried out using *Chlorella pyrenoidosa* (UNH strain)

and buffered agar plates as previously described[1]. Approximately 20 μ. of test solution were applied to 0.25 inch sterile paper disks (Difco) and the disks placed on the *Chlorella*-seeded agar surface. After several days exposure to continuous fluorescent lighting, zones of inhibition appeared as colorless areas against a surrounding green background.

Collection, drying, and grinding of Chondrus crispus

Chondrus crispus was collected year-round in the inter-tidal zone at Rye Beach, New Hampshire. Freshly collected fronds were taken to the laboratory as soon as possible, where they were washed with cold tap water, blotted, and set out to air-dry at room temperature for several days. Air-dried fronds were ground to a powder (No. 16 mesh) in a Wiley Mill and stored in a freezer prior to extraction.

Extraction of Chondrus crispus

To a 100-g sample of air-dried ground *Chondrus crispus* fronds was added 1000 ml of 0.1 M sodium phosphate buffer (pH 6.8). The mixture was kept at 5 °C for 1–2 days, during which time it was shaken periodically by hand. The mixture was then filtered through cheesecloth using gentle suction, the filtrate being collected in an ice-cooled flask. The residue, although still containing some activity, was discarded. The extract was further clarified by centrifugation at 20 000 × *g* for 30 min to give a bright red–orange supernatant.

Purification of the Chondrus crispus enzyme

All steps during purification were carried out at 0–5 °C unless otherwise stated.

Step 1. n-Butanol extraction. The 20 000 × *g* supernatant was mixed with an equal volume of *n*-butanol. The mixture was allowed to stand for several min, then centrifuged at 10 000 × *g* for 30 min. This treatment, as described by Leibo and Jones[11], caused the deposition of unwanted photosynthetic pigment phycocyanin at the interface. The aqueous phase, red–orange in color due to the presence of phycoerythrin, was removed and the butanol fraction discarded.

Step 2. $(NH_4)_2SO_4$ precipitation. To the butanol-treated extract solid $(NH_4)_2SO_4$ at 45 g/100 ml was added slowly with shaking. After standing for several hours the mixture was centrifuged at 12 000 × *g* for 20 min. The precipitate was dissolved with stirring in 50–100 ml of 0.01 M sodium phosphate buffer (pH 6.8). This solution was transferred to dialysis tubing and dialyzed against a minimum of four 2-l changes of distilled water over a period of 2–3 days. Insoluble material in the retentate was removed by centrifugation at 10 000 × *g* for 10 min and to the supernatant sufficient sodium phosphate was added to make the solution 0.1 M (pH 6.8).

Step 3. DEAE-cellulose chromatography. A DEAE-cellulose column (1.5 cm × 12 cm) was prepared using 10 g of Whatman DE 52 ion-exchange cellulose and equilibrated with 0.1 M sodium phosphate (pH 6.8). The sample, in the same buffer, was placed on the column and the column washed with 500 ml of the same buffer. The enzyme was eluted from the column with the same buffer containing 0.3 M NaCl. Fractions from the DE-52 column (Fig. 1) showing activity in the *Chlorella* assay were pooled and dialyzed overnight against several changes of distilled water.

Step 4. Pepsin–trypsin treatment. The retentate was adjusted to pH 3.5 with dilute HCl (final volume approx. 80 ml), 20 mg pepsin (3 times crystallized) added,

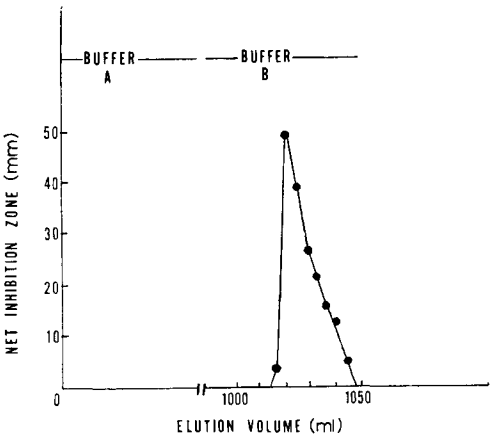

Fig. 1. DEAE-cellulose chromatography of *Chondrus* hexose oxidase. Sample volume was about 500 ml. Buffer A, 0.1 M sodium phosphate (pH 6.8); Buffer B, was composed of Buffer A containing 0.3 M NaCl. Fraction volume was 3–4 ml with a flow rate of 15–20 ml/h for a 1.5 cm × 12 cm column. Net inhibition zone = total zone diameter *minus* disk diameter.

and the mixture incubated at 37 °C with shaking for 5 h. The reaction was stopped by adjusting the pH to 6.8 with dilute NaOH. Sodium phosphate was added to make the digest 0.01 M (pH 6.8) with respect to phosphate. The mixture was treated with 20 mg trypsin (2 times crystallized) for 5 h with shaking at 37 °C, and the digest then freeze-dried.

Step 5. Gel filtration. The freeze-dried digest was suspended in 3 ml of distilled water and applied to a column (2.5 cm × 96 cm) of Sephadex G-200 and the column developed with 0.1 M sodium phosphate (pH 6.8). Fractions showing activity in the

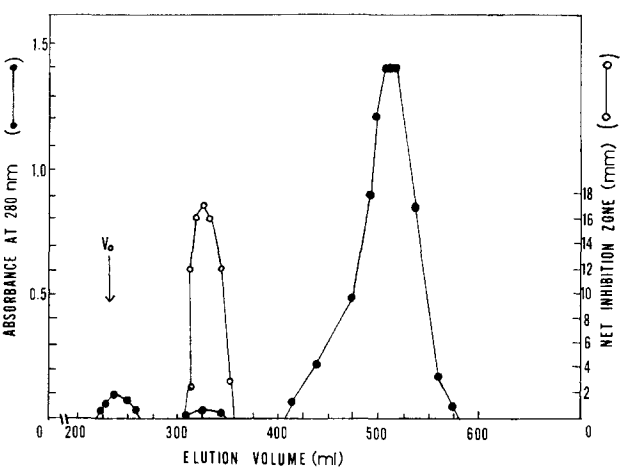

Fig. 2. Gel filtration of *Chondrus* hexose oxidase on Sephadex G-200. V_0, void volume determined with blue dextran. Eluant was 0.1 M sodium phosphate (pH 6.8). Fraction volume was 3–4 ml with a flow rate of 10–12 ml/h for a 2.5 cm × 96 cm column.

Chlorella assay (Fig. 2) were pooled, dialyzed extensively against 2-l changes of distilled water, and freeze-dried.

RESULTS

Purification of Chondrus hexose oxidase

The procedure described for the purification of hexose oxidase from *Chondrus crispus* has purified the enzyme 117-fold with a recovery of 11% of the activity (Table I). Approximately 10 mg of purified enzyme were obtained from 100 g of air-dried

TABLE I

PURIFICATION OF HEXOSE OXIDASE FROM *Chondrus crispus*

Stage of purification	*Volume (ml)*	*Total protein (mg)*	*Total carbohydrate (mg)*	*Total activity (units)*	*Spec. act. (units/mg protein)*	*Yield (%)*
20 000 × *g* supernatant	690	2277	5520	81 420	35	100
$(NH_4)_2SO_4$ ppt.	425	468	527	69 700	149	85
DEAE-cellulose	84	76	29	49 340	650	66
Sephadex G-200	59	2	7	8 190	4095	11

ground fronds. Disc gel electrophoresis of the purified enzyme showed only a single band staining with Coomassie blue (Fig. 3). The *Chondrus* enzyme was not affected by the pepsin–trypsin digestion as shown by no loss in biological activity in the *Chlorella* assay and no alteration in molecular size when examined by gel filtration on Sephadex G-200. This digestion step was necessary in order to remove the red pigment phycoerythrin which otherwise persisted as an impurity in the enzyme preparation.

Composition and molecular weight of Chondrus hexose oxidase

The enzyme showed a carbohydrate content of approximately 70% by the anthrone method using D-galactose as standard and 20% protein by the method of Lowry *et al.*[3] based on bovine serum albumin. Moisture may account for approx. 10% of the weight of the lyophilized enzyme. The carbohydrate composition of *Chondrus* hexose oxidase was determined on a 1-mg sample of enzyme which was hydrolyzed with 1 ml of 1 M H_2SO_4 for 4 h in a boiling water bath. Solid $BaCO_3$ was added to the hydrolysate until the pH was approx. 5 and the mixture was then centrifuged. The supernatant solution and washings from the $BaSO_4$ precipitate were combined and concentrated, and the concentrate was chromatographed along with known sugars on Whatman No. 1 paper in the following systems: *n*-butanol–ethanol–water (2:1:1, by vol.)[4], benzene–*n*-butanol–pyridine–water (1:5:3:3, by vol.)[4], *n*-butanol–pyridine–water (45:25:40, by vol.)[12], and ethyl acetate–pyridine–wat er(2:1:2, by vol.)[12]. The chromatograms were sprayed with either aniline hydrogen phthalate or aniline hydrogen oxalate[13]. Galactose and xylose were identified as the principal sugars in the *Chondrus* enzyme. Galactose appeared to be the predominant sugar, because, based on a galactose standard, the carbohydrate content of the enzyme was

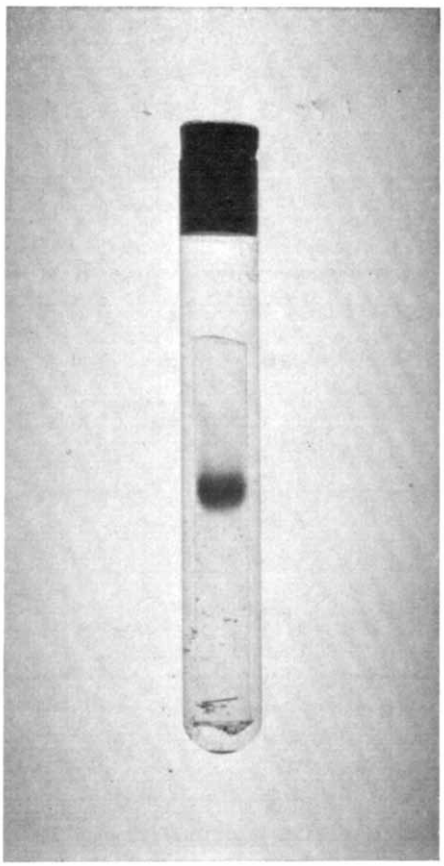

Fig. 3. Disc gel electrophoresis of purified *Chondrus* hexose oxidase.

estimated at 70%, whereas, based on a xylose standard, the carbohydrate content calculated out as 115%, due to a lower color yield from xylose. The amino acid composition was determined with a Spinco Amino Acid Analyzer on a 4.7-mg sample of enzyme which had been hydrolyzed in 6 M HCl at 110 °C for 24 h (Table II). It appeared rich in aspartic acid, threonine, serine, glutamic acid, glycine, alanine, and valine, and low in the basic amino acids (lysine, histidine, arginine), the sulfur-containing amino acids (cysteine, methionine), and the aromatic amino acids (tyrosine, phenylalanine). Tryptophan was not determined. Without corrections for loss or degradation, the total weight of amino acids was calculated from the analysis to be 605 μg or approx. 13% of the sample weight which showed agreement with the low value from the determination by the method of Lowry *et al.*[3].

The glycoprotein nature of *Chondrus* hexose oxidase was further demonstrated by staining with Alcian Blue following cellulose acetate electrophoresis[14]. By this procedure both the *Chondrus* enzyme and glucose oxidase showed a blue band against a pale blue background. Sections from an unstained cellulose acetate strip coinciding with the stained band were excised and placed in the *o*-dianisidine–peroxidase mixture (see Methods and Materials). The rapid formation of a yellow–orange color

TABLE II

AMINO ACID COMPOSITION OF *Chondrus* HEXOSE OXIDASE

Amino acid	*μmole/mg enzyme*	*Molar ratio**
Lysine	0.0447	5
Histidine	0.0083	1
Ammonia	—	—
Arginine	0.0247	3
Aspartic acid	0.1689	20
Threonine	0.0851	10
Serine	0.1223	14
Glutamic acid	0.1647	20
Proline	0.0723	9
Glycine	0.1483	18
Alanine	0.1140	14
Half-cystine	0.0264	3
Valine	0.0832	10
Methionine	0.0179	2
Isoleucine	0.0357	4
Leucine	0.0621	8
Tyrosine	0.0198	2
Phenylalanine	0.0459	6
Tryptophan	—	—

* Obtained by normalizing values relative to histidine = 1.

indicated the association of glucose oxidase activity with the band stained for glycoprotein. Staining a developed strip containing *Chondrus* enzyme with Ponceau S[15] resulted in a pink–red band against a pink background having the same mobility as those sections having enzyme activity and staining with Alcian Blue. *Chondrus* hexose oxidase failed to stain with Schiff's reagent[16] which is not uncommon for glycoproteins rich in carbohydrate[14].

An emission spectrum of the enzyme showed copper and a trace of sodium as the only metals present. Copper determinations on two different batches of enzyme by the atomic absorption method and the dithizone method showed a copper content of 0.6%.

Qualitative determination for FAD was done by the method of Pazur and Kleppe[17], which involved treatment of the enzyme at 45 °C for 15 min with pyridine.

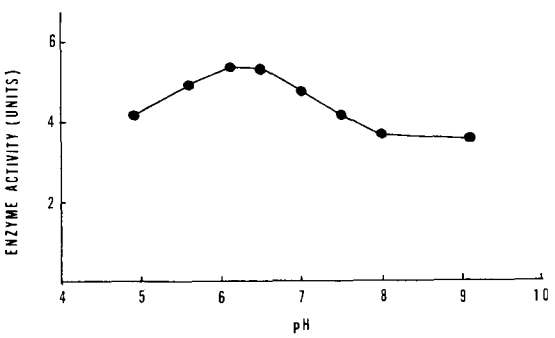

Fig. 4. Effect of pH on *Chondrus* hexose oxidase activity.

TABLE III

SUBSTRATE SPECIFICITY OF *Chondrus* HEXOSE OXIDASE

*Substrate**	*Relative rate*
D-Glucose	100
D-Galactose	82
Maltose	40
Cellobiose	32
Lactose	22
D-Glucose 6-phosphate	10
D-Mannose	8
2-Deoxy-D-glucose	8
2-Deoxy-D-galactose	6
D-Fucose	2
D-Glucuronic acid	2
D-Xylose	1

* Sugars not oxidized: L-glucose, D-fructose, D-gluconic acid lactone, γ-D-galactonolactone, dulcitol, D-gluconic acid, D-arabinose, xylitol, sucrose.

Under these conditions the presence of FAD in a glucose oxidase preparation could be readily demonstrated by an intensely fluorescing spot under ultraviolet light after paper chromatography. A lyophilized 2-mg sample of *Chondrus* enzyme (very pale green), treated similarly, showed no traces of FAD. The same result was found with a 5–10 min treatment in a boiling water bath. A featureless visible spectrum which showed no discernable peaks, even in the 380 and 460 nm regions characteristic of FAD[18], also indicated the absence of FAD in the *Chondrus* enzyme.

An approximate molecular weight was obtained by gel filtration on Sephadex G-200. A column (2.5 cm × 43 cm) was equilibrated at 5 °C with 0.1 M sodium phosphate (pH 6.8) and several proteins of known molecular weights were used as standards (see Methods and Materials). Blue dextran was used to determine the void volume (V_0). The elution volume for the *Chondrus* enzyme corresponded to a mol. wt of approx. 130 000.

Properties of Chondrus hexose oxidase

The pH optimum of the enzyme was determined using 0.1 M sodium phosphate

TABLE IV

COMPARISON OF SUBSTRATE SPECIFICITY OF *Euthora* AND *Chondrus* ENZYMES AND GLUCOSE OXIDASE

Substrate	*Relative rate*		
	*Euthora enzyme**	*Chondrus enzyme*	*Glucose oxidase*
D-Glucose	100	100	100
D-Galactose	95	82	0
Maltose	32	40	1
Cellobiose	95	32	2
Lactose	51	22	0

* Partially purified sample obtained from DEAE-cellulose column (see Methods and Materials).

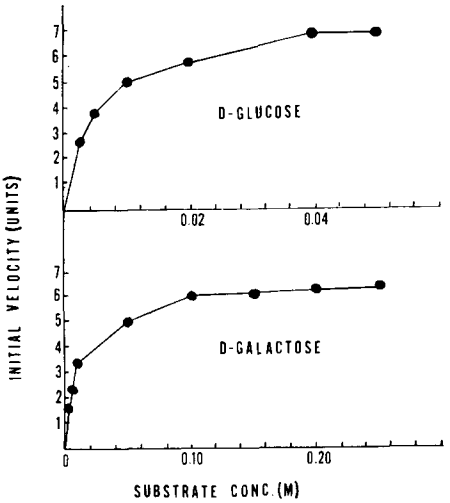

Fig. 5. Effect of substrate concentration on reaction velocity of *Chondrus* hexose oxidase.

buffers ranging in pH from 4.9 to 9.1. The optimum pH appeared to be approx. 6.3 (Fig. 4). The enzyme was most active at 25 °C. Heat stability of the enzyme was determined by heating for 5 min in 0.1 M sodium phosphate buffer (pH 6.3) at various temperatures, chilling in an ice bath, and assaying with the peroxidase–*o*-dianisidine system. A sudden drop in stability occurred between 50 and 60 °C.

Substrate specificity of the enzyme was determined using a number of sugars at a final concentration of 0.1 M (Table III). The substrates most readily oxidized were D-glucose, D-galactose, maltose, cellobiose, and lactose. L-Glucose was not oxidized. The five substrates oxidized by *Chondrus* enzyme were also tested with a partially purified extract of *Euthora cristata* and with glucose oxidase (Table IV). The

TABLE V

EFFECT OF VARIOUS INHIBITORS ON *Chondrus* HEXOSE OXIDASE

*Inhibitor**	*Concentration (M)*	*Inhibition (%)*
Sodium diethyl-dithiocarbamate	10^{-4}	95
	10^{-5}	22
NaCN	10^{-3}	61
	10^{-4}	15
Hydroxylamine·HCl	10^{-2}	100
	10^{-3}	96
	10^{-4}	26
Sodium azide	10^{-1}	85
	10^{-2}	78
	10^{-3}	65
Sodium acetate	10^{-1}	56
	10^{-2}	13
Sodium pyruvate	10^{-1}	43

* Showed no inhibition at 10^{-2} M: sodium pyruvate, sodium benzoate, D-gluconic acid, D-gluconic acid lactone and D-glucuronic acid.

TABLE VI

PAPER CHROMATOGRAPHY OF PRODUCTS FROM THE *Chondrus* HEXOSE OXIDASE REACTION

*Sample**	R_F *values***	
	Solvent A	*Solvent B*
Glucose	0.14	0.35
δ-D-Gluconolactone	0.37	0.54
Chondrus enzyme product from glucose	0.37	0.46
Glucose oxidase product	0.37	0.46
Galactose	0.13	0.42
γ-D-Galactonolactone	0.32	0.46
Chondrus enzyme product from galactose	0.32	0.44

* Glucose and galactose were detected with aniline hydrogen phthalate spray[12,13] and the lactones and oxidation products by spraying with hydroxylamine and ferric chloride[20].

** Run on Whatman No. 1 paper. Solvent systems as used by Bean *et al.*[21]. System A, *n*-butanol–acetic acid–water (52:13:35, by vol.); System B, phenol–water (80:20, v/v).

Euthora extract gave essentially the same results as *Chondrus* enzyme, but glucose oxidase attacked only D-glucose at any significant rate. In order to determine the possibility that free glucose might be present in the disaccharide samples, 1% solutions of each sugar were chromatographed on Whatman No. 1 paper in either ethyl acetate–pyridine–water (120:50:40, by vol.) or isopropanol–water (4:1, v/v) and the chromatograms sprayed with aniline hydrogen phthalate and heated at 100 °C. A trace of glucose was detected only in the sample of maltose.

The effect of increasing substrate concentration on initial reaction velocity was determined with D-glucose and D-galactose (Fig. 5). From this data the Michaelis constant (K_m) of the enzyme for D-glucose was calculated by the Lineweaver–Burk[19] method to be 0.004 M and that for D-galactose to 0.008 M. The K_m of *Iridophycus* hexose oxidase for D-glucose has been reported as 0.0025 M[2].

The effect of various inhibitors on the *Chondrus* enzyme was determined (Table V). The most potent inhibitor was sodium diethyldithiocarbamate, effective at 10^{-5} M. This substance also inhibited glucose oxidase at this level. The enzyme was also inhibited by sodium azide, hydroxylamine·HCl, NaCN, and sodium acetate. The inhibition found with sodium acetate was not as high as reported by Bean and Hassid[2] for the *Iridophycus* enzyme.

Products of Chondrus hexose oxidase

That H_2O_2 was a product of the *Chondrus* enzyme reaction was shown by the fact that when peroxidase was omitted from the assay mixture, *o*-dianisidine was only very slowly oxidized to a colored product. In the presence of peroxidase, H_2O_2 specifically oxidizes the *o*-dianisidine. That H_2O_2 was the substance that was toxic to *Chlorella* when paper disks containing *Chondrus* hexose oxidase were placed on *Chlorella*-seeded agar plates was shown by adding an excess of catalase to the paper disks. Only in the absence of catalase was inhibition observed. Any H_2O_2 formed was destroyed by the catalase. The toxic nature of H_2O_2 to *Chlorella* was also shown by inhibition produced by paper disks treated with H_2O_2. H_2O_2 at a level of 10 mg/ml gave a net zone of inhibition of 3.8 cm (disk diameter subtracted from the total dia-

meter of the inhibition zone), a net zone of 1.6 cm at 1 mg/ml, and a net zone of 0.2 cm at 0.1 mg/ml. Glucose oxidase, when placed on the disks, also inhibited *Chlorella*.

In order to determine the product formed from hexose in the *Chondrus* hexose oxidase reaction, enzyme *plus* excess catalase were incubated with 2 ml of 0.1 M glucose in 0.1 M sodium citrate buffer (pH 6.3) at 25 °C for 12 h. Glucose oxidase was also reacted under the same conditions. Examination of the reaction mixtures by paper chromatography (Table VI) showed that D-gluconolactone was formed from D-glucose and D-galactonolactone from D-galactose.

DISCUSSION

The *Chondrus crispus* hexose oxidase on the basis of its ability to oxidize not only D-glucose, but also D-galactose, maltose, cellobiose, and lactose, and on the basis of its oxidizing the hexoses to the corresponding hexonolactones and its inhibition by acetate, appears to be similar to the hexose oxidase of the red alga *Iridophycus flaccidum* described by Bean and Hassid[2]. The K_m values for both enzymes are also of the same order of magnitude.

Although *Chondrus* hexose oxidase resembles fungal glucose oxidase in its molecular weight (130 000 as compared to 150 000 for glucose oxidase), it differs markedly from glucose oxidase in that it lacks FAD but contains copper, and it contains 70% carbohydrate compared to 17% for glucose oxidase[22]. In addition, the carbohydrate constituents of fungal glucose oxidase are principally mannose with small amounts of galactose and glucosamine[22] while *Chondrus* hexose oxidase contains galactose and xylose. On the basis of a mol. wt of 130 000 and a copper content of 0.6%, it is calculated that the enzyme contains approximately 12 g atoms of copper per mole. This is considerably more than the 1 g atom of copper per mole found in galactose oxidase[23]. The *Chondrus* enzyme also differs from galactose oxidase in that it oxidizes hexose to the hexonolactone while galactose oxidase oxidizes galactose at the C-6 position to form a dialdehyde[23].

The *Chondrus* hexose oxidase, and apparently also the hexose oxidase of *Iridophycus*, is therefore unique in that it is a high-copper-containing enzyme which oxidizes D-glucose, D-galactose, and their disaccharides. Preliminary studies indicate that the red alga *Euthora cristata* also contains the same enzyme.

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