Phytochemistry, 1973, Vol. 12, pp 2645 to 2648. Pergamon Press. Printed in England.

PEROXIDASE ACTIVITY IN THE BROWN ALGA *LAMINARIA DIGITATA*

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(*Received* 16 *April* 1973. *Accepted* 15 *May* 1973)

Key Word Index—*Laminaria digitata*; Phaeophyceae; brown alga; peroxidase; iodotyrosines.

Abstract—Cell-free extracts of the brown alga *Laminaria digitata* catalyse the oxidation of *o*-dianisidine and of iodide, as well as the formation of iodoamino acids. The enzyme(s) requires hydrogen peroxide for these activities, which are strongly inhibited by cyanide and azide. It is suggested that the activity may be due to a haem-containing peroxidase which, in extracts, is strongly bound, possibly to alginate.

INTRODUCTION

A PEROXIDASE from the green alga *Enteromorpha linza* was shown capable of catalysing the oxidation of iodide as well as the formation of iodoamino acids,[1] thus contrasting with peroxidase from a species of red alga.[2] Iodinated compounds have been reported in green[3] and brown algae;[4–6] in other organisms peroxidases are known to be involved in the formation of such compounds[7–9] and this led us to seek evidence for peroxidatic activity in the brown alga *Laminaria digitata.*

RESULTS AND DISCUSSION

Cell-free extracts of *Laminaria digitata* catalysed oxidation of the electron donors *o*-dianisidine and iodide ion. The pH optima of the two systems were 6 and 7, and optimum hydrogen peroxide concentrations were 0·25 and 0·33 mM, respectively. In these properties, as well as in its thermostability, the brown algal enzyme parallels the peroxidase of *Enteromorpha linza* which was studied in a more purified state.[1]

Aliquots (0·1 ml) of a typical cell-free extract from *L. digitata* catalysed the oxidation of 0·1 μmol iodide/min/3 ml of reaction mixture (see Experimental). In the absence of added hydrogen peroxide the reaction rate was negligible. The addition of catalase (100 μg/3 ml of the complete reaction mixture) completely inhibited the reaction, showing that iodide oxidation requires hydrogen peroxide. The cell-free extract of *L. digitata* was incubated with ^{125}I for periods varying from 30 to 60 min (see Tong and Chaikoff[4] and Experimental). After TLC and autoradiography, radioactively-labelled monoiodotyrosine and a little

[1] MURPHY, M. J. and Ó hEOCHA, C. (1973) *Phytochemistry* **12,** 61.
[2] MURPHY, M. J. and Ó hEOCHA, C. (1973) *Phytochemistry* **12,** 55.
[3] SCOTT, R. (1954) *Nature* **173,** 1098.
[4] TONG, W. and CHAIKOFF, I. L. (1955) *J. Biol. Chem.* **215,** 473.
[5] KLEMPERER, H. (1957) *Biochem. J.* **67,** 381.
[6] ROCHE, J. and YAGI, Y. (1952) *Compt. Rend.* **146,** 642.
[7] COVAL, M. L. and TAUROG, A. (1967) *J. Biol. Chem.* **242,** 5510.
[8] HOSOYA, T. and MORRISON, M. (1967) *J. Biol. Chem.* **242,** 2828.
[9] MORRIS, D. R. and HAGER, L. P. (1966) *J. Biol. Chem.* **241,** 1763.

diiodotyrosine were seen to be formed from added iodide and tyrosine, particularly so in the presence of a hydrogen peroxide generating system (glucose/glucose oxidase) (Fig. 1B). Some reaction took place in the absence of added hydrogen peroxide (Fig. 1A), but not in the presence of catalase (100 μg), thus suggesting that there was some endogenous hydrogen peroxide in the cell-free extract. Such qualitative experiments indicate that iodination in this alga may be catalysed by a peroxidase.

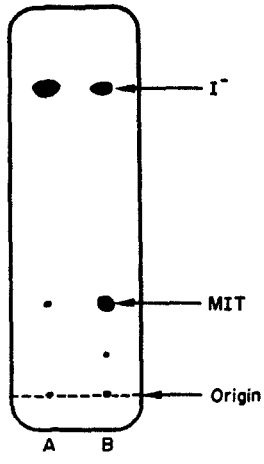

FIG. 1. AUTORADIOGRAPHS OF THE REACTION PRODUCTS FORMED AFTER INCUBATION OF *Laminaria digitata* CELL-FREE EXTRACT WITH ^{125}I AND TYROSINE, AS DESCRIBED IN THE EXPERIMENTAL.
(A) The reaction products in the absence of a hydrogen peroxide-generating system. (B) The reaction products in the presence of a hydrogen peroxide-generating system.

The peroxidase activity in dialysed and concentrated sample of cell-free extract was completely excluded when subjected to gel-filtration on Sephadex G200, equilibrated with 0·05 M-glycine–NaOH buffer, pH 9·0. Chromatography on agarose separated the activity into two components (Fig. 2), both of which catalysed the oxidation of *o*-dianisidine and of iodide. The smaller component, which was excluded at the probable void volume of the agarose column, was removable by high speed centrifugation of the extract (100 000 *g* for 1 hr) and is probably associated with particulate material.

The elution volume on agarose of the main fraction of peroxidase activity suggested that its size falls between blue dextran (MW 2×10^6) and *R*-phycoerythrin (MW $2{\cdot}9 \times 10^5$). However, the enzyme is unlikely to have as high a MW as this result would indicate. Unsuccessful efforts were made to solubilize the activity with reagents such as cholate, deoxycholate, Triton X100, digitonin and a variety of high molarity buffers. Incubation of the cell-free extract with trypsin (0·5 mg/ml for 1 hr at 37°) did not release a low MW form of the peroxidase; there was no significant loss in enzyme activity as a result of this incubation.

The possibility that the enzyme was attached to alginate, a characteristic polysaccharide of brown algae,[10] was explored by studying the characteristics of cytochrome *c* and horseradish peroxidase in the presence of either sodium alginate or cell-free extract of *L. digitata.*

[10] PERCIVAL, E. and MCDOWELL, R. H. (1967) *Chemistry and Enyzmology of Marine Algal Polysaccharides*, Academic Press, New York.

A typical result is shown in Fig. 3; the bulk of the added cytochrome *c* was eluted at the void volume of the column, with a smaller fraction at the normal elution volume of this protein, suggesting that much of it was bound. Using carbazole-sulphuric acid, alginate was shown present in concentrated enzymically-active, extracts.

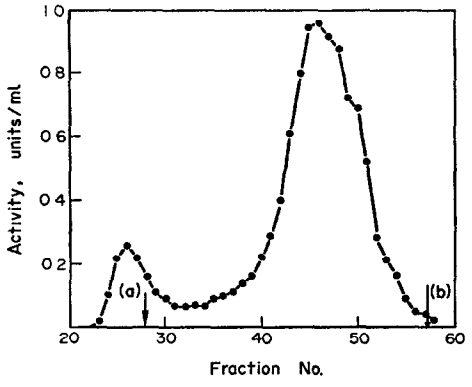

FIG. 2. CHROMATOGRAPHY OF A CONCENTRATED SAMPLE OF *Laminaria digitata* CELL-FREE EXTRACT ON AGAROSE A-50M (BIO-RAD LABORATORIES). The sample (1 ml) was applied to an agarose column equilibrated with 0·05 M glycine–NaOH buffer, pH 9·0, and 3 ml fractions were collected. The arrows refer to the position of maximal elution of (a) blue dextran and (b) R-phycoerythrin; these values were determined in separate experiments under identical conditions.

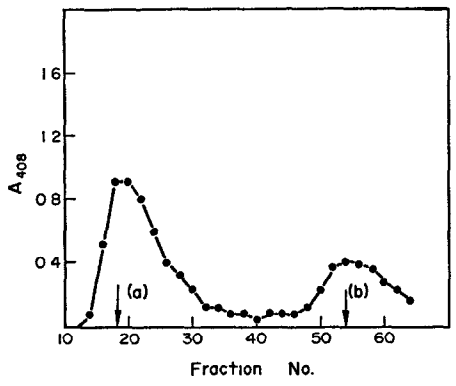

FIG. 3. CHROMATOGRAPHY OF FREE AND COMPLEXED CYTOCHROME-*c* ON SEPHADEX G200. Cytochrome-*c* was incubated overnight at 4° with *Laminaria digitata* cell-free extract and applied to a Sephadex G200 column equilibrated with 0·05 M glycine–NaOH buffer, pH 9·0. 3 ml fractions were collected and cytochrome-*c* monitored spectrophotometrically at 408 nm. The arrows refer to the positions of maximal elution of (a) blue dextran and (b) cytochrome-*c*; these values were determined in separate experiments.

The main active fraction from agarose chromatography was treated with various reagents which might affect its activity (see Table 2, Ref. 1). Neither acriflavine nor 2,4-dinitrophenol caused inhibition, suggesting that neither flavin nor metal ions participate in the activity.[1] On the other hand, 50% inhibition of *o*-dianisidine oxidation was caused by KCN (1×10^{-5} M), NaN_3 (5×10^{-4} M), KSCN ($1{\cdot}5 \times 10^{-5}$ M) and NaF ($1{\cdot}5 \times 10^{-3}$ M), results to be expected from a haem-containing peroxidase. (Inhibition was not monitored by iodide oxidation, as products can react with inhibitors, thereby negating their effect).[12]

Contrary to the conclusion of Tong and Chaikoff[4] who used cell-free extracts of the brown alga *Nereocystis lutkeana*, our preliminary studies suggest that iodination reactions in *Laminaria digitata* may involve peroxidase action, the enzyme being similar in properties to that from the green alga *Enteromorpha linza*.[1]

EXPERIMENTAL

Preparation of cell-free extracts. Laminaria digitata was collected on the shores of Galway Bay. Fronds, which were washed thoroughly and freed from stipes, were stored at −30° until required. Cell-free extracts were prepared by a method similar to that of Tong and Chaikoff.[4] Fronds were chopped and 100 g quantities were homogenized in a food mixer for a few min with 1 l. of dist. H_2O. After settling, the supernatant was decanted and 10 mM $NaHCO_3$, pH 8·3 (300 ml), added and the material again blended. The resulting suspension was stored at −30° overnight. After thawing, small quantities were treated in a Potter–Elvehjem

[11] DISCHE, Z. (1955) in *Methods of Biochemical Analysis*, (GLICK, D., ed.), Vol. 2, p. 313, Academic Press, New York.

[12] ROCHE, J. ,THAOI, N-v. and LAFON, M. (1949) *Compt. Rend.* **143**, 1327.

homogenizer with a high-speed Teflon plunger. The pooled homogenates were again frozen and thawed and homogenized further, this cycle being repeated several times. The homogenate was centrifuged at 35 000 *g* for 30 min, and the supernatant constituted about 250 ml of cell-free extract. Its protein concentration, as determined by the method of Potty,[13] was about 0·4 mg/ml.

Enzyme assays. These were carried out as described previously.[1] The final concentration of H_2O_2 in the *o*-dianisidine standard assay was 0·167 mM, while the final concentrations of iodide and H_2O_2 in the iodide assay were 5 and 0·167 mM, respectively.

Radioactive experiments. Aliquots of *Laminaria digitata* cell-free extract (0·1 ml) were incubated at 37° in a reaction mixture (3 ml) containing phosphate buffer, pH 7·0 (150 μmol), L-tyrosine (3 μmol), ^{125}I (50 μCi) in NaI (0·03 μmol). When an H_2O_2 generating system was used it consisted of glucose (3 mg) and glucose oxidase (20 μg). At the end of the incubation period the reaction was terminated by the addition of 2-thiouracil to a final concentration of 1 mM. The reaction products were chromatographed according to the procedure of Ouellette and Balcius.[14]

Acknowledgements—M. J. M. thanks the Department of Education, Dublin, Republic of Ireland, for a Research Maintenance Grant.

[13] POTTY, V. H. (1969) *Anal. Biochem.* **29,** 535.
[14] OUELLETTE, R. P. and BALCIUS, J. F. (1966) *J. Chromatog.* **24,** 465.