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Enzymic degradation of phenolic materials in peatlands – measurement of phenol oxidase activity

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Abstract

The model substrate L-dihydroxy phenylalanine (L-DOPA) was used to measure the activity of phenol-oxidase (PO) in peat from a Welsh riparian wetland. The sensitive and relatively simple technique measured the rate of formation of the red coloured compound 2-carboxy-2,3-dihydroindole-5,6-quinone from the enzymic oxidation of L-dopa. The method was used to test the hypothesis that the large exports of phenolic materials from peatlands into aquatic systems were caused by low phenolic-degrading enzyme activities within the peat matrix. The low oxygen availability and acidic pH of the peat soil were found to be sub-optimal for PO activity. Furthermore, a depth-dependent decline in PO activity was inversely correlated with phenolic concentrations. Thus, the findings supported the above hypothesis.

Introduction

Peatlands occur in areas that are waterlogged as a consequence of high rainfall and/or poor drainage. They can be defined as unbalanced ecosystems, in which the rate of production of organic material exceeds the rate at which those compounds are degraded (Moore and Bellamy, 1974). Their persistence is generally considered to be due to a marked decline in microbial activity with increasing depth within the peat profile (Dickinson, 1979; Gammelgaard et al., 1992).

Microbial metabolism within the unusual physical conditions of the peat environment leads to the release of soluble humic (phenolic) and organic acids (Dickinson, 1979; Sikora and Keeney, 1977) and also gaseous products such as carbon dioxide and methane (Clymo and Reddaway, 1971). Of the soluble products, phenolics are of particular interest for two reasons. First, they are enzyme inhibitors (Appel, 1993; Wetzel, 1992) and thus we would suggest that they have the potential to reduce the rate of degradation of the peat matrix. Secondly, they are released in substantial quantities into the aquatic systems that drain our wetlands (sensu Wetzel, 1992) where their inhibitory activity may continue (Freeman et al., 1990; Wetzel, 1992).

The long residence times of waters passing through peatlands (cf. Gafni and Brooks, 1990), would be expected to present ample opportunity for the enzymic degradation of phenolic compounds prior to their export into aquatic systems. However, the substantial quantities of phenolics that are released, suggests that such enzymic degradation is severely limited. As a test of that hypothesis, we have investigated the activity of the enzyme *phenol oxidase* within peat from a Welsh riparian wetland.

Extracellular enzymes have been investigated widely in other soil environments (Burns, 1978) and have traditionally been assayed using colorimetric methods (Ladd, 1984). However highly sensitive and simple methods using model-substrates have recently been developed (e.g. Goulder, 1990; Marxsen and Witzel, 1990) and this is also true for phenol oxidase (PO). The phenolic compound L-dihydroxy phenylalanine (L-DOPA) has great potential as a model substrate in studies of enzymic degradation of peat phenolics. In the presence of PO it is rapidly converted into the compound 2-carboxy-2,3-dihydroindole-5,6-quinone with a readily detectable absorption maxima of 460nm. The technique has recently been employed in studies of phenol oxidase within river biofilms (Sinsabaugh and Linkins, 1988, 1990), and more extensively in studies of animal physiology (e.g. Pomerantz and Li, 1970; Canicatti and Seymour, 1991). We now describe its use within peatlands.

Materials and methods

Peat samples were collected from a riparian-gully mire at the Llanberis pass, north Wales (Nat. grid ref. SH 638 56, altitude 150 m). The water-table was near the surface at the time of sampling, with a pH of approximately 5. An homogenate was prepared under nitrogen, in the proportion 1 cm^3 peat per 2 mL distilled water. A stomacher (Seward Colworth model 400) was adopted for sample homogenisation in order to minimise cell disruption. The homogenate was diluted 1:1 with distilled water, and 4.5 mL aliquots containing 0.2 grams peat (dry-weight) were transferred to replicate 10 mL Nalgene centrifuge tubes. A 10 m*M* concentration solution of L-DOPA (Sigma) was used throughout (due to poor solubility at higher concentrations). To each tube, 4.5 mL of L-DOPA solution or 4.5 mL distilled water (control) were added, and the tubes incubated in a shaker for 1 or 3 minutes, at 11 °C (typical of field temperatures). The reaction was terminated by immediate centrifugation at 12000 r.p.m for 5 minutes at 5 °C, and no further increase in absorbance was found after centrifugation. The supernatant was filtered through a Whatman GF/C filter and the absorbance measured at 460nm. Samples were dried at 55 °C to constant mass for determination of the dry weight used in each assay. Finally, activity was expressed in terms of uMOL 2, 3-dihydroindole-5,6-quinone-2-carboxylate (hereafter referred to as diqc) per minute per g peat (dry weight), by using Beers Law and the difference in absorbance between incubation times of 1 and 3 minutes and a molar absorbancy coefficient for diqc of 3.7×10^4 (Mason, 1948).

Several factors were varied to determine the optimal conditions for the assay. These included: 1) Incubations over 1 to 6 minutes to determine the optimal incubation duration. 2) Manipulation of the pear content of the final L-DOPA homogenate from 0.11 to 0.44 mg dry weight. 3) Variation in the reaction pH (through pH 1.8, 4.8, 8.0, 9.6) by dropwise addition of 40% NaOH or conc. H_2SO_4. (After incubation at pH's >6.5, it was found necessary to return the pH to 5 with 50 m*M* acetate buffer to avoid artifacts from pH-induced shifts in the absorbance spectrum.) Finally, we investigated the phenol oxidase activity within a core of peat from the riparian wetland, and attempted to relate the PO activities to the concentrations of phenolic materials and oxygen found down the peat profile. The core of peat was collected within a thin walled P.V.C. cyclinder (10 cm in diameter and 50 cm length). Sections were taken out at various depths (0, 2, 4, 5, 8, 10, 15, 20, 30 and 40 cm) and stored under nitrogen in polythene bags at 11 °C until measurement (<24 hours). Approximately 25 cm^3 was used to produce a peat homogenate for the enzyme assay, while the remaining material was drained of its waters (by compression) and these waters analysed for their oxygen content, and also phenolic content using the Folin-Ciocalteau phenol reagent (Box, 1983).

Results and discussion

Our preliminary assays showed the rate of L-DOPA oxidation to approximate towards linearity over short incubation periods of less than 5 minutes (Fig. 1). Therefore, subsequent assays were conducted over 3 minute incubation periods, while all initial measurements were

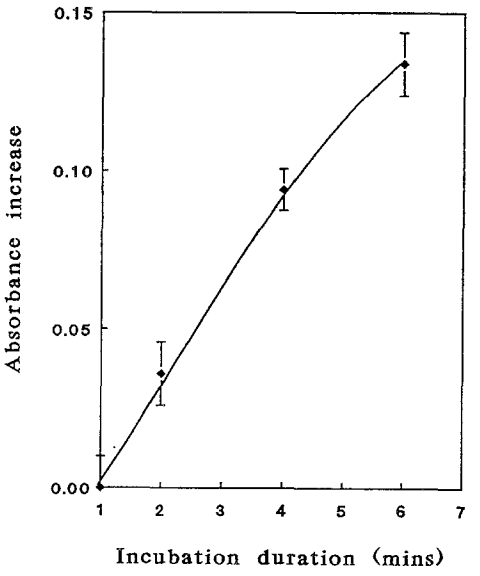

Fig. 1. Effect of incubation duration on phenol oxidase activity (mean ± S.E., n = 5).

made after 1 minute (the minimum period in which reproducible homogenisation, aliquot removal and preparation for centrifugation, could be accomplished). Increasing the peat content of the final homogenate of peat/L-DOPA, failed to produce linear increases in phenol oxidase activity (Fig. 2). This may indicate that the substrate/product concentrations may have influenced the reaction rate, and suggests that the weight of added peat should be rigorously standardised between assays. The concentration of hydrogen ions also had a pronounced effect on the rate of L-dopa oxidation (Fig. 3), with optimal rates pH 8 and pH 9.6. This result suggested that the acidic pH of many peatlands was likely to be relatively unfavourable for the degradation of phenolic-material.

Investigation of the spatial distribution of phenol oxidase activity within the peat profile, showed activities to fall rapidly with depth (Fig. 4). Studies of *gross* microbial activity within peatlands have shown similar inverse relationships with depth (Gammelgaard et al., 1992). Activities within the peat were strongly correlated with oxygen availability (Fig. 5) and approximated towards an exponential model ($R^2 = 0.83$; $p < 0.01$). Thus, it seems likely that low oxygen availabilities may limit PO activities at depth. Furthermore, those lower PO activities may have allowed phenolic materials to accumulate, as suggested by the strong inverse correlation between phenolic concentration and enzyme activity (Fig. 6; $R^2 = 0.67$; $p < 0.01$, multiplicative model). However, that inverse relationship could also be explained by the ability of phenolics to inactivate many enzymes through

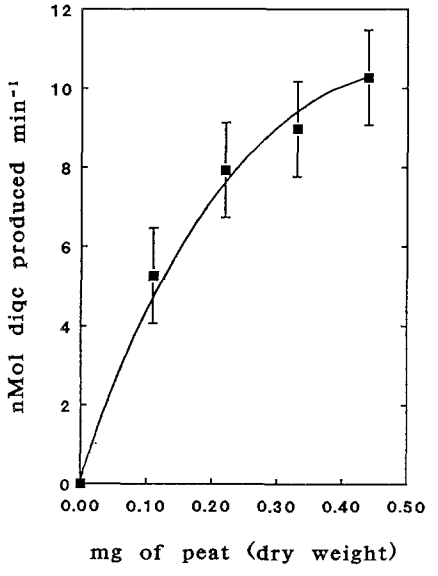

Fig. 2. Effect of increasing the peat content of the final peat: L-DOPA homogenate on phenol oxidase activity (mean ± S.E., n = 5).

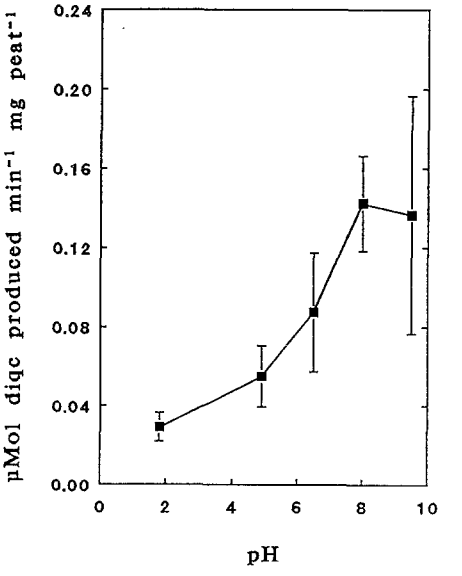

Fig. 3. Effect of pH on phenol oxidase activity (mean ± S.E., n = 5).

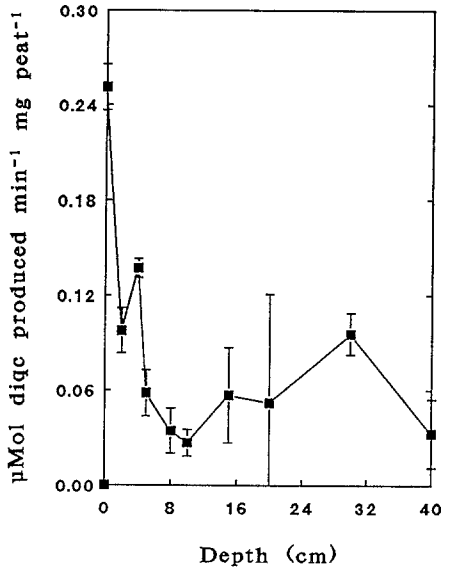

Fig. 4. Spatial distribution of phenol oxidase activity within the peat profile (0–50 cm) (mean ± S.E., n = 5).

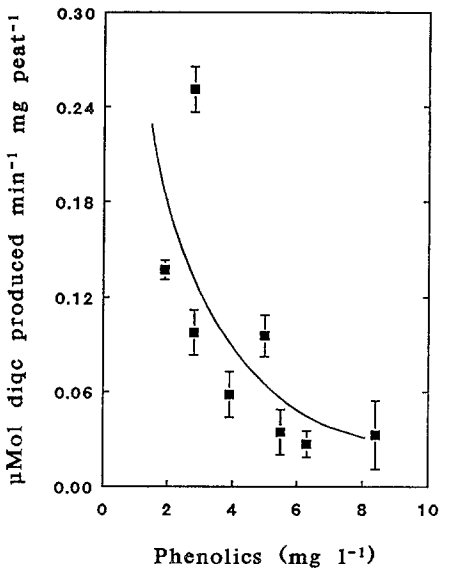

Fig. 6. The inverse relationship between phenol oxidase activity and phenolic concentrations within the soil profile.

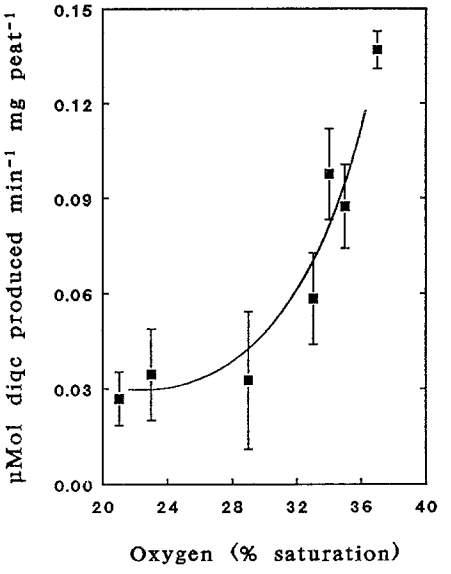

Fig. 5. Relationship between phenol oxidase activity and dissolved oxygen concentration within the soil profile.

complexation reactions (Wetzel, 1992). An interesting adjunct to the possible presence of inactivated phenolic:enzyme complexes, is that those complexes could be transported within aquatic ecosystems to sites where they may be re-activated by photolysis (Stewart and Wetzel, 1982). Our findings suggest that re-activation of these enzymes in the more favourable pH and oxygen levels to be found outside of the wetland, could result in a subsequently increased rate of biodegradation of the phenolic materials in the receiving waters.

However, in the wetland itself, our study has shown phenol oxidase enzymes to be subject to sub-optimal pH and dissolved oxygen levels, possibly together with inhibition of enzyme activity throughout all but the surface of the peat profile. Our data, therefore support the hypothesis that the substantial export of phenolics from wetlands may be due to low rates of phenolic degradation. Moreover, the L-DOPA assay, has been shown to be sensitive and relatively simple to perform, while yielding information that can aid our understanding of wetland biogeochemical processes. As such, the technique is likely to prove of great value in future wetland studies.

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