Phytochemistry, Vol. 22, No. 11, pp. 2427–2430, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

PEROXIDASE FROM CULTURED PEANUT CELLS AND FUNGAL MYCELIUM AS AUXIN RECEPTORS

J. ŁOBARZEWSKI and A. DAWIDOWICZ*

Department of Biochemistry and *Physical Chemistry, M. Curie-Skłodowska, Lublin, Poland

(*Received* 23 *February* 1983)

Key Word Index—*Arachis hypogaea*; Leguminosae; *Trametes versicolor*; peroxidase; affinity; auxin.

Abstract—The electrophoretic patterns of peroxidase isozymes from cultured peanut cells and from the mycelium of *Trametes versicolor* showed minor differences. These differences were also observed on affinity chromatography using naphthyl-1-acetic acid as a ligand. The results suggest specific interactions between peroxidases and auxins.

INTRODUCTION

An association between peroxidase and plant cell walls has frequently been postulated [1–3]. Since peroxidase is readily released by cells [4–6], it can either be transported across the cell wall or remain in the cell wall. That peroxidase mediates in the polymerization of coniferyl alcohol to lignin [4, 7] is a well-established concept. Similarly, part of the biodegradation of lignin has been linked to peroxidase activity [8–10]. In addition to these functions, there may be others [11] which are as yet not well defined [4, 5].

Some plant membrane proteins have an affinity for auxins [12] and could be potential receptor sites for hormones. A schematic arrangement of these receptor sites has been proposed [13]. Probably the aromatic ring of indoleacetic acid meets with the receptor molecule in the membrane. Optimal binding occurs apparently between pH 5 and 6 [14]. Moreover, auxin receptor proteins may also play a role in the proton pump involved with cell elongation [15]. But whatever the function of the auxin receptor may be, only structural proteins have been suggested to play a role so far.

The results of the following study suggest that enzymes, and particularly plant peroxidase of peanut and fungal origin, may possibly act as auxin receptors.

RESULTS AND DISCUSSION

In the following, particular attention was focused on the affinity of naphthyl-1-acetic acid (NAA) when used as a ligand for affinity chromatography, and some forms of plant peroxidase. In contrast to other studies [12], it was possible to dissociate the auxin and their receptor molecules by carefully controlled conditions. As the receptor sites, peroxidases (donor: H_2O_2 oxidoreductase; EC 1.11.1.7) from peanut (*Arachis hypogaea*) cell cultures and the mycelium of a wood rot fungus (*Trametes versicolor*) were used.

Two forms of peanut peroxidase were bound by NAA linked to a glass bead column at pH 5.4 and were independently eluted from the column by a double gradient (pH 5.4–3 followed by 0–0.7 M $(NH_4)_2SO_4$) as shown in Fig. 1. Elution with a gradient employing an increase of pH from 5.4 to 7 did not result in a separation (Fig. 1).

In parallel experiments using fungal material, only one form of peroxidase could be eluted regardless of whether the initial pH gradient increased or decreased (Fig. 2). However, other proteins than peroxidase in the crude mycelial extract behaved like the second peroxidase form of peanut. This indicates that the fractionation capacity of the column was unimpaired. These results agree with published [14] reports that maximal affinity occurs at pH values between 5 and 6. And because of this similarity of pH responses in binding of auxin with receptor sites to those with peroxidase, a proposal is made that the latter may fulfil the role of the former. When indole-3-acetic acid (IAA) was added exogenously to a sample of peanut cultures it was noted that over a 5 hr period less peroxidase was released by the cells (Fig. 3). The pH of the medium during growth is usually close or slightly above 5 [16]. Therefore, it is not improbable that upon the addition of exogenously added IAA, peroxidase or its transport out of the cells is temporarily associated with the auxin and serves as a receptor site. Cultured peanut cells usually grow more rapidly than the cells in the intact plant [16] and peroxidase is produced at higher rates than normal [4].

A comparison between the isozymal patterns of peroxidase from peanut and from the fungus was made using electrophoresis and isoelectric focusing (Figs. 4 and 5). Isoelectric focusing gave eight peroxidase bands for the unfractionated peanut peroxidase (Fig. 4B) while there were only seven bands for the extract from the fungal mycelium (Fig. 5B). An increase in the number of forms on isoelectric focusing has also been reported for peroxidases from tobacco [17]. In addition, Figs. 4 and 5 show that a single isozymal form could be isolated by affinity chromatography by the second elution with a gradient of ammonium sulphate. Moreover, this form was anionic as shown by the electrophoretic mobility.

Finally, it was noted from Figs. 4 and 5 that both in the case of peanut cells and fungal mycelium the peroxidases had an isoelectric point between pH 3 and 3.5, since they could be dissociated at that pH from NAA, the substitute for the plant auxins. It is therefore concluded that some, mainly anionic, forms of plant peroxidases may play an

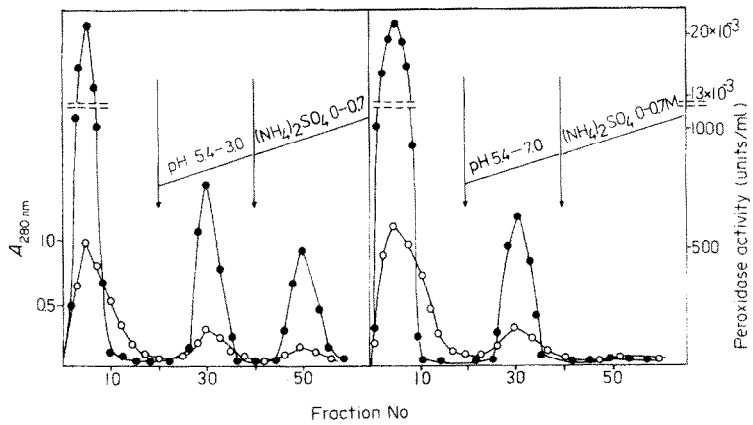

Fig. 1. Affinity chromatography of crude peanut cell culture peroxidase on naphthyl-1-acetic acid–alkilamine–carbodiimide porous glass beads. The column (1 × 6 cm) was equilibrated with 0.02 M sodium acetate buffer, pH 5.4, before the addition, in the same buffer, of 12 mg of protein. The unbound proteins were eluted from the column using the equilibrating buffer. (A) Linked proteins eluted from the column with a linear pH gradient from 5.4 (0.02 M acetate buffer) to 3.0 (0.02 M glycine buffer) (100 ml) and then with an ammonium sulphate gradient, 0–0.7 M (100 ml). (B) Linked proteins eluted from the column using a linear gradient of pH from 5.7 to 7.0 (100 ml), the second gradient as in A. ○——○, $A_{280\,nm}$; ●——●, peroxidase activity (units/ml).

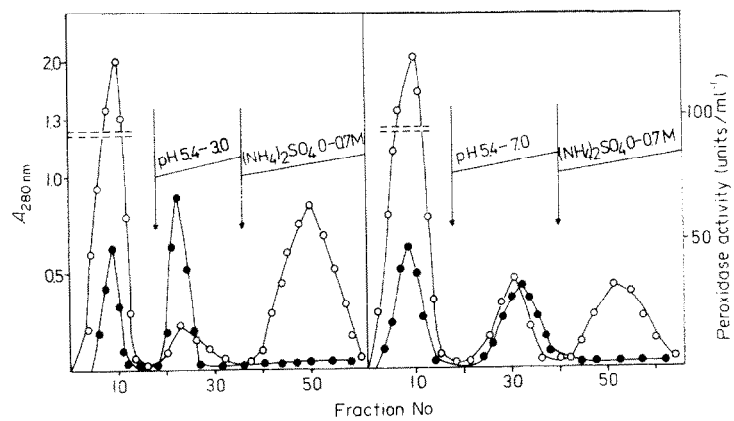

Fig. 2. Affinity chromatography of crude peroxidase preparation from the mycelium of *T. versicolor* on naphthyl-1-acetic acid–alkilamine–carbodiimide porous glass beads. Chromatographic conditions as in Fig. 1, except that 50 mg of protein was applied to the column.

important physiological role in the response of plant cells to auxins. Peroxidase is known to destroy IAA but according to Gaspar *et al.* [5], this is brought about by the cationic rather than the anionic peroxidases. It is noteworthy that some peroxidases only exhibit IAA oxidase activity after purification [5].

EXPERIMENTAL

Peanut cells were cultured in suspension medium as described [18] and the peroxidase extracted as usual [19]. In a specific case, *ca* 5 g (7-day-old) cells was resuspended in 50 ml fresh medium containing 0.2 mM IAA and incubated for 1–5 hr. Peroxidase measurements were made as detailed in ref. [20]. The growing conditions and the subsequent extraction of the mycelium of *Trametes versicolor* (Lex Fr, Quel) have been described [6, 21].

The matrix of the affinity chromatography was porous 96% silica glass beads (150–200 Å average pore diameter 180–220 mesh). The beads were silanized with γ-aminopropyltriethoxysilane (Pierce Chem. Co., Rockford, IL, U.S.A.) according to the procedure by Wilchek [22]. The alkilamine beads were activated using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide HCl (Sigma Chem. Co., St. Louis, MO, U.S.A.) as described by Lappi *et al.* [23]. Naphthyl acetic acid (Chemapol Praha, Czechoslovakia) in batches of 100 mg per 4 g glass beads was linked in the presence of H_2 for 3 hr at ambient temp. and 12 hr in the refrigerator. The use of the matrix is described in Figs. 1 and 2.

Disc PAGE (Merck, Darmstadt, West Germany) was carried out as described for the alkaline [24] and the acidic [20] buffer systems. Isoelectric focusing was carried out in 0.9 × 13 cm glass tubes using Pharmalyte pH 3–10 (Pharmacia, Uppsala, Sweden) following the procedure of Laas and Fast-Johansson [25]. Peroxidase staining was carried out as described in ref. [24].

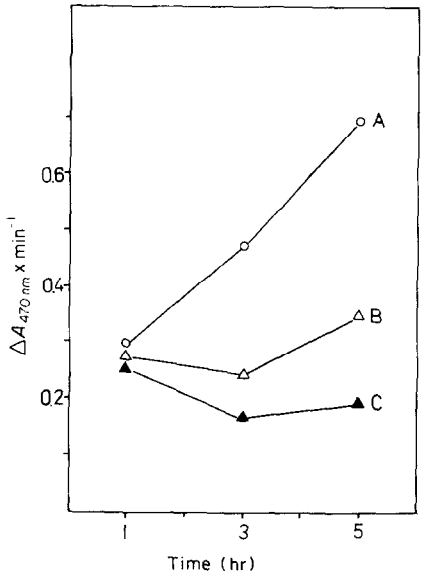

Fig. 3. Changes in peroxidase activity in 50 ml of medium containing 5 g (fr. wt) of peanut cells. (A) Control; (B) cells preincubated for 1 hr with 0.2 mM indole-3-acetic acid; (C) cells preincubated for 5 hr with 0.2 mM of indole-3-acetic acid.

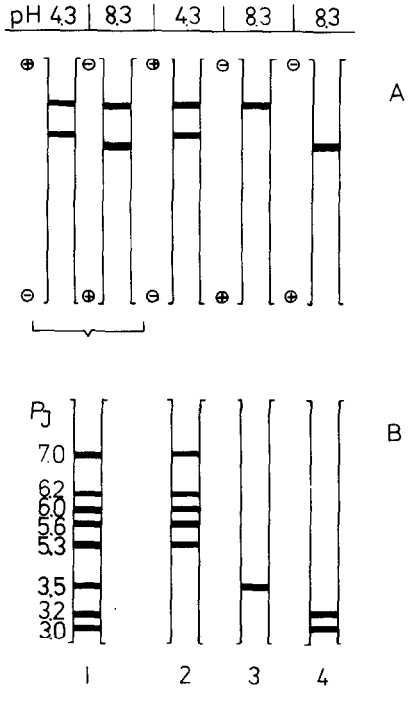

Fig. 4. Disc PAGE (A) and isoelectric focusing (B) of peanut cell peroxidase. 1, Crude peroxidase preparation; 2, peroxidase eluted from the column using buffer pH 5.4; 3, peroxidase eluted from the column using gradient pH 5.4–3.0; 4, ammonium sulphate gradient 0–0.7 M. Peroxidases stained with benzidine–H_2O_2.

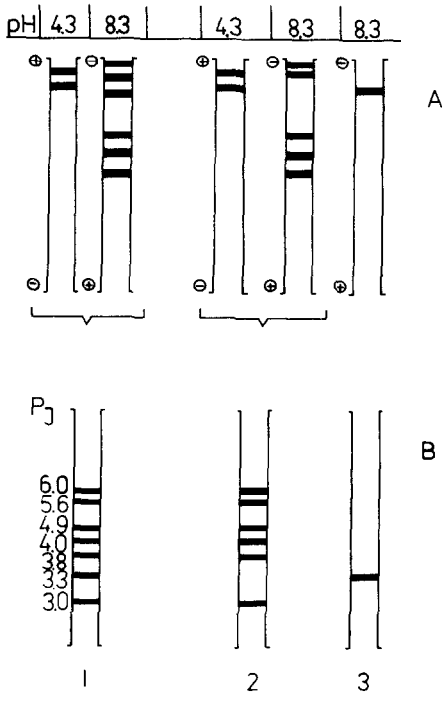

Fig. 5. Disc PAGE (A) and isoelectric focusing (B) of fungal peroxidase. 1, Crude peroxidase preparation; 2, peroxidase eluted from the column using buffer pH 5.4; 3, peroxidase eluted from the column using gradient pH 5.4–3.0. Peroxidases stained with benzidine–H_2O_2.

Acknowledgement—The peanut cell culture and the extracted peroxidase from the culture medium were graciously provided by Dr. R. B. van Huystee, Department of Plant Sciences, University of Western Ontario, Canada.

REFERENCES

1. Birecka, H. and Miller, A. (1974) *Plant Physiol.* **53**, 569.
2. Mäder, M. (1976) *Planta* **131**, 11.
3. Łobarzewski, J. (1983) *Holzforschung* (in press).
4. van Huystee, R. B. and Cairns, W. L. (1982) *Phytochemistry* **21**, 1843.
5. Gaspar, Th., Penel, Cl., Thorpe, T. and Greppin, H. (1982) *Peroxidases* 1970–1980 Université de Geneve-Centre de Botanique, Geneve, Switzerland, p. 73.
6. Łobarzewski, J. (1974) *Ann. Univ. M. Curie-Skłodowska, Lublin, Sec. C* **29**, 41.
7. Grisebach, H. (1977) *Naturwissenschaften* **64**, 619.
8. Crawford, D. L. and Crawford, R. L. (1980) *Technology* **2**, 11.
9. Kirk, T. K. (1975) *Biological Transformation of Wood by Microorganisms*, p. 153. Springer, Berlin.
10. Łobarzewski, J., Trojanowski, J. and Wojtaś-Wasilewska, M. (1982) *Hozforschung* **36**, 173.
11. Whitmore, F. W. (1976) *Phytochemistry* **15**, 375.
12. Tappeser, B., Wellnitz, D. and Klämbt, D. (1981) *Z. Pflanzenphysiol.* **101**, 295.
13. Katekar, G. K. and Geissler, A. E. (1982) *Phytochemistry* **21**, 257.
14. Batt, S. and Venis, M. A. (1976) *Planta* **130**, 15.
15. Venis, M. A. (1977) *Nature (London)* **266**, 268.
16. Verma, D. P. S. and van Huystee, R. B. (1971) *Exp. Cell. Res.* **69**, 402.

17. Nessel, A. and Mäder, M. (1977) *Z. Pflanzenphysiol.* **82**, 235.
18. Kossatz, V. C. and van Huystee, R. B. (1976) *Can. J. Botany* **54**, 2089.
19. van Huystee, R. B. (1976) *Bot. Gaz.* **137**, 325.
20. Łobarzewski, J. and van Huystee, R. B. (1982) *Plant Sci. Letters* **26**, 39.
21. Łobarzewski, J. (1981) *Int. J. Biol. Macromol.* **3**, 77.
22. Wilchek, J. (1974) in *Methods in Enzymology*, Vol. 34, p. 59. Academic Press, New York.
23. Lappi, D. A., Stolzenbach, F. E. and Kaplan, N. O. (1976) *Biochem. Biophys. Res. Commun.* **69**, 878.
24. Łobarzewski, J. and Trojanowski, J. (1979) *Acta Biochim. Pol.* **26**, 309.
25. Laas, T. and Fast-Johansson, A. (1979) Reprinted from *Protides of Biological Fluids*, 27th Colloquium, p. 693. Pergamon Press, Oxford.