Characterization and Purification of Herbicide Naproanilide Receptor from Tobacco Mesophyll Callus

Yei-Shung Wang,* Kuan-Lang Hwang,† and Yar-Ning Hsieh

Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China

Herbicide naproanilide receptor proteins in tobacco mesophyll callus were isolated and characterized. The reaction between naproanilide and its receptor proteins is a specific binding with dissociation constant $K_d = 1.8 \times 10^{-7}$ M. The optimum conditions for the binding reaction are 0−4 °C at pH 5.8−7.8 for 30−45 min. Purification of naproanilide receptor proteins on a chromatograph with Sepharose 6B gel filtration, DEAE-Sephacel ion exchange and NOP-ϵ-L-lysine-Sepharose 4B affinity columns in sequence revealed the molar mass 48 kDa. Purified naproanilide receptor exhibited no stimulation on RNA synthesis, and the results indicated that the receptor may be only a carrier for absorption and translocation of plant hormone but cannot enter nuclei for RNA synthesis and regulation of gene expression.

Keywords: *Naproanilide; receptor protein; binding activity; chromatography; purification method*

INTRODUCTION

Naproanilide [2-(2-naphthoxy)propionanilide] is extensively used as a selective herbicide to control most annual and perennial weeds in paddy fields. This chemical is rapildy degraded in soil (Oyamada et al., 1980), in aqueous solution on irradiation with UV (Wang et al., 1988) or sunlight, and in surface water of flooded soil (Oyamada and Kuwatsuka, 1986). 2-(2-Naphthoxy)propionic acid (NOP) and methyl 2-(2-naphthoxy)-propionate (NOPM) are major degradation products. The herbicide is absorbed through the roots, but little is translocated to the upper part of rice plants (Oyamada and Kuwatsuka, 1982), *Cyperus serotinus* (Kobayashi and Ichinose, 1987), and tobacco (*Nicotiana tabacum* cv. Wisconsin 38; Wang et al., 1994). The rate of absorption by roots was much greater in the tolerant rice plant than in two susceptible plants, smallflower umbrella plant (*Cyperus difformis* L.) and *C. serotinus* (Kobayashi and Ichinose, 1984). Naproanilide and its metabolites in shoots were more abundant in a susceptible plant, but in roots they were more abundant in tolerant rice plants (Kobayashi and Ichinose, 1984). Naproanilide is rapidly metabolized in the plant and NOP, NOPM, and conjugates (glycoside) are major metabolites (Oyamada and Kuwatsuka, 1982; Kobayashi and Ichinose, 1984, 1987; Wang et al., 1992, 1994; Oyamada et al., 1986). This herbicide remarkably stimulated RNA synthesis in a susceptible plant such as smallflower umbrellaplant, *C. serotinus* (Kobayashi et al., 1983), and tobacco (Hwang et al., 1995), indicating that RNA synthesis may be a primary site of naproanilide action in plants. The disturbance of the RNA metabolism might induce the observed abnormal growth, and the subsequent result of suppressed plant growth was reported by Kobayashi et al. (1983). They also suggested that naproanilide inhibited tuber initiation through its action on the RNA synthetic process relative to the development of rhizome into tuber. Inhibition of tuberization implies that this herbicide may be a hormone-type herbicide (Kusanagi, 1978).

* Corresponding author (fax +886-2-3620155).

† Present address: Refining & Manufacturing Research Center, Chinese Petroleum Corp., Chiayi, Taiwan, Republic of China.

The present work is designed to isolate and to purify the naproanilide receptor by using varied column chromatography in sequence and to prove the presence of a receptor in tobacco mesophyll callus.

MATERIALS AND METHODS

[^{14}C]Naproanilide uniformly labeled at the naphthalene ring with a specific activity 1.65 *mCi*/mmol and a radiochemical purity greater than 99% was used. The labeled materials and unlabeled naproanilide of purity greater than 99.9% were provided by Mitsui Toatsu Chemical Inc. (Tokyo, Japan). [5,6-^{3}H]UTP with a specific activity 41 *mCi*/mmol and radiochemical purity greater than 98% was purchased from Amersham International plc (Buckinghamshire, England). Seeds of tobacco (*N. tabacum* cv. Wisconsin 38) were provided by the Department of Botany, National Taiwan University.

Suspension Culture of Tobacco Mesophyll and Isolation of Membrane Protein from Tobacco Mesophyll Callus. Details of the method for the suspension culture of tobacco mesophyll are given in our previous report (Hwang, 1995). Extraction of membrane protein from tobacco mesophyll calli followed the steps of Oostrom et al. (1980). Tobacco mesophyll calli were poured into an equal volume of TME buffer solution (composed of 40 mM Tris-HCl, 2 mM 2-mercaptoethanol, and 0.2 mM EDTA, pH 7.8) and homogenized for 30 s. After the calli were filtered with doubled nylon net to discard the scrap, the filtrates were centrifuged (4800*g*, Himac Centrifuge SCR20BA) for 10 min to exclude the cell fragments and macro-organelles. Cellular membranes in the liquid, including plasma membrane, tonoplast, endoplasmic reticulum, Golgi, outer membranes of plastids and mitochondria, probably fragments of broken mitochondria and plastids, etc., were separated with an ultracentrifuge (80000*g*, SCP-85 H, Hitachi Co.) for 30 min, and membrane proteins were extracted with Triton X-100 (1%, w/v).

Binding Activity Determination. Naproanilide was added to membrane protein extracts (2 mL, containing 1−5 μg of protein/mL). For testing in higher concentration, [^{14}C]-naproanilide (5 nmol) with unlabeled chemical (5, 25, 50, 100, or 200 nmol) was added. In the case of naproanilide concentrations less than 5.0 nmol, only the labeled compound was added (0.25, 0.50, 1.0, and 5.0 nmol). The tests were conducted in triplicate. The samples were incubated at 4 °C for 30 min and then applied to disposable column (PD-10) prepacked with Sephadex G-25M (Pharmacia Co.). The columns were eluted with TME buffer, and the eluates from the second to the sixth (bound naproanilide) were collected. Portions (1 mL) of the eluates were placed in glass scintillation vials (20 mL) contain-

S0021-8561(96)00590-0 CCC: $14.00

ing scintillation cocktail (10 mL, composed of 0.4% PPO, 0.02% POPOP, and 6% naphthalene in a mixture of dioxane, methanol, and ethylene glycol (88:10:2), *i.e.*, Bray's solution). The radioactivity was measured for 3 min using Tri-Carb 4430 Scintillator (Packard Co.), and the binding activity of protein bound to [^{14}C]naproanilide was obtained. According to the formula from Scatchard (1949)

$$B/F = K(n - B)$$

in which B and F were bound and free [^{14}C]naproanilide, respectively, K is the binding constant, and n is the number of binding sites. The number of binding sites and the dissociation constant K_d (slope $= -1/K_d$) were derived from plots of B *vs* B/F. Otherwise, the dextran-coated charcoal method (Bogers et al., 1980; Oostrom et al., 1975) was used for examination of binding activity.

Purification of Membrane Protein. Membrane protein extracts (40 mL) were applied to a filtration column (Sepharose 6B gel, 60 × 5 cm) and eluted with TME buffer solution at the rate of 60 mL/h. The eluates were collected in fractions (each 10 mL) and the fractions with absorption at 280 nm were combined and tested binding activity. Further purification was performed on introducing the above primary purified protein into an ion exchange column (DEAE-Sephacel, 20 × 1.6 cm). The column was eluted with TME buffer solution overnight and then eluted with same buffer solution containing NaCl with concentration gradient (0−2 M) at 25 mL/h. Eluates were collected in fractions (each 5 mL), and the fractions with absorption at 280 nm were combined and tested for binding activity as above.

Preparation of NOP-ϵ-L-lysine-Sepharose 4B. NOP-ϵ-L-lysine-Sepharose 4B was prepared by the following three steps in sequence: (1) synthesis of L-lysine copper chelate followed the method of Neuberger and Sanger (1943), (2) synthesis of NOP-ϵ-L-lysine followed the method of Hutzinger and Kosuge (1968), and then (3) synthesis of NOP-ϵ-L-lysine-Sepharose 4B followed the method of Cuatrecasas et al. (1968).

NOP-ϵ-L-lysine-Sepharose 4B Affinity Column Chromatography. Crude membrane protein extracts or after purification on columns (Sepharose-6B and DEAE-Sephacel) were adjusted to solution in NaCl (0.1 M) and applied to NOP-ϵ-L-lysine-Sepharose 4B affinity column (10 × 1 cm). The column was eluted with TM buffer solution containing NaCl (0.1 M) at 200 mL/h and 4 °C until the eluates showed no absorption at 280 nm. The column was then eluted with NaCl (1 M, 30 mL), and eluates were collected in fractions (each 2 mL). NaCl in the column was washed with H_2O (30 mL), and the column was then eluted with KOH (2 mM, 30 mL, pH 11.2). The eluates were collected in test tubes containing a mixture (0.02 mL) of 1 M Tris-HCl (pH 7.5) and 2-mercaptoethanol (1 M) in fractions (each 2 mL). The fractions with absorption at 280 nm were combined to determine the stimulation of the protein to RNA synthesis.

Determination of the Stimulation of Naproanilide Receptor to RNA Synthesis. The reaction mixture (0.25 mL) containing of Tris-HCl (20.0 μmol, pH 8.0), Mg^{2+} (3.0 μmol), Mn^{2+} (0.125 μmol), ATP, GTP, and CTP (0.1 μmol), dithiothreitol (0.3 μmol), EDTA (0.01 μmol), UTP (0.005 μmol), [^{3}H]UTP (7.5 μ*Ci*), glycerol (1.25%), heat-denatured calf thymus DNA (20.0 μg), and RNA polymerase (2.0 units) was reacted with separated membrane protein (50 μg) for 15−30 min at 37 °C. A mixture of $Na_2P_2O_7$ (10 mM), SDS (5%, 0.5 mL), and TCA (10%, 3 mL, cold) was added to quench the reaction. The precipitates were collected on Whatman GF/A filter, washed three times with quenching solution, and then dried and counted.

Electrophoresis. SDS−PAGE was performed according to Laemmli (1970) using a stacking gel (0.375%) and a running gel (10%). The Mighty Small II electrophoresis unit (Hoefer SE 250) was used to operate the gel at 100 V. Proteins were stained with Coomassie Blue (Fairbanks et al., 1971). IEF electrophoresis was performed between pH 3 and 10 using the mini IEF cell (Bio-Rad model 11) according to the manufacturer's instructions.

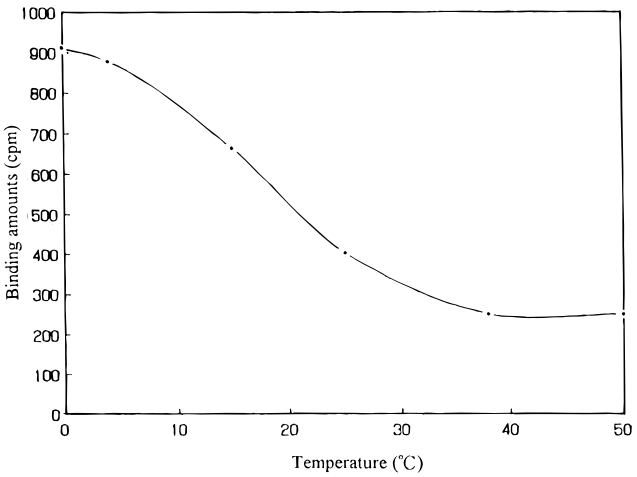

Figure 1. Effect of temperature on the binding of naproanilide to tobacco callus membrane proteins (pH 7.8, 30 min, 5.0 × 10^{-6} M).

RESULTS AND DISCUSSION

That naproanilide stimulates RNA synthesis in tobacco mesophyll cells is known from previous work (Hwang et al., 1995). Naproanilide receptor protein, therefore, may be present in plant cells and may have similar actions as of 2,4-D (2,4-dichlorophenoxyacetic acid). After separating cytoplasm and cellular membrane by ultracentrifugation, we examined the binding of naproanilide to its receptor protein by the method of dextran-coated charcoal. Binding activity was observed in the protein extracts from the cellular membrane and were not found in cytoplasm, indicating that naproanilide receptor protein was located at the cellular membrane.

Extracts were brownish during extraction of protein from tobacco callus with TME buffer, even when the concentration of TME was raised to 60 mM Tris-HCl, 3 mM 2-mercaptoethanol, and 0.3 mM EDTA. The effect was not found in extracts from tobacco plant under exactly the same procedure, indicating either that many phenolic substances or the strong activities of phenol oxidases were present in tobacco callus. The brownish materials had not only loss of enzyme activity but also the product of chemiluminescence which seriously interfered with radioactivity measurement. To avoid this interference, we modified experiments by diluting the protein extracts and elevating the counts of sample.

The effects of temperature, reaction duration, and pH on binding of naproanilide with its receptor protein in tobacco callus membrane are shown in Figures 1−3. The optimum conditions for the binding reaction are temperature 0−4 °C (Figure 1, pH 7.8, 30 min), reaction duration 30−45 min (Figure 2, 4 °C, pH 7.8), and pH 5.8−7.8 (Figure 3, 4 °C, 30 min) when the reaction proceeded with a 5.0 × 10^{-6} M concentration of naproanilide.

The effects of naproanilide concentration on binding [^{14}C]naproanilide to the receptor protein are shown in Table 1. The results showed that binding activity decreased with increasing concentration of naproanilide on adding unlabeled chemicals. Ray (1977) defined specific binding as thermally sensitive with high affinity, and saturable binding; on the other hand, nonspecific binding is thermally stable with lower less affinity and lack of saturable binding. Sussman and Kende (1978) defined saturable binding as binding activity that is decreased upon addition of more growth hormone or

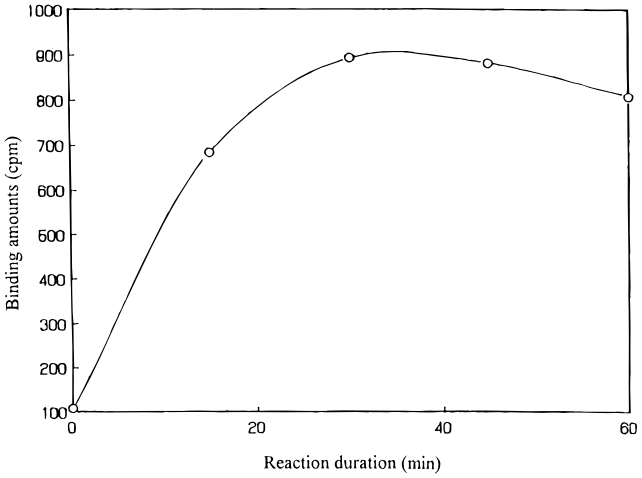

Figure 2. Effect of reaction duration on the binding of naproanilide to tobacco callus membrane proteins (pH 7.8, 4 °C, 5.0×10^{-6} M).

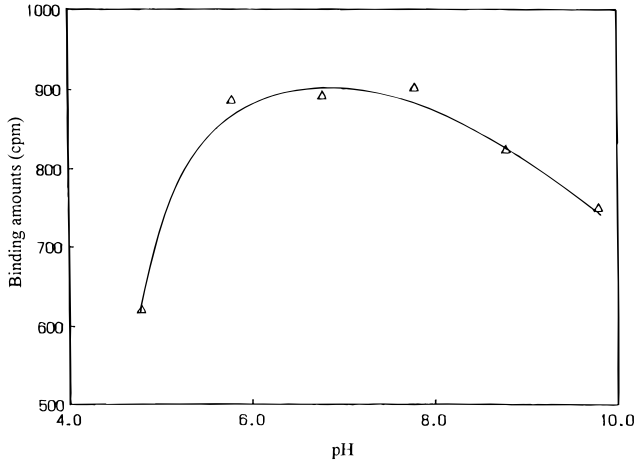

Figure 3. Effect of pH on the binding of naproanilide to tobacco callus membrane proteins (pH 7.8, 4 °C, 5.0×10^{-6} M).

Table 1. Effect of Concentration on Binding [^{14}C]Naproanilide to Receptor Proteins

naproanilide added (*n* mol)			naproanilide concentration	binding
^{14}C-labeled	unlabeled	total	in protein extracts (M)	activity (dpm)
0.25		0.25	1.25×10^{-7}	185
0.50		0.50	2.50×10^{-7}	287
1.0		1.0	5.00×10^{-7}	394
5.0		5.0	2.50×10^{-6}	1103
5.0	5	10	5.00×10^{-6}	980
5.0	25	30	1.50×10^{-5}	721
5.0	50	55	2.55×10^{-5}	669
5.0	100	105	5.25×10^{-5}	584
5.0	200	205	1.03×10^{-4}	447

similar compounds into the system and is unaffected by nonsaturable binding. The binding studies with [^{14}C]-naproanilide show clearly saturation behavior, which is typical for specific receptors (Table 1). However, these experiments were carried out with a crude membrane protein preparation rather than with the highly purified receptor protein and contained large amounts of non-receptor proteins (Figures 6 and 7). It seems highly probable that the [^{14}C]naproanilide was metabolized to NOP by enzymes in the crude preparation as was the case in whole plant tissue (Takasawa et al., 1975), which then is bound to an auxin receptor through which the biological activity of naproanilide is usually brought about. The dependence on time (Figure 2) and pH

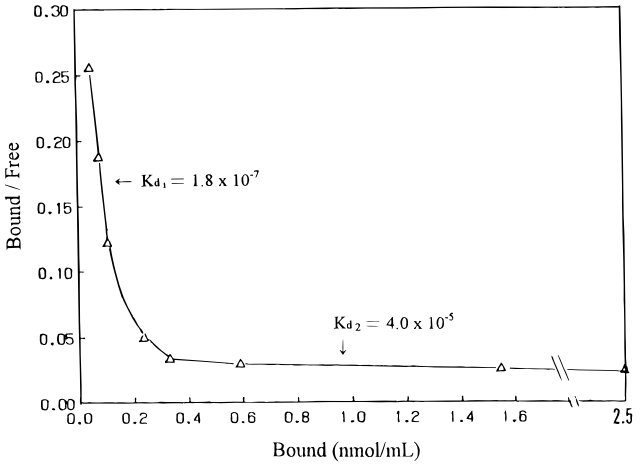

Figure 4. Scatchard plot of naproanilide binding to extracts from tobacco callus.

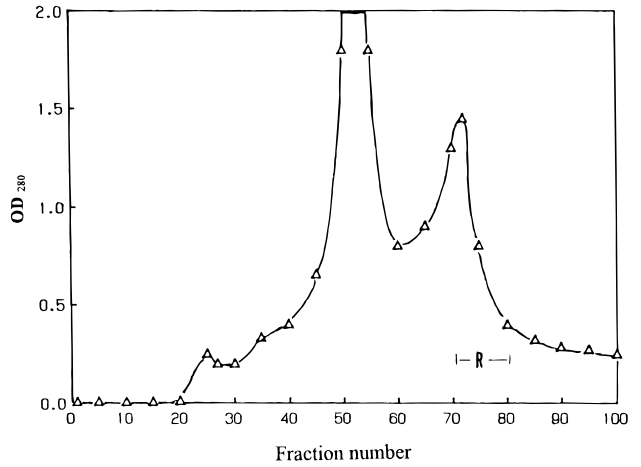

Figure 5. Elution pattern for tobacco callus membrane protein on Sepharose 6B gel filtration column. "|−R−|" is the range of naproanilide-bound protein fraction.

(Figure 3) supports this assumption. The temperature dependence (Figure 1) could be interpreted in terms of weaker binding (faster dissociation) at higher temperature or by reduced decomposition by further metabolic steps. The binding between [^{14}C]naproanilide and its receptor protein is therefore a specific binding. According to Scatchard's equation plot of bound *vs* bound/free [^{14}C]naproanilide appears in Figure 4. Two binding sites with dissociation constants $K_{d_1} = 1.8 \times 10^{-7}$ M and $K_{d_2} = 4.0 \times 10^{-5}$ M were observed. With more specific binding, K_{d_1} may be the binding site for naproanilide, and the more nonspecific binding of K_{d_2} may be for other substrates. Batt and Venis (1976) demonstrated that auxin binding sites of two classes were present in corn coleoptile membranes with K_d values of 1.8×10^{-7} M (site I) and 14.5×10^{-7} M (site II).

According to these results, the naproanilide receptor was present in tobacco callus and located at the cellular membrane. Further separation and purification of this receptor was performed on a chromatograph. The elution pattern for tobacco callus membrane extracts through a gel filtration column (Sepharose 6B) is shown in Figure 5. [^{14}C]Naproanilide-bound protein eluted between fractions 70 and 80. On further purification on an ion exchange column (DEAE-Sephacel), [^{14}C]-naproanilide receptor protein was elutable with NaCl (0−03 M) (Figure 6). NOP-ϵ-L-lysine-Sepharose 4B affinity column was prepared for further purification. Mass and IR (KBr pellet) spectra of NOP-ϵ-L-lysine

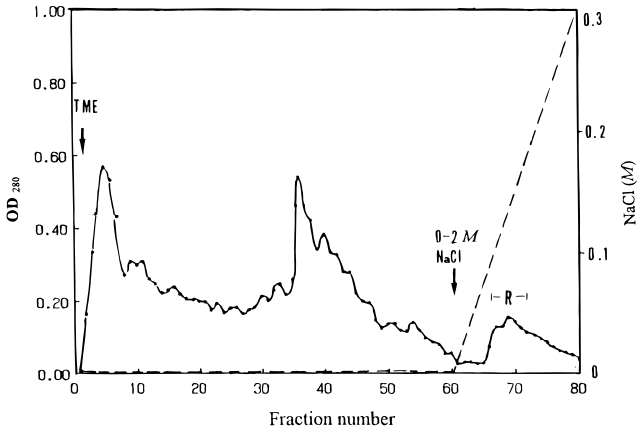

Figure 6. Elution pattern for tobacco callus membrane protein on DEAE-Saphacel ion exchange column. "|−R−|" is the range of naproanilide-bound protein fraction.

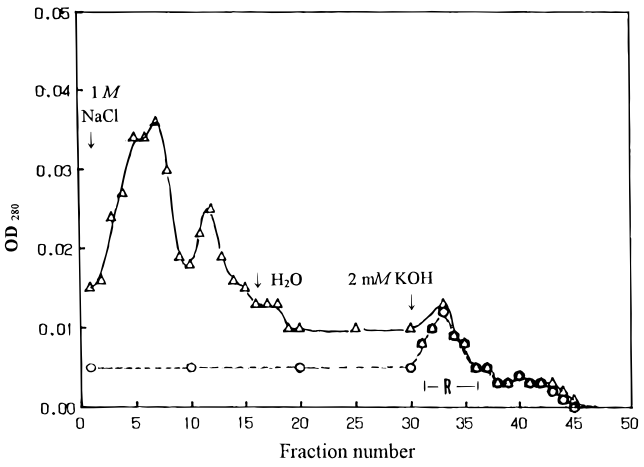

Figure 7. Elution pattern for naproanilide receptor on NOP-ϵ-L-lysine-Sepharose 4B affinity column. (△) Crude membrane protein extracts; (○) after purification by Sepharose-6B and DEAE-Sephacel. |−R−| is the range of naproanilide-bound protein fraction.

showed a molar mass of 344 and characteristic −CONH absorption at wavenumber 1654 cm^{-1}, demonstrating that NOP was associated with a covalent bond at ϵ-NH_2 of L-lysine, because the α-NH_2 and −COOH groups were blocked by Cu^{2+} (Cuatrecasas et al., 1968). NOP instead of naproanilide was used in this work, because no amine and carboxyl groups on naproanilide molecule exist to attach on Sepharose 4B using L-lysine as bridge. Takasawa et al. (1975) reported that naproanilide itself showed no lethal activity on the plant; the activity was exhibited after absorption by the plant and transformation to NOP. The elution pattern for naproanilide receptor on a NOP-ϵ-L-lysine affinity column chromatograph (Sepharose 4B) is shown in Figure 7. Two proteins implying nonspecific binding were eluted from crude protein extracts with NaCl (1 M), and afterward, naproanilide receptor was eluted with KOH (2 mM). The isolation of the receptor by affinity chromatography was carried out with a NOP anchor group, also specific for auxins, but this study does not provide strong evidence for a specific naproanilide receptor.

At each stage of purification, the eluates were examined with polyacrylamide gel electrophoresis (Figure 8). Many impure substances were observed at primary purification with a gel filtration column (Sepharose 6B), but the purity was greatly increased after purification on an ion-exchange column (DEAE-Sephacel); several bands of proteins were still found. On further purification with an NOP-ϵ-L-lysine-Sepharose 4B affinity column, only one protein band with molar mass about 48 000 Da was observed. Oostrom et al. (1980) purified IAA receptor from tobacco callus; at least 100 kinds of protein in the eluates were found after column chromatography (Sepharose 6B), and homogeneous protein was not obtained when further purification was performed on an ion-exchange column chromatograph (DEAE-Sephacel).

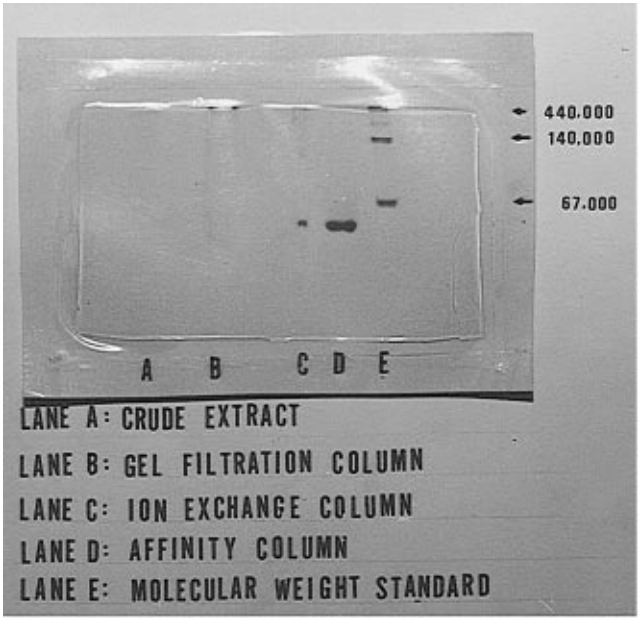

Figure 8. SDS−PAGE for naproanilide receptor at different purification stages. Crude fractions (lane A) show only light staining, many impure substances are observed in crude gel filtration (lane B) and ion exchange (lane C) preparations, the purity is greatly increased in lane C, while a single band is found in lane D. This work is to show purification, as surely equivalent amounts of protein are not determined.

Purified naproanilide receptor exhibited no stimulation of RNA synthesis in this work, indicating that naproanilide receptor may not be exactly the same kind of protein purified by Venis (1971) or Rizzo et al. (1977). They reported that proteins isolated from pea and corn cell were demonstrated to stimulate RNA transcription after purification on a 2,4-D-ϵ-L-lysine-Sepharose 4B affinity column chromatograph. Naproanilide receptor purified from tobacco callus membrane in this work and plant cellular membrane receptor discovered in other work may be only a carrier for absorption and translocation of plant hormone but cannot enter nuclei for RNA synthesis and regulation on gene expression. In the other words, the action of the receptor protein on RNA synthesis cannot be expected because the protein is membrane bound and hence is not accessible to the nucleus. Membrane-bound receptors, when activated, always act indirectly on the gene expression by secondary messengers. Because much information has not yet been obtained, the role that naproanilide receptor plays on tobacco callus is not discriminative.

ACKNOWLEDGMENT

We thank Mitsui Toatsu Chemical Inc., Tokyo, Japan, for providing pure and ^{14}C-labeled naproanilide for this experiment and the National Science Council of the Republic of China for support.

LITERATURE CITED

Batt, S.; Venis, M. A. Separation and localization of two classes of auxin binding sites in corn coleoptile membranes. *Planta* **1976**, *130*, 15−21.

Bogers, R. J.; Kulescha, Z.; Quint, A.; van Vliet, Th. B.; Libbenga, K. R. The presence of a soluble auxin receptor and the metabolism of 3-indoleacetic acid in tobacco pith explants. *Plant Sci. Lett.* **1980**, *19*, 311−317.

Cuatrecasas, B. P.; Wilchek, M.; Anfinsen, C. B. Selective enzyme purification by affinity chromatography. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *65*, 636−643.

Fairbanks, G.; Steck, T. L.; Wallack, D. F. H. Electrophoretic analysis of the major polypeptides of human erythrocyte membrane. *Biochemistry* **1971**, *10*, 2606−2617.

Hutzinger, O.; Kosuge, T. Microbial synthesis and degradation of indole-3-acetic acid. III. The isolation and characterization of indole-3-acetyl-ϵ-L-lysine. *Biochemistry* **1968**, *7*, 601−605.

Hwang, K. L.; Hsieh, Y. N.; Wang, Y. S. Effect of naproanilide on CO_2 fixation and RNA, protein and lipid biosynthesis in tobacco cell. *J. Chin. Agric. Chem. Soc.* **1995**, *33*, 665−672.

Kobayashi, K.; Ichinose, K. Absorption, translocation and metabolism of root-applied naproanilide in rice and cyperaceous weeds. *J. Pestic. Sci.* **1984**, *29*, 38−44.

Kobayashi, K.; Ichinose, K. Absorption, translocation and metabolism of naproanilide in *Cyperus serotinus* Rottb. *Weed Res., Jpn.* **1987**, *32*, 46−53.

Kobayashi, K.; Ichinose, K.; Hyakutake, H.; Ishizuka, K. Effect of naproanilide on turberization and RNA synthesis of *Cyperus serotinus* Rottb. *Weed Res., Jpn.* **1983**, *28*, 43−50.

Kusanagi, T. Biology and control of perennial paddy weeds. *J. Pestic. Sci.* **1978**, *3*, 485−497 (in Japanese with English summary).

Laemmli, U. K. Cleavage of structural proteins during assembly of the head of bacteria phage T4. *Nature* **1970**, *227*, 680−685.

Neuberger, A.; Sanger, F. The availability of the acetyl derivatives of lysine for growth. *Biochem. J.* **1943**, *37*, 515−519.

Oostrom, H.; van Loopik-Detmers, M. A.; Libbenga, K. R. A high affinity receptor for indoleacetic acid and in cultured tobacco pith explants. *FEBS Lett.* **1975**, *59*, 194−197.

Oostrom, H.; Kulescha, Z.; Van Vliet, Th. B.; Libbenga, K. R. Characterization of a cytoplasmic auxin receptor from tobacco pith callus. *Planta* **1980**, *149*, 44−47.

Oyamada, M.; Kuwatsuka, S. Absorption, translocation and metabolism of the herbicide naproanilide, 1-(2-naphthoxy)-propionanilide, in rice plants. *J. Pestic. Sci.* **1982**, *7*, 9−14.

Oyamada, M.; Kuwatsuka, S. Photodegradation of the herbicide naproanilide in aqueous solution and in surface water of flooded soil. *J. Pestic. Sci.* **1986**, *11*, 179−187.

Oyamada, M.; Igarashi, K.; Kuwatsuka, S. Degradation of the herbicide naproanilide, 1-(2-naphthoxy)propionanilide, in flooded soil under oxidative and reductive conditions. *J. Pestic. Sci.* **1980**, *5*, 495−501.

Oyamada, M.; Tanaka, T.; Takasawa, Y.; Takematzu, T. Metabolic fate of the herbicide naproanilide in rice plants (*Oryza sativa* L.) and *Sagittaria pygmaea* Miq. *J. Pestic. Sci.* **1986**, *11*, 197−203.

Ray, P. M. Specificity of auxin-binding sites on maize coleoptile membranes as possible receptor sites for auxin action. *Plant Physiol.* **1977**, *60*, 585−591.

Rizzo, P. J.; Petersen, K.; Chent, J. H. Stimulation of transcription by a soluble factor isolated from soybean hypocotyl by 2,4-D affinity chromatography. *Plant Sci. Lett.* **1977**, *8*, 205−211.

Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660−672.

Sussman, M. R.; Kende, K. In vitro cytokinin binding to a particulate fraction of tobacco cells. *Planta* **1978**, *140*, 251−259.

Takasawa, Y.; Igarashi, K.; Takamatsu, T. MT-101: A selective herbicide for rice. *Proc. Asian-Pacific Weed Sci. Soc. Cong., 5th*, **1975**, 96−98.

Venis, M. A. Stimulation of RNA transcription from pea and corn DNA by protein retained on Sepharose coupled 2,4-dichlorophenoxyacetic acid. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1824−1827.

Wang, Y. S.; Lu, C. H.; Chen, Y. L. Photodecomposition of the herbicide naproanilide in aqueous solution. *Weed Sci. Bull. (Taiwan, ROC)* **1988**, *9*, 1−9.

Wang, Y. S.; Hwang, K. L.; Hsieh, Y. N.; Chen, Y. L. Fate of the herbicide naproanilide in a rice-paddy model ecosystem. *J. Pestic. Sci.* **1992**, *17*, 161−167.

Wang, Y. S.; Hwang, K. L.; Chen, Y. L. Absorption, translocation and metabolism of herbicide naproanilide in tobacco. *Pestic. Sci.* **1994**, *42*, 53−58.

Received for review August 5, 1996. Revised manuscript received April 1, 1997. Accepted April 4, 1997.[⊗] This work was supported by the National Science Council of the Republic of China (NSC 79-0409-B002-53).

JF960590D

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1997.