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Characteristics of Nitrate Reductase-inactivating Proteins Obtained from Corn Roots and Rice Cell Cultures[1]

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ABSTRACT

Nitrate reductase (NR)-inactivating proteins from corn roots (Wf-9 × 38-11) and rice cell suspension cultures were tested against a partially purified NR obtained from corn leaves (W64A × W182E). The corn protein was purified 921-fold and the rice protein, 1,660-fold using standard purification procedures. Approximate molecular weight values were 75,000 for the corn protein, and 150,000 for the rice protein as determined by Sephadex G-100 gel filtration. The Sephadex-treated proteins were characterized by electrophoresis on polyacrylamide gels. With a running pH of 9.4 the corn protein remained at the origin whereas the rice protein migrated with an R_F value of 0.49. With a running pH of 4.0 the corn protein migrated with an R_F value of 0.25. With the corn protein the activities of NR inactivation and hydrolysis of azocasein were detected in the same protein band. The rice protein, however, had no associated protease activity. From sodium dodecyl sulfate gel electrophoresis, there was one major protein band with an estimated molecular weight of 66,000 in corn protein. In rice protein four bands were observed with estimated molecular weights of 73,000, 66,000, 62,500, and 58,500, respectively.

Both inactivators had an inhibitory effect on NADH-NR and NO_3^- induced NADH-cytochrome *c* reductase activities but they had less influence on the activities of $FMNH_2$-NR and reduced methylviologen-NR. Inactivation of rice cell NR by rice inactivator was reversed by addition of NADH. Inactivation of corn leaf NR by rice inactivator was inhibited by the simultaneous addition of NADH, but rice inactivator-inactivated corn leaf NR could not be reactivated by NADH.

Rapid fluctuations of NADH-NR[4] and its constituent activities in higher plants indicate effective *in vivo* regulatory mechanisms. For example, in corn roots NR is induced by the substrate NO_3^- and there is a rapid loss of NR when NO_3^- is removed from the medium. The induction is more rapid in root tips, whereas the loss in activity is more rapid in mature root sections (2, 10). This later observation is seen both *in vivo* and in cell-free extracts (10). The enhanced rate of inactivation in mature root sections could be controlled by specific inactivating proteins (12). However, there are also other probable explanations and some of these have recently been reviewed by Hewitt (5).

NR-inactivating proteins have been isolated from corn roots (12–14), rice cell cultures (18, 19), rice seedlings (8, 20), and soybean leaves (7). These inactivators preferentially inactivate NADH-NR and its constituent activities. The inactivators from corn and rice cells appear to be localized in the cytoplasm where the NADH-NR is also thought to be located (13, 19). These results suggest that the NR inactivators are concerned with one of the systems which regulate NR in plant tissues.

Partially purified corn inactivator has proteolytic activity toward casein and azocasein (13, 15). Its activity is inhibited by PMSF which is an inhibitor of serine-dependent enzyme, suggesting that the corn inactivator is a serine-protease (13). This inactivator inactivated each of the activities of the NR complex from corn scutellum but the NADH-CR component was more sensitive than $FADH_2$-NR activity. This observation suggested that the NADH-CR component is the main site of the action (14). On the other hand, partially purified rice cell inactivator had no protease activity and had no influence on the activity of reduced MV-NR from rice cells (19). The inactivation of NADH-NR from the cells by the rice inactivator was reversed by the addition of NADH during the reaction and this protective effect of NADH was canceled by the addition of NO_3^- or Cyt *c* together with NADH (21). These results suggested that the rice cell inactivator is a binding protein for the oxidized form of NR in rice cells. The soybean inactivator purified to an electrophoretic homogeneous protein also has no protease activity, and in addition it does not inhibit NADH-CR from soybean leaves. Changes in the mobility of soybean inactivator through Sephadex G-75 with or without NR suggested that it was also a specific binding protein (7). Similarly, the rice root inactivator is probably also not a proteolytic enzyme and it has no effect on the reduced MV-NR component (8, 20). Mol wt of the various inactivators were estimated to be 44,000 (13), 200,000 (19), and 31,000 (7) for corn, rice cells, and soybean leaf, respectively. The NR inactivators studied to date seem to have different characteristics. This paper extends comparisons of the characteristics of highly purified corn and rice NR inactivators against a partially purified NR obtained from corn leaves.

MATERIALS AND METHODS

Plant Materials. The seeds of *Zea mays* L. (var. Wf-9 × 38-11) were surface-sterilized by 1% NaOCl and 0.01 N HCl (1). They were then planted on 0.9% agar made up with a modified 0.1-strength Hoagland solution containing 10 mM KNO_3 and 0.08 μM of molybdenum as described previously (10). After 3 days at 26 C, the seedlings were transferred to liquid culture containing the same medium without agar and grown for another 5 days at 26 C with 12-h light photoperiod. The mature root region of the seedlings (primary root minus 0 to 1-cm tip and basal regions) were used in preparing the NR inactivator. NR was prepared as described previously (2) from primary leaves of *Zea mays* L. (var. W64A × W182E). Rice cells were cultured in liquid R-2 medium for 10 days as reported before (19).

Purification of Inactivating Proteins. A 106-g sample of corn

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[4] Abbreviations: NR: nitrate reductase; MV: methylviologen; CR: cytochrome *c* reductase; $FMNH_2$: reduced flavin adenine mononucleotide; PMSF: phenylmethylsulphonyl fluoride.

roots and an 85-g sample of rice cells were frozen immediately in liquid N_2, then extracted with 50 mM K-phosphate (pH 7.5) containing 0.5 mM EDTA and 5 mM cysteine. Standard purification procedures involved precipitation with $(NH_4)_2SO_4$, fractionation at pH 4.0, and adsorption on CM-cellulose were used as described previously (13, 19). Inactivator fractions obtained with CM-cellulose were concentrated by precipitation with 80% $(NH_4)_2SO_4$, redissolved in a small volume of 10 mM K-phosphate (pH 7.0), and dialyzed in the same buffer for 15 h. Then the solutions (3 ml) were applied to Sephadex G-100 column (1.2 × 90 cm) equilibrated with 10 mM K-phosphate (pH 7.0) containing 0.1 M KCl and eluted with the same buffer. The active fractions were pooled, concentrated with solid sucrose, and filtered a second time with the same column. The inactivator was then concentrated by lyophilization and stored at −20 C until required.

Polyacrylamide Disc Gel Electrophoresis. Analytical disc gel electrophoresis of the inactivators from corn and rice cells was performed at 4 C using a Tris-glycine buffer system (4). Gels (7.5% acrylamide) were prepared from a stock solution of 30% acrylamide and 0.8% bisacrylamide. The purity of the corn inactivator was estimated using 7.5% gels and a pH of 4.0 with a β-alanine-KOH buffer system (11). Stacking gels (2.5%) were used in each case. A drop of glycerol was added to each sample (50 μl) and gels were run at 2 mamp per tube for about 4 h.

SDS-polyacrylamide gel electrophoresis of the purified inactivators was prepared as described by Weber *et al.* (16). Gels (10% acrylamide) were prepared from the stock solution of 30% acrylamide and 0.8% bisacrylamide. The lyophilized protein was incubated at 100 C for 2 min in 50 μl of 10 mM Na-phosphate at pH 7.2 containing 1% SDS and 1% 2-mercaptoethanol. For the corn inactivator, 2.5 μl of 0.6% of PMSF was added in the SDS solution. As references, 5 μg per tube of BSA (mol wt 68,000), ovalbumin (45,000), glyceraldehyde-3-P dehydrogenase (36,000), trypsin (23,300), and Cyt *c* (12,800) were used. Protein was stained with Coomassie brilliant blue R and each gel was scanned at 550 nm with a Gilford spectrophotometer 2400.

Partial Purification and Assay Methods of NR. Primary leaves of corn (W64A × W182E) were extracted with a cold mortar and pestle in 4 volumes of 0.1 M Hepes (pH 7.4) containing 0.5 mM EDTA and 5 mM cysteine as described previously (2). NR was partially purified by precipitation with 40% saturation (24.3 mg/100 ml) $(NH_4)_2SO_4$ and subsequent centrifugation at 30,000*g* in a Sorvall RC-2 centrifuge. In some studies a special extraction procedure described by Wallace (14) for isolating NO_3^--induced NADH-CR was adopted. The extraction medium was 0.05 M Hepes containing 0.4 M sucrose, 1% (w/v) BSA, 0.5 mM EDTA, 0.1 mM $MgCl_2$, and 5 mM cysteine (pH 7.5). The extracts were centrifuged at 10,000*g* for 10 min. The supernatant was further centrifuged at 272,000*g* for 60 min. NR, in the final supernatant solution, was again partially purified by precipitation with 40% $(NH_4)_2SO_4$. The pellet was taken up in a known volume of 0.1 M Hepes containing 0.5 mM EDTA and 50% glycerol (pH 7.0). In either case the NR fraction could be stored at −20 C for several months.

NADH-NR and NADH-CR were assayed as described previously (2). $FMNH_2$-NR was assayed according to the method outlined by Wray and Filner (17) except that flushing the assay tubes with N_2 was unnecessary. The assay system for reduced MV-NR was as follows: Hepes (pH 7.5, 130 μmol), EDTA (1 μmol), MV (0.1 μmol), KNO_3 (20 μmol), in a total volume of 1.5 ml. The activity was assayed in the same way as $FMNH_2$-NR. A unit of NADH-NR, $FMNH_2$-NR, or reduced MV-NR was defined as that amount of activity which produced 1 nmol NO_2^- per min at 28 C. A unit of NADH-CR was defined as that amount which caused an increase of 1.0 in *A* at 550 nm per min.

Assay of Inactivating Protein Activity. The corn leaf NR stored in glycerol in a freezer was dialyzed in 0.1 M Hepes buffer containing 0.5 mM EDTA (pH 7.5) for 2 h. Then it was diluted 20 times with 0.1 M K-phosphate (pH 7.0) for corn inactivator and pH 7.5 for rice inactivator, and 0.1 ml of diluted NR fraction was used as a substrate. Inactivating protein fraction (10–100 μl) was incubated with the NR fraction for 60 min at 28 C. The inactivating reaction was stopped by the dilution with the NR assay mixture minus NADH or sodium dithionite. The NR reaction was then started with the appropriate addition. When the action of NADH on NR or the NR-inactivating proteins was studied the reaction was started by adding NO_3^-. The inactivating activity was calculated from the difference in the NR or its associated activities with or without inactivation. A unit of inactivator was defined as that amount which inhibited 1% NR activity per 60 min at 28 C.

Protease Activity and Protein Determinations. Standard assay procedures were used for the assay of azocasein-degradating activity (3). Aliquots of inactivating protein (0.25 ml) were incubated for 3 h at 37 C with 1.75 ml of 2.5% azocasein dissolved in 0.1 M K-phosphate buffer, pH 7.0 for corn inactivator and pH 7.5 for rice inactivator. The reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid to 1 ml of supernatant and after centrifugation it was diluted with 1 ml of 0.5 N NaOH. After 20 min the activity was measured at 440 nm. BSA-degradating activity was assayed as reported before (19) and the content of amino acid released was determined by a ninhydrin procedure using alanine as a standard (23).

Protein content was determined by the method of Lowry *et al.* (9), as described previously (2).

RESULTS

Characterization of Inactivating Proteins by Gel Electrophoresis. The corn-inactivating protein was purified 921-fold and the rice protein, 1,660-fold as shown in Table I. Recovery of both inactivators increased in the CM-cellulose chromatography step, indicating that an inhibitor of the inactivators is removed in this step. A heat-stable inhibitor of the corn protein was found in corn

Table I. *Summary of Purification of NR-inactivating Proteins from Corn Roots and Rice Cells*

The activities of inactivating proteins from corn roots and rice cells were assayed with partially purified NADH-NR from corn leaves which formed 1.61 nmol nitrite per min in the assay without the inactivator. CMC = carboxymethyl cellulose.

Step	Total Activity		Recovery		Specific Activity		Purification	
	Corn	Rice	Corn	Rice	Corn	Rice	Corn	Rice
	units × 10^{-3}		%		*units/mg protein* (×10^{-3})		*fold*	
20,000*g* supernatant	320	52	100	100	0.9	0.07	1	1
40–60% $(NH_4)_2SO_4$ ppt	231	24	72	47	3.1	0.08	3.5	1.4
pH 4.0 supernatant	245	27	77	52	4.2	0.16	4.6	2.7
CMC eluate	384	120	120	231	97.4	13.9	108.0	240.0
Sephadex G-100 eluate (1st)	250	51	78	98	356.9	42.5	397.0	733.0
(2nd)	207	61	65	117	828.6	96.3	921.0	1660.0

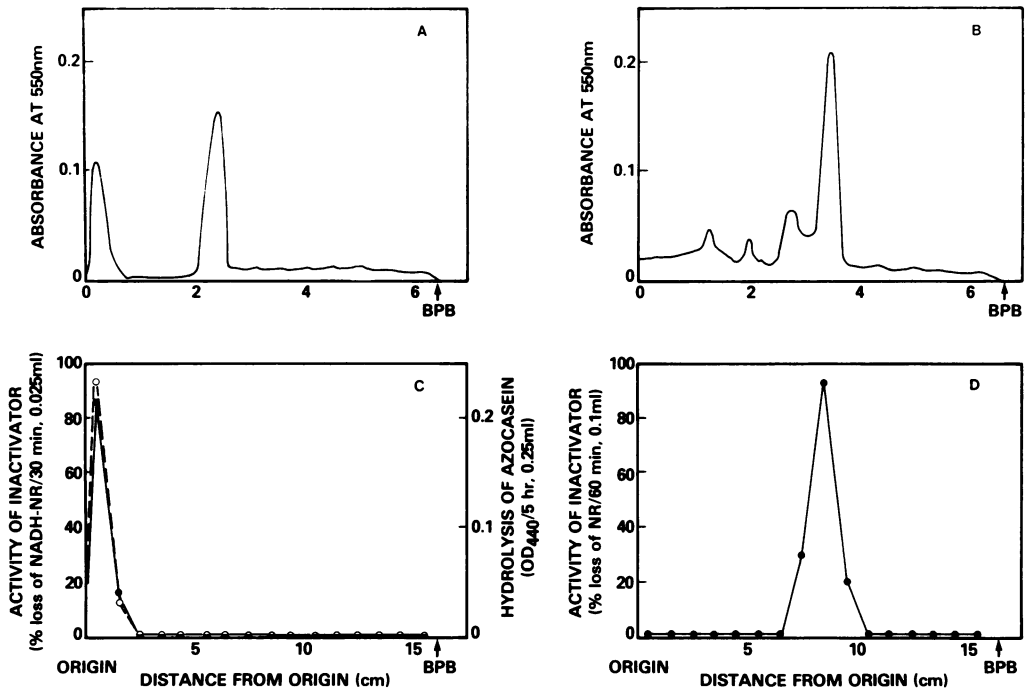

FIG. 1. Polyacrylamide gel electrophoresis of NR-inactivating proteins from corn roots and rice cells with the Tris-HCl buffer system. Inactivating proteins from corn roots (10 μg of protein; 40,068 units of inactivator) and from rice cells (27 μg of protein; 3,387 units of inactivator) were applied to the gels. A: scan of protein from corn inactivator preparation at 550 nm; B: scan of protein from rice inactivator preparation at 550 nm; C: activities of NADH-NR inactivation (●) and hydrolysis of azocasein (○) by corn inactivator (6.1 units); D: activities of NADH-NR inactivation by rice inactivator.

root tips (15) and a factor which inhibits both inactivators was also obtained in the 80% ethanol-soluble fraction from corn scutellum (Yamaya and Oaks, in preparation).

The second filtrate from the Sephadex G-100 (2.5 μg of protein) of corn inactivator was as effective as 0.4 μg of trypsin in hydrolyzing azocasein. Rice inactivator (24 μg), which had almost the same NR-inactivating activity as the corn inactivator, had no detectable proteolytic activity against either azocasein and BSA.

Purified inactivator fractions were characterized by electrophoresis on 7.5% polyacrylamide gels. With a running pH of 9.4 in a Tris-glycine buffer system, the corn protein remained at the origin (R_F value of 0.03) whereas the major band of the rice protein migrated with an R_F value of 0.49 (Fig. 1). Although the corn system had two protein bands with R_F values of 0.03 and 0.34, both the NR inactivator activity and the hydrolysis of azocasein were associated with the slow moving protein band. Recovery of the activities of NR inactivation and hydrolysis of azocasein in corn inactivator was 34 and 41%, respectively. Recovery of the NR inactivator from rice cells was 71%.

The corn inactivator was extracted from the gels with 0.1 M K-phosphate buffer (pH 7.0), dialyzed in 10 mM K-phosphate (pH 7.0), and concentrated by lyophilization. Purity of the lyophilized corn inactivator was confirmed by electrophoresis on polyacrylamide gels using a running pH of 4.0. In this case the gel scan showed one protein band with an R_F value of 0.25. NR inactivation and hydrolysis of azocasein were associated with this protein band (Fig. 2). Recoveries of 32 and 29%, respectively, were found.

Mol Wt Estimation of Inactivating Proteins. The NR-inactivating proteins from corn roots and rice cells had an approximate mol wt of 75,000 and 150,000 by comparing their elution volume from Sephadex G-100 gel filtration with five proteins of known mol wt. From SDS-gel electrophoresis, one major protein band was observed with an estimated mol wt of 66,000 for the corn protein. For the rice protein four protein bands were found (Fig. 3). The estimated mol wt were 73,000, 66,000, 62,500, and 58,500.

Effect of NADH on Inactivation of NADH-NR by Inactivators.

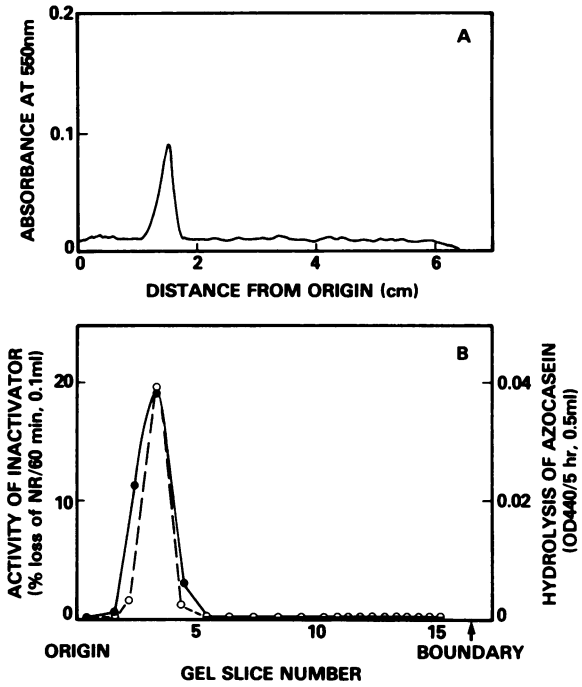

FIG. 2. Polyacrylamide gel electrophoresis of corn inactivator with the β-alanine-KOH buffer system. Corn inactivator, which had 1,425 units of NADH-NR inactivation activity and 0.40 units of azocasein hydrolysis activity, was applied to the gel. A: scan of protein at 550 nm; B: activities of NADH-NR inactivation (●) and hydrolysis of azocasein (○).

In the rice system preincubation of NADH-NR with NADH results in a 60% loss in NR activity (21). In addition, preincubation with NADH completely inhibited the action of the rice inactivator (21). Preincubation of corn leaf NADH-NR with NADH resulted

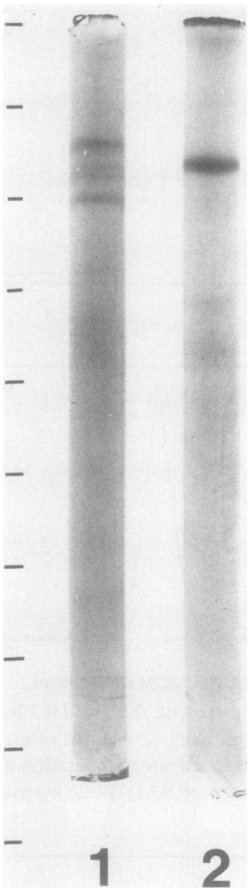

FIG. 3. SDS-polyacrylamide gel electrophoresis. Inactivating enzymes were extracted from the polyacrylamide gels with 0.1 M K-phosphate buffer (pH 7.0). These extracts were dialyzed in 10 mM Na-phosphate buffer (pH 7.2) and concentrated by lyophilization. The corn inactivator (4,272 units) and rice inactivator (2,107 units) were treated with SDS. Photograph of gels: left rice inactivator, right corn inactivator. The R_F values of the reference proteins were: BSA, 0.21; ovalbumin, 0.36; glyceraldehyde-3-P dehydrogenase, 0.42; trypsin, 0.59; and Cyt *c*, 0.82. The R_F values of polypeptides from inactivators of corn were 0.23 and of rice were 0.19, 0.24, 0.245, and 0.27.

in 65% inhibition of NR activity when the preincubation was conducted at pH 8.0 but not with a pH of 7 (Table II). When corn inactivator was included in the reaction its activity was not affected by the addition of NADH at either pH. With the rice inactivator the addition of NADH inhibited the inactivation of corn leaf NADH-NR at both pH 7.0 and pH 8.0.

In Table III the effect of NADH on NADH-NR from corn and rice and on the corn and rice inactivator systems is examined. Corn inactivator inactivated corn NADH-NR more efficiently than rice NADH-NR, suggesting a functional difference either in the inactivating proteins or in the NADH-NRs. Again, the activity of the corn inactivator was not affected by addition of NADH whereas the activity of the rice inactivator was completely inhibited by this treatment. When NADH-NR from rice cells is used as a substrate for rice inactivator, NADH is effective whether it is added initially or close to the completion of the reaction, *i.e.* its effect is reversible. With the corn leaf NADH-NR, on the other hand, NADH inhibits the activity of the rice inactivator but does not reverse its affect.

Susceptibility of NADH-NR and Its Associated Activities from Corn Leaves and Root Tips to NR-inactivating Proteins. The isolated corn- and rice-inactivating proteins inhibited NADH-NR and NADH-CR components from corn leaves but they had less influence on the activities of $FMNH_2$-NR and reduced MV-NR (Table IV). The corn inactivator also inhibited the $FMNH_2$-NR activity from root tips but had no effect on the reduced MV-NR

Table II. *Effect of Preincubation of NADH-NR from Corn Leaves with NADH on Inactivation of NR by Inactivators*

The Sephadex G-100 (first) fraction of corn roots (0.2 μg protein) and that of rice cells (2 μg protein) were used as the inactivating protein.

Amounts of NADH Preincubated	Assay pH[a]	NR Activity of Control	Inactivator Activity	
			Corn	Rice
μmol		*units*	*units*	
0.0	7.0	1.99	69.1	69.9
0.2	7.0	1.82	67.8	0.0
0.5	7.0	1.79	68.5	1.1
0.0	8.0	2.01	63.7	81.4
0.2	8.0	0.70	57.6	2.4
0.5	8.0	0.63	57.7	0.0

[a] Corn NR was diluted with 0.1 M Tris-HCl buffer (pH 7.0 or 8.0) after dialysis in 0.1 M Hepes buffer (pH 7.5) containing 0.5 mM EDTA. NADH-NR (0.1 ml, 66 μg protein) was preincubated with 50 μl of NADH solution for 20 min at 28 C, then 50 μl of inactivating protein was added.

Table III. *Influence of Addition of NADH on Inactivation of NADH-NRs from Corn Leaf and Rice Cells by Inactivators*

The Sephadex G-100 (first) fraction was used as the inactivating proteins. NADH (0.5 μmol) was added at the time indicated. NADH-NR of 2-day-old rice cells was partially purified with 40% $(NH_4)_2SO_4$. Inactivators (50 μl) were incubated with 0.1 ml of NADH-NR in 0.1 M Tris-HCl buffer (pH 8.0) as described in Table II.

Time of NADH Addition	Source of NADH-NR	NADH Activity of Control	Inactivator Activity	
			Corn	Rice
min		*units*	*% loss*	
Not added	Corn leaf	1.97	67.0	86.9
0	Corn leaf	0.76	67.8	0.0
40	Corn leaf	1.17	72.7	52.7
Not added	Rice cells	2.81	12.0	46.5
0	Rice cells	1.68	11.0	0.9
40	Rice cells	2.66	11.2	2.3

Table IV. *Effects of NR-inactivating Proteins on NADH-NRs and Its Associated Activities from Corn Leaves and Root Tips*

Nitrate-induced NADH-CR was isolated from corn leaves as described under "Materials and Methods." Root tip NADH-CR was largely the constitutive enzyme. The Sephadex G-100 (first) fraction was used as the inactivator; protein amounts of corn and rice inactivator were 0.2 and 2 μg, respectively.

Enzyme	Enzyme Source	Enzyme Activity of Control	Inactivator Activity	
			Corn	Rice
		units	*% loss*	
NADH-NR	Leaf	1.88	61.6	71.1
	Root tip	1.51	38.4	7.7
$FMNH_2$-NR	Leaf	3.75	20.8	6.1
	Root tip	1.08	23.0	6.9
MVH-NR	Leaf	5.96	10.4	4.2
	Root tip	0.57	1.6	3.1
NADH-CR	Leaf	0.30	55.2	64.0
	Root tip	1.65	18.2	8.1

activity. Rice inactivator had less influence on the activities of NADH-NR and its associated enzymes obtained from root tips.

DISCUSSION

Previous studies of NR-inactivating proteins from corn roots (13, 14) and rice cells (19, 20) have suggested that they have different characteristics: the corn inactivator was apparently an NR-specific degrading protein whereas the rice inactivator was apparently a specific binding protein. In these earlier investigations partially purified preparations were used to measure inactivator activity. In the current investigation highly purified inactivator proteins were obtained by using disc gel electrophoresis as the ultimate purification step. Corn protein obtained from a G-100 Sephadex column had two protein bands associated with it. The minor band with an R_F of 0.03 had both NR-inactivating activity and azocasein-protease activity associated with it. When this protein was extracted and rerun in an acid buffer system, the protein band migrated into the gel and the two activities were still associated with it. This observation suggests that the corn protein is, indeed, a protease which preferentially degrades NR and hence supports Wallace's earlier contention (13–15).

The corn inactivator had an approximate mol wt of 66,000 to 75,000 by Sephadex G-100 gel filtration and SDS gel electrophoresis. In earlier work the corn protein was estimated to have a mol wt of 44,000 by Sephadex G-200 filtration (13). The lower value is probably caused by the low ionic strength buffer used by Wallace (13). Under these conditions the protein would be absorbed to the Sephadex G-200 because of the basic surface charges of the corn-inactivating protein.

The rice protein eluted from Sephadex G-100 had one major protein band after electrophoresis and this protein band had NR-inactivating activity but no protease activity associated with it. The rice cell inactivator was a protein of 150,000–200,000 mol wt. After treatment with SDS it had four major polypeptide bands with approximate mol wt of 73,000, 66,000, 62,500, and 58,000. The relative amounts of polypeptides 1 and 4 were approximately equal and were more than of polypeptides 2 and 3.

Jolly and Tolbert (7) have also found an NR-inactivating protein in soybean leaf. This protein did not migrate into high pH gels and it had no protease activity associated with it. It had an approximate mol wt of 31,000 with two identical subunits of estimated mol wt of 18,000.

These observations suggest that there are likely to be a number of proteins which will affect nitrate reductase preferentially. Of the three proteins identified to date there are different net surface charges, and subunit structures. They are also different with respect to associated protease activity. The rice and corn proteins selectively inactivate the NADH-CR portion of the NADH-NR complex (14, 19). Recent work with trypsin (6, 22) also suggests that the NADH-CR portion of the native protein is most sensitive to attack. In contrast to this the soybean leaf inactivator had no selective effect on the NADH-CR activity (7).

In the accompanying paper we describe the effects of the NR-inactivating proteins from corn roots and rice cells on a purified NR from *Chlorella* (22). Although NADH-NR and NADH-CR were inactivated, the reduced MV-NR was not inactivated by either inactivating protein. With this system shifts in the NR protein band were observed after disc gel electrophoresis after treatment with the corn inactivator protein (22). The rice inactivator, on the other hand, had no influence on the migration of the protein band. These results indicate that the corn protein inactivates the NADH-NR and NADH-CR components of the NR complex by limited proteolysis whereas the rice inactivator does not.

There is also apparently a unique pattern of interactions between NADH, the NR obtained from rice, corn leaves and corn roots and the NR-inactivating proteins obtained from corn and rice. Rice NR was less sensitive to corn inactivator than was corn leaf NR. Corn root tip NR was also relatively insensitive to the corn inactivator but this probably reflects the presence of an inhibitor of corn NR inactivator found in root tip preparations (15). In addition, the inactivation of NADH-NR from rice cells by rice inactivator was reversed by the addition of NADH whereas the action of the rice inactivator on corn leaf NR was irreversibly inhibited by NADH. Thus, at this stage it is difficult to generalize from one NR system to another. It is hoped that with a clearer definition of the cellular location of the inactivators and NR and with a better characterization of the corn root NR that we will be able to interpret the *in vivo* significance of the NR-inactivating proteins.

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