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Molecular biology and biotechnology of higher plant nitrate reductases

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Nitrate reductase (NR) catalysing the reduction of nitrate to nitrite is the first and rate limiting enzyme in the assimilation of nitrate by the plants. At least two of its isoforms; a NADH specific and a NAD(P)H bispecific have been characterized. In this article, the physico-chemical properties of NADH specific isoform, which is the principal isoform of nitrate assimilation, have been described. The properties of cloned genes and the production of transgenic plants with altered NR activity, and mechanism of induction of NR by nitrate and cytokinins, repression by glutamine and post-translational modification of NR protein through reversible phosphorylation by light–dark transitions have also been accounted for. The characterization of NAD(P)H : NR gene and some regulatory aspects of this isoform have also been described. The article demonstrates that it is possible to produce mutants and transgenics with altered structure and function of NR, with an ultimate aim to affect qualitative and quantitative improvement of crop plants.

NITRATE reductase (NR) catalysing the reduction of nitrate to nitrite is the first enzyme in the assimilation of nitrate by plants. The activity is considered to be the rate-limiting step in nitrate assimilation, which is often positively correlated with the total protein and nitrogen contents and sometimes also with the overall productivity of the plants[1]. Three isoforms of this enzyme have been described from soybean[2], viz. (i) A nitrate inducible NADH : NR (E.C.1.6.6.1) with a pH optimum of 7.5, (ii) A constitutive or inducible bispecific NAD(P)H : NR (E.C.1.6.6.2) with a optimum pH of 6.5, and (iii) A constitutive NADH : NR (E.C. number not yet assigned), with a pH optimum of 6.5. The inducible NADH : NR and the bispecific NAD(P)H : NR are usually found in close association with various plants and perhaps both are involved in nitrate assimilation. The NAD(P)H : NR however, is considered to be associated with some other functions also, such as with the transport of nitrate across the membranes and with the dissimilatory release of oxygen in anaerobic environment[3]. The evidences for these alternate functions are yet to be known, although the observation that NAD(P)H : NR isoform was localized principally on the plasma membrane[4] is a strong indicator of its role in nitrate acquisition and transport. NR has been used as a biotechnological tool/product also. Mellor

et al.[5] have evolved a bioreactor that employs immobilized NR from maize along with some bacterial enzymes to reduce nitrate to nitrogen gas. The bioreactor may be used for reducing nitrate contamination of groundwater which is otherwise rendered unfit for human consumption because of toxic nitrate contamination. Similarly, the accumulation of toxic levels of nitrate in crop plants can also be restricted by *in vivo* regulation of NR through genetic manipulations. For example, Quillere *et al.*[6] have demonstrated that genetically transformed *Nicotiana plumbaginifolia* plants with a 25 to 150% higher NR activity have a 32 to 47% lower nitrate contents as compared to wild type controls. Thus, the interest in the study of NR has been generated with different objectives.

Physico-chemical nature of the enzyme

Although the NR from higher plants was characterized for the first time in the early fifties[7] its purification could be achieved only in the seventies[8,9], primarily because the enzyme is very unstable and it requires special care for extraction, isolation and purification. The *in vivo* experiment showed that the protein is short lived with a half life of only 3 to 5 hours[10]. The enzyme has been purified from a variety of species including barley, maize, soybean, spinach, squash, tobacco, etc. by affinity chromatography and electrophoresis. Monoclonal antibodies of the enzyme have been raised[11,12]. Nitrate reductase (E.C. 1.6.6.1) from higher plants is a homodimer with each sub unit of 100 to 120 kD molecular mass of 881 (bean) to 926 (spinach) amino acids[13]. In each sub unit, acidic amino acids are more abundant than the basic ones; the isoelectric point of a squash NR has been determined[14] to be 5.7. There are 9 to 19 cysteine residues in each sub unit and the two sub units are linked through at least three disulphide bonds of cysteine residues[15]. Each sub unit contains at least three prosthetic groups, FAD, heme (cytochrome b_{557}) and Mo-pterin (Mo-CO). The NADH/NADPH binding region and the FAD domain are at C-terminal while Mo-CO is at N-terminal of the polypeptide. The cytochrome heme domain occupies a central part in the polypeptide. The Mo-pterin domain is a complex of Mo atom linked to a pterin (a heterocyclic compound) molecule via a thiol group (Figure 1). The three domains of the polypeptide are apparently linked through two hinges; hinge I (H I) between Mo-Co and cytochrome and hinge II (H II) between the cytochrome and the FAD. As described later H I plays an important role in the enzyme modulation by phosphorylation–dephosphorylation[16]. The three regions of the polypeptide can be separated by treatment of the enzyme with appropriate proteases. Three separated domains show partial activities of the enzyme. The Mo-Co domain is believed to be involved in the dimerization of sub units.

The reductant used in the reduction of nitrate to nitrite is NADH. The bispecific NAD(P)H : NR however, uses either NADH or NADPH; the preference being for the latter. The initial acceptor of the electron from NADH/NADPH is FAD of the enzyme. Then the electron flows through cytochrome b_{557} site to Mo-pterin site in the enzyme and ultimately to the nitrate, which is reduced to nitrite;

$$\text{NAD(P)H} \rightarrow \text{FAD} \rightarrow \text{Cyt } b_{557} \rightarrow \text{Mo} \rightarrow NO_3^- .$$

Active site amino acid mapping of the enzyme has been done by using appropriate enzyme inhibitors. Histidine[17] and arginine & lysine[18] are involved in NADH binding with the enzyme. A difference in the amino acid sequence between bispecific NAD(P)H : NR of birch (*Betula pendula*) and that of monospecific NADH : NR of many species have been found in the FAD domain of the enzyme[19]. Arginine residues are involved in the catalytic function of FAD and Mo-CO domain[20]. Specific inhibitors binding these amino acids block the flow of electrons from NADH to nitrate and hence the catalytic activity of NR. Cysteine also appears to be actively participating in the electron transfer. Dwivedi *et al.*[21] replaced each of the five cysteine residues of recombinant cytochrome reductase domain from maize leaf NR with other amino acid analogues using site-directed mutagenesis. The enzyme had intact NADH-binding sites but had reduced ferricyanide reductase activity. The authors have concluded that cysteine is essential for a highly efficient catalytic transfer of electrons from pyridine nucleotides to flavins.

Gene cloning and transgenics

Genetic experiments including the study of NR deficient mutants have helped in the identification of genes coding for the synthesis of NR protein. In barley, two structural loci *Nar 1* and *Nar 7* have been identified, which

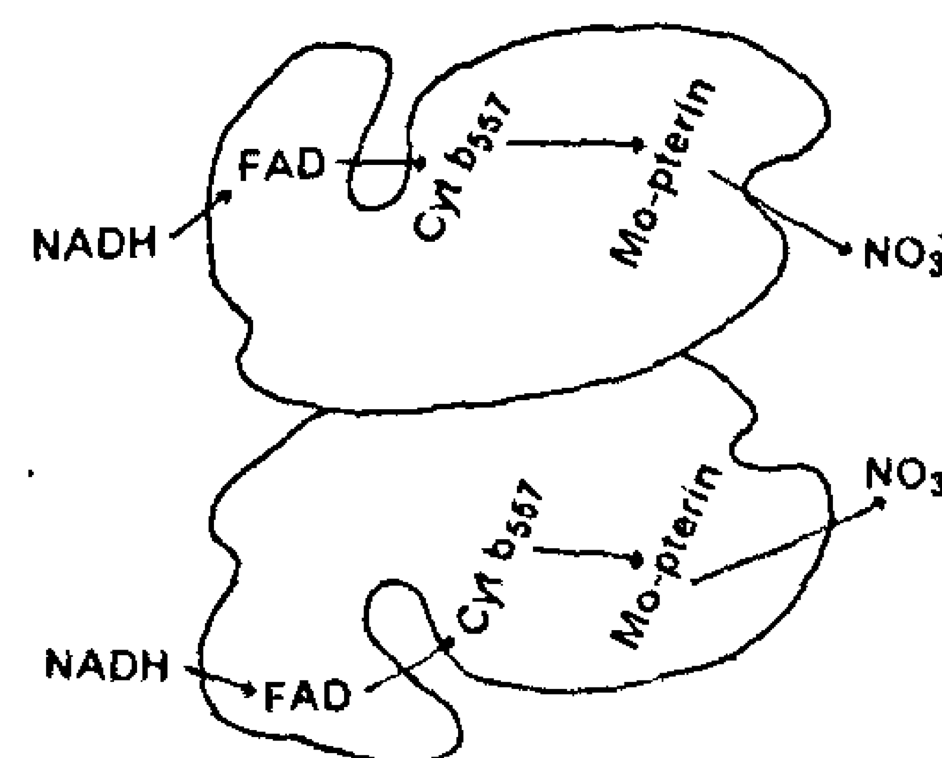

Figure 1. Diagrammatic representation of the structure of nitrate reductase showing path of electron transfer.

apparently code for inducible NADH : NR and NAD(P)H : NR respectively[22]. Two genes *NR 1* and *NR 2* have been identified in *Arabidopsis thaliana*[23] and in tobacco[24]. Zhou *et al.*[25] have described the presence of only one gene for NADH : NR in cultivated barley, while two genes in diploid wheat *Triticum monococcum* and in hexploid wheat *Triticum aestivum*. They have suggested that during evolutionary events NADH : NR gene duplicated before the divergence of wheat and barley and that a deletion resulted in the loss of one NADH : NR gene from cultivated barley.

Both the NADH and NAD(P)H specific NR genes have been now cloned from a variety of species from either genomic library or from c-DNA (Table 1). The complete sequence of the nucleotides is known from *Arabidopsis*, birch (NAD(P)H : NR), barley, bean, rice, tobacco and tomato[13]. The NADH : NR gene is about 3000 to 5000 bp long and contains about one to three introns. The genome of barley NADH : NR is however, longer, about 7300 nucleotides and the NAD(P)H : NR from the same species is 3538 bp with two introns. A transposable element of about 565 bp in the first exon of NR genome of a NR-deficient mutant (obtained by γ-irradiation) of tobacco has been detected[26]. It occurs as a single copy in the genome and is apparently lacking in *Lycopersicon* and *Petunia.*

Transgenic tobacco with altered NR activity has been developed[27–31]. Constructs carrying the entire or part of the tobacco c-DNA cloned between the promoter and terminator sequence of the 35 S RNA of cauliflower mosaic virus were introduced in tobacco[27]. A few NR-deficient transgenics were obtained and a few with increased NR m-RNA and NR protein were also obtained. In another attempt, NR deficient tobacco plants were transformed to high NR activity types through the transfer of NR c-DNA with 35 S constitutive promoter from cauliflower mosaic virus[28]. The transgenics accumulated higher amounts of glutamine and reduced levels of nitrate in their leaves. Transgenic tobacco have helped in visualizing the physiological significance of the inducibility of NR protein by nitrate. The study has shown that the constitutive expression of NR does not influence

Table 1. Some characteristics of nitrate reductase genes cloned from higher plants

Plant species and the organ	Clone	Some characteristics	Reference
A. NADH : NR gene			
Arabidopsis thaliana leaves	Genomic	3829 bp, two introns	83
Cucurbita maxima (squash) cotyledons	c-DNA	2754 bp plus a untranslatable region of 135 nucleotides at 3′ end and poly A tail of 16 nucleotides, coding for 918 amino acids	84
Glycine max (soybean) leaves	c-DNA	*NR 1*; 2661 bp, coding for 886 amino acids	46
		NR 2: 2673 bp, coding for 891 amino acids	
Hordeum vulgare (barley) leaves	Genomic	7300 bp, one large intron of 2700 bp, coding for 915 amino acids	41
Licopersicon esculentum (tomato) leaves	Genomic	5300 bp, three introns, coding for 911 amino acids	85
Nicotiana tabaccum (tobacco) leaves	Genomic	6000 bp, three introns coding for 904 amino acids	86
Oryza sativa (rice) leaves	Genomic	5400 bp, three introns coding for 916 amino acids	87
Phaseolus vulgaris (bean) – roots	Genomic	4600 bp, three introns, coding for 881 amino acids	88
– leaves	Genomic	Four introns, coding for 890 amino acids	89
Spinacea oleracea (spinach) leaves	c-DNA	3284 bp, two introns, coding for 926 amino acids	90
B. NAD(PH) : NR gene			
Betula pendula (birch) leaves	c-DNA	3031 bp, coding for 898 amino acids	91
Hordeum vulgare (barley) leaves	Genomic	3538 bp, coding for 891 amino acids	42

the foliar protein and chlorophyll contents, the assimilatory products of nitrate, under any circumstances[29]. Further, under the conditions of nitrogen deficiency, the NR protein is actively degraded.

Transgenics have also been created with a view to understanding some of the regulatory aspects of NR. Nussaume *et al.*[31] have transformed a NR deficient tobacco by introducing a full length *Nicotiana* c-DNA with an internal deletion of 168 bp in the 5′ end fused to cauliflower mosaic virus 35 S promoter and appropriate termination signals. In transgenic plants expressing this construct, NR activity was restored and normal growth resulted. However, *in vitro* NR activity from these transformants was not modulated by ATP and light. A heterogenous expression system has been developed to examine the regulatory mechanism of NR through phosphorylation–dephosphorylation[32]. Wild type and mutant forms of NR c-DNA from *Arabidopsis thaliana* have been inserted and expressed in the yeast *Pichia pastoris*. This allowed to produce mutant forms of NR for various regulatory studies. The technology may also be used in the production of plant NR in bulk for X-ray crystallography and other biophysical investigations.

Transcriptional control of NR gene expression

Nitrate reductase is subjected to a multivalent control at gene expression level[33]. Some of the regulators of the enzyme are structurally or metabolically related to nitrate, the substrate of the enzyme, or to one of the assimilatory products of nitrate; while others are general regulators of NR and of other enzymes.

Induction by nitrate

Induction of NADH : NR gene has been demonstrated by nitrate, cytokinin and light. Nitrate is the substrate of the enzyme and also a specific inducer of NR activity, a property which has been demonstrated in almost all the investigations[1]. Different levels of nitrate are required for optimum induction of NR in different species, which is perhaps linked to the differential rates of uptake and/or mobilization of nitrate to the genomic level[1]. When the entire plant or its excised organs are incubated in a nitrate-containing medium, the NR activity in the plant or organ starts increasing after a lag of about 15–45 min and goes on increasing almost linearly until 180–400 min. Thereafter, a steady state level is maintained. However, if nitrate is withdrawn from the medium, the steady state level declines. Further, the linear increase in enzyme activity is abolished in the presence of transcriptional and translational inhibitors. Chemical isolation of RNA, Northern blotting and c-DNA isolation and hybridization experiments performed in a variety of plant systems have demonstrated that nitrate supply in fact increased the synthesis of m-RNA. For example, in *Cucurbita maxima* (squash) an increased apoprotein synthesis in response to nitrate was linked with the increase in m-RNA[34]. This increased transcription required intact plastids in the cotyledons[35]. In barley, when nitrate-starved seedlings were transferred to a nitrate-containing medium, NR m-RNA increased rapidly from essentially zero to high levels within a few hours in both roots and shoots[36]. In nitrate cultured tobacco and tomato seedlings also, further supply of nitrate increased NR m-RNA, although at relatively slow rate[37]. In maize roots, the increase in NR activity in response to nitrate is correlated with the increase in NR m-RNA[38,39]. Transcriptional assays with isolated soybean nuclei also indicate that induction of NR m-RNA is due to *de novo* synthesis of the transcript and not due to reduced RNA degradation.

In a recent study, a nitrate independent transcription of two NR isogenes in androgenic haploid embryos of *Brassica napus* (rape seed) has been demonstrated[40]. However, in the leaf, NR gene expression responds to nitrate supply as in most other systems. This demonstrates the uniqueness of the regulation of NR gene expression in haploid embryogenesis.

The effect of nitrate supply on NAD(PH) : NR has also been examined. In barley, the genes for both NADH : NR and NAD(PH) : NR have been isolated and sequenced[41,42]. There is a high degree of sequence similarity between the two genes. However, there is a significant divergence at 5′ end of the gene and thus it has been possible to differentiate between the m-RNAs of two genes. The divergence also indicates that there was a possibility of differential regulation of the expression of two genes. In soybean, nitrate supply has no effect on NAD(P)H : NR activity although it induces NADH : NR[2,43]. However, in barley, NADH : and NAD(P)H : NR m-RNAs are at detectable levels in both roots and leaves within 15 to 30 min after exposure of seedling roots to nitrate[44]. Nitrate depletion triggers a rapid decrease in both m-RNAs and in the activities of both the isoforms. It indicates that in barley at least both the genes, *Nar 1* and *Nar 7* coding for NADH : NR and NAD(P)H : NR respectively are under the control of the same promoter/regulator. Vaucheret and Caboche[30] by using transgenic *Nicotiana plumbaginifolia* have demonstrated that the promoter for *Nia* gene is specific for the gene. In transgenic plants, the expression of a reporter gene neomycin phosphotransferase driven by a 1.4 kb *Nia* promoter had a negative correlation with the induction of *Nia* gene[30]. Positive response of NAD(P)H : NR to nitrate supply has been demonstrated in maize also[18], although the species has a significant level of constitutive expression also (Shankar and Srivastava, unpublished).

The mechanism of signal transduction in nitrate induction of NADH or NAD(P)H : NR gene is not known. Presumably *cis* acting elements and *trans* acting proteins are involved in the induction. In transgenic tobacco plants containing *Arabidopsis* NR genes, the DNA sequence responsive to nitrate has been identified to be at 5′ end, a 238 or a 188 bp sequence in *NR 1* or *NR 2* genes respectively[45]. The promoter region for the two genes in tobacco, however seems to be larger[24].

Regulation by glutamine

Inhibition of NR activity by the addition of glutamine has been demonstrated in many species. That the inhibition is at transcription level has been demonstrated in soybean[40,46], tobacco[47,48], maize[10] and in cultured spinach cells[49]. The inhibition is partially overcome by the addition of glucose in tobacco leaves[48]. Endogenous glutamine level also seems to be controlling NR gene expression. In tobacco, inhibition of glutamine synthetase enzyme leads to a sharp decline in glutamine level, which resulted in an increased NR m-RNA[47]. In maize seedlings, the root enzyme is more sensitive to exogenously supplied glutamine than the shoot enzyme, which is apparently because of the more sensitive NAD(P)H : NR (primarily a root enzyme) than the NADH : NR[39]. However NR m-RNA was almost equally inhibited by glutamine in both roots and shoots. Thus, there might be some additional aspect of down regulation of active NR protein formation by the glutamine in the roots.

Induction by cytokinins

Exogenous supply of cytokinins invariably increases NR activity, although the magnitude of increase depends upon the species and the concentration and nature of the cytokinin. Apparently, the endogenous cytokinin level is an important factor in determining the effects of exogenous cytokinins. As with nitrate, cytokinins also appear to be acting at transcription level in inducing NR activity[50,51]. Adenine or adenosine have no effect on NR activity[51]. The induction by cytokinins is usually higher in the presence of nitrate than in its absence[52], although the two inducers do not seem to be acting synergistically[51]. Samuelsson *et al.*[51] have also demonstrated that nitrate supply elevated cytokinin zeatin riboside level in barley roots and shoots. This could determine the influence of externally supplied cytokinin on NR activity in the presence of nitrate. The exact mechanism of induction of transcription by cytokinins is not known. However, Suty *et al.*[53] have reported that cytokinin-mediated transcription in tobacco cell suspensions involved m-RNA polyadenylation.

The plant hormone abscissic acid represses NR gene expression in barley, which is partially recovered by equal concentration of benzyladenine[50]. Thus, abscissic acid also might be acting at transcription level. Bueno *et al.*[54], however, have suggested that kinetin-mediated increase in NR activity in *Cicer areitenum* cotyledons is both through synthesis and activation of the enzyme.

Effect of light and sugars

Light is another factor regulating NR activity in upward direction. Enzyme extracted from the organs of the plants raised in light is several folds more active than those from dark grown. The positive response of light is apparent in non-photosynthetic organs such as roots, endosperm and scutella also[55]. In many systems, the effect of light appears to be at transcription level, although post transcriptional modifications of NR protein by light is also known.

In a study with five-day-old maize seedlings, Lillo[56] observed a four-fold increase in NR m-RNA level within one hour exposure to light. This was reported to be due to increased transcriptional activity. In another study with maize, Huber *et al.*[57] also reported a significant increase in leaf NR m-RNA within two hours of illumination of previously darkened seedlings.

It has been often suggested that the light/dark effects on NR activity were responsible for observed diurnal variation in enzyme activity. However, in a few investigations, an increase in NR m-RNA and in NR activity has been observed during the night. This is apparently because sucrose and glucose replace light requirements in the induction of m-RNA as has been reported in *Arabidopsis*[23] and tobacco[48]. In tobacco leaves, fructose also induces transcription of NR m-RNA while ribose or mannitol has no effect[48]. This has led these investigators to believe that up-regulation of NR gene transcription by light is mediated via carbohydrate synthesis in green leaves.

There is also some indication of post transcriptional modification of NR m-RNA in plants. In *Arabidopsis*, the expression of *Nia 2* gene shows circadian oscillations[58]. The m-RNA accumulation also shows rhythm even up to five days in continuous darkness after the plants have been grown in light/dark cycle.

Translational control

The modification of NR protein synthesis at translation level has not been demonstrated although it is often assumed that many factors controlling transcription have effect on translation also. There is some circumstantial evidence for translational control of NR synthesis by oxygen, which is known to inhibit NR activity in oat

leaves[59]. In detached maize leaves, NR activity and NR protein appearance were partially inhibited at 100% oxygen, but the m-RNA levels as measured by leaf NADH : NR c-DNA hybridization tests were the same in air and 100% oxygen-treated leaves[60].

Post-translational modification of NR protein

Some of the regulators of NR activity have very fast effects, the activity responding the regulator as quickly as in a few minutes. These regulators apparently act through the modulation of NR protein. For example, in spinach leaves, the NR activity decreases rapidly during darkening of the leaves, reaching to about 15% of the control value in only about two minutes[61].

The processing of the nascent NR protein into an active NR molecule itself is a step which is affected by some regulators. Molybdenum cofactor is inserted in the NR protein after it has been fully synthesized. In the absence of Mo, no active NR is formed. Tungsten, a metal classified with Cr, Mo and U in the periodic table competes with Mo, and produces a NR protein which is inactive in nitrate reduction[62].

Reversible phosphorylation

The reversible phosphorylation of NR protein appears to be the most important mechanism of enzyme regulation in both prokaryotes and eukaryotes. It allows a fast modulation of enzymic protein with the change in its micro-environment. For NR, it has been now demonstrated that phosphorylation–dephosphorylation was involved in dark–light inactivation/activation of the enzyme. Upon transition of plant or plant organs from darkness to light, the NR protein is in dephosphorylated (active) form, whereas phosphorylation of specific serine residues is increased on transition to darkness[63,64]. The phosphorylation is catalysed by specific protein kinases; two protein kinases, NR : PK I and NR : PK II have been isolated from spinach leaves[65]. NR : PK I has a broader specificity and can phosphorylate sucrose phosphate synthase also. NR : PK II however, which has an apparent *Mr* of 160 kD, is specific for NR only. The NR specific PK (PK II) is a Ca^{2+}-dependent and uses ATP as phosphorylation substrate. *In vitro* experiments have revealed that $r^{32}P$ of ATP is incorporated into NR protein from *Brassica campestris*[66]. Analysis of phosphoamino acids in phosphorylated NR from *Arabidopsis* has revealed that serine at position 308 in Mo-Co domain is required for phosphorylation of NR and also apparently for normal activity of the enzyme[67]. When serine is replaced by aspartic acid, both the processes, the normal activity and the phosphorylation of the NR protein are disrupted. However, in spinach, phosphorylation of Ser-543 has been demonstrated by using recombinant DNA fragments[24]. In another investigation with *Arabidopsis* NR expressed in *Pichia pastrois*, Ser-534 is shown to be phosphorylated[32]. In both these systems, Ser-543 or Ser-534 are located in hinge I region and occupy equivalent position in the polypeptide. Further, transgenic tobacco containing a 168 bp deletion at 5′ end of the *Nia* are insensitive to modulation by ATP or light[31]. However, it is not clear whether this deletion results in the deletion of the critical serine residue or in some other kind of amino acid changes. The phosphorylation of NR protein in dark is apparently not itself enough for complete enzyme inactivation. The inhibition in fact is affected by another protein known as inhibitor protein (IP; *Mr* 110 kD) which in the presence of Mg binds to the phosphorylated NR and inactivates it[68,69] (Figure 2). Magnesium application is known to inhibit *in vitro* NADH : NR activity but not the methyl viologen activity[70]. Apparently, the electron flow from NADH to the flavin is inhibited in the presence of Mg. The NR-inhibiting protein from spinach has been characterized by Yoshimura *et al.*[71], which is a dimer with sub unit *Mr* of approximately 53 kD.

Divalent cations Ca^{2+} and Mg^{2+} are key regulators of NR activity through their involvement as cofactors for either protein kinases or inhibitor protein. The inhibition of NR activity by these ions as reported in many species appears to be a hysteric property, at least for the enzyme

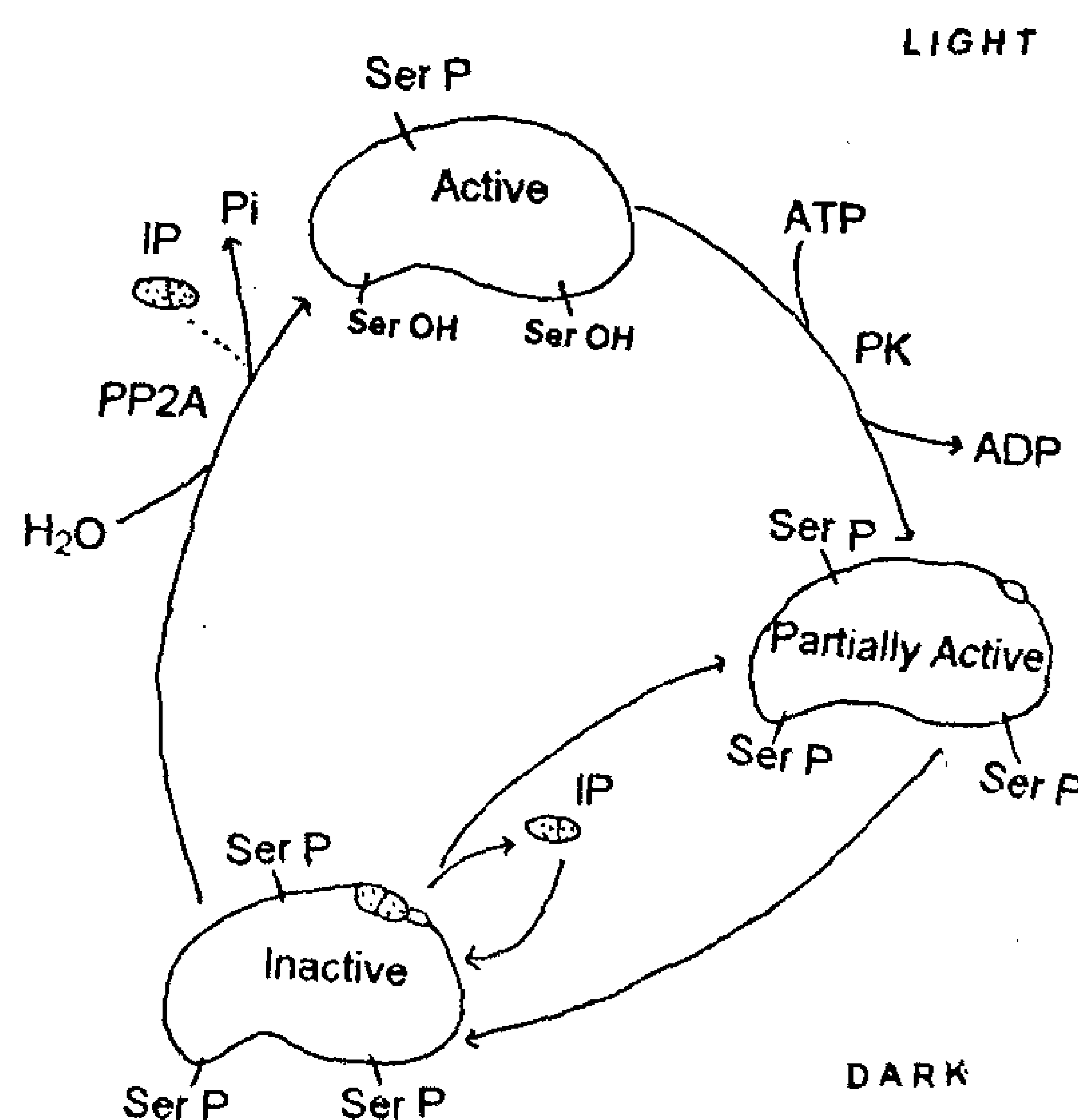

Figure 2. Mechanism of reversible phosphorylation of nitrate reductase during dark-light transition (IP = inhibitor protein; PK = protein kinase; PP 2A = protein phosphatase 2A).

from spinach leaves[72]. The enzyme extracted from darkened leaves is in low activity or low affinity form, while the one extracted from illuminated leaves is in high activity or in high affinity form[72,73]. When extracted from darkened leaves, the enzyme which is in low affinity form is inhibited more strongly by Ca^{2+} and Mg^{2+}, than when extracted from illuminated leaves, where the enzyme is in high affinity form. Little inhibition of enzyme is seen when it is converted to high affinity form by preincubation of the low affinity enzyme preparation with the substrate[72] or with Pi, 5′AMP and FAD[73]. The low affinity form has a optimum pH of 7.5, while high affinity form has a optimum pH of 7.8 (ref. 74). The conversion of enzyme to a high affinity form in light apparently involves dephosphorylation of the enzyme protein by phosphatases. In *in vivo* experiments, the reactivation of inactivated (darkened plants) enzyme is prevented by okadoic acid, an inhibitor of certain phosphatases[75]. Further, in *in vitro* experiments, the NR that had been inactivated in the presence of NR kinase, ATP-Mg and inhibitor protein could be reactivated either by dephosphorylation with protein phosphatase 2A or by dissociation of inhibitor protein from NR[69].

Signal transduction

Attempts have been made to trace the path of signal transduction in NR regulation by light at least. Light signals for NR induction is apparently received by the phytochrome, at least in etiolated plants[36,58,76]. Sopory and his associates have suggested the involvement of Ca^{2+} and phosphoinositide cycle as second messengers in the transduction of signal from active phytochrome to NR gene expression[77–79]. In fact, Raguram and Sopory[80] have demonstrated that the involvement of phosphoinositide cycle and protein phosphorylation by protein kinase type enzyme, is a common strategy in signal transduction through phytochromes. Bergarche *et al.*[81] have also demonstrated that the phytochrome was effective only when free Ca^{2+} was available. In addition, a signal originating from chloroplasts is also required for the control of NR expression by light, in green tissues[28].

Recently, the presence of a specific receptor for nitrate has been suggested in the plasma membrane, which besides binding with the nitrate may also bind with the respiratory inhibitors azide and cyanide[82]. The significance of the binding of the inhibitors is not understood at the moment.

Concluding remarks

Significant achievements have been made in the study of the molecular biology of higher plant NR during the last 10 years or so. The story is not complete; the mysteries are absorbing and the prospects of deep inquisitiveness and indulgence are inevitable. The successful creation of transgenics with altered NR structure and function have opened up the possibilities of manipulating this enzyme for qualitative and quantitative improvement of crops and also for the reduction of nitrate contamination of food and feed products. A commercially viable technology for using NR preparation for reduction of nitrate contamination of ground water is to be developed; the probabilities are excellent.

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