Identification by mutational analysis of four critical residues in the molybdenum cofactor domain of eukaryotic nitrate reductase

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Abstract **The nucleotide sequence of the nitrate reductase (NR) molybdenum cofactor (MoCo) domain was determined in four *Nicotiana plumbaginifolia* mutants affected in the NR apoenzyme gene. In each case, missense mutations were found in the MoCo domain which affected amino acids that were conserved not only among eukaryotic NRs but also in animal sulfite oxidase sequences. Moreover an abnormal NR molecular mass was observed in three mutants, suggesting that the integrity of the MoCo domain is essential for a proper assembly of holo-NR. These data allowed to pinpoint critical residues in the NR MoCo domain necessary for the enzyme activity but also important for its quaternary structure.**

Key words: Nitrate reductase; Molybdenum cofactor; Molybdoenzyme; Mutant; *Nicotiana plumbaginifolia*

1. Introduction

Molybdenum-containing enzymes are ubiquitous in living organisms and play a major role in several metabolic pathways such as nitrogen assimilation (nitrate reductase and nitrogenase), sulfur catabolism (sulfite oxidase) or purine metabolism [1]. All molybdoenzymes, except nitrogenase, contain a molybdenum cofactor (MoCo) which consists of the metal ion bound to a pterin moiety called molybdopterin [2]. So far X-ray structural informations are not available for MoCo-containing enzymes and, although these enzymes have been the subject of many biochemical and spectroscopic studies [3], little is known of their structure/function relationships.

Assimilatory nitrate reductase (NR, EC 1.6.6.1/3) catalyzes the first step of nitrate assimilation, the reduction of nitrate to nitrite, and is one of the best known MoCo-containing enzymes. It has been shown that higher plants NR is a homodimeric enzyme of which each subunit is probably organized in three domains containing the three NR prosthetic groups, namely FAD, a b_5-type cytochrome (cytochrome b_{557}) and MoCo [4,5]. Electrons travel from the physiological electron donor (NAD(P)H) to nitrate through, successively, FAD, heme and MoCo. This electron flow can be shunted by, either artificial electron acceptors (like cytochrome *c*) or artificial electron donors (reduced viologens or flavins). This makes possible the measurement of partial catalytic activities involving only one or two of the NR prosthetic groups. Many eukaryotic NR amino acid sequences are now available from plants, algae and fungi [5,6]. Sequence comparisons with cytochrome b_5 reductase (a flavoprotein), cytochrome b_5, and the MoCo-containing enzyme sulfite oxidase, suggested a N to C termini arrangement of the MoCo, heme and FAD domains [7,8]. X-ray absorption fine structure spectroscopy of sulfite oxidase suggested that one or two cysteine residues might be involved in molybdenum binding [9]. Two cysteine residues are indeed conserved among NR and sulfite oxidase sequences [10,11]. It was also shown that arginine residue(s) may be important for the MoCo domain functionality [12]. But, to our knowledge, there are no other data on structural features which would be involved in MoCo or nitrate binding.

In the diploid species *Nicotiana plumbaginifolia*, we have obtained, by selection for chlorate resistance, a collection of 65 NR-deficient mutants affected in the apoenzyme gene (*nia* mutants) [13,14]. They were classified in four groups corresponding to mutants without any detectable NR protein and activities (class 1) and to, respectively, mutants presumably affected in the FAD (class 2), the MoCo (class 3) and the heme (class 4) domains [15]. Sequencing of class 4 mutants confirmed that they were indeed affected in the heme domain thus validating our working hypothesis [16].

In the *N. plumbaginifolia nia* mutant *NA* 36, the mutation was ascribed to the MoCo domain but the nucleotide sequence of the mutation was not determined [20]. Up to now only two mutations in the MoCo domain have been localized by sequencing, both of them in *Arabidopsis*. One, in the *NIA1* NR structural gene, converted Ala 192 from the conserved CAGNRR motif into Thr and resulted in an inactive NR [17]. The other one (B29) was shown to change Gly 308 into Asp in the *NIA2* gene and interestingly to affect both NR activity and phosphorylation [18,19].

We present in this paper the sequence analysis of four null *nia* mutants of *N. plumbaginifolia*, i.e. completely devoid of NADH:NR activity, and presumed to be defective in the NR MoCo domain. Moreover structural analysis of the mutated NR by gel filtration suggested that in some of these *nia* plants the NR quaternary structure is affected.

2. Materials and methods

2.1. Plant material

The E77, E122, F19 and H22 *nia* mutants were obtained from haploid protoplasts of *N. plumbaginifolia* (var Viviani) by selection for chlorate resistance [14] and were previously characterized [15]. Mutants E77, E122 and F19 were of spontaneous origin whereas the mutant H22 was obtained after γ-ray mutagenesis [14]. Leaf material was obtained as previously described [15].

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Abbreviations: NR, nitrate reductase; MoCo, molybdenum cofactor; PCR, polymerase chain reaction; CcR, NADH:cytochrome *c* reductase activity; FPLC, fast protein liquid chromatography.

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2.2. Oligonucleotide synthesis

The oligonucleotides were synthesized in a 381A DNA synthesizer (Applied Biosystems). Primers Ol2, Ol3, Ol5, Ol7 and Ol9 [16] as well as primers Ol6 and Ol8 [21] were previously described and were all derived from the tobacco *Nia2* NR gene sequence (Fig. 1). The other primers that were used for PCR amplification and sequencing were Ol18 (20-mer) 5′-AACGAATTCCCTTCTAGAGA-3′ (nucleotides 649–668), Ol21 (20-mer) 5′-GCTTACATGCAGAACGGAGA-3′ (nucleotides 949–968), OlPG1 (20-mer) 5′-TGGCAAGTTAGCACACTAGA-3′ (nucleotides 3237–3256), Olmag1 (20-mer) 5′-TGAAACGGCAGCGGCGCCCCAG-3′ (reverse, nucleotides 762–743), OlX (15-mer) 5′-CTCCCTTGTGAGGCT-3′ (reverse, nucleotides 4265–4251). The numbering of the nucleotides is taken from tobacco *Nia2* genomic sequence.

2.3. RNA isolation, cDNA synthesis and sequencing

Total RNA preparations were obtained from leaves as previously described [16]. First strand cDNA synthesis, PCR amplification of the NR cDNA and gel-purification of PCR products were performed as described [16,22]. PCR cycling conditions were the following: 3 min of single strand cDNA denaturation at 94°C then 30 cycles of DNA denaturation (45 s at 94°C), annealing of the primers (30 s at 50°C) and extension (2 min at 72°C). Gel-purified PCR products were used as templates for the sequencing reactions which were performed with the Dye Terminator Sequencing kit (Applied Biosystems) and then analyzed on a 370A Automatic DNA Sequencer (Applied Biosystems). Each mutation was sequenced at least two times on each strand and verified on a different amplified cDNA preparation. Protein and DNA sequence analysis was carried out using the UWGCG package (University of Wisconsin Genetics Computer Group). The structure predictions using the PHD neural network were performed at the EMBL (Heidelberg, Germany).

2.4. NR protein analysis

Leaf soluble proteins were prepared from grafted mutants and ammonium sulfate-precipitated as described in [23]. NADH:NR and NADH:cytochrome *c* reductase (CcR) activities were measured, respectively, by nitrite accumulation and cytochrome *c* reduction followed at 550 nm [15]. Units of both activities were defined as the reduction or production of, respectively, 1 nmol of cytochrome *c* or nitrite per min. NADH:NR activity reconstitution experiments were performed as previously described with mutant D51 extracts [24]. The CcR assay was adapted for microtitration plates by measuring the activity in 110 μl of 100 mM potassium phosphate buffer (pH 7.5) containing 100 μg horse heart cytochrome *c* (Sigma) and an aliquot of the extract. The reaction was initiated with 40 μl of a 1 mg/ml NADH solution and the reduction of cytochrome *c* was followed in a UVmax microplate reader (Molecular Dynamics). Ammonium sulfate-precipitated leaf extracts of the mutants were analyzed by gel filtration on a TSK 3000SW column (Tosohaas, 30 cm × 7.5 mm) installed on a FPLC equipment (Pharmacia). The column was calibrated with Biorad molecular mass markers. Approximately 100 μl of extract were injected on the column equilibrated with a 100 mM potassium phosphate buffer (pH 7.5) containing 100 mM sodium sulfate and eluted with the same buffer at a flow rate of 0.5 ml/min. Fractions of 250 μl were collected, immediately put on ice and supplemented with 25 μl of 100 mM potassium phosphate buffer (pH 7.5) containing FAD (final concentration 20 μM), leupeptin (final concentration 4 μM), Pefablock (Interchim, final concentration 400 μM) and DTT (final concentration 1 mM) in order to preserve NR activity. Protein elution was monitored by following the absorbance at 280 nm and each fraction was assayed for CcR or NADH:NR activity.

3. Results

3.1. NR cDNA amplification from nia mutants

Four *nia* mutants presumably affected in the NR MoCo domain [15]were retained for this study (E122, E77, F19 and H22). Northern blot analysis, using a NR cDNA as probe, showed that, in these mutants, the NR mRNA was overexpressed and of the same size as the wild type one [25]. In order to measure more accurately the size of their coding sequences

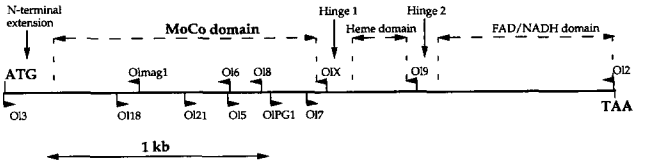


Fig. 1. Structure of the NR coding sequence with the corresponding protein domains. The oligonucleotides used in this work are indicated with their 5′ to 3′ orientation.

we synthesized a first strand cDNA using the tobacco NR specific primer Ol2 (Fig. 1). The NR coding sequence was then amplified by PCR with the primers Ol2 and Ol3. No differences in size were observed between the mutants and the wild type (data not shown). The same first strand cDNAs were then used for PCR amplification of the MoCo and heme domains with primers Ol3 and Ol9 (Fig. 1). The obtained PCR products were purified and used as templates for different sequencing reactions with each of the nested primers located inside the MoCo domain (Fig. 1).

3.2. Identification of sequence changes

Sequencing of the MoCo domain of the four mutants revealed only one base change in each case when we compared the mutant sequences to the wild type one (Fig. 2). In mutant E122 the transversion (GGA→GTA) converted a glycine residue (Gly 293) into a valine, in mutant E77 the transversion (AGA→ATA) converted an arginine residue (Arg-298) into an isoleucine, in mutant F19 the same transversion (TGG→TTG) converted a tryptophane residue (Trp-459) into a leucine, and in H22 the transition (GGA→GAA) converted a glycine residue (Gly-463) into a glutamic acid (Fig. 3). All the mutated residues are conserved among all known plant NR sequences (Fig. 3) but also with algal and fungal NR sequences with the exception of Gly-463 which is replaced by a serine in the fungus *Ustilago maydis* [26]. Moreover, as shown in Fig. 3, the same residues are also conserved in the MoCo domain of sulfite oxidases. Secondary structure prediction by the PHD neural network [27,28] based on multiple sequence alignment suggested that the residue affected in mutant E77 has about 90% of chance of being in an α-helix extending from Gly-297 to Arg-305 and that Gly-293 (mutant E122) might be in a loop connecting a β-strand to the former α-helix (80% of chance). No reliable secondary structure prediction could be made for the region corresponding to the mutations of F19 and H22.

3.3. NR activities in the mutants and gel filtration

We retained the mutants E77, E122 and H22 for further analysis of the biochemical properties of the mutated NR enzymes. For the mutant F19, indeed, we were not able to get enough material from grafted plants. We prepared ammonium sulfate-precipitated extracts of soluble leaf proteins from these three mutants. The NR-linked NADH:cytochrome *c* reductase (CcR) activities were estimated by the difference between the total CcR activity and the residual activity of the extract measured after inhibition by saturating amount of the NR-specific monoclonal antibody 96(9)25 [15]. For the three mutants, NR-linked CcR activities were 205 units per mg of protein for E77, 270 for E122 and 155 units per mg of protein for mutant H22. As described before [15], these mutants overexpressed the CcR activity when compared to the wild type activity (around 100

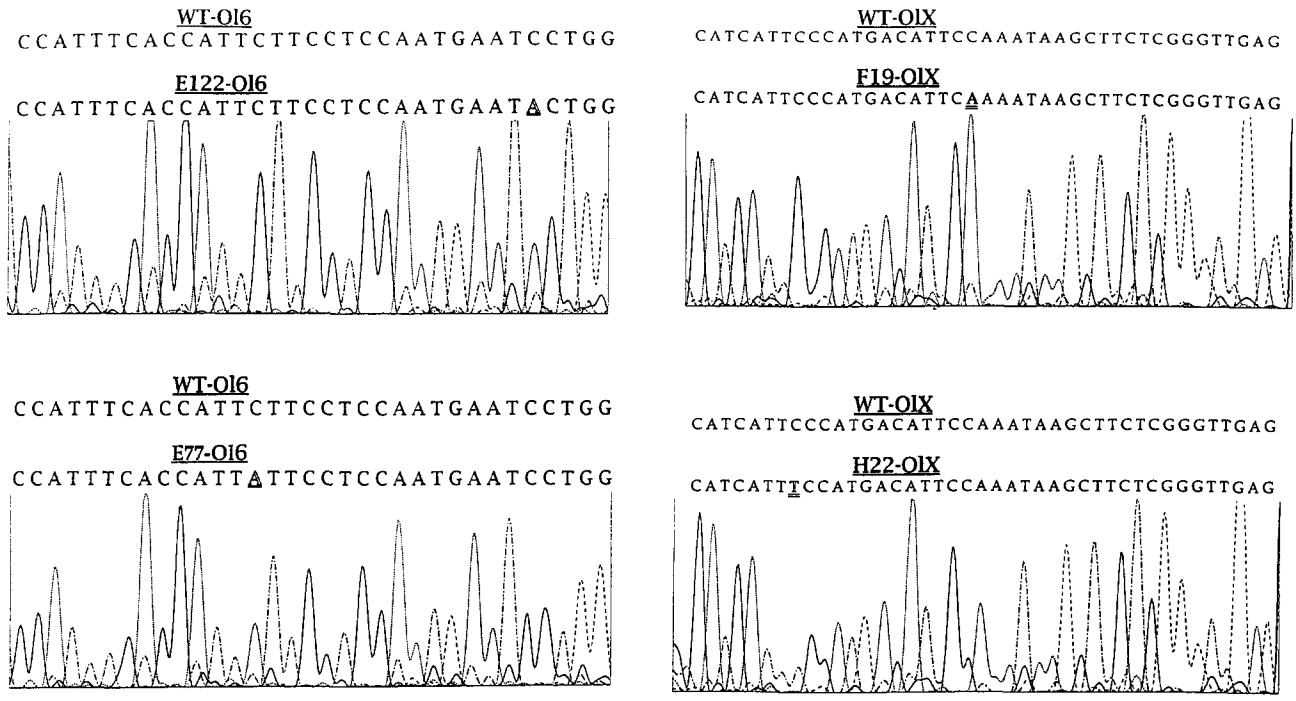


Fig. 2. DNA sequence histograms showing the base changes found in the mutants E122, E77, F19 and H22. The sequence of the complete MoCo domain was determined for each mutant and for the wild type. As the mutations are clustered in two small regions, the same sequence fragment is shown for the mutants E122 and E77 as well as for the mutants F19 and H22. The wild type sequence (WT) is shown on the top of each mutant sequence. The two primers used to generate the sequence data were O16 and OIX. Sequences are in the 5′ to 3′ direction on the complementary strand.

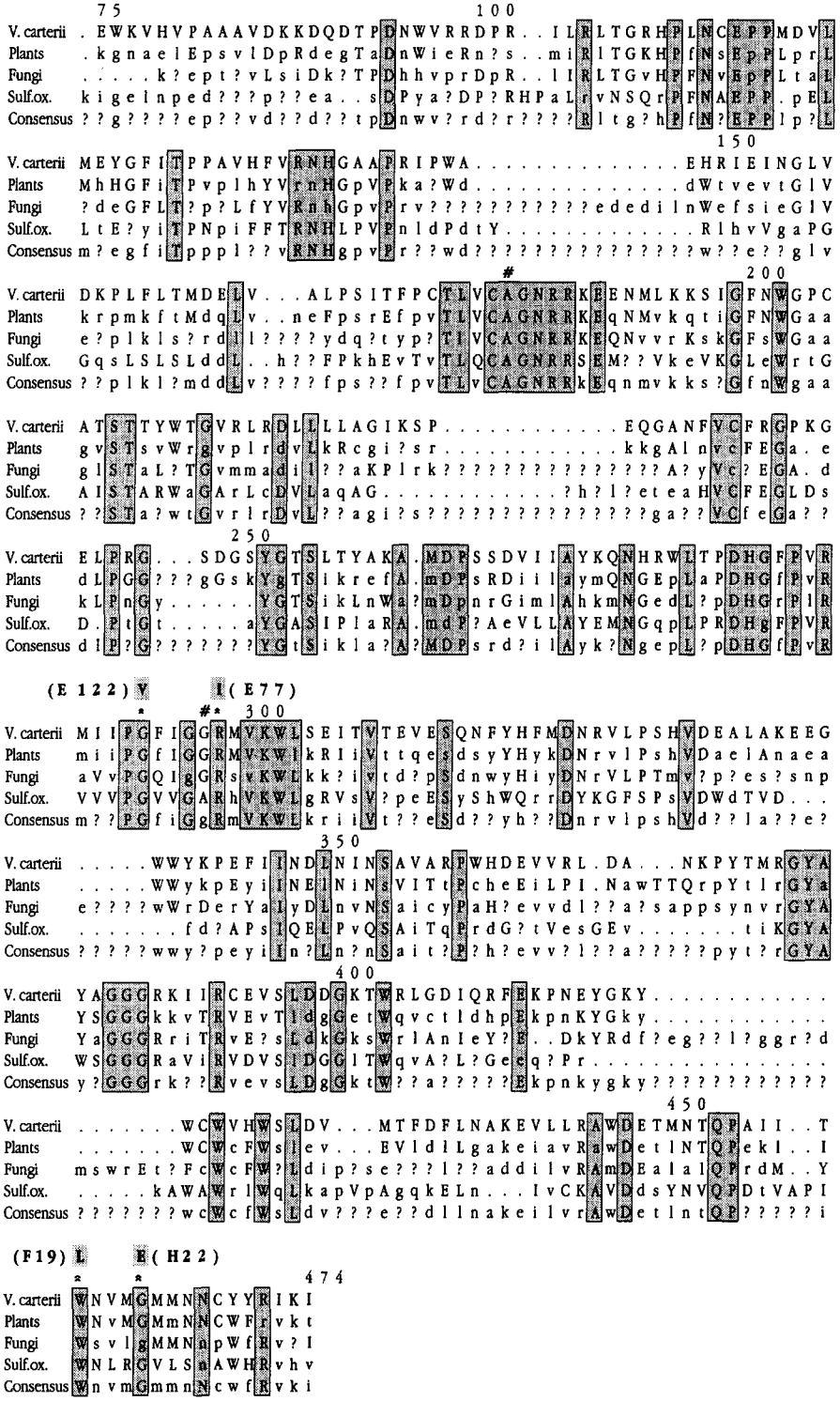
units per mg of protein). We have previously shown that *nia* mutants affected in different domains of NR can sometimes present intragenic complementation when crossed together [24]. Restoration of NADH:NR activity was also observed in vitro when mixing extracts from different mutants. We tested the appearance of NADH:NR activity after mixing the E77, E122 and H22 extracts with an ammonium sulfate-precipitated extract of mutant D51 affected in the FAD domain. The mutant E122 extract did not allow any reconstitution of NADH:NR activity after mixing whereas extracts from mutants E77 and H22 led to a NADH:NR activity of, respectively, 3.3 and 0.23 units per mg of protein. The level of reconstituted NADH:NR activity was very low but reproducibly above background for mutant H22. We investigated then the distribution of CcR activities after separation of mutant extracts by gel filtration chromatography (Fig. 4). In the wild type extract, a peak of NADH:NR activity which co-eluted with a broad peak of CcR activity was observed with a predicted molecular mass of around 220 kDa (fractions 8 and 9). This corresponds to the observed molecular mass of native NR[29]. For the mutants E77 and H22, a sharper peak of NR-linked CcR activity was observed corresponding to an apparent molecular mass of 120 kDa. On the other hand the NR-linked CcR activity peak in the mutant E122 almost eluted with the excluded volume of the column (Fig. 4). This peak corresponds to an apparent molecular mass of around 520 kDa and may correspond to a tetramer, or even higher degree of multimerization, of NR.

4. Discussion

A previous study on four *nia* mutants affected in the NR heme domain revealed that only one mutant out of the four carried a missense mutation [16]. Surprinsingly all four mutants defective in the MoCo domain presented missense mutations (Figs. 2 and 3). Furthermore analysis of mutants with defect in both the *Arabidopsis NIA1* and *NIA2* NR structural genes showed that they were presenting missense mutations in the MoCo domain [17,19]. This can be explained by the fact that these mutants were initially characterized by the presence of a residual CcR activity, which demands a functional FAD and heme domain. Thus nonsense or frameshift mutations in the N-terminal MoCo domain were not retained or classified in the same group because they would lead to a loss of all NR-linked activities.

The complete NR coding sequence was not determined for each of the mutants, thus we cannot exclude the presence of mutations in the other domains. However, the frequency of double mutation in a single locus is very low compared to the one of a single mutational event. Moreover the four mutants still displayed NR-linked CcR activity which suggests that the FAD and heme domains, which are required for this activity, are not mutated. In addition some mutants like E77 are capable to complement mutants affected in both the FAD and heme domains [24].

Alignment of the NR amino acid sequences has already allowed to pinpoint several conserved residues in plant, but also fungal and algal MoCo domain [3,5]. For some of these residues, the conservation extends also to the MoCo domain sequences of animal sulfite oxidases [30]. Nevertheless knowledge about the structural and functional features of the MoCo domain is hitherto rather scarce compared to the FAD domain which structure has been recently solved [31] or to the heme domain for which the structure can be predicted by homology



to cytochrome b_5 [16]. Furthermore it is difficult to determine which among the conserved residues are critical for function and/or structure of the MoCo domain. The study of *nia* mutants with a null phenotype highlights such residues which mutation totally abolishes NR activity. We have localized four of these residues so far and it appears that they are clustered in two regions of the MoCo domain (Fig. 3).

The first region, corresponding to mutants E77 and E122, is highly conserved among all NRs and also sulfite oxidases. The main difference between these two mutants was that the first one is able to complement other *nia* mutants whereas E122 never presented any intragenic complementation [24]. Nevertheless the corresponding mutations are very close on the NR sequence (Fig. 3). To address this question we have first looked to the quaternary structure of NR by analysing the elution profile of NR-linked CcR activity in E77 and E122. This study

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Fig. 3. Alignment of the MoCo domain amino acid sequences from eukaryotic NRs and animal sulfite oxidases. Consensus sequences were first derived from the alignment of, respectively, plant NRs, fungi NRs and animal sulfite oxidases ('Sulf.ox.') MoCo domain sequences. These consensus sequences were then aligned together with the only complete algal NR sequence known (from *Volvox carterii*). This way a final consensus sequence was determined ('Consensus'). Boxed and shaded residues correspond to conserved amino acids among NR and sulfite oxidase sequences. Residues in uppercase in the primary consensus sequences ('Plants', 'Fungi',...) indicate conserved residues in the corresponding alignments whereas residues in lowercase correspond to the most represented amino acid at this position. The positions in the final alignment where the same amino acid is found are shown in the final consensus by letters in uppercase. Introduced gaps in the alignments are represented by dots in the consensus sequences. Question marks correspond to positions where no majority amino acid was found. Amino acid changes introduced by the mutations are shown on the top of the alignment. Positions where mutations were found in the MoCo domain of Arabidopsis NRs are also indicated (#). The protein sequences that were used for this alignment are, for plants, the *Arabidopsis* NIA1 and NIA2 gene products, the tobacco NIA1 and NIA2 gene products, the petunia, tomato, squash, birch, rice, soybean and *Lotus japonicus* NRs, the leaf and root bean NRs, and the barley NAR1 and NAR7 gene products. The fungi sequences were from *Aspergillus niger*, *A. nidulans*, *Neurospora crassa*, *Leptosphaeria maculans*, *Ustilago maydis* and *Fusarium oxysporum*. The sulfite oxidases sequences are from rat, man and chicken. All the sequences were obtained from the Genbank database and in [3,5] The numbers above the alignment refer to the tobacco NR protein sequence (NIA2).

suggested that NR is at least tetrameric in mutant E122. This could explain why E122 is unable of complementing other defective NRs, indeed one of the mechanism of restoration of NR activity may involve formation of heterotetramers (two dimers from the two different mutants) and/or heterodimers which would allow intermolecular electron flow from NADH to nitrate. Thus if NR from mutant E122 self-aggregates, it could be impaired in the interaction with other NR molecules. On the other hand the apparent molecular mass of NR from mutant E77 and H22 is compatible with the enzyme being monomeric. A similar conclusion has been reached for the *Arabidopsis* mutant B29 [18]. Another hypothesis would be that NR is present as a partly degraded dimer but this is not supported by the fact that we can still measure a CcR activity, which needs both the FAD and heme domains. Moreover the presence of a truncated NR molecule carrying only these two domains is not compatible with the apparent molecular mass of the CcR elution peak. Indeed limited proteolysis experiments have led to a model where intersubunit interactions in the NR dimer occur in the MoCo domain [32]. The presence or not of the MoCo itself in the mutant NRs has not been investigated so far since leaf material from *nia* mutants is rather limited. Thus, at this stage, we cannot differentiate between a direct effect of the mutations on MoCo binding and an effect of the mutations on tertiary structure which would affect indirectly the structure and activity of NR. But we hope to be able to address this important question by expressing mutant enzymes in heterologous systems.

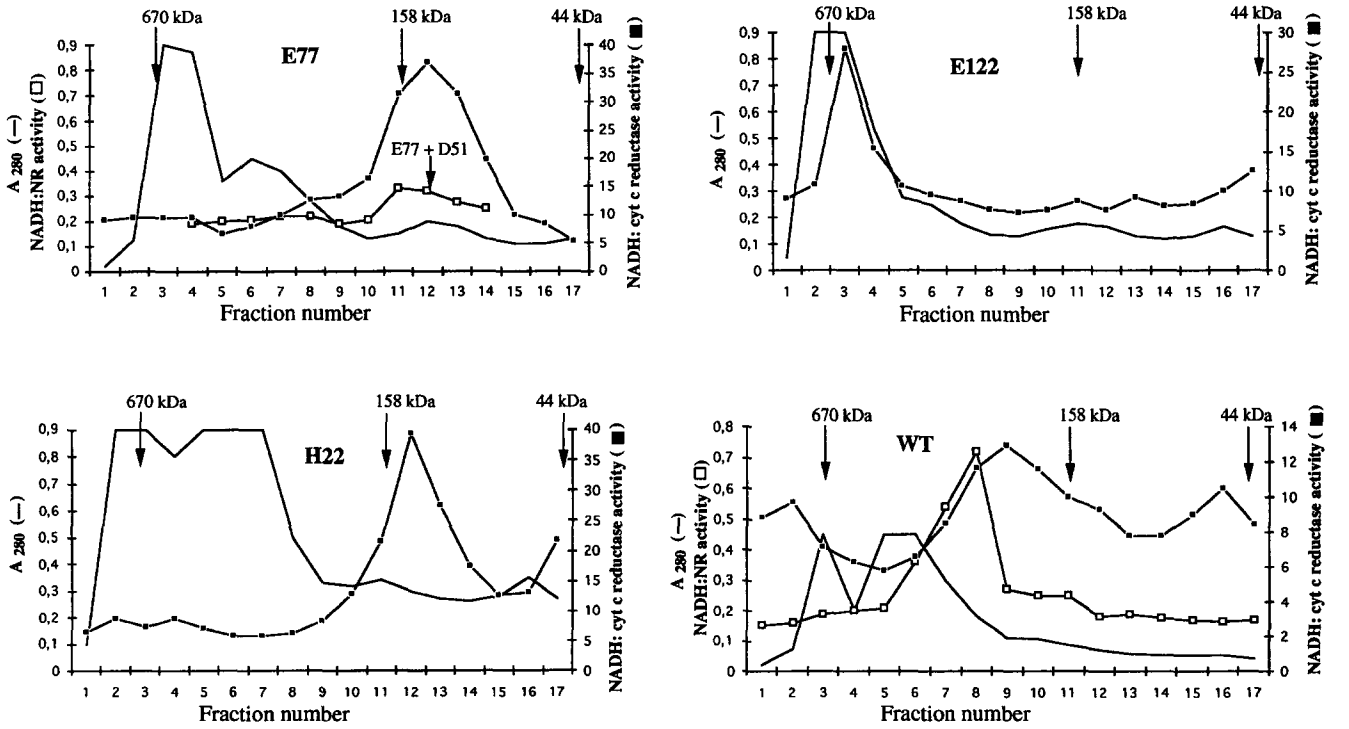

Fig. 4. Analysis by FPLC gel filtration of the mutant extracts. Mutant and wild type leaf extracts were prepared as described in section 2. The absorbance at 280 nm (A_{280}) and the CcR activity elution profiles are presented for the wild type and the mutants E77, E122 and H22. For the wild type the elution profile of the NADH:NR activity is also shown. For the mutant E77, fractions were mixed with mutant D51 extract and the level of reconstituted NADH:NR activity is indicated for each fraction. Arrows indicate the elution peaks, monitored at 280 nm, of thyroglobulin (670 kDa), g-globulin (158 kDa) and ovalbumin (44 kDa). CcR and NADH:NR activities are expressed, respectively, in nmol of cytochrome *c* reduced or nitrite produced per min and per ml of elution fraction.

The critical residues that we and others have identified in the NR MoCo domain could now serve as a basis for a site-directed mutagenesis of this domain in order to get some insight in the structure/function relationships of molybdoenzymes.

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References

[1] Coughlan, M.P. (1980) Molybdenum and molybdenum-containing enzymes, Pergamon Press, Oxford.
[2] Johnson, J.L. and Rajagopalan, K.V. (1982) Proc. Natl. Acad. Sci. USA 79, 6856–6860.
[3] Wootton, J.C., Nicolson, R.E., Cock, J.M., Walters, D.E., Burke, J.F., Doyle, W.A. and Bray, R.C. (1991) Biochim. Biophys. Acta 1057, 157–185.
[4] Redinbaugh, M.G. and Campbell, W.H. (1985) J. Biol. Chem. 260, 3380–3385.
[5] Rouzé, P. and Caboche, M. (1992) in: Inducible Plant Proteins (J.L. Wray ed.) pp. 45–77, Cambridge University Press, Cambridge.
[6] Solomonson, L.P. and Barber, M.J. (1990) Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 225–253.
[7] Calza, R., Huttner, E., Vincentz, M., Rouzé, P., Galangau, F., Vaucheret, H., Chérel, I., Meyer, C., Kronenberger, J. and Caboche, M. (1987) Mol. Gen. Genet. 209, 552–562.
[8] Campbell, W.H. and Kinghorn, J.R. (1990) Trends Biochem. Sci. 15, 315–319.
[9] George, G.N., Kipke, C.A., Prince, R.C., Sunde, R.A., Enemark, J.H. and Cramer, S.P. (1989) Biochemistry 28, 5075–5080.
[10] Barber, M.J. and Neame, P.J. (1990) J. Biol. Chem. 265, 20912–20915.
[11] Garrett, R.M. and Rajagopalan, K.V. (1994) J. Biol. Chem. 269, 272–276.
[12] Baijal, M. and Sane, P.V. (1988) Phytochemistry 27, 1969–1972.
[13] Hoff, T., Truong, H.N. and Caboche, M. (1994) Plant Cell Environ. 17, 489–506.
[14] Gabard, J., Marion-Poll, A., Chérel, I., Meyer, C., Müller, A. and Caboche, M. (1987) Mol. Gen. Genet. 209, 596–606.
[15] Chérel, I., Gonneau, M., Meyer, C., Pelsy, F. and Caboche, M. (1990) Plant Physiol. 92, 659–665.
[16] Meyer, C., Levin, J.M., Roussel, J.-M. and Rouzé, P. (1991) J. Biol. Chem. 266, 20561–20566.
[17] Wilkinson, J.Q. and Crawford, N.M. (1993) Mol. Gen. Genet. 239, 289–297.
[18] Braaksma, F.J. and Feenstra, W.J. (1982) Theor. Appl. Genet. 64, 83–90.
[19] Labrie, S.T. and Crawford, N.M. (1994) J. Biol. Chem. 269, 14497–14501.
[20] De Vries, S.E., Dirks, R., Mendel, R.R., Schaart, J.G. and Feenstra, W.J. (1986) Plant Sci. 44, 105–110.
[21] Pouteau, S., Spielmann, A., Meyer, C., Grandbastien, M.-A. and Caboche, M. (1991) Mol. Gen. Genet. 228, 233–239.
[22] Meyer, C., Pouteau, S., Rouzé, P. and Caboche, M. (1994) Mol. Gen. Genet. 242, 194–200.
[23] Chérel, I., Marion-Poll, A., Meyer, C. and Rouzé, P. (1986) Plant Physiol. 81, 376–378.
[24] Pelsy, F. and Gonneau, M. (1991) Genetics 127, 199–204.
[25] Pouteau, S., Chérel, I., Vaucheret, H. and Caboche, M. (1989) The Plant Cell 1, 1111–1120.
[26] Banks, G.R., Shelton, P.A., Kanuga, N., Holden, D.W. and Spanos, A. (1993) Gene 131, 69–78.
[27] Rost, B. and Sander, C. (1993) J. Mol. Biol. 232, 584–599.
[28] Rost, B. and Sander, C. (1994) Proteins 19, 55–72.
[29] Moureaux, T., Leydecker, M.-T. and Meyer, C. (1989) Eur. J. Biochem. 179, 617–620.
[30] Neame, P.J. and Barber, M.J. (1989) J. Biol. Chem. 264, 20894–20901.
[31] Lu, G.G., Campbell, W.H., Schneider, G. and Lindqvist, Y. (1994) Structure 2, 809–821.
[32] Solomonson, L.P., Barber, M.J., Robbins, A.P. and Oaks, A. (1986) J. Biol. Chem. 261, 11290–11294.