

Regulation of Nitrogenase-2 in *Azotobacter vinelandii* by Ammonium, Molybdenum, and Vanadium

SUSANNE JACOBITZ AND PAUL E. BISHOP*

Agricultural Research Service, U.S. Department of Agriculture, and Department of Microbiology,
North Carolina State University, Raleigh, North Carolina 27695-7615

Received 21 January 1992/Accepted 8 April 1992

Under diazotrophic conditions in the absence of molybdenum and in the presence of vanadium, *Azotobacter vinelandii* reduces N_2 to NH_4^+ by using nitrogenase-2, a V-containing enzyme complex encoded by *vnfH* (the gene for dinitrogenase reductase-2), and *vnfD* (the genes for dinitrogenase-2 subunits). Accumulation of the *vnfH* and *vnfD* transcripts occurred under Mo-deficient conditions in the presence and absence of V; however, in the case of *vnfD*, the protein products only accumulated in the presence of V. This suggests that V is required for translation of the *vnfD* transcripts. In addition, expression of *vnfH-lacZ* and *vnfD-lacZ* transcriptional fusions was only partially repressed in the presence of NH_4^+ . Transcripts hybridizing with *vnfH* (1.4 and 1.0 kb), *vnfD* (3.4 and 1.8 kb), and *vnfK* (3.4 kb) were detected in RNA extracted from wild-type cells cultured with NH_4^+ in the presence or absence of V. However, nitrogenase-2 subunits were not detected in extracts of cells derepressed for nitrogenase-2 in the presence of NH_4^+ . These results indicate that this nitrogen source acts at the posttranscriptional level as well as at the transcriptional level. *vnf* transcripts were not detected in the presence of Mo (with or without NH_4^+).

Azotobacter vinelandii harbors three genetically distinct nitrogenases (4). The expression of these nitrogenases is regulated by the availability of ammonium, molybdenum, and vanadium in the growth medium. Nitrogenase proteins do not accumulate in the presence of NH_4^+ . Nitrogenase-1 (the conventional Mo-containing nitrogenase) is made in the absence of a fixed nitrogen source when Mo is available, whereas nitrogenase-2 (an alternative V-containing nitrogenase) accumulates under Mo-deficient conditions in the presence of V. In the absence of both Mo and V, an alternative nitrogenase that does not appear to contain either Mo or V (nitrogenase-3) accumulates (4; for a review, see reference 5). The regulatory genes *nifA*, *vnfA*, and *anfA* are required for the expression of nitrogenase-1, -2, and -3, respectively (14). *NifA* binds to an upstream activator sequence (5'-TGT-N₁₀-ACA-3') and activates transcription of *nif* operons preceded by this sequence (6). The binding sites for *VnfA* and *AnfA* are unknown. An additional regulatory gene designated *nfrX* has been identified and is required for diazotrophic growth in the presence or absence of Mo (26). The nucleotide sequence of *nfrX* has been determined, and its predicted protein product has an N terminus that is highly homologous to the uridylyltransferase-uridylyl-removing enzyme encoded by *glnD* of *Escherichia coli* (10).

In contrast to the structural genes for nitrogenase-1 (*nifHDK*) and nitrogenase-3 (*anfHDK*), the structural genes for nitrogenase-2 are organized into two separate transcriptional units, *vnfH* and *vnfD* (16). This allows dinitrogenase reductase-2 (the *vnfH* gene product) and dinitrogenase-2 (encoded by *vnfD*) to be expressed independently of each other. Thus, dinitrogenase reductase-2 is expressed under Mo-deficient conditions in the presence or absence of V, whereas dinitrogenase-2 is expressed only in the presence of V (3, 8, 24). Recently it was shown that dinitrogenase reductase-2 is required for expression of nitrogenase-3 (18).

In the present study we investigate the effects of NH_4^+ , Mo, and V on the transcription of *vnfH* and *vnfD* in wild-type and mutant strains of *A. vinelandii*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains of *E. coli* and *A. vinelandii* used in this study are listed in Table 1. *E. coli* K-12 71-18, S17-1, and JC5466 were cultured in Luria-Bertani medium. *E. coli* C-600 was grown in Y broth (10 g of Bacto-Tryptone [Difco Laboratories], 2.5 g of NaCl, and 0.1 g of yeast extract per liter). Maltose was added to a concentration of 0.2%. *Azotobacter* strains were grown in modified Burk medium (29) as previously described (2). Ampicillin and tetracycline, when required, were added to final concentrations of 50 and 20 µg/ml, respectively. When required, Na_2MoO_4 and V_2O_5 were added, each at a final concentration of 1 µM. Fixed N was added as ammonium acetate (final concentration, 28 mM).

Construction of *vnfA-lacZ*, *vnfH-lacZ*, and *vnfD-lacZ* transcriptional fusion strains. Plasmids containing *vnfA*, *vnfH*, and *vnfD* were transformed into the suppressor-negative strain *E. coli* S17-1 and mutagenized with Tn5-B21 (27) as described by Joerger et al. (18). Plasmids containing Tn5-B21 insertions were linearized and transformed into *A. vinelandii* (23); then tetracycline-resistant (Tc^r) transformants were selected. Tc^r transformants resulting from double-crossover events were identified by screening for ampicillin sensitivity. Strains containing *vnfA-lacZ*, *vnfH-lacZ*, or *vnfD-lacZ* fusions were grown in Mo- and V-deficient modified Burk medium to a cell density of approximately 150 Klett units. Cells were harvested by centrifugation, washed once with potassium phosphate buffer (pH 7.0, 7 mM), and resuspended in the appropriate media. These media were Mo-deficient Burk medium containing ammonium acetate (28 mM), N-free Burk medium containing 1 µM Na_2MoO_4 or 1 µM V_2O_5 , Burk medium containing ammonium acetate plus Mo, and Mo- and V-deficient Burk medium. The resuspended cells were derepressed for β-galactosidase for 6

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain, plasmid, or phage	Relevant characteristics or genotype	Reference or source
<i>E. coli</i>		
K-12 71-18	$\Delta(lac-pro)$ F' <i>lac-9 lac-7 M15 pro⁺ supE</i>	20
C600	F ⁻ <i>thi-1 thr-1 leuB6 lacYI tonA21 supE44 lambda⁻</i>	25
S17-1	<i>thi pro</i> HsdR ⁻ HsdM ⁺ <i>recA</i> RP4 2-TC::Mu-kan::Tn7 (integrated plasmid)	28
JC5466	<i>trp his recA56 rplE</i>	C. Kennedy
<i>A. vinelandii</i>		
CA	Wild type	7
DJ143	$\Delta nifA$	D. Dean
CA11	$\Delta nifHDK$	1
CA11.81	$\Delta nifHDK$, <i>vnfH81::Tn5-B21</i>	This work
CA11.82	$\Delta nifHDK$, <i>vnfD82::Tn5-B21</i>	This work
CA46	<i>vnfA46::Tn5</i>	14
CA46.81	<i>vnfA46::Tn5</i> , <i>vnfH::Tn5-B21</i>	This work
CA46.82	<i>vnfA46::Tn5</i> , <i>vnfD82::Tn5-B21</i>	This work
CA66	$\Delta anfA66::kan$	14
CA66.81	$\Delta anfA66::kan$, <i>vnfH81::Tn5-B21</i>	This work
CA66.82	$\Delta anfA66::kan$, <i>vnfD82::Tn5-B21</i>	This work
CA71	$\Delta anfDGK71::kan$	15
CA71.81	$\Delta anfDGK71::kan$, <i>vnfH81::Tn5-B21</i>	This work
CA71.82	$\Delta anfDGK71::kan$, <i>vnfD82::Tn5-B21</i>	This work
CA30	<i>nifB30::Tn5</i>	17
CA30.81	<i>nifB30::Tn5</i> , <i>vnfH81::Tn5-B21</i>	This work
CA30.82	<i>nifB30::Tn5</i> , <i>vnfD82::Tn5-B21</i>	This work
CA81	<i>vnfH81::Tn5-B21</i>	This work
CA82	<i>vnfD82::Tn5-B21</i>	This work
CA85	<i>vnfA85::Tn5-B21</i>	This work
Plasmids		
pVASJ1	pUC18 containing 2.1-kb <i>Sma</i> I fragment of <i>vnfA</i>	This work
pVHSJ1	pUC9 containing 0.6-kb <i>Pst</i> I- <i>Eco</i> RI fragment of <i>vnfH</i>	This work
pVDSJ1	pUC9 containing 1.4-kb <i>Pst</i> I- <i>Sal</i> I fragment of <i>vnfDG</i>	This work
pVKSJ1	pUC18 containing 0.8-kb <i>Sal</i> I- <i>Pst</i> I fragment containing <i>vnfK</i>	This work
Phage		
$\lambda::Tn5-B21$	<i>b221 cI857 Pam80</i>	28

h. Samples (50 μ l) were taken from these cultures, and the accumulation of β -galactosidase activity was determined as described by Miller (21). The cells were made permeable by adding 50 μ l of 0.1% sodium dodecyl sulfate and 30 μ l of chloroform to the assay buffer.

Northern blot analysis. *A. vinelandii* CA was grown under diazotrophic conditions in the presence of 1 μ M V₂O₅ to a cell density of 150 Klett units. The cells were harvested by centrifugation, washed once with phosphate buffer, and resuspended in medium containing V or Mo in the presence or absence of NH₄⁺, as described above. After incubation for 6 h, total RNA was isolated as described by Krol et al. (19). RNA samples (approximately 60 μ g) were glyoxylated and electrophoresed on a 1.1% agarose gel in 10 mM sodium phosphate buffer (pH 7.2). RNAs were transferred from gels to GeneScreen hybridization membranes and hybridized as suggested by the suppliers of GeneScreen (NEN). Probes (a 0.6-kb *Pst*I-*Eco*RI fragment containing *vnfH*, a 1.4-kb *Pst*I-

TABLE 2. Activities of *vnfH-lacZ* and *vnfD-lacZ* fusions in *A. vinelandii*

Strain	Pregrowth conditions ^a	Derepression time (h)	% β -Galactosidase activity ^b			
			-Mo+N	+Mo-N	+V-N	-Mo-N
CA81	NH ₄ ⁺	0	30	ND ^c	ND	ND
		6	50	31	100 ^d	92
		24	67	8	112	64
	Mo	0	ND	10	ND	ND
		6	7	7	100 ^e	53
		24	34	2	132	75
CA82	NH ₄ ⁺	0	13	ND	ND	ND
		6	16	12	100 ^f	58
		24	47	13	133	92
	Mo	0	ND	7	ND	ND
		6	19	14	100 ^g	29
		24	21	8	142	46

^a Cells were precultured in the presence of 28 mM NH₄⁺ acetate or 100 nM Na₂MoO₄.

^b Percent β -galactosidase activity for cells cultured in the presence of NH₄⁺ and in the absence of Mo (-Mo+N), in the presence of Mo and the absence of NH₄⁺ (+Mo-N), in the presence of 1 μ M V₂O₅ and the absence of NH₄⁺ (+V-N), or in the absence of Mo, V, and NH₄⁺ (-Mo-N). Each value represents the average of three or more experiments.

^c ND, not determined.

^d 100% = 9,835 \pm 695 Miller units.

^e 100% = 10,960 \pm 1,911 Miller units.

^f 100% = 15,007 \pm 1,163 Miller units.

^g 100% = 9,696 \pm 548 Miller units.

*Sal*I fragment containing *vnfDG*, and a 0.8-kb *Sal*I-*Pst*I fragment containing *vnfK*) were labeled with ³²P by random-primed labeling as described by the supplier of the probes (Amersham). Marker RNAs of different sizes (0.24 to 9.5 kb) were blotted onto GeneScreen and stained with 0.1% methylene blue dissolved in 0.5 M sodium acetate (pH 5.2).

Two-dimensional protein gel electrophoresis. *A. vinelandii* CA, CA81, and CA82 were derepressed for nitrogenase-2 for 6 h under the conditions described above. Cell extracts were prepared as previously described by Bishop et al. (2). Isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins in cell extracts were conducted as described by O'Farrell (22) with the modifications of Bishop et al. (2).

RESULTS

Expression of *vnfH-lacZ* and *vnfD-lacZ* transcriptional fusions. Table 2 summarizes the β -galactosidase activities observed after 6 h of derepression of the *vnfH-lacZ* (strain CA81) and *vnfD-lacZ* (strain CA82) transcriptional fusion strains. Activities for strain CA81 were highest after derepression in the presence of V with cultures that were pregrown in Mo-deficient medium containing NH₄⁺ or in medium containing Mo. High activities were also observed after derepression in Mo- and V-deficient media, conditions under which nitrogenase-3 is expressed. Surprisingly, β -galactosidase activity also accumulated in the presence of NH₄⁺. Cells grown in the presence of Mo and subsequently derepressed in Mo-deficient medium in the presence or absence of NH₄⁺ showed reduced β -galactosidase activities (Table 2). These reduced activities probably resulted from carryover of previously accumulated Mo, even though the concentration of Na₂MoO₄ (100 nM) in the preculture growth medium was 10-fold lower than that normally used for Mo-dependent diazotrophic growth. For strain CA82, the

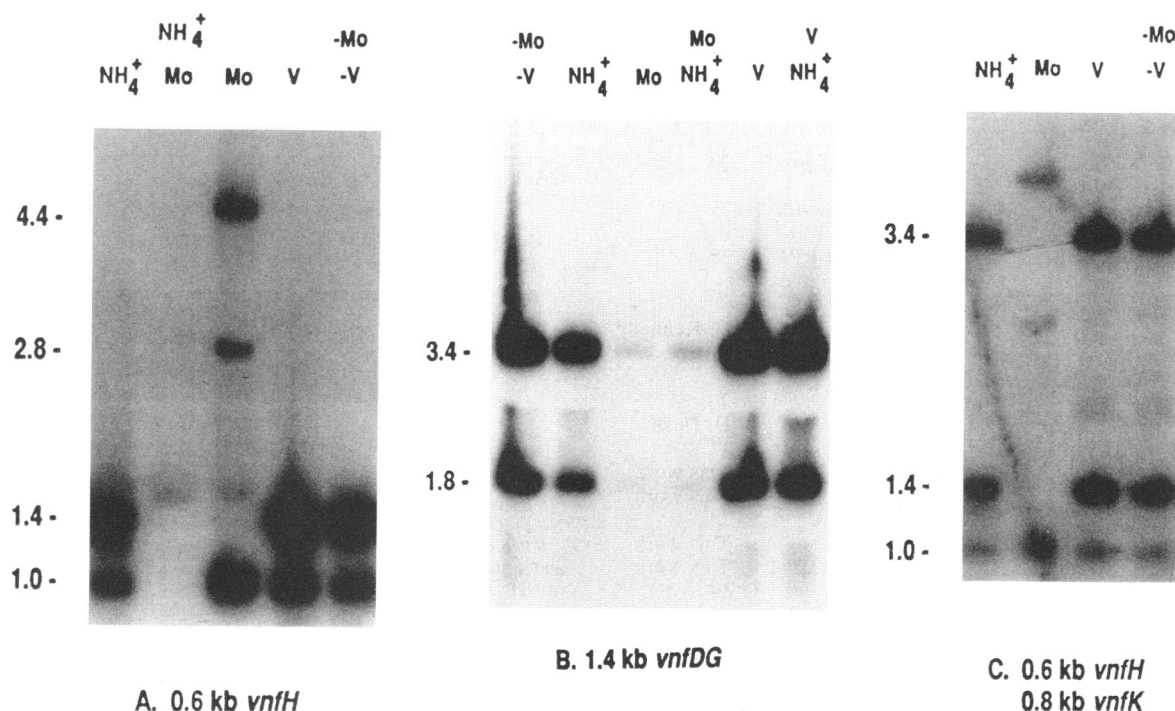


FIG. 1. Identification of *A. vinelandii* CA transcripts hybridizing to *vnfH* (A), *vnfDG* (B), and *vnfH* and *vnfK* (C). For the experiment shown in panel C, hybridization was conducted with the *vnfH* probe first and then with the *vnfK* probe.

highest β -galactosidase activities were also observed under V-sufficient conditions and lower activities were seen in the absence of V. Again, as with CA81, these activities were not repressed by NH_4^+ . The fusions carried by strains CA81 and CA82 were also expressed at low levels when cells were derepressed in the presence of Mo. After preculture in the presence of Mo, the β -galactosidase activities for strains CA81 and CA82 were reduced to 2 and 8%, respectively, after a 24-h derepression period.

***vnfH*-, *vnfDG*-, and *vnfK*-hybridizing mRNA species in the wild-type strain CA.** Since the *lacZ* fusion strains CA81 and CA82 are inactivated for *vnfH* and *vnfD*, respectively, we considered the possibility that repression by NH_4^+ requires the products of these structural genes. We therefore used Northern RNA blot analysis to look for the presence or absence of *vnf* transcripts in RNA from the wild-type strain CA. With *vnfH* as a probe, two mRNA species (1.4 and 1.0 kb) were detected after growth in Mo-deficient medium in the presence or absence of V or NH_4^+ (Fig. 1A). The amounts of these species were somewhat reduced in RNA from cells cultured in the presence of NH_4^+ as compared with those in RNA from cells cultured in the absence of NH_4^+ . The 1.0-kb mRNA species contains the structural *vnfH* gene without the ferredoxinlike *orf*, whereas the 1.4-kb mRNA species represents the full-length *vnfHorfFd* transcript (12, 16). A third mRNA species of about 1.3 kb was also visible in RNA from cells cultured in the presence of NH_4^+ . However, the band representing the 1.4-kb mRNA species from cells cultured under N-free conditions was very intense, making it difficult to resolve the 1.3-kb mRNA species. After growth in the presence of Mo, mRNA species of 4.4, 2.6, and 1.0 kb hybridized with the *vnfH* probe. Based on the similarity between *vnfH* and *nifH* sequences (88.5% identity [16]), the origin of these mRNA species is likely to be the *nifHDK* operon (12). After growth in the presence of

NH_4^+ plus Mo, no *vnfH*-hybridizing mRNA species were detected (Fig. 1A). *vnfD*-hybridizing mRNAs of 3.4 and 1.8 kb were abundant in RNA from cells cultured in Mo-deficient medium in the presence or absence of V (Fig. 1B). The amounts of *vnfD*-hybridizing mRNA species in RNA from cells cultured in the presence of NH_4^+ and the absence of V were slightly reduced. These mRNA species were very faint in RNA from cells grown in the presence of Mo (Fig. 1B).

With a *vnfK*-specific probe, a single mRNA species (3.4 kb) was observed in RNA from cells cultured in Mo-deficient medium in the presence or absence of V (Fig. 1C). Again this species was reduced in amount, but still present, in RNA from cells cultured in medium containing NH_4^+ .

Cells were cultured for 24 h in the presence or absence of V and NH_4^+ . *vnfH* and *vnfDGK* transcripts were present in cells grown in the absence of Mo, V, and NH_4^+ and in the presence of V with or without NH_4^+ . However, in the presence of NH_4^+ , transcripts were not detected in the absence of V (data not shown). This indicates that prolonged growth in the absence of V and in the presence of NH_4^+ results in the disappearance of *vnf* transcripts.

Presence of nitrogenase-2 proteins. From the results presented above, it is apparent that transcription of the *vnfHorfFd* and *vnfDGK* operons is only partially inhibited by the presence of NH_4^+ , whereas little or no accumulation of transcripts occurs in the presence of Mo. To determine whether the expression of nitrogenase-2 is posttranscriptionally regulated by NH_4^+ , we used two-dimensional protein gel electrophoresis to determine the presence or absence of dinitrogenase reductase-2 and dinitrogenase-2 subunits in cell extracts. Subunits of dinitrogenase-2 and dinitrogenase reductase-2 were absent when strain CA was incubated in the presence of Mo with or without NH_4^+ (Table 3). These subunits were present in extracts of cells incubated in

TABLE 3. Presence of protein subunits of nitrogenase-2 under different conditions of derepression^a

Strain	Addition(s)	Presence of nitrogenase-2 subunits:		
		α	β	γ
CA	NH ₄ ⁺ , Mo	—	—	—
	NH ₄ ⁺	—	—	—
	Mo	—	—	—
	V	+	+	+
	None	—	—	+
CA81	NH ₄ ⁺ , Mo	—	—	—
	V	+	+	—
CA82	NH ₄ ⁺ , Mo	—	—	—
	V	—	—	+

^a Cells were grown in Mo-free Burk medium containing 28 mM ammonium acetate, harvested, resuspended in Burk medium containing 28 mM ammonium acetate, 1 μ M Na₂MoO₄, or 1 μ M V₂O₅, and derepressed for nitrogenase-2 for 6 h.

Mo-deficient medium in the presence of V. The dinitrogenase reductase-2 subunit was the only subunit present in the absence of V, and nitrogenase-2 subunits were not detected in extracts of cells incubated in Mo-deficient medium containing NH₄⁺. As expected, strain CA81 (*vnfH-lacZ*) did not accumulate dinitrogenase reductase-2 and strain CA82 (*vnfD-lacZ*) did not accumulate dinitrogenase-2. However, each strain accumulated the complementary nitrogenase component not affected by the Tn5-B21 insertion.

Expression of *vnfH-lacZ* and *vnfD-lacZ* transcriptional fusions in different mutant backgrounds. *vnfH-lacZ* and *vnfD-lacZ* fusions in the VnfA[−] strains CA46.81 and CA46.82 were not expressed under any condition tested (Table 4). In the AnfA[−] strain CA66.81, the level of *vnfH-lacZ* expression in the presence of NH₄⁺ was much lower than that under the same conditions in the AnfA⁺ strain CA81. Expression was also reduced in N-free Mo-deficient medium with and without V. However, Northern blot analysis of RNA from strain CA66 indicated the presence of large amounts (comparable to those found for the wild type) of the *vnfH*-hybridizing mRNA species (1.4 and 1.0 kb) in cells grown in Mo-deficient medium in the presence or absence of V or NH₄⁺ (data not shown). The NifA[−] strains (DJ143.81 and DJ143.82) and strains containing deletions in the structural genes for nitrogenase-1 and -3 (strains CA11.81, CA11.82, CA71.81, and CA71.82) were relatively unaffected in the accumulation of β -galactosidase activity (Table 4). These results indicate that regulatory and structural genes required for nitrogenase-1 and nitrogenase-3 are not significantly involved in the expression or repression of nitrogenase-2. Finally, *nifB*, a gene that is essential for diazotrophic growth under all conditions (13, 17) and is known to be involved in FeMo cofactor synthesis, does not appear to be required for transcription of the *vnfHorFfd* and *vnfDGK* operons (strains CA30.81 and CA30.82) (Table 4).

DISCUSSION

Expression of the *vnfHorFfd* and *vnfDGK* operons in *A. vinelandii* seems to be regulated at least at two levels. Transcription of both operons is strongly repressed by Mo. On the other hand, the presence of NH₄⁺ only partially repressed transcription of these operons. This latter observation is in contrast to the situation with the structural gene

TABLE 4. Activities of *vnfH-lacZ* and *vnfD-lacZ* fusions in different mutant background *A. vinelandii*

Strain	Relevant genotype	% β -Galactosidase activity ^a			
		−Mo+N	+Mo−N	+V−N	−Mo−N
CA81 ^b		70	25	100 ^c	117
CA46.81	<i>vnfA46::Tn5</i>	2	1	2	2
CA66.81	<i>ΔanfA66::kan</i>	19	10	47	55
CA71.81	<i>ΔanfDGK71::kan</i>	66	13	104	94
CA11.81	<i>ΔnifHDK</i>	78	25	108	71
DJ143.81	<i>ΔnifA</i>	72	29	57	60
CA30.81	<i>nifB30::Tn5</i>	73	20	74	85
CA82 ^d		32	15	100 ^c	51
CA46.82	<i>vnfA46::Tn5</i>	2	1	8	10
CA66.82	<i>ΔanfA66::kan</i>	29	6	46	19
CA71.82	<i>ΔanfDGK71::kan</i>	28	16	77	45
CA11.82	<i>ΔnifHDK</i>	34	17	67	31
DJ143.82	<i>ΔnifA</i>	34	18	58	46
CA30.82	<i>nifB30::Tn5</i>	35	20	63	63

^a Percent β -galactosidase activity for cells precultured in the presence of 28 mM ammonium acetate and resuspended in the presence of NH₄⁺ and the absence of Mo (−Mo+N); in the presence of 1 μ M Na₂MoO₄ and the absence of NH₄⁺ (+Mo−N); in the presence of 1 μ M V₂O₅ and the absence of NH₄⁺ (+V−N); or in the absence of Mo, V, and NH₄⁺ (−Mo−N). The resuspended cells were incubated for 6 h.

^b CA81 mutant strains contain *vnfH::Tn5B21*.

^c 100% = 9,068 \pm 720 Miller units. Percentages represent the averages of two to five independent experiments.

^d CA82 mutant strains contain *vnfD::Tn5B21*.

^e 100% = 9,596 \pm 696 Miller units. Percentages represent the averages of two to five independent experiments.

operons encoding the other two nitrogenases, in which NH₄⁺ almost completely represses transcription (12, 18; unpublished data). The results of the experiments with *lacZ* fusions and Northern blots indicate that transcription of *vnfHorFfd* and *vnfDGK* in the presence of NH₄⁺ is at least 50% of that present under V sufficiency in the absence of NH₄⁺. On the other hand, no accumulation of nitrogenase-2 subunits occurred in the presence of NH₄⁺. In addition, preliminary results with translational *vnfH-lacZ* and *vnfD-lacZ* fusions indicate that β -galactosidase does not accumulate in the presence of NH₄⁺ (unpublished data). Transcription of the *vnfENX* operon also takes place in the presence of NH₄⁺ (30). This indicates that some of the regulation by NH₄⁺ occurs at the posttranscriptional level in at least three different *vnf* operons (*vnfHorFfd*, *vnfDGK*, and *vnfENX*). VnfA is required for the expression of the *vnfHorFfd*, *vnfDGK*, and *vnfENX* operons; as might be expected, a transcriptional *vnfA-lacZ* fusion also accumulates β -galactosidase in the presence of NH₄⁺ (unpublished data). In *Klebsiella pneumoniae*, *nifL* is thought to regulate *nif* operons in response to NH₄⁺ and O₂ by destabilizing *nif* transcripts (9) or inactivating NifA by binding of NifL to NifA (11). However, a *nifL*-like gene linked to *vnf* operons has not been found. One might speculate that the absence of a factor comparable to NifL in *K. pneumoniae* could lead to relatively stable *vnf* transcripts in the presence of NH₄⁺. Lack of translation of these transcripts in cells grown in the presence of NH₄⁺ suggests that translational repression may occur under these conditions. *vnf* transcripts are absent after a longer (24-h) incubation period in the presence of NH₄⁺ and the absence of V. *vnfDGK* transcripts are still present after 24 h of incubation in the absence of NH₄⁺, Mo, and V, whereas no dinitrogenase-2 subunits are present under these conditions. Therefore, the requirement of V for expression

of dinitrogenase-2 seems to be at the level of translation. Alternatively, in the absence of V, dinitrogenase-2 subunits may undergo rapid proteolytic degradation.

In conclusion, regulation of the structural genes encoding nitrogenase-2 in *A. vinelandii* occurs at both the transcriptional and posttranscriptional levels. Molybdenum blocks transcription, whereas NH_4^+ appears to act at both the transcriptional and posttranscriptional levels. In the absence of Mo and V, conditions under which nitrogenase-3 is expressed, the *vnfH* gene product is expressed, since dinitrogenase reductase-2 is required for transcription of *anfH-DGK* (18). However, dinitrogenase-2 does not appear to accumulate in the absence of V, even though *vnfD* transcripts are made in the absence of this metal. One might speculate that regulation of nitrogenase-2 at the posttranscriptional level might give *A. vinelandii* an advantage in a microenvironment (such as a microbial community) with rapidly fluctuating NH_4^+ and/or V concentrations. For example, after depletion of NH_4^+ , *A. vinelandii* cells would be able to express nitrogenase-2 in the presence of V relatively quickly, since transcripts are already present.

ACKNOWLEDGMENTS

We thank Chris Kennedy for providing the *vnfH-lacZ* and *vnfD-lacZ* plasmids and strain JC5466 and for communicating unpublished results. We also thank Dennis Dean for providing strain DJ143.

This work was supported by U.S. Department of Agriculture grant 88-37120-3872 and by postdoctoral fellowship Ja 512/1-1 from Deutsche Forschungsgemeinschaft to S.J.

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