Transcriptional repression of gdhA in *Escherichia coli* is mediated by the Nac protein

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Abstract

In this work we show that the *nac* gene from *Escherichia coli* is transcriptionally active, and that its expression is dependent on NRI (NtrC) and sigma-54. Northern blot experiments show a monocistronic *nac*-specific mRNA that is detected when wild-type cells are grown in nitrogen-limiting conditions. Our data also show that in nitrogen-limiting conditions Nac is involved in the transcriptional repression of the *gdhA* gene (encoding glutamate dehydrogenase) except when l-glutamine is used as the only nitrogen source. Moreover, the high level of GDH activity observed in a *nac* mutant strain is reduced when a wild-type *nac* gene is introduced under control of the *lac* promoter in N-limiting conditions, but not in l-glutamine or N-excess. These results suggest the existence of an additional mechanism responsible for overcoming repression by Nac.

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1. Introduction

Glutamate provides α-amino groups for the synthesis of purines, pyrimidines and amino acids. In *Escherichia coli*, it is predominantly synthesized by glutamate dehydrogenase (GDH) and glutamate synthetase (GOGAT). The mechanisms that control the synthesis and activity of these enzymes are only partially understood [1].

It has been shown that in *E. coli*, GDH activity is two-fold lower in cells grown in 0.5 mM NH₄Cl (an N-limiting condition) than in 15 mM NH₄Cl (an N-excess condition). This reduction is the consequence of a low transcription level of *gdhA* in 0.5 mM NH₄Cl conditions [2]. These results strongly suggested the presence of an unknown mechanism able to regulate *gdhA* transcription in *E. coli*.

In *Klebsiella aerogenes*, Nac protein is able to repress *gdhA* transcription during N-limiting conditions [3]; however, GDH activity is not repressed in *Salmonella typhimurium* [4]. These results suggest that enterobacteria have evolved different ways to adjust GDH activity.

In *K. aerogenes*, besides GDH, some enzymes involved in nitrogen metabolism are controlled by Nac. Histidase and proline oxidase are positively controlled, whereas GDH is repressed. Since *nac* transcription is dependent on both RNA polymerase associated with sigma-54 (Eσ⁵⁴) and NRI-P all sub-
sets of Nac-controlled genes are coordinated with the Ntr system (nitrogen regulated system) [1,5].

In contrast, the K. aerogenes hutC gene is only slightly regulated by nitrogen conditions in E. coli [6]. In addition, there are conflicting reports regarding GDH regulation by N-availability [2,7–11], making it difficult to conclude if histidase and GDH are regulated in E. coli.

In the course of this work, Muse and Bender [12] identified the nac gene of E. coli and showed that a nac mutant strain grown in 0.2% L-arginine has a two-fold increase of GDH activity. Our results confirm and extend Muse and Bender’s analysis concerning the transcriptional control of gdhA by both N-availability and Nac.

2. Materials and methods

2.1. Bacterial strains and plasmids

MX614 [(lac-pro)Δ galE ilv-680 thi-1]; MX1019, MX902 and MX848 are derivatives of MX614; glnL83, glnG74::Tn5 and rpoN73::Tn5, respectively, were a gift from Dr. F. Bastarrachea [13,14]. MC1061 [15] was used to construct MX4005 (see Section 2.6). Plasmid pPC36 carries the nac gene from K. aerogenes [16]. pKO3 is a suicide plasmid, unable to replicate at 42°C.

2.2. Culture conditions

Bacterial strains were grown at 37°C in NN-minimal medium [14]. Amino acids were used at 50 µg/ml. Glucose was used at 0.2%. 15 mM or 0.5 mM NH₄Cl were used as N-excess or N-limiting conditions. l-Glutamate at 0.2%, or l-glutamine at 0.1% were added when indicated as the only nitrogen source.

2.3. Enzyme assays

GDH activity was measured as described [17].

2.4. Cloning and sequence of nac gene

Degenerated oligonucleotides were designed from the amino acid sequence of Nac from K. aerogenes (Nac_Ka). A PCR was carried out using total chromosomal DNA from MX614 cells or a 11.5-kb gel-purified fraction of EcoRI-digested DNA; this fraction was identified to carry a nac gene by hybridization against a fragment from nac_Ka obtained from pPC36. The PCR product was gel purified and the nucleotide sequence was obtained. Cloning of the complete nacEc (from E. coli) gene was done using this PCR product as a probe.

2.5. Northern blot

RNA extraction and Northern blots were performed as described [18].

2.6. Isolation of the nac::kan insertion

A 900-bp fragment containing nac was cloned into pKO3 plasmid. The resulting construction was digested with BgII, whose recognition site is located in the middle of the predicted coding region of Nac. A BamHI fragment obtained from pUC4K (KanR) (Pharmacia P-L Biochemicals) was ligated into the BgII site of pKO3nac. This plasmid carrying nac::kan was transformed into MC1061 cells. The plates were incubated overnight at 42°C. Isolated colonies were grown at 30°C in the presence of sucrose and kanamycin. Five KanR CmS colonies were further analyzed. The presence of nac::kan was confirmed by Southern blot in the nac::kan isolate MX4005.

3. Results

3.1. Regulation of gdhA transcription in E. coli

The GDH activity of wild-type strain MX614 was determined in different nitrogen sources (Table 1). The highest activities were observed when using 15 mM NH₄Cl or 0.1% l-glutamine, while a decrease of approximately two-fold was detected in 0.5 mM NH₄Cl, as previously reported by Riba et al. [2]. The lowest activity was observed with 0.2% glutamate as nitrogen source [7]. Since it is not known whether this reduced activity is due to glutamate-specific inhibition of GDH, or if transcriptional repression of gdhA is involved, we decided to analyze the transcription of gdhA in this condition. For this, a
A gdhA-specific probe was used to detect gdhA transcripts in Northern blots of total RNA from MX614 cells grown in either 15 mM NH₄Cl or 0.2% glutamate. As can be observed in Fig. 1, a strong hybridization signal was detected in conditions of N-excess; in contrast, no signal could be detected when glutamate was used as the only nitrogen source. These results show that transcriptional repression was the main cause for the low GDH level detected in this condition. Since gdhA transcription was also repressed when MX614 cells were grown in other N-limiting conditions, like 0.5 mM NH₄Cl (Fig. 1) or 0.2% arginine (data not shown), it can be concluded that gdhA transcription is reduced in N-limiting conditions, suggesting that the Ntr system might be involved in the control of gdhA transcription.

Transcription of genes under Ntr-control is directly or indirectly regulated by NRI [1]. Therefore, we decided to explore if GDH activity as well as gdhA transcription were affected by the absence of NRI.

Strain MX902 carrying an insertion in the glnG (ntrC) gene (glnG74::Tn5), which encodes for the NRI protein (NtrC), was used to determine GDH activity in N-excess or N-limiting conditions. It can be observed (Table 1) that MX614 cells as well as MX902 cells showed the same level of GDH activity when grown with 15 mM NH₄Cl; however, only MX902 showed GDH activity when grown with glutamate (Table 1). The same regulation was observed for gdhA-specific mRNA (Fig. 1). These results suggest that NRI is involved in the repression of gdhA transcription in N-limiting conditions.

Since no NRI-P binding sites have been detected in the gdhA regulatory region [2], direct repression of the gdhA promoter by NRI is unlikely. One possible explanation is that the Nac protein may be the actual repressor of gdhA transcription in E. coli.

Analysis of the gdhA regulatory region showed a consensus Nac-binding sequence 19 bp upstream of the putative start codon. In addition, three more sequences resembling the Nac binding consensus were located, at +25, at −25, overlapping the functional P3 promoter [2], and at −81. Although the last two putative binding sites have a mismatch in one out of six bases when compared to the consensus sequence reported for binding of Nac from [19], they may be functional.

### 3.2. nac transcription is N-regulated in E. coli

We cloned the nac gene from the E. coli chromosome as described in Section 2. The sequence of this...
gene proved to be identical to those previously reported [12,20].

To determine if nac transcription is regulated by N-availability, Northern blots of total RNA from MX614 cells grown in either N-excess or N-limiting conditions were probed with a fragment of the nacEc gene. As shown in Fig. 2, nacEc is only expressed in N-limiting conditions. Only a single transcript of approximately 1 kb was detected, suggesting that this gene is monocistronic. As expected, nacEc is not expressed in MX902 or MX848 (rpoN73::Tn5) cells (data not shown). Unexpectedly, a slight but reproducible reduction of the nac transcripts was observed in cells grown in 1-glutamine as compared with 0.2% glutamate, or with 0.5 mM NH₄Cl.

3.3. Does Nac repress gdhA transcription in E. coli?

To obtain direct evidence that NacEc is involved in repression of gdhA transcription, the chromosomal nac gene was disrupted with a kanamycin resistance cassette (see Section 2). The strain MX4005, carrying the allele nac::kan, showed a derepressed GDH activity in glutamate containing medium (Table 1). MX4005 cells were then transformed with a plasmid (pAO10) that allowed nac expression from the lac promoter. GDH repression was restored when these cells were grown in the presence of 0.2% glutamate and 10 μM IPTG. This activity correlated with a reduction in the amount of gdhA-specific mRNA present in MX4005/pAO10 cells (Fig. 3). These data support the idea that in E. coli gdhA transcription is repressed by Nac. Surprisingly, no GDH repression was detected in MX4005/pAO10 cells grown in 15 mM NH₄Cl even when higher IPTG concentrations were tested. This result, together with the high level of GDH activity detected in wild-type cells grown in 1-glutamine, strongly suggests that in some conditions, repression of the gdhA promoter by Nac can be overcome by a still unknown mechanism.

In order to obtain additional evidence to support this hypothesis, gdhA transcription was analyzed in a strain carrying the glnL83 mutation, which makes the Ntr system constitutively active.

As expected for an Ntr-constitutive mutant, the nac-specific transcript was detected in all the N-sources tested (Fig. 4A). In contrast, GDH activity was not repressed when 1-glutamine or 15 mM NH₄Cl were used as N-source, whereas a five-fold reduction in GDH activity was observed with 1-glutamate (Table 1). The same regulation was observed for gdhA-specific mRNA (Fig. 4B).

4. Discussion

In the course of this work, nacEc gene was identified and it was suggested that Nac is involved in GDH repression [12]. We also identified this gene and in addition we have shown that nac is expressed in an Ntr-dependent fashion as a monocistronic mRNA.

Our data show that Nac is involved in the transcriptional repression of the gdhA gene in N-limiting conditions.

Fig. 3. Northern blot of gdhA transcript. Total RNA from MX4005 (lanes 1 and 2) or MX4005/pAO10 (lanes 3 and 4) was hybridized against a 32P-labelled gdhA fragment. RNA was extracted from cells grown with 15 mM NH₄Cl, lanes 1 and 3; or with 0.2% 1-glutamate, lanes 2 and 4.

Fig. 4. Northern blot of nac and gdhA transcripts. Total RNA from MX1019 strain was probed against a 32P-labelled nac fragment (panel A) or against a gdhA fragment (panel B). RNA was extracted from cells grown with the following nitrogen sources: 15 mM NH₄Cl, lanes 1; 1% 1-glutamine, lanes 2; and 0.2% 1-glutamate, lanes 3.
conditions except when l-glutamine is used as N-source. These results contribute to clarify the controversy on GDH regulation by N-availability. In some cases GDH activity was determined in cells grown in l-glutamine as N-limiting condition [8,9,11], whereas in others a low amount of NH₄Cl or l-glutamate was used [2,7,10]. The molecular mechanism that avoids gdhA repression in cells grown in l-glutamine is still unknown.

As has been suggested for K. aerogenes, the repression of GDH through Nac may allow GS to assimilate ammonium preferentially when it is scarce. However, the following evidence suggests that E. coli possesses an additional mechanism for overcoming repression by Nac. (i) In wild-type strain, gdhA is not repressed in l-glutamine. (ii) In the nac::kan strain expressing the nac gene from the lac promoter, gdhA is not repressed when growing in 15 mM NH₄Cl as the only N-source. (iii) In a glhL-constitutive mutant, although the nac transcript is detected, gdhA is not repressed in cells grown in 15 mM NH₄Cl or in l-glutamine.

Based on our results, we hypothesized that the putative mechanism responsible for overcoming repression by Nac might be related to a low intracellular amount of glutamate. It has been reported that the intracellular pool of this amino acid in cells grown in 15 mM NH₄Cl or in l-glutamine is 5-fold lower that in 0.5 mM NH₄Cl [21]. Moreover, a stronger repression of gdhA is observed when cells grow in N-sources whose degradation produces l-glutamate.

Whether this mechanism acts to directly modulate Nac, or involves only gdhA transcription, remains to be elucidated.

Our results partially agree with those reported by Muse and Bender [12]; both works indicate that GDH is repressed by Nac. However, two differences should be noted. (i) In our strains, GDH activity changes appreciably in response to nitrogen limitation. (ii) The nac mutant reported here does not show GDH levels significantly higher than those observed in wild-type cells grown in 15 mM NH₄Cl. Therefore, it is difficult to conclude whether this work and that of Muse and Bender [12] are describing the same mechanism that prevents the repression of gdhA by Nac.

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References


