NOTES

Negative Regulation of the Gene for Fe-Containing Superoxide Dismutase by an Ni-Responsive Factor in *Streptomyces coelicolor*

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In *Streptomyces coelicolor*, transcription of the *sodF* genes, encoding Fe-containing superoxide dismutases, is negatively regulated by nickel. Gel mobility shift assays with *sodF1* promoter fragments and cell extracts from the A3(2) strain indicate the presence of a nickel-responsive DNA-binding protein, most likely a transcriptional repressor. The boundary for the Ni-responsive cis-acting region was identified both in vitro and vivo. Ni does not regulate the level of the putative repressor but only the binding competence of this protein.

All aerobically growing organisms encounter toxic derivatives of molecular oxygen and thus are equipped with defense systems against oxidative stress (5, 8). Superoxide dismutase (SOD) is an important component of this protective system, disproportionating superoxide anion into dioxygen and hydrogen peroxide (6). Based on the metal ions present in active sites, four groups of SODs have been distinguished; CuZnSOD, MnSOD, FeSOD, and NiSOD (6, 13, 20). Many organisms possess more than one type of SOD. For example, aerobically grown *Escherichia coli* contains MnSOD and FeSOD in the cytosol and CuZnSOD in the periplasm (1, 17). The regulation of *sod* gene expression has been best demonstrated for the MnSOD gene (*sodA*), which is under the control of a number of transcription factors, including SoxRS, Fur, ArcA, Fnr, and IHF (3, 17).

*Streptomyces coelicolor* Müller contains two types of SOD: NiSOD, encoded by the *sodN* gene, and FeSOD, encoded by the *sodF* gene (11, 12). In *S. coelicolor* A3(2), two FeSOD polypeptides are produced from two separate genes: *sodF1*, which is identical to the *sodF* gene of the Müller strain, and *sodF2*, which differs from *sodF1* by about 12% of its nucleotide sequence (2). Expression of these *sod* genes is differentially regulated by nickel, which increases the expression of the *sodN* gene at both the transcriptional and posttranscriptional levels and represses the transcription of the *sodF* genes (2, 11, 12). The details of the regulation of SOD gene transcription by various metals have been studied primarily in *E. coli* (regulation by manganese and iron) and in yeasts (regulation by copper) (7, 16). However, the antagonistic regulation of two *sod* genes by a single metal is most pronounced in *S. coelicolor*.

Ni-dependent transcriptional regulation has been reported in the expression of the hydrogenase gene (*hup*) in *Bradyrhizobium japonicum* (14, 15), and a nickel-specific transport system (encoded by *nikABCDE*) in *E. coli* (4), in which the *nikA* operon has been suggested to be repressed by a nickel-responsive regulator, NikR (4). In this study, we investigated the metal specificity of the *sodF1* gene regulation in *S. coelicolor* A3(2) and report the involvement of a nickel-responsive DNA-binding protein, most likely a repressor, in the regulation of *sodF1* gene expression.

**Effects of various transition metals on *sodF1* gene expression.** To examine whether transition metals other than nickel regulate SOD expression, the amount of FeSODs in *S. coel-

![FIG. 1. Effects of various transition metals on the expression of FeSODs. *S. coelicolor* A3(2) M145 cells were grown for 4 days on NA plates containing various transition metals (NiCl$_2$, CoCl$_2$, FeCl$_2$, MnCl$_2$, and ZnCl$_2$) at the indicated concentrations. (A) Cell extract containing 20 µg of proteins was analyzed for the amount of SodF1 and SodF2 proteins by Western blotting using antibodies against the SodF protein of the Müller strain as described previously (2). (B) The amount of SodF proteins was analyzed in parallel by using antibodies against the SodF protein of the Müller strain (12). (C) RNAs prepared from the above-described cells were analyzed for *sodF1* mRNA by S1 nuclease mapping using a *sodF* probe labeled at the 5’ position of the *BglII* end at position +479 relative to the transcription start point (11). (D) The *sodF* mRNA was analyzed in parallel by using a *sodN* probe labeled at the 5’ position of the *BglII* end at position +402 relative to the transcription start point (12).**
protein from M145 cells grown in the presence of 100 mM EDTA–4 mM dithiothreitol–5 mM MgCl2–20 mM KCl–bovine serum albumin at 20 °C. In contrast, production of NiSOD was increased at concentrations at which SodF1 started to decrease and SodN started to increase were 10 nM NiCl2 and 1 μM CoCl2 (data not shown). The total amount of SodF1 and SodN polypeptides from cells grown at 100 μM CoCl2 was comparable to that at 100 nM NiCl2, implying that nickel is more effective than cobalt by at least 2 orders of magnitude in regulating sodF1 and sodN gene expression.

**Ni-responsive protein binding to sodF1 promoter.** To search for the presence of transcriptional regulators responsive to nickel, gel mobility shift assays were performed with cell extracts and sodF1 promoter fragments. Cell extracts were prepared from A3(2) cultures grown with or without added NiCl2 in YEME medium (9). Two different sodF1 promoter fragments of different lengths were generated by PCR using two sets of primers: SODF1N1 (5′-CCG AAC AAC-3′ [the HindIII site at position −130 relative to the transcription start site is underlined]) and SODF1Bam (5′-CAT GGC GGA TCC CTC CGG-3′ [the BamHI site at position +30 is underlined]) were used to generate the longer fragment (−130 to +30), and SODF1N2 (5′-CCG GGA TCC CTC CGG-3′ [the BamHI site at position +30 is underlined]) were used to generate the shorter fragment (−60 to +30).

Using the two sodF1 promoter fragments, two distinct complexes were formed by extracts from an Ni-supplemented cul-

**FIG. 2.** Binding of Ni-responsive protein to sodF1 promoter region. (A) Gel mobility shift assays used cell extracts prepared from S. coelicolor A3(2) M145 cells grown for 2 days in YEME with (lanes 5 to 7 and 11 to 13) or without (lanes 2 to 4 and 8 to 10) 100 μM NiCl2. The indicated amount of cell extract was incubated with the sodF1 DNA probe spanning the region between nt −130 (lanes 1 to 7) or −60 (lanes 8 to 14) and nt +30 relative to the transcription start point. Each binding reaction mixture contained about 10 fmol (0.6 to 1.0 ng) of 32P-labeled DNA fragment (about 104 cpm) in 4 mM Tris–HCl (pH 8.0)–1 mM EDTA–4 mM dithiothreitol–5 mM MgCl2–20 mM KCl–bovine serum albumin at 0.3 μg/μl–10% (vol/vol) glycerol–1 μg of poly(dI-dC). FP denotes free probe DNA, and arrows indicate retarded bands sensitive to nickel. (B) Specificity of binding to sodF1. The shorter promoter probe was incubated with 50 μg of protein from M145 cells grown in the presence of 100 μM NiCl2, as for panel A. The binding reaction was challenged with a 5- to 60-fold molar excess of either unlabeled promoter fragments (Specific; lanes 3 to 5) or HpaII restriction fragments of pGEM-3Zf DNA (Non-specific; lanes 7 to 9) in the binding buffer.

*S. coelicolor* A3(2) cells grown on a nutrient agar (NA) plate supplemented with various metals was analyzed by immunoblotting (Fig. 1A). Nickel effectively repressed the production of SodF1 even at 1 μM and that of SodF2 at 100 μM (Fig. 1A, lanes 2 and 3). Cobalt suppressed SodF1 production partially at 100 μM and SodF2 production only marginally (Fig. 1A, lane 4). Other transition metals did not affect the production of either SodF1 or SodF2. In all cases, the level of FeSOD activity correlated well with the amount of SodF polypeptides (data not shown). In contrast, production of NiSOD was increased at 1 μM NiCl2 and 100 μM CoCl2 but was not affected by other metals (Fig. 1B).

The change in levels of sodF1 transcripts in these cells was analyzed by S1 nuclease mapping (Fig. 1C), and the effects of various metals on transcription correlated reasonably well with those on polypeptide production. Therefore, we suggest that sodF1 transcription is very sensitive to inhibition by nickel and less sensitive to inhibition by cobalt. As a comparison, changes in sodN mRNA were analyzed in parallel, and this gene exhibited regulation by nickel and cobalt opposite to that of the sodF genes (Fig. 1D). Comparison of the concentrations of these two metals required for regulation indicated that the minimum concentrations at which SodF1 started to decrease and SodN started to increase were 10 nM NiCl2 and 1 μM CoCl2 (data not shown). The total amount of SodF1 and SodN polypeptides from cells grown at 100 μM CoCl2 was comparable to that at 100 nM NiCl2, implying that nickel is more effective than cobalt by at least 2 orders of magnitude in regulating sodF1 and sodN gene expression.
ture (Fig. 2A, lanes 5 to 7 and 11 to 13). The proportion of the slower-migrating complex increased with greater amounts of cell extract, suggesting that the bound protein is the multimeric form of that in the faster-migrating complex. The binding pattern of the longer and shorter DNA fragments were almost the same, indicating that the binding site is located within the boundary of the shorter fragment. Given that Ni represses sodF1 gene transcription, the Ni-sensitive binding pattern suggests that the bound factor functions as a repressor for the sodF1 promoter. The sodF1 complexes were strong enough to compete out more easily, consistent with the results in Fig. 1, only cobalt allowed in vitro activation of the sodF1-binding protein, although much less efficiently than did nickel (Fig. 4B).

Our results demonstrate the presence of a sodF1 promoter-binding protein, most likely a transcriptional repressor, whose binding activity was enhanced greatly in response to low levels of nickel and also in response to much higher levels of cobalt. The cis-acting negative regulatory site located between nt −60 and +30 of the sodF1 promoter and the nickel-sensitive binding of a trans-acting factor to this region constitute the nickel-dependent negative regulatory system of sodF1 gene transcription. Various reports suggest that not only the deficiency but also the overexpression of SOD is toxic to cells (18, 19). The antagonistic production of FeSOD and NiSOD regulated by nickel could therefore be a kind of homeostatic regulatory mechanism to maintain the total SOD activity in S. coelicolor within an optimal range. The modulation of the DNA-binding activity of a pre-existing regulator by Ni ensures a rapid response, keeping the total SOD activity relatively constant.

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REFERENCES