

Investigating *Escherichia coli* NikR's second metal-binding site

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Escherichia coli NikR is a nickel-responsive transcription factor that regulates nickel ion homeostasis. In the presence of nickel, NikR binds to the promoter of the *nik* operon, which encodes the nickel membrane transporter and blocks its transcription (1, 2). Previous studies suggest that repression is elicited by binding of nickel to two metal-binding sites in NikR: a high-affinity and a low-affinity site (3, 4). The putative low-affinity site has not been localized or characterized in detail. To locate the low-affinity site, chemical modification and mass spectrometry were used to identify residues involved in nickel binding. Mutations of a potential second metal-binding site were constructed and the effect of these mutations on the metal-responsive DNA-binding activity of NikR was investigated by mobility-shift assay and DNase footprinting. These experiments provide the first step towards understanding the mechanism of the nickel-selective activity of this prokaryotic metalloregulator.

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