CbnR, a LysR-type transcriptional regulator from *Cupriavidus necator* NH9, activates the transcription of chlorocatechol-degradative enzymes. To activate the transcription, CbnR needs to bind not only to the *cbnA* promoter but also to the inducer. In this study, the transcriptional activity and DNA-binding activity of twenty-five mutants of CbnR were analyzed. Of the 17 mutants of the DNA-binding domain, 11 mutants lost their ability to activate transcription. While most mutants without transcriptional activation did not show DNA-binding activity, Asn17Ala, Gln29Ala, and Pro30Ala retained DNA-binding activity, suggesting that transcriptional activation by CbnR requires more than its binding to promoter DNA. Of the 8 mutants of the regulatory domain, 6 mutants changed their responses to the inducer, when compared with wild-type CbnR. Interestingly, Arg199Ala and Val246Ala induced constitutive expression of the *cbnA* promoter without the inducer, suggesting that these mutations brought about a conformational change mimicking that induced by the inducer molecule.

Mutational analysis of a LysR-type transcriptional regulator, CbnR, revealed critical amino acids in regulatory domain (A and B) and DNA-binding domain (C and D).