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Properties of the Conserved Histidine of 5'-Adenylylsulfate Reductases from *Pseudomonas aeruginosa* and *Enteromorpha intestinalis*

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[Introduction]

Plants assimilate sulfate for biosynthesis of many sulfur containing compounds including cysteine, methionine, glutathionine and secondary metabolites such as glucosinolates. The adenosine 5'-phospho sulfate (APS) reductase of sulfur assimilation mechanism contains four conserved cysteine residues that are absent from the [4Fe-4S] center and the phosphoadenosine 5'-phospho sulfate (PAPS) reductase. In this report, we investigated conserved histidine amino acids that blocked iron-sulfur clusters of APS reductase from *P. aeruginosa* and *Enteromorpha intestinalis*, and investigated the role of this particular histidine residue to identify site-specific mutations. The goal of this study was to gain insight into the function of conserved histidine residues in bacterial and plant type APS reductase.

[Materials and Methods]

The pET30b-PaAPR and pET30b-EiAPR (Gao et al., 2000, Kim et al., 2006) constructs were used as templates for site-specific mutagenesis. Site specific mutations of PaAPR and EiAPR were performed using the QuikChange kit (Stratagene) according to the manufacturer's instructions. Using the Shimadzu model UV-2401 PC spectrophotometer, the absorbance spectra of the visible and ultraviolet regions were measured at room temperature with a 0.5-nm spectral resolution. The enzymatic activity of PaAPR and variants with *E. coli* thioredoxin were measured by coupling the PaAPR-catalyzed oxidation of thioredoxin to the reduction of thioredoxin by NADPH catalyzed by *E. coli* NTR. The enzymatic activity of EiAPR and variants were measured by coupling the EiAPR-catalyzed oxidation of glutathione to the reduction of GSH by NADPH catalyzed by yeast glutathione reductase (Sigma).

[Resulfts and Dicussion]

we have investigated specific role of conserved histidine (His) closed iron-sulfur cluster of APS reductases in *Pseudomonas aeruginosa* and *Enteromorpha intestinalis*. Iron-sulfur contents and *Vmax* of His/Ala variants of PaAPR (thioredoxin as an electron donor) or EiAPR (glutathione as an electron donor) were exhibited lower than that of their wild-type. However, these His/Arg variants showed similar results obtained from that of wild-type. These results implicate that conserved positive charged His position at 136 in PaAPR and 162 in EiAPR play important role for iron-sulfur ligation and substrate APS binding or enzyme structure.

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