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Designing Active Sites on Amicyanin Using Histidine S Plus Cobalt, and Measuring Their Functional Activity

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Abstract : There is a growing interest in introducing a desired functional group on enzymes in the field of protein engineering. In here, various redox centers were newly created using histidine tag, which is widely used for protein purification, plus cobalt in one of cupredoxins, amicyanin. The coordination of Cobalt-His tag and reactivity of the Co^{2+} loaded His-tag also were characterized. 3xHis-tag, 6xHis-tag, and 9xHis-tag were introduced on amicyanin by site-directed mutagenesis, and then Co^{2+} was loaded on each His-tagged amicyanin. The spectral changes at 330 nm corresponding to cobalt binding on His-tag site indicated the binding ratio of 3xHis-tag, 6xHis-tag, and 9xHis-tag to cobalt as 1:1, 1:2, 1:3 respectively. Based on kinetic studies of binding cobalt to 3xHis-tag, 6xHis-tag, and 9xHis-tagged amicyanin, the nature of the sites was elucidated. In addition, internal electron transfer properties between Cu^{1+} site and engineered site of amicyanin were determined. These results provide insight into improvement of metal coordination and alternation of the redox properties of metal as a new catalytic site on proteins.

Keywords : amicyanin, cobalt, histidine, protein engineering
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