412a Correlation between the Protein Binding Strength on Immobilized Metal Affinity Chromatography and the Surface Accessibility of Its Histidine

Lakshmi P. Pathange, David R. Bevan, Timothy J. Larson, and Mike (Chenming) Zhang

Immobilized metal affinity chromatography (IMAC), in which proteins interact with the immobilized metal ions via the histidines on their surface, has become an important tool for protein purification and characterization. Here, we studied whether or not there is a direct correlation between the protein binding strength on IMAC and the surface accessibility of its histidine. The model protein is T4 lysozyme, and the static surface accessibility of each amino acid on T4 lysozyme was calculated from the crystal structure (1L63) by using SYBYL 6.7 software. Six amino acid sites were selected based on their surface accessibility, and they were substituted by histidine via site-directed protein mutagenesis to generate six T4 lysozyme variants, each containing only one histidine on its surface with different surface accessibility. Wild type T4 lysozyme and all its variants had comparable enzyme activity, indicating the global structure of all variants was unaffected by the amino acid substitution. IMAC was then used to experimentally quantify each protein's interaction with immobilized copper ion. A direct correlation was shown between the protein binding affinity and its histidine's surface accessibility. In addition, in variant K147H, the histidine was found to be involved in a hydrogen bond with the Tyrosine (Tyr 139). However, our results showed that the protein still binds to the IMAC column, although at reduced strength comparing with the variant containing a histidine with similar surface accessibility.