A Critical Histidine in *Klebsiella aerogenes* UreG

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ABSTRACT. Urease is a nickel-containing enzyme found in several microorganisms and plants. Assembly of the urease metallocenter requires three accessory proteins (UreD, UreF and UreG) and is facilitated by a fourth (UreE)[1]. Urease apoprotein forms a complex that includes UreD, UreF and UreG, but UreG also is present as monomeric protein in the cell. UreG exhibits a nucleotide-binding motif and possesses GTPase activity when it is part of the UreD-UreF-UreG-apourease complex, but does not bind or hydrolyze GTP as the monomeric protein[2]. In this work, we describe a new purification protocol making use of biotin-tagged UreG. In addition, we present a structural model of UreG, built by using a distant sequence homolog as a template structure. UreG contains a highly conserved His residue that is also found in the close homolog HypB, necessary for nickel insertion into hydrogenases; however UreG does not bind nickel with physiologically significant affinity[2]. The codon for UreG His74 was mutated to assess its importance. When His74 was replaced by Ala, Asn or Cys, almost no urease activity was detected in crude extracts, suggesting that this residue is critical for UreG's role in metallocenter assembly. The fact that Asn was unable to restore partial activity indicates that His74 is not just involved in protein-protein interactions. In our structural model, His74 is placed in a large loop that could modulate the GTP binding/hydrolysis activity of UreG, but further experiments are required to test this hypothesis.

References:

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