

Biochemistry CUME May 2016

Topic: Protein Structure and Function

Biotin-dependent carboxylases belong to a family of widely distributed enzymes that have important functions in the metabolism of fatty acids, amino acids, carbohydrates and other compounds.

- 1) **(20 points)** The reactions catalyzed by biotin-dependent carboxylases proceed in two major steps and involve three main protein components. Please describe and/or illustrate the essential steps of biochemical reactions catalyzed by biotin-dependent carboxylases and clearly state the substrate, enzyme component, co-factor(s) and product in each catalytic step.

- 2) The 3D architecture of a biotin-dependent carboxylase offers a structural framework for understanding how a sequence of biochemical reactions are coordinated and regulated in a multi-subunit or multi-domain complex. The authors have applied two major biophysical methods to investigate the tertiary and quaternary structures of biotin-dependent carboxylases holoenzymes and/or isolated domains.
 - a) **(20 points)** Describe the general strategy and specific aim in each method.
 - b) **(20 points)** Discuss the strengths and limitations of each method.
 - c) **(10 points)** Are the structural results from these two methods fully consistent? If not, provide explanations for any discrepancy.
 - d) **(10 points)** What experiment(s) other than the structure data itself are helpful for determining the quaternary structure (dimer vs. hexamer) of a biological assembly?

- 3) **(20 points)** Summarize two major findings from structural investigation of the single-chain multi-domain long-chain acyl-CoA carboxylase holoenzyme from bacteria (MapLCC), and explain what structural features of this elaborate hexameric structure allow us to speculate on how biotin-dependent carboxylases work at the molecular level.