Study of Structural Change in SCOT upon Binding CoA

Stephanie Kernaghan, Marie Fraser, *University of Calgary.* E-mail: sdkernag@ucalgary.ca

Succinyl CoA:3-ketoacid transferase (SCOT, EC 2.8.3.5) allows cells to utilize ketone bodies [1] and individuals with low SCOT activity suffer from disease that can be fatal [2]. There has been decades of work done on this enzyme; it is known to form dimers and tetramers in solution [3] the substrates have been characterized [1] and the energetics of binding have also been calculated. Experiments in the 1990s showed that there is a large change in binding energy associated with a small region of the CoA molecule (pantoic acid domain) [4], and we are exploring what may contribute to this phenomenon.

SCOT is known to form a covalent thiolester intermediate with coenzyme A [5], and there is evidence for a structural change when CoA binds as noted by White et al. [6]. A structural change was postulated because SCOT is more readily inactivated by DTNB binding when the enzyme is bound to CoA. The specific cysteine being labeled was identified by Rochet [7] to be Cys28. We have studied the importance of this residue using site directed mutagenesis, kinetics as well as X-ray crystallography. The mutants constructed are C28S, C28A and C28W. C28A and C28S have be crystallized in P21 with dimensions a=63 Å, b=263 Å, c=59 Å, β=110º and both have diffracted to better then 2.3 Å.

[1] Stern *et al.*, *J. Biol. Chem*., 1956, **221**, 1. [2] Niezen-Koning *et al.*, *Eur. J. Pediatr*, 1997, **156,** 870. [3] Rochet *et al.*, *Biochemistry*, 2000, **38**, 11291. [4] Whitty *et al.*, *Biochemistry*, 1995, **34**, 11678. [5] Soloman, Jencks, *J Biol Chem*, 1969, **244**, 1079. [6] White *et al., J. Biol. Chem.*, 1976, **251**, 1700. [7] Rochet, *Thesis*, 1998, 392.

Keywords: structure-function enzymes, catalysis structure of intermediates, x-ray crystallography of proteins