PS04.02.28 SITE-DIRECTED MUTAGENESIS OF HUMAN CARBONIC ANHYDRASE I: STRUCTURE AND FUNCTION. K.K. Kannan, A.K. Mohanty, M.V. Hosur, M.B. Satyamurty, A.V.S.S. Narayan Rao, S.K. Mahajan, Bhabha Atomic Research Centre, Bombay-400 085, India

Human carbonic anhydrase, a zinc metalloenzyme, catalyses the reversible hydration of carbon dioxide. The proposed catalytic mechanism comprises a proton shuttle through a hydrogen bond network among residues T199, E106 (Kannan et al., FEBS LETTS, 73: 115-119, 1977). To verify this proposal we have undertaken site-directed mutagenesis studies of human carbonic anhydrase I (HCAI). Total RNA was prepared from an HEL cell line and used to prepare cDNA using AMV reverse transcriptase and an hCAI specific primer. The cDNA was amplified by PCR and cloned into the expression vector pET-3a. The gene was induced by IPTG, and the protein purified by affinity chromatography was found to be as active as the RBC HCAI. DNA sequencicg revealed that the cloned gene differed from the published RBC hCAI sequence (Barlow, J.H., Nucl. Acid. Res, 15:2386, 1987) with two start codons (ATG) and five other base changes. At the protein level there are two N-terminus methionines and two mutations V31I and V218A. Site-directed mutagenesis (T199V) of this gene was done by a PCR technique using mutated oligonucleotides and confirmed by gene sequencing. Specific activity of the purified mutant protein was assayed and found to be 2% of the wild type. The mutant protein is being crystallized. The recombinant protein crystallized in thick walled glass capillary tubes (Kannan et al., JMB, 63:601-604,1972) are of a different morphology compared to the RBC HCAI. The structure of the recombinant protein and its comparison to the RBC HCAI will be reported.