SITE-DIRECTED MUTAGENESIS AND X-RAY CRYSTALLOGRAPHIC STUDIES OF HUMAN BETA-1 ALCOHOL DEHYDROGENASE

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Submitted to the faculty of the Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Biochemistry,
Indiana University

March 1990

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

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February 9, 1990

ABSTRACT

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Site-Directed Mutagenesis and X-ray Crystallographic Studies of Human Beta-1 Alcohol Dehydrogenase

Based on the crystal structure of the horse EE ADH, Arg-47 has been suggested to be important in coenzyme binding. In order to gain a better understanding of the role of Arg-47 in the human $\beta_1\beta_1$ enzyme, Lys, His, Gln, and Gly were substituted for Arg-47 in $\beta_1\beta_1$ using site-directed mutagenesis. The mutant enzymes were expressed in E. coli, purified to homogeneity, and characterized using steady-state kinetics. The substitution of Arg-47 by Lys, His, and Gln resulted in enzymes with decreased affinity for NAD^+ and higher V_{max} values versus $\beta_1\beta_1$. The largest effects were seen with the Gln-47 mutant where the affinity for NAD^+ decreased 12-fold and the V_{max} increased 220-fold, the highest V_{max} reported for a mammalian ADH. The substitution of Gly for Arg-47 resulted in an enzyme which exhibited a V_{max} one-half that of $\beta_1\beta_1$ and affinity for NAD^+ which was 8-fold lower than $\beta_1\beta_1$. The results of product inhibition studies on the Gly-47 enzyme are consistant with a Rapid Equilibrium Random Bi Bi mechanism. All other enzymes obey the Ordered Bi Bi mechanism. The recombinant $\beta_1\beta_1$ enzyme was crystallized from solutions containing PEG 8000 and NAD^+, and X-ray diffraction data was collected to 2.9Å resolution on these crystals. In the presence of NAD^+, the

human enzyme crystallizes in the same space group as the horse EE enzyme (triclinic) and has similar cell dimensions. The structure of the human enzyme was solved using the method of molecular replacement and the solution was refined to 3.3Å resolution. There are no significant differences between the structures of horse EE and human $\beta_1\beta_1$ at this level of resolution.

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TABLE OF CONTENTS