EFFECT OF AMINO ACID SUBSTITUTIONS IN HUMAN LIVER ALCOHOL DEHYDROGENASE ON INACTIVATION BY IODOACETATE

Ann R. Kusnadi

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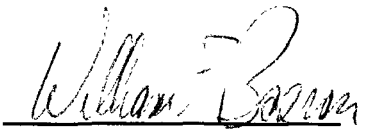
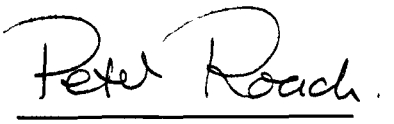


William F. Bosron, Ph.D.

(Committee chairman)

David W. Crabb, M.D.

(Member)



Peter J. Roach, Ph.D.

(Member)

August 9, 1989.

ABSTRACT

Horse and human liver ADH are irreversibly inactivated by iodoacetate. The inactivation is the result of the alkylation of Cys-46 in the case of horse liver ADH, or Cys-174 in the case of human liver $\beta_1\beta_1$ and $\beta_2\beta_2$ ADH isoenzymes. The rate of inactivation follows a first-order kinetics, and examination of the dependency of the first-order rate constant on IAA concentration indicates that it follows Michaelis-Menten kinetics. This is consistent with a mechanism where a reversible enzyme-iodoacetate complex is formed prior to the alkylation of the Cys residue. The basic Arg-47 and Arg-369, which are located in the coenzyme binding domain, are postulated to be important in anion binding. Accordingly, these residues could be critical in binding and directing the acidic iodoacetate to alkylate the Cys residue. The importance of Arg-47 and Arg-369 in mediating the binding and kinetics of inactivation by iodoacetate was examined by comparing the rate of inactivation of $\beta_1\beta_1$ (with Arg-47 and Arg-369), $\beta_2\beta_2$ (with His-47 and Arg-369), and $\beta_3\beta_3$ (with Arg-47 and Cys-369). Additionally, single amino acid substitutions of β_1 were performed by oligonucleotide-directed mutagenesis: Arg-47 was substituted by Lys, Gln, and Gly, and Arg-369 by Ser. The pseudobimolecular rate constant for variants with Arg, Lys, His, Gln, and Gly at position 47 are 19, 56, 8.3, 34, and 16 $min^{-1}M^{-1}$, respectively; for variants with Cys and Ser at position 369 the pseudobimolecular rate constants are 41 and 15 $min^{-1}M^{-1}$, respectively. Based on the study of alkylation of a model compound (zinc-mercaptoethanol complex) by IAA vs. alkylation of Cys-46 in horse liver ADH, a rate enhancement of about 60 fold would be expected if a specific anion binding site were to mediate the alkylation. Thus, the 7-fold variation in the rate of alkylation for the seven enzymes examined is substantially less than expected if Arg-47 or Arg-369 were to mediate IAA modification. (Kinetic parameters for β369-C variant are rather difficult to interpret because the Cys-369 itself could be alkylated in addition to Cys-174 or Cys-47). Moreover, no direct correlation between the pseudobimolecular rate constant of iodoacetate inactivation and the pK_a of the substituted amino acid residue is observed. These observations suggest that Arg-47 and Arg-369 are not critical for the binding of iodoacetate prior to the alkylation of the Cys residue. To have a better understanding, it is important to determine which Cys residue(s) is(are) modified. It is possible that the active site zinc is the positively charged element that involves in the binding of iodoacetate. Based on the three dimensional structure, the active site zinc is in a proper position to orient the iodoacetate toward either Cys-46 or Cys-174.

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