Crystallographic evidence for Tyr157 functioning as the active site base in human UDP-galactose 4-epimerase

James B. Thoden[‡], Travis M. Wohlers[^], Judith L. Fridovich-Keil[§], and Hazel M. Holden[‡]
[‡]*Department of Biochemistry, University of Wisconsin, Madison, WI 53705 USA*
[^]*Graduate Program in Genetics and Molecular Biology, Emory University School of Medicine, Atlanta, GA 30322 USA*
[§]*Department of Genetics, Emory University School of Medicine, Atlanta, GA 30322 USA*

Introduction

The human enzyme UDP-galactose-4-epimerase (hGALE), hereafter referred to as epimerase, normally catalyzes the second step in the Leloir pathway of galactose metabolism, namely the interconversion of UDP-galactose and UDP-glucose as shown in Figure 1. Impairment of this enzyme results in the complex disorder of epimerase-deficiency galactosemia that affects as many as 1/6700 individuals, at least in some ethnic groups. Clinically, epimerase-deficiency galactosemia can range from benign to severe. The molecular basis of this variance remains unclear, although allelic heterogeneity observed in the patient population raises the intriguing possibility of a genotype/phenotype correlation.

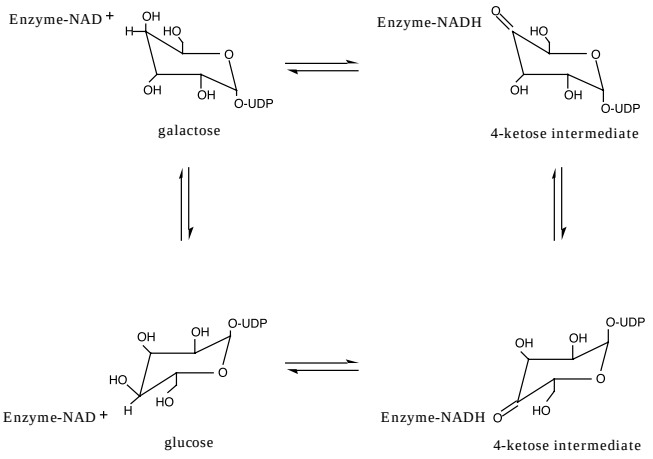

Figure 1: The second step in the Leloir pathway of galactose metabolism, namely the interconversion of UDP-galactose and UDP-glucose.

We have determined the structure of human epimerase in a ternary complex with bound NADH and UDP-glucose to 1.5 Å. A ribbon diagram of one monomer of the homodimeric enzyme is shown in Figure 2. The amino acid side chains responsible for anchoring the NAD+ to the protein include Asp33, Asn37, Asp66, Tyr157, and Lys161. The glucosyl group of the substrate is bound to the protein via the side chain carboxamide groups of Asn187 and Asn207. Additionally, Og of Ser132 and Oh of Tyr157 lie within 2.4 Å and 3.1 Å, respectively, of the 4´-hydroxyl group of the sugar. Comparison of the polypeptide chains for the resting enzyme and for the protein with bound NADH and UDP-glucose demonstrates that the major conformational changes that occur upon substrate binding are primarily limited to the regions defined by Glu199 to Asp240 and Gly274 to Tyr308. Additionally, this investigation reveals, for the first time, that a conserved tyrosine (namely Tyr157) is in the proper position to interact directly with the 4´-hydroxyl group of the sugar substrate and to thus serve as the active site base. A low barrier hydrogen bond between the 4´-hydroxyl group of the sugar and Og of Ser132 facilitates proton transfer from the sugar 4´-hydroxyl group to Oh of Tyr157.

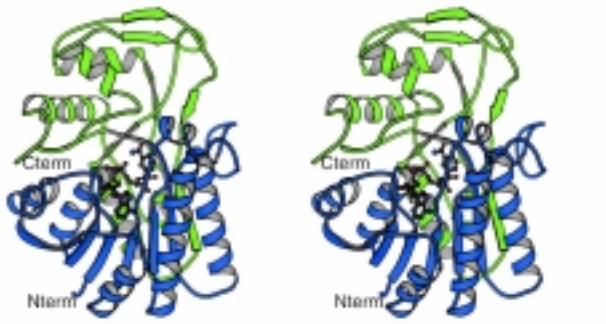

Figure 2: Ribbon diagram of one monomer of the the homodimeric enzyme.