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Crystal Structure of Δ-3-Ketosteroid Isomerase From Pseudomonas testosteroni in Complex with Equilenin Settles the Correct Hydrogen Bonding Scheme for Transition-State Stabilization

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Bacterial Δ-3-ketosteroid isomerase (KSI) catalyzes the conversion of Δ- to Δ-3-ketosteroids via enolate formation, which is also found in the synthesis of all steroid hormones in mammals. In Pseudomonas testosteroni, KSI Asp38 (pKa ~ 4.7) was identified as the general base which abstracts the steroid C4b-H (pKa ~ 12.7) to form the dienolate intermediate. A key issue involved in the proton abstraction is how the energy required for the unfavorable proton transfer is provided at the active site of KSI and/or how the thermodynamic barrier is drastically reduced by the stabilization of the reaction intermediate. In order to address the question, favorable interactions between the enzyme and the reaction intermediate has to be correctly predicted. However, this has been a controversial issue. We present the crystal structure of Pseudomonas testosteroni KSI in complex with a reaction intermediate analogue equilenin at 2.26 Å resolution, which clearly shows that the Tyr14 OH and Asp99 COOH provide direct hydrogen bonds to the oxyanion of equilenin. The structure in conjunction with mutational, kinetic, and nuclear magnetic spectroscopic analyses indicates that the Tyr14 OH provides a strong low-barrier hydrogen bond to the dienolic oxyanion of the intermediate. This is supported by ab initio calculations.