The enzyme urate oxidase (UO) is involved in nitrogen metabolism of many organisms including bacteria, plants, and mammals. Most oxidase enzymes require either a metal ion or cofactor for catalysis. UO is unique in that it is able to catalyze the oxidation of urate without the aid of a metal ion or cofactor. Because of this unique property, the chemical mechanism of UO has been investigated to determine how the oxidation chemistry takes place. Proton inventory (PI), Raman spectroscopy, and kinetic isotope effects (KIE) methods have been utilized to further investigate how UO accomplishes activation of urate for oxidation. A PI study was conducted to determine the number of protons in flight during each round of catalysis. From this study it was determined that more than one proton is in flight during catalysis. This observation supports the proposed catalytic diad being involved in the formation of a dianion intermediate. Raman spectroscopy has been used to look at structural changes that occur to the substrate when bound to UO. This study gives evidence for the formation of a dianion intermediate. To further study how UO activates the substrate, new methods for measuring kinetic isotope effects (KIE) are being developed. The use of continuous flow mass spectrometry to measure carbon isotope effects (IE) at positions C4 and C5 of urate will give insight into the transition state structure of the reaction. Continuous flow MS has the advantage of measuring the naturally abundant isotopic ratios of solid samples. Another new technique is the use of isotope depleted samples to measure carbon IE’s of multi-carbon molecules. These new techniques have the potential to be employed in the study of many enzymatic reaction mechanisms.