Bioorganic Chemistry **27,** 351–362 (1999)
Article ID bioo.1999.1142, available online at http://www.idealibrary.com on IDEAL®

Theoretical Study of Intermediates in the Urate Oxidase Reaction

Kalju Kahn

Department of Biochemistry, University of Missouri at Columbia, Columbia, Missouri 65211

Received September 15, 1998

The energetics of the urate oxidase reaction has been studied employing HF, MP2, and DFT calculations. Gas-phase energies of possible urate oxidase reaction intermediates were calculated; radical species and dehydrourate had high relative energies while 5-hydroperoxyisourate was stable in the gas phase. Significant stabilization of the initial radical pair relative to reactants occurred in an aqueous environment, suggesting that oxidation of urate in water proceeds via a single-electron transfer pathway. The possibility of enzyme catalysis through proton abstraction from urate has been investigated by comparing the calculated properties of uric acid, urate monoanion, and urate dianion. Results indicate that ionization of uric acid is accompanied by polarization of the central carbon–carbon double bond and weaker binding of valence electrons.

INTRODUCTION

Urate oxidase functions in the purine degradation pathway and catalyzes the reaction between urate monoanion and dioxygen to give 5-hydroxyisourate and hydrogen peroxide (Scheme 1) (*1, 2*). Oxidation of urate to more soluble compounds is required for the excretion of excess nitrogen in most mammals (*3, 4*); the same reaction plays an important role in the transport of *de novo* fixed nitrogen throughout the plant in soybean and some other tropical legumes (*5*). Soybean root nodule urate oxidase does not contain or require any organic cofactors or transition metals and the enzyme may activate urate by proton abstraction (*6*). The overall two-electron oxidation of singlet urate by triplet dioxygen is spin-forbidden (*7*) and the reaction most likely proceeds via an initial single electron transfer followed by spin inversion and radical recombination, similar to the well-characterized reaction between reduced flavin cofactor and dioxygen (*8*, *9*). Two distinct enzymatic intermediates were recently observed in the urate oxidase reaction, and based on their spectral characteristics the structures of urate dianion and 5-hydroperoxyisourate were assigned to these intermediates (*10*). Two other intermediates, urate radical anion and dehydrourate, probably occur in the reaction path but are too short-lived for detection in the stopped-flow time scale.

This paper reports computational studies on the mechanism of urate oxidation. In the first part, the relative gas-phase energies of putative intermediates and the reaction product are calculated to evaluate their stabilities and obtain the energy profile for the uncatalyzed reaction, hence providing information about the intrinsic energetics of the reaction, which is crucial for the understanding of the catalytic power of urate

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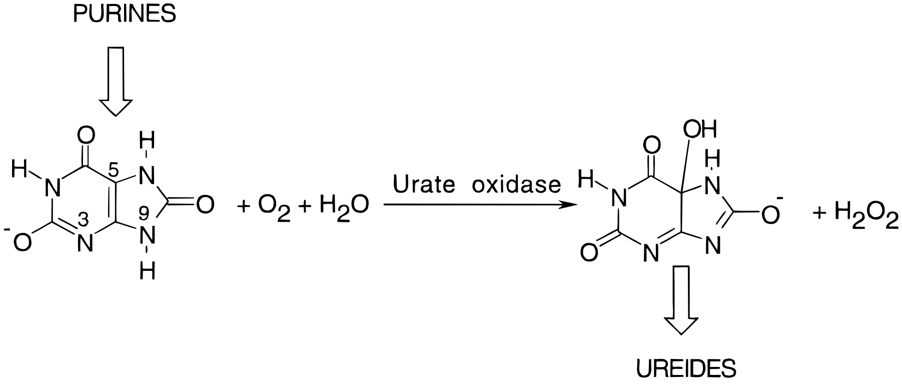

SCHEME 1. The urate oxidase reaction.

oxidase. In the second part, the effect of environment on the reaction is studied by calculating the reaction profile in a simulated aqueous environment. Finally, one possible role of the enzyme is investigated by comparing the electronic structures and electron donating abilities of uric acid, urate anion, and urate dianion.

METHODS

All calculations were performed with Gaussian 92 and Gaussian 94 (*11*) programs using MP2(Full)/6-31+G(d,p) optimized geometries unless otherwize specified. The HF/6-31+G(d,p) minima were used as starting points for MP2 optimizations after performing frequency and wave function stability analyses. Geometry optimizations of urate dianion, 5-hydroxyisourate, 5-hydroperoxyisourate, and hydrogen peroxide were performed with no constraints. Other molecules were kept planar during MP2 optimizations as the planar forms were found to be the true minima at the HF/6-31+G(d,p) level. Reported energy differences were corrected for the zero-point vibrational energy (ZPVE) differences after scaling ZPVEs by a factor of 0.92 (*12*).

To choose a suitable level of theory for the description of relative energies of reaction intermediates, three test systems were studied (Table 1). These were (i) adiabatic electron affinity of molecular dioxygen, (ii) adiabatic ionization potential of uric acid, and (iii) two-electron oxidation of (*E*)-2-butene to *trans*-1,3-butadiene with concomitant reduction of dioxygen to hydrogen peroxide. The weakness of ab initio methods in calculating the electron affinity of dioxygen is well documented (*13, 14*) and I chose to use the experimental value (-10.4 ± 0.2 kcal/mol) as the energy difference between oxygen and superoxide in the description of the energy profile. The performance of MP2 and B3LYP ab initio methods in calculation of accurate ionization energies (*15, 16*) and gas-phase basicities (*17–19*) has been reported previously. Based on the results of test systems and published performance indicators, UMP2/6-31++G(2d,p) and B3LYP/6-311++G(2d,2p) methods were chosen to describe the relative energies of reaction intermediates and product.

The total energies of putative intermediates and products of the urate oxidation

TABLE 1

Performance of Theoretical Methods in Calculation of Reaction Energies[a]

Method	Test 1	Test 2	Test 3
UHF/6-31+G(d,p)	−14.8	166.0	14.5
PUHF/6-31+G(d,p)	−16.7	160.7	21.5
ROHF/6-31+G(d,p)	−8.4	170.0	0.2
UMP2/6-31+G(d,p)	2.3	189.5	3.7
UMP2/6-31++G(2d,p)	3.4	189.9	1.6
UMP2/6-311++G(d,p)	−0.8	188.8	3.6
PMP2/6-31+G(d,p)	1.6	185.2	8.2
PMP2/6-31++G(2d,p)	2.5	185.8	6.3
PMP2/6-311++G(d,p)	−1.5	184.4	8.1
B3LYP/6-311++G(2d,2p)	13.1	185.1	−0.9
ZPVE-corrected Exp.[b]	9.7	188.0	−3.4

[a] Calculated energies (in kcal/mol) were obtained at the indicated theory level using MP2/6-31+G(d,p) geometries.

[b] Experimental reaction enthalpies were obtained using data from the NIST Chemistry Webbook (*28*). ZPVE and thermal corrections amount to −0.7, +0.1, and +0.5 kcal/mol, for test reactions 1, 2, and 3, respectively.

relative to the substrates were calculated considering only intermediates for which the total charge of the system is −1. All intermediates that were studied are metastable molecules; no calculations of transition states were attempted. The interaction energy between molecules, mostly of ion-dipole nature, was neglected. The structure of the enzyme was not available until recently (*20*), and the inclusion of active-site residues to model the environment around reacting species was thus outside the scope of the present study. The data presented here will serve as a useful reference point for future studies in which active-site residues are explicitly included.

Solvent effects were studied using two methods. The solvation energies of all species were calculated with the self-consistent isodensity polarized continuum model (SCIPCM) (*21–23*) at the HF/6-31+G(d,p) level of theory, and density matrix was used to evaluate electron density. The relative energies of radical intermediates in the aqueous phase were also calculated from the relative gas-phase energies, gas-phase proton affinities, aqueous pK_a values, and experimentally known solvation energies with the help of appropriate thermodynamic cycles (*24*). The gas-phase basicity of urate was obtained by adding thermal and entropic corrections to the electronic energy difference between the base and the protonated molecule at the MP2/6-31+G(2d,p) level. The values 1.48 kcal/mol and 26.04 cal/(K mol) were used for the integrated heat capacity and entropy of the proton at 298.15 K, respectively (*25*). Correction for the basis set superposition error (2.4 kcal/mol) was also made by the counterpoise method in the calculation of gas-phase basicity of urate (*19*).

The comparison of properties of uric acid, urate monoanion, and urate dianion was done at MP2(FU)/6-31+G(d,p) and MP2/(FU)/6-31++G(2d,p) levels. Adiabatic ionization potentials were obtained as ZPVE-corrected UMP2/6-31++G(2d,p) energy differences between the radical and the parent molecule in their respective energy minima. It was found that partial charges derived from the Mulliken population analysis were strongly basis set dependent; fitting to electrostatic potential (CHelpG scheme) was used to obtain more representative partial atomic charges. Population analyses were performed using MP2 densities. The electron density and HOMO localization were visualized using the program MOLDEN (*26*). Natural bond orbital analysis (*27*) was performed at the MP2(FU)/6-31+G(2d,p) level of theory.

RESULTS AND DISCUSSION

Energies of putative intermediates in urate oxidase reaction in the gas phase. The oxidation of urate is likely to proceed through the initial single-electron transfer followed by radical recombination to give 5-hydroperoxyisourate (*10*). The isotope tracer studies have shown that both atoms of H_2O_2 are derived from molecular oxygen and that the hydroxyl group in the C5 position of 5-hydroxyisourate is derived from water (*2, 29*), suggesting that the conversion of 5-hydroperoxyisourate to 5-hydroxyisourate during the enzymatic turnover occurs via a short-lived dehydrourate anion.

The UMP2 and B3LYP energies and HF ZPVEs of substrates, putative intermediates, and products of the reaction are given in Table 2. Frequency calculations at the UHF/6-31+G(d,p) level indicated that planar forms of uric acid, urate, uric acid radical cation, urate radical, and urate radical anion were minimum energy conformations. Radical species showed moderate spin contamination with $s^2_{0,\mathrm{UMP2}}$ values being 0.83, 0.86, and 0.78 for the cation, neutral, and anion, respectively. The spin contamination in radicals was reduced to s^2_1 values 0.80 (cation), 0.82 (neutral), and 0.77 (anion) by annihilation of the first spin component at the MP2/6-31++G(2d,p) level. Annihilation was more effective at the PUHF/6-31++G(2d,p) level at which all s^2_1 values were below 0.76. ZPVE-corrected energy profiles calculated at UMP2(FU)/

TABLE 2

Ab Initio Total Energies of Compounds Considered in This Study

Compound	UMP2 energy[a]	B3LYP energy[a]	HF ZPVE[b]
Uric acid	−636.274244	−637.885740	73.89
Urate anion	−635.757121	−637.366633	65.14
Uric acid radical cation	−635.971621	−637.590769	73.82
Urate radical	−635.630337	−637.249312	65.11
Urate radical anion	−635.118581	−636.729514	56.31
5-Hydroperoxyisourate	−785.795154	−787.745740	70.80
Dehydrourate	−634.521450	−636.111495	49.26
5-Hydroxyisourate	−710.817701	−712.592571	68.58
Dioxygen	−150.015611	−150.372828	2.83
Superoxide anion	−150.021037	−150.393673	2.03
Hydroperoxyl radical	−150.581126	−150.963842	5.64
Hydrogen peroxide	−151.231444	−151.608416	18.51
Water	−76.2687716	−76.4620309	14.51

[a] Single-point UMP2(FU)/6-31++G(2d,p) and B3LYP/6-311++G(2d,2p) energies at the MP2(FU)/6-31+G(d,p) minima.

[b] From HF/6-31+G(d,p) minima.

6-31++G(2d,p) and B3LYP/6-311++G(2d,2p) levels are shown in Fig. 1. With the exception of urate radical anion, the agreement between these two methods was quite good; in contrast, UHF/6-31+G(d,p) energies (data not shown) differed considerably due to the neglect of electron correlation. The energy profile indicates that 5-hydroperoxyisourate is relatively stable and can accumulate during the reaction, consistent with the interpretation of the stopped-flow data (*10*) that the second observable enzymatic intermediate is 5-hydroperoxyisourate. Both radical intermediates and dehydrourate anion were highly unstable in the gas phase; these results suggest that the radical species and dehydrourate probably do not accumulate during the chemical oxidation or enzymatic turnover.

It is likely that the energy barriers associated with decomposition of 5-hydroperoxyisourate and hydration of dehydrourate are significantly smaller than the energy required to transfer a single electron from urate to dioxygen. Thus, in the mechanism outlined in Fig. 1, the transfer of a single electron from urate to dioxygen is probably the rate-limiting step. The enzyme could lower this energy barrier by (i) promoting the transfer of a proton simultaneously with the electron transfer (giving urate anion radical and hydroperoxy radical), (ii) abstracting a proton from urate monoanion prior to the electron transfer step, or (iii) stabilizing radical intermediates relative to reactants via specific and nonspecific interactions. The concerted transfer of an electron and a proton would avoid the high energy superoxide–urate radical intermediate, lowering the gas-phase energy barrier to the level approximately equal to the relative energy of the hydroperoxyl–urate radical anion pair (assuming that the transition state is close in energy to the radical intermediate, as was found in the case of reaction between triplet dioxygen and methane (*30*)). The two other modes of lowering the

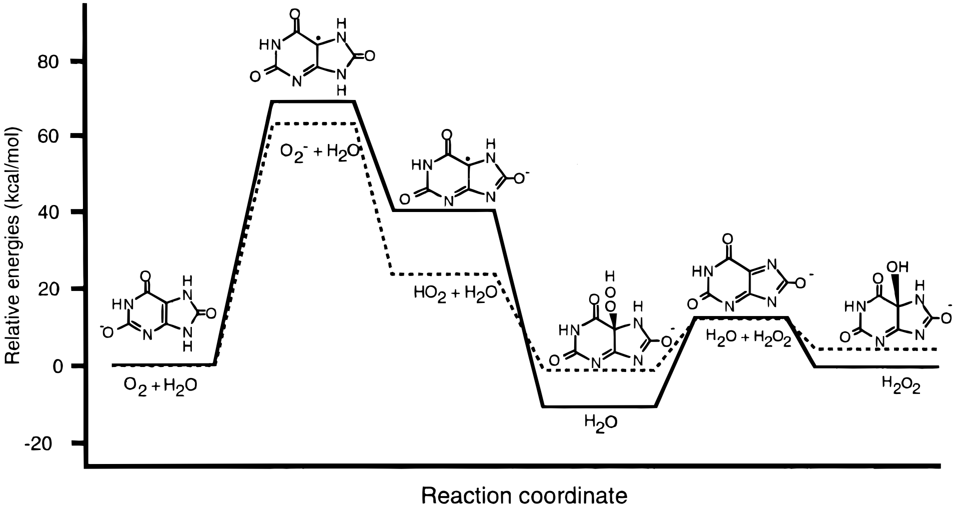

FIG. 1. ZPVE-corrected relative energies of possible intermediates and products in the urate oxidase reaction in the gas phase. Solid line—UMP2/6-31++G(2d,p) results; dotted line—B3LYP/6-311++G(2d,2p) results. Relative energies were calculated from data in Table 2 along with the experimental electron affinity of dioxygen.

energy barrier, abstraction of the proton and the environment effects, are discussed in the next sections.

Energies of putative intermediates in urate oxidase reaction in aqueous solution. Solvent effects are expected to play an important role in determining the relative stabilities of reaction intermediates in the urate oxidase reaction since in the first step an apolar oxygen molecule is reduced to superoxide anion. The free energy of reaction in aqueous solution can be calculated directly by employing classical ensemble treatments (*15, 31*) or indirectly from the corresponding gas-phase value when the solvation free energy difference between reactants and products is known. The latter can be estimated from ab initio reaction field calculations (*21*) or, when aqueous pK_a values are available, through the use of a thermodynamic cycle that links gas-phase and solution basicities (*24*).

The reaction field calculation was employed to estimate solvation energies of reaction intermediates. Figure 2 shows the B3LYP energy profile corrected for the HF solvation energies. The strong solvation of superoxide relative to dioxygen was responsible for a large solvent effect (44.7 kcal/mol) in the initial single-electron transfer step. The SCIPCM results suggest that one-electron oxidation of urate becomes feasible in aqueous solution, that 5-hydroperoxyisourate is a possible metastable oxidation intermediate, and that the overall oxidation of urate to 5-hydroxyisourate is accompanied by a small decrease in reaction free energy. Because explicit interactions with solvent molecules are not treated by the SCIPCM model, the energy of specific hydrogen bonds between solvent and solute is not included, such that comparison with an alternative approach is desirable.

The free energy of single-electron transfer from urate monoanion to dioxygen in

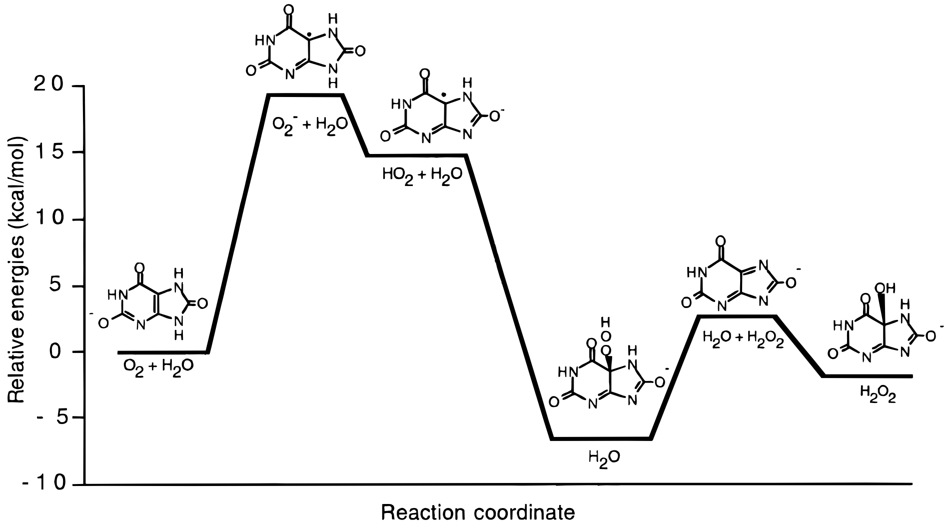

FIG. 2. Calculated reaction profile for the nonenzymatic oxidation of urate by molecular oxygen in aqueous solution. The profile was obtained by correcting B3LYP/6-311++G(2d,2p) gas-phase energies for solvation effects at the HF/6-31+G(d,p) level using the SCIPCM model.