Study of solid-state catalytic isotope exchange of hydrogen in L-hydroxyproline under the action of spillover tritium

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Reaction of high-temperature solid-state catalytic isotope exchange (HSCIE) of hydrogen in L-hydroxyproline was studied by *ab initio* quantum-chemical calculations. A one-center synchronous mechanism of isotope exchange between the amino acid and the H_3O^+ model acidic center was considered. The structures of transition states of the reaction and the activation energies were determined. Relative reactivity of the C—H bonds in the hydroxyproline molecule under conditions of HSCIE was studied. The results obtained are in agreement with experimental data on the stereoselectivity and regioselectivity of the HSCIE reaction, *viz.*, the lower the calculated activation energy of isotope exchange, the larger the portion of hydrogen substituted by tritium in a given position of the amino acid molecule. The enhancement of the reactivity under conditions of solid-state isotope exchange can be associated with additional interaction between the exchanging H atoms and the electron-donor O and N atoms of the amino acid molecule in transition state.

Key words: *ab initio* quantum-chemical calculations, activation energy, hydrogen isotope exchange, hydrogen spillover.

Reactions of solid-state hydrogenolysis are used to obtain organic compounds labeled with hydrogen isotopes. The reaction, based on applying high-temperature solid-state catalytic isotope exchange (HSCIE) appears to be particularly efficient for the synthesis of tritium-labeled biologically active compounds.[1,2] The reaction proceeds with retention of configuration of the asymmetric carbon atoms and in some instances it is possible to achieve virtually complete substitution of hydrogen atoms. It has been shown experimentally that the HSCIE reaction is based on the phenomenon of hydrogen spillover (HS).[3] A correlation between the local proton affinities of amino acids and the capability of hydrogen atoms to be substituted in the HSCIE reaction has been found.[4]

Hydrogen spillover can be considered as separate transfer of a proton and electron over the surface of an inorganic support. In this case the proton and electronic conductivity are simultaneously observed.[5] Water adsorbed on the surface of the support is a cocatalyst of HS,[6] which suggests the formation of complexes of the type H_3O^+ on the surface. A one-center synchronous mechanism with participation of a strongly acidic proton surface center was suggested for the hydrogen exchange reaction in CH_4.[7] This work is dedicated to studying the reaction of solid-state isotope exchange of hydrogen by tritium in hydroxyproline.

Experimental

Solid-state isotope exchange of hydrogen by tritium in L-hydroxyproline. Activated carbon (Norit A, Serva, 25 mg) and an aqueous solution of L-hydroxyproline (Sigma, 0.5 mL) were mixed. Water was removed at a reduced pressure at 20 °C. Activated carbon coated with the amino acid was mixed with 5 mg of catalyst (5% Rh/Al_2O_3, Fluka). The solid mixture containing 0.5 mg of L-amino acid was placed into a 10 mL ampule. The ampule was evacuated and filled with gaseous tritium until a pressure of 250 Torr was reached. The reaction was carried out at 150 °C for 60 min. The ampule was cooled, evacuated, and blown with hydrogen. The amino acid was desorbed with 0.1 *N* aqueous ammonia containing 20% EtOH. The solution of tritium-labeled amino acid was evaporated to dryness and then additionally evaporated twice with 20% aqueous ethanol to remove the labile tritium. The purification of L-Hyp was performed by ligand exchange chromatography using chiral sorbents.[8] The tritium distribution in the labeled amino acid was determined by ^3H NMR spectroscopy.[9] The extent of tritium incorporation into the amino acid was established by liquid scintillation analysis.

Calculation procedure. Calculations of isolated molecular systems, fragments of the potential energy surfaces, and transition states (TS) of the isotope exchange reaction were performed by the Hartree—Fock (HF) method and according to the second-order Møller—Plesset (MP2) level of perturbation theory[10] in the 6-31G* basis set. The geometry of the systems studied was optimized using analytical gradients without considering the point symmetry group. The search for TS was

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Table 1. Interatomic distances (*d*) and total energies (*E*) of the structures of TS of the hydrogen exchange reaction in β-position of alanine molecule with participation of H_3O^+ ion, and activation energies (E_a) of this reaction calculated by the RHF/6-31G* and MP2/6-31G* methods

Parameter	Transition state	
	β1	β2
d/Å		
N(1)—C(2)	1.45	1.45
C(2)—C(3)	1.54	1.54
C(2)—C(4)	1.52	1.52
C(4)—O(5)	1.18	1.18
C(4)—O(6)	1.34	1.33
C(3)—H(13)	1.21	1.20
C(3)—H(14)	1.22	1.20
H(13)—H(14)	0.86	0.88
O(15)—H(13)	2.08	2.00
O(15)—H(14)	1.99	1.96
O(6)—H(13)	2.93	2.33
N(1)—H(14)	2.40	2.69
−*E*/au (RHF/6-31G*)	398.1151	398.1144
−*E*/au (MP2/6-31G*)	399.2124	399.2126
E_a/kcal mol^{-1} (RHF/6-31G*)	22.8	23.3
E_a/kcal mol^{-1} (MP2/6-31G*)	16.6	16.4

Note. TS β1 and TS β2 were calculated with full geometry optimization and with fixed geometry of the $C(3)H_2O(15)$ fragment, respectively.

carried out using the quasi-Newtonian method of synchronous transition.[11] Calculations were performed using the GAUSSIAN-94[12,13] and GAMESS[14] programs on a CRAY J-90 supercomputer (Berkeley, California, USA).

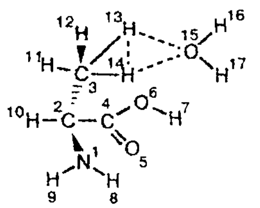
Results and Discussion

Previously, the reaction of hydrogen exchange between methane and a model acidic H_3O^+ center was studied by the Hartree—Fock method in different basis sets. The transition state was found and the activation energy of this reaction was determined. *Ab initio* calculations of TS of reactions with participation of much more complex molecules than the methane molecule require enormous computational resources even for modern supercomputers.

We attempted to find a simplified computational procedure for calculations of TS that would substantially reduce the computing time. Using the Hartree—Fock and MP2 methods in the 6-31G* basis set, we calculated the TS of the reaction of hydrogen exchange in the methyl group of the alanine molecule (at the β-C atom) with participation of the H_3O^+ ion. The results of calculations of geometric parameters of this TS (TS β1), the total energy of its formation, and the activation energy of the hydrogen exchange reaction are listed in Table 1. The numbering of atoms is shown in Scheme 1.

Then, we calculated this system assuming a fixed geometry of the $C(3)H_2O(15)$ fragment containing exchanging H atoms and free variation of other internal coordinates of the molecular system (TS β2). The bond lengths and bond angles for the $C(3)H_2O(15)$ fragment were taken from the results of calculations of TS of the reaction of hydrogen exchange in methane.[7] From the data listed in Table 1 it follows that the approximation we used is valid since the geometric parameters of TS β1 and TS β2 are close and their total energies virtually coincide. The results obtained make it possible to use this approximation in calculations of TS of the hydrogen exchange reactions in much more complex amino acids.

Scheme 1

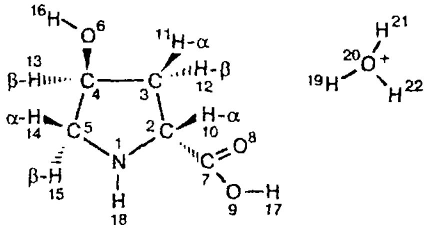


The reactivity of H atoms toward spillover hydrogen in organic compounds can be predicted on the basis of the results of quantum-chemical calculations of interactions between these compounds and a model acidic H_3O^+ center. L-Hydroxyproline (L-Hyp) was chosen as an example for such a study. The numbering of atoms is shown in Scheme 2.

Table 2. Interatomic distances (*d*) in the L-Hyp molecule and in TS structures of the reaction of hydrogen exchange with the H_3O^+ ion calculated by the RHF/6-31G* method

d/Å	L-Hyp	Transition state					
		2α	3α	3β	4β	5α	5β
N(1)—C(2)	1.44	1.41	1.44	1.48	1.51	1.47	1.47
C(2)—C(3)	1.53	1.59	1.53	1.52	1.52	1.55	1.54
C(3)—C(4)	1.54	1.52	1.56	1.58	1.59	1.52	1.53
C(4)—C(5)	1.55	1.53	1.55	1.55	1.59	1.59	1.59
N(1)—C(5)	1.45	1.48	1.47	1.48	1.46	1.42	1.42
C(4)—O(6)	1.41	1.45	1.44	1.43	1.42	1.44	1.42
C(7)—O(8)	1.19	1.19	1.19	1.19	1.22	1.19	1.19
O(6)—H(16)	0.95	0.97	0.97	0.97	0.97	0.97	0.97
N(1)—H(19)	—	2.06	3.27	2.66	3.21	2.12	2.10
O(6)—H(19)	—	1.86	1.96	3.47	2.12	1.81	3.14
O(8)—H(19)	—	2.98	3.85	3.60	2.51	4.79	4.40

Scheme 2



The structures of transition states of the reaction of L-Hyp with the model acidic H_3O^+ center are shown in Scheme 3. The exchanging hydrogen atom: 5α, α-H atom at C(5); 5β, β-H atom at C(5); 3α, α-H atom at C(3); 3β, β-H atom at C(3); 2α, α-H atom at C(2); and 4β, β-H atom at C(4).

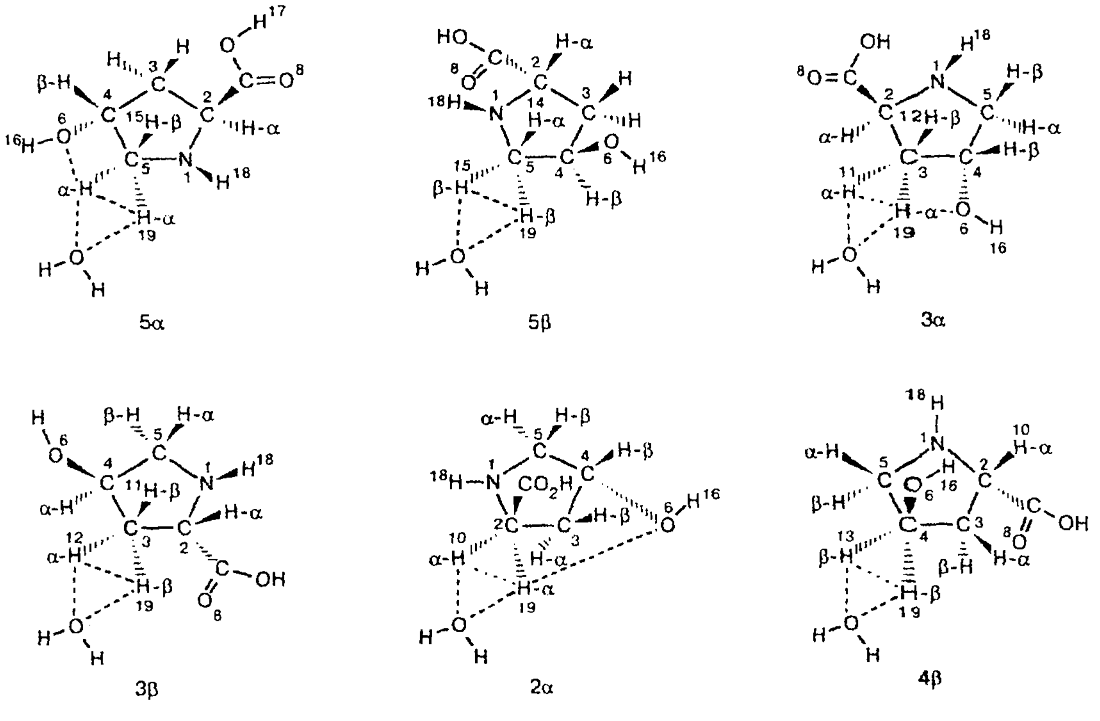
The geometric parameters of these structures are listed in Table 2 and the charge distribution is presented in Table 3.

There are several properties common to transition states of exchange reactions of different hydrogen atoms in the L-Hyp molecule. For instance, in all TS the C—C bond adjacent to the exchanging H atom is lengthened by ~0.05 Å. The charge on the C atom at which hydrogen exchange occurs is increased by ~0.3 au and that on the exchanging H atom is decreased by ~0.25 au. The charges on the H atoms bonded to the N(1), O(6), and O(8) atoms decrease by 0.05 au.

The ability of hydrogen atoms of the L-Hyp molecule to be exchanged depends on their positions in the molecule. For those positions in which substitution is the easiest to occur, the exchanging H atoms in TS are brought closer to the electron-donor O(6) or N(1) atoms. For instance, in the initial amino acid the α-H atom virtually does not interact with the O atom of the hydroxyl group and the O(6)—H(10) distance is 3.24 Å. This hydrogen atom is highly reactive in the HSCIE reaction and the O(6)—H(19) distance in TS 2α is shortened to 1.86 Å, which implies a strong interaction between the O atom of the hydroxyl group and the exchanging H atom.

Shortened distances between the electron-donor O(6) and N(1) atoms and the H—H(19) fragment are also observed in TS 5α, 5β, and 3α. These positions of the L-Hyp molecule are characterized by the highest rate of the hydrogen exchange reaction. Interaction between the hydroxyl group of the amino acid and the exchanging H atoms in TS 2α, 3α, and 5α occurs with a slight increase in the charge on the O(6) atom, while the O(6)—H(16) bond length and the charge on the H(16) atom remain unchanged. In the TS studied, the carboxyl group does not interact with exchanging H atoms, except for the TS 4β in which the distance O(8)—H(19) is 2.51 Å and the charge on the O(8) atom is increased to 0.63 au. This interaction is likely responsible for the enhanced reactivity of the hydrogen atom in the TS 4β compared with that for TS 3β, in which similar interaction with the N(1), O(6), and O(8) atoms is even weaker.

Scheme 3



5α 5β 3α

3β 2α 4β

Table 3. Effective charges (au) on the atoms of the L-Hyp molecule and in TS structures of the reaction of hydrogen exchange with the H_3O^+ ion calculated by the RHF/6-31G* method

Atom	L-Hyp	Transition state					
		2α	3α	3β	4β	5α	5β
N(1)	−0.72	−0.73	−0.78	−0.77	−0.71	−0.73	−0.73
C(2)	−0.08	−0.34	−0.02	−0.09	−0.12	−0.03	−0.03
C(3)	−0.32	−0.34	−0.66	−0.60	−0.34	−0.36	−0.36
C(4)	0.14	0.08	0.11	0.10	−0.13	0.15	0.12
C(5)	−0.14	−0.09	−0.07	−0.10	−0.12	−0.42	-0.39
O(6)	−0.75	−0.80	−0.77	−0.74	−0.70	−0.78	−0.74
C(7)	0.77	0.84	0.82	0.84	0.83	0.79	0.79
O(8)	−0.56	−0.49	−0.50	−0.49	−0.63	−0.53	−0.53
O(9)	−0.70	−0.72	−0.77	−0.78	−0.69	−0.72	−0.72
α-H(10)	0.19	0.48	0.26	0.29	0.28	0.26	0.26
α-H(11)	0.21	0.27	0.44	0.35	0.30	0.25	0.25
β-H(12)	0.19	0.27	0.34	0.41	0.26	0.23	0.22
β-H(13)	0.17	0.26	0.26	0.25	0.41	0.26	0.25
α-H(14)	0.16	0.22	0.21	0.22	0.24	0.47	0.34
β-H(15)	0.18	0.21	0.23	0.22	0.23	0.32	0.40
H(16)	0.44	0.49	0.49	0.48	0.49	0.49	0.48
H(17)	0.47	0.51	0.50	0.51	0.50	0.50	0.49
H(18)	0.36	0.43	0.42	0.41	0.40	0.44	0.42
H(19)	—	0.40	0.43	0.44	0.44	0.40	0.41
O(20)	—	−0.92	−0.92	−0.92	−0.91	−0.91	−0.92
H(21)	—	0.48	0.49	0.49	0.49	0.49	0.49
H(22)	—	0.48	0.48	0.48	0.48	0.48	0.49

The above data suggest the existence of a correlation between the reactivity of the C—H bonds of the amino acid molecule in the HSCIE reaction and the calculated values of activation energies of the hydrogen exchange reaction. The distribution of isotope label in the labeled amino acid is determined by the ratio of the rates of hydrogen isotope exchange in corresponding positions of the molecule. In fact, an antiparallel dependence is observed: the lower the calculated E_a value, the higher the ability of hydrogen atoms to be exchanged by spillover tritium in the HSCIE reaction. This ability also increases in the case of additional stabilization of TS. The reason for such a stabilization and the observed stereoselectivity of substitution in the HSCIE reaction could be interaction between the exchanging H atoms and the negatively charged O or N atoms.

The results of the experimental study of isotope exchange in the L-Hyp molecule and theoretical calculations of the ability of H atoms of this amino acid molecule to be exchanged are listed in Table 4. The α-H atom at the C(5) atom is the most reactive. The interaction between exchanging H atoms at the O(6) and N(1) atoms is observed in TS 5α, which corresponds to the substitution of this hydrogen atom. The β-H atom at this carbon atom can not interact with the hydroxyl group at the C(4) atom and stabilization of TS 5β occurs only with participation of the N(1) atom. The α-H atom at the C(3) atom in TS 3α interacts only with the O(6) atom; all heteroatoms are too distant from the β-H atom to stabilize this TS.

The α-H(5) atom is the most easily involved in the HSCIE reaction and this position is characterized by the lowest activation energy (18.1 kcal mol^{-1}). The least reactive is the β-H(3) atom, characterized by the highest E_a value, which approaches the activation energy of the hydrogen exchange reaction in CH_4 calculated in the same basis set. For all positions in the L-Hyp molecule a correlation is observed between the calculated activation energy of hydrogen exchange and the extent of hydrogen substitution by tritium in the HSCIE reaction.

Thus, all of the most active positions in the L-Hyp molecule are characterized by stabilization of TS with

Table 4. Tritium distribution in the L-[^{3}H]Hyp molecule after conducting the HSCIE reaction at 150 °C and the activation energies (E_a) of the hydrogen exchange reaction calculated by the RHF/6-31G* method

Position	Extent of tritium incorporation (%)	E_a /kcal mol^{-1}
α-H(2)	9	23.6
α-H(3)	6	23.7
β-H(3)	1	26.2
β-H(4)	4	25.6
α-H(5)	59	18.1
β-H(5)	21	22.0

Note. The E_a value for the reaction of hydrogen exchange in methane is 27.7 kcal mol^{-1} (data of RHF/6-31G* calculations).[7]

participation of O and N atoms. Regularities of stereoselectivity and regioselectivity of hydrogen substitution in the HSCIE reaction can be well described using a model acidic H_3O^+ center.

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