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New Development in the Solid-State Isotope Exchange with Spillover Hydrogen in Organic Compounds

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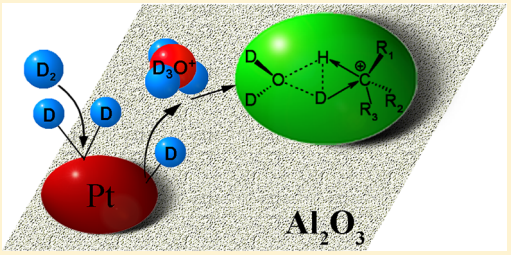
ABSTRACT: Solid-state catalytic isotope exchange of hydrogen under the action of spillover hydrogen in organic compounds applied on a nonorganic carrier has been studied. The deuterium-labeled peptide [D]dalargin containing 14 deuterium atoms, [D]melatonin, and [D]histamine with the 72−92% substitution degree of all C−H bonds were prepared by this reaction. The activation energy of hydrogen isotope exchange with T_2 and D_2 in glycine and α-aminoisobutyric acid was obtained experimentally. It was shown that for the studied reaction the kinetic isotopic effect is 1.2−1.4 by the Hartree−Fock method, which is several times smaller than one in the liquid-phase reactions. Quantum chemical calculations of the isotope shift values in the electronic spectra of deuterium-labeled metal ion complexes were performed using the RB3LYP/LanL2DZ and CIS/LanL2DZ methods for calculation of the ground and the excited states. It was shown for deuterium-labeled histamine complexes $[D_{12}]Pd(him)_2Cl_2$ and $[D_{12}]Cu(him)_2Cl$ that the isotopic effect of UV spectra was 1600 and 1800 cal/mol, respectively. The measurement of isotopic shifts for electronic transitions potentially can be utilized as a new informative method for the investigation of complex formation. The deuterium-labeled compounds were shown to be useful as internal standards for quantitative mass spectroscopy (MS) analysis.



INTRODUCTION

The phenomenon of hydrogen spillover has been investigated for more than forty years, and the number of relevant articles has exceeded ten thousand.[1,2] One of the major problems of the research concerned with spillover hydrogen (SH) is a lack of experimental techniques capable of measuring this effect directly. In spite of the abundance of publications on catalytic processes allegedly involving SH, no universal judgment about the nature of this active particle has been formed. According to various hypotheses, hydrogen can migrate in the form of a solvated proton, as a proton−electron pair, or as atomic hydrogen. The hydrogen spillover phenomenon at ambient temperature is of interest because it can be used for the development of adsorbents on porous carbon surfaces for storage application.[3,4]

Numerous publications have been aimed at DFT calculations of hydrogen interactions with small clusters of transient metals of M4 and M7 type on the zeolite surface.[5,6] The migration of atomic hydrogen onto a semiconductor nonorganic carrier is shown to be accompanied by TiO_2 protonation and the transition of the electron into the conduction band.[7−9] DFT calculations of SH formed over the surface of nonreducible metal oxide showed that hydrogen atoms are unable to migrate in the nonorganic framework and that the migration of a hydrogen atom from a metal particle to the surface of a nonreducible support is too slow to explain the observed hydrogenation of molecules adsorbed on the support by spillover of H atoms.[10] Transition-state theory ascribes the high reactivity of SH to the possibility of active hydrogen formation on a metal center and its migration into the gaseous phase.[11,12]

At the time when our studies were initiated, no publications describing the chemical interaction between organic compounds and spillover hydrogen had been available. We suggested the high-temperature solid-state catalytic isotope exchange (HSCIE) reaction based on special separation of a solid organic compound and heterogeneous catalyst.[13] The HSCIE reaction has been shown to proceed on new Brønsted-type acidic centers formed under the action of SH. After theoretical and experimental exploration of isotope exchange in amino acids, we described the mechanism of the reaction between spillover hydrogen and organic compounds for the first time.[14] The transition state of this reaction is characterized by the formation of a pentacoordinated carbon and a three-centered bond between the carbon and the incoming and outgoing hydrogen atoms.

Quantum chemical calculations of hydrogen exchange in amino acids gave the same stereoselectivity, regioselectivity, and values of activation energy similar to the experimental ones. The virtually complete absence of racemization in HSCIE reactions makes this reaction a valuable preparative method for the production of evenly tritium- or deuterium-labeled organic compounds. We analyzed the role of the structure of peptide

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chains and of their structural interactions on the hydrogen spillover reaction in peptides and proteins.[15,16] Using the HSCIE reaction, highly tritium- and deuterium-labeled ligands have been obtained for the subtypes of glutamate, serotonin, dopamine, nicotine, and melatonin brain receptors. They are employed for the screening and analysis of the molecular mechanisms underlying the action of the prospective medical drugs.[17,18]

The HSCIE reaction makes it possible to produce evenly tritium-labeled peptides for use both in receptor binding analysis and for the determination of their peptidase biodegradation paths in vivo and in vitro.[19−21] Using this reaction between an organic compound applied onto an inorganic carrier and SH activated on the catalyst, it is possible to substitute hydrogen for tritium almost quantitatively in organic compounds and at the same time completely retain their physiological activity. There are no other reactions existing capable of producing similar compounds. Using highly tritium-labeled compounds, we discovered for the first time and developed a theoretical description of isotopic effects in electron spectroscopy.[22]

The chemical interaction between solid organic compounds and spillover hydrogen was analyzed in review.[23] It has been shown that spillover hydrogen interacts with organic compounds as a positively charged particle and not as an atomized one. New data obtained in the continuing studies of the reaction between the spillover hydrogen and organic compounds are presented. Kinetic isotopic effects of the HSCIE reaction were studied for the first time as well as the isotopomeric composition in labeled peptides and biogenic amines prepared by means of solid-state isotope exchange and isotopic effects in the electron spectra of metal complexes with labeled biogenic amines.

EXPERIMENTAL SECTION

2.1. Solid-State Isotope Exchange with Deuterium and Tritium in Amino Acids. The HSCIE reaction proceeds under the action of deuterium or tritium at elevated temperatures in a solid mixture formed from an organic compound, an inorganic support, and a heterogeneous catalyst of platinum group metal.[3] Aluminum oxide (Serva, 20 mg) was added to a solution of the amino acid (1.0 mg) in water (0.5 mL), and the mixture was evaporated under reduced pressure. Aluminum oxide with the applied amino acid was mixed with 5% Rh/Al_2O_3 catalyst (Fluka, 10 mg). The resulting solid mixture was loaded in a 10 mL ampule, evacuated, and filled with gaseous deuterium containing 0.15% tritium up to a pressure of 25 kPa. The reaction was conducted at the temperature 140−260 °C during 2−30 min. The ampule was cooled, evacuated, flashed with hydrogen, and evacuated again. The amino acids were desorbed by 20% aqueous ethanol. To remove labile tritium and deuterium, the amino acids were dissolved two times more with aqueous ethanol (20%), and the resulting solution was concentrated. To measure the substitution degree of hydrogen for its isotopes in Gly and Aib, labeled amino acids were transformed to their phenylthiocarbamoyl (PTC) derivatives after HSCIE reaction. The labeled PTC-amino acids were purified by HPLC on a Kromasil C18 column (8 × 150 mm) in a gradient of aqueous methanol (10→50%) with 0.1% TFA; the flow rate was 3 mL/min. HPLC profiles of labeled PTC-amino acids and the ratios of UV absorbances at 220/254 nm are identical to those for unlabeled PTC-amino acids.

2.2. Solid-State Isotope Exchange with Deuterium in Dalargin. The HSCIE reaction was used for the preparation of highly tritium-labeled peptides with retention of their physiological activity.[24] Aluminum oxide (20 mg, Serva) was mixed with 1.0 mg of dalargin (DALG, Tyr-(D-Ala)-Gly-Phe-Leu-Arg) in aqueous solution. Then water was removed under reduced pressure. Aluminum oxide with the applied peptide was mixed with 10 mg of catalyst (5% $Pd/BaSO_4$, Fluka). The resulting solid mixture was placed in a 10 mL ampule which was evacuated and then filled with gaseous deuterium up to a pressure of 25 kPa. The mixture was heated to 190 °C and kept at this temperature for 20 min. The ampule was cooled, evacuated, flashed with hydrogen, and evacuated once again. The labeled peptide was desorbed with 50% aqueous ethanol, and labile deuterium was removed by repeated dissolution in 50% aqueous ethanol and followed by evaporation. The labeled peptide was purified by HPLC on a Kromasil C18 column (8 × 150 mm) in a gradient of aqueous acetonitrile (30→50%) with 0.1% TFA; the flow rate was 3 mL/min. HPLC profiles of labeled peptide and ratios of UV absorbances at 220/254 nm are identical to those for starting unlabeled peptide.

2.3. Solid-State Isotope Exchange with Deuterium or Tritium in Melatonin and Histamine. The HSCIE reaction was used for synthesis of tritium- and deuterium-labeled ligands of the brain receptors.[17] Deuterium- and tritium-labeled melatonin and histamine were prepared by the HSCIE reaction with gaseous deuterium or tritium. Aluminum oxide (20 mg, Serva) was added to a solution of the biogenic amine (1 mg) in alcohol (0.5 mL), and the solution was evaporated under reduced pressure. Aluminum oxide with the applied biogenic amine was mixed with 10 mg of catalyst (5% Rh/Al_2O_3, Fluka). The resulting solid mixture was placed into a 10 mL ampule. The ampule was evacuated and filled with gaseous deuterium or tritium at 25 kPa. The reaction was carried out at 190 °C for 10 min. The ampule was cooled, evacuated, and flashed with hydrogen and evacuated once again. The labeled biogenic amines were extracted from the solid reaction mixture with ethanol (2 × 3 mL). The resulting solutions were combined and evaporated. The procedure was repeated twice with 50% ethanol to remove labile tritium or deuterium. The labeled biogenic amine was purified by HPLC on a Kromasil C18 column (8 × 150 mm) in a gradient of aqueous acetonitrile (10→40%) with 0.1% TFA; the flow rate was 3 mL/min. HPLC profiles of labeled biogenic amines and ratios of UV absorbances at 220/254 nm are identical to those of starting unlabeled compound.

2.4. Calculation Methods. The calculations of organometallic molecules and of its isotope-substituted isomers were performed by the restricted Hartree−Fock method (RHF),[25] the restricted Becke−Lee−Young−Parr modification (RB3LYP) of the density functional theory,[26,27] and the method of configuration interaction (RCIS).[28] The Dunning−Hay D95 atomic basis sets were used for the optimization of the geometry and for the calculation of frequencies of normal vibrations; 6-31G*, for the calculation of one-electron energies.[29] We calculated excited states using the configuration interaction method RCIS and the D95 basis set. Zero-point energies (ZPE) were calculated by a combined application of the three methods mentioned (RB3LYP, RHF, RCIS). The RHF method was used for definition of ZPE of the ground state and RCIS for definition of the exited states of organometallic complexes.

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RESULTS AND DISCUSSION

Using the HSCIE reaction, it becomes possible to substitute hydrogen for deuterium or tritium almost quantitatively in a series of amino acids without racemization.[3] Isotopic substitution is a widely used tool for mechanistic analysis of chemical reaction. To analyze the difference in values of the HSCIE activation energy with deuterium and tritium, the reactions with a mixture of deuterium and tritium were used. Deuterium incorporation to PTC derivatives of labeled amino acids was measured with the help of HPLC and MS analysis (Figure 1). Tritium incorporation into amino acids was

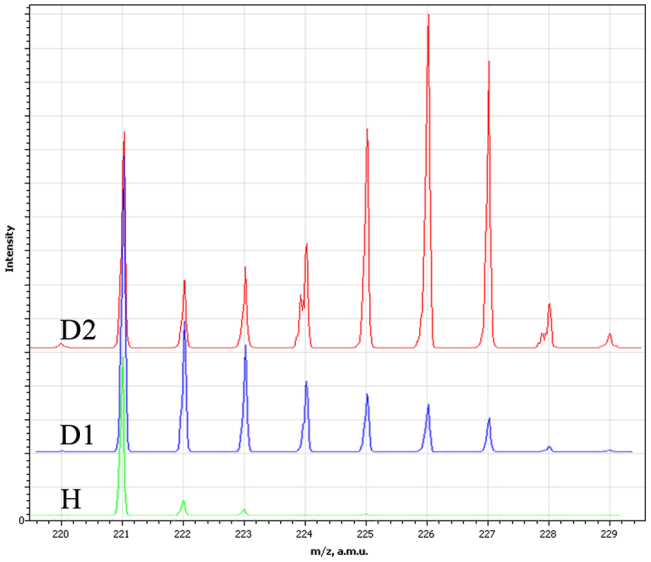

Figure 1. MS analysis of phenylthiocarbamoyl derivatives of [^{2}H]Aib prepared with the HSCIE reaction at 160−260 °C. (H) Aib-PTC light; (D1, D2) [G-^{2}H] Aib-PTC heavy. Average degree of substitution of ^{1}H for ^{2}H for D1 is 1.55 and for D2 is 3.64 atoms per molecule.

measured with the help of liquid scintillation counting. Investigation of the kinetic isotopic effects (K_{IE}) of the HSCIE reaction has been conducted for the first time. E_{act} in glycine (Gly) and α-aminoisobutyric acid (Aib) were calculated using temperature-dependent data of the rate of tritium hydrogen and deuterium hydrogen exchange with help of the SigmaPlot Ver12 program (Figure 2). E_{act} of the substitution of

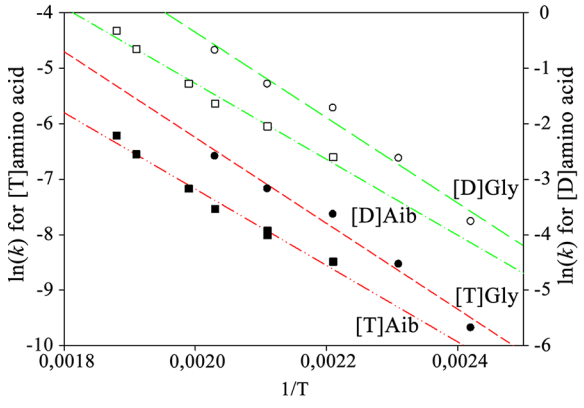

Figure 2. Kinetic isotopic effects of the HSCIE reaction. The dependence of ln(k) on T^{-1} for the HSCIE reaction with deuterium and tritium in Gly and Aib.

hydrogen by deuterium and tritium in Aib was found to be 13.6 and 13.7 kcal/mol, respectively. In Gly, the same values were 15.3 and 15.4 kcal/mol, respectively. The coefficients of variation for these data were 5−7%. It can be concluded from these data that values of activation energy in the HSCIE reaction with deuterium and tritium are similar, and hydrogen isotopes react at virtually the same rate.

Quantum chemical calculations of the K_{IE} of the solid-state isotope exchange reaction were performed using the one-center mechanism by the RHF method. The structure of the transition state of the hydrogen exchange reaction between the methyl group of Ala and the acidic center (represented by H_3O^+ ion) is shown in Figure 3. The activation energy of the hydrogen

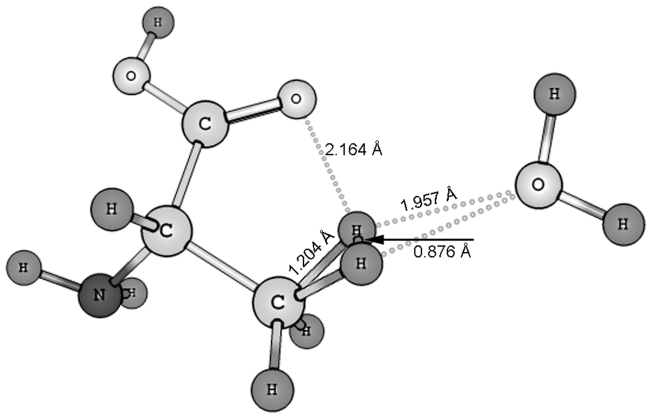

Figure 3. Structure of the transition state of the hydrogen exchange reaction between the acidic center (H_3O^+ ion) and Me group of Ala.

substitution reaction for the methyl group of Ala was found to be 16.19 kcal/mol (RHF 6-31G*). The experimentally measured activation energy of the HSCIE reaction with tritium for the methyl group of Ala was found to be 14 kcal/mol[30] which agrees with this theoretical calculation. The difference in activation energies of hydrogen−deuterium and hydrogen−tritium exchange in the methyl group of Ala was found to be 0.08 kcal/mol (RHF 6-31G*), and K_{IE} for this hydrogen exchange reaction was 1.23 (RHF 6-31G*, 450 K) according to the Eyring theory of absolute reaction rates.

Quantum chemical calculations of E_{act} of the exchange reaction of hydrogen in Gly and Aib for deuterium and tritium were performed. E_{act} of the exchange reaction of hydrogen in Aib for hydrogen, deuterium, and tritium was found to be 12.05, 12.24, and 12.36 kcal/mol (RHF 6-31G*), respectively. The difference between the activation energies of hydrogen−deuterium and hydrogen−tritium exchange was found to be 0.12 kcal/mol (HF 6-31G*), and the value of K_{IE} was 1.32 (RHF 6-31G*, 450 K). For Gly, E_{act} of the exchange reaction of hydrogen with hydrogen, deuterium, and tritium was found to be 24.54, 24.85, and 25.04 kcal/mol (RHF 6-31G*), respectively. The difference in activation energies of hydrogen−deuterium and hydrogen−tritium exchange was found to be 0.19 kcal/mol (RHF 6-31G*), and K_{IE} was 1.42 (RHF 6-31G*, 450 K).

Good agreement was observed between the results of quantum chemical calculations and experimental data for the isotopic effect of activation energy of hydrogen exchange. Quantum chemical calculations of the kinetic isotopic effects performed with the use of a one-center mechanism correlate with experimental data. It was shown that the kinetic isotopic

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effect of solid-state reaction of hydrogen exchange with SH is 1.2−1.4. It is several times lower than that of liquid state reactions of protium and deuterium transfer which has values in the range from 3 to 30. The K_{IE} of the enzymatic hydrogen transition reaction for the C(2) position of deuterated alanine under the action of deuterated pyridoxal enzyme racemase is about 3.[31] It can be mentioned that extremely high kinetic isotope effects in the range from 10^2 to 10^4 were found for the reactions of muonium and muonic helium with H_2.[32] Good agreement was observed between our experimental data and the results of quantum chemical calculations of hydrogen exchange on model acidic centers like $H^+(H_2O)_n$. The obtained data indicate that in the HSCIE reaction K_{IE} is small, and hydrogen isotopes react at virtually the same rate.

The solid-state isotope exchange reaction conducted at elevated temperatures was shown to be effective for the introduction of isotopes into peptides and proteins. Deuterium-labeled peptide [^{2}H]DALG was produced with 14 atoms of deuterium by the HSCIE reaction (Figure 4). Deuterium

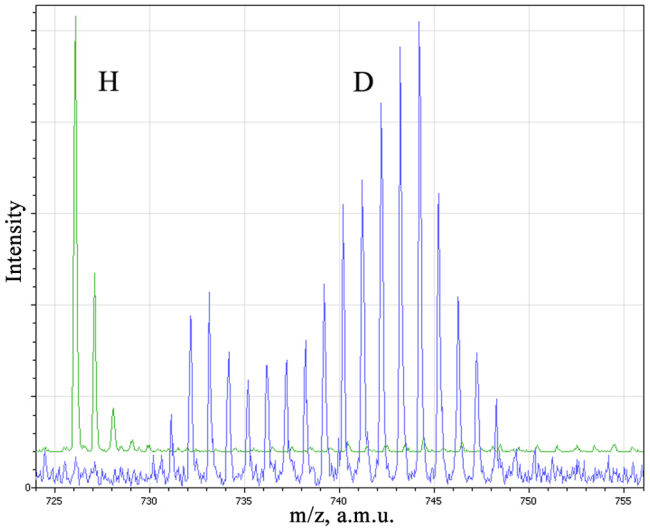

Figure 4. Solid-state isotope exchange reaction with D_2 in DALG (Tyr-(D-Ala)-Gly-Phe-Leu-Arg). MS analysis of [G-^{2}H]DALG with average incorporation of 14.6 deuterium atoms.

distribution in [^{2}H]DALG was calculated by comparison of peak intensities in the one- and two-dimensional (^{1}H/^{1}H-TOCSY with mixing time of 60 ms) ^{1}H NMR spectra of ^{2}H-labeled and unlabeled DALG peptides (Figure 5, Table 1). Measurements have been done using an NMR spectrometer Varian NMR System 700 MHz. It has been shown that the isotope labels were distributed over the whole molecule of peptide [^{2}H]DALG allowing the monitoring in all the fragments formed during its proteolysis with the use of mass spectrometry. The tritium-labeled dalargin was produced with a molar radioactivity of 138 Ci/mmol by the HSCIE reaction at 180 °C with retention of its physiological activity.[14] Tritium distribution in [^{3}H]DALG was analyzed using ^{3}H NMR. Hydrogen isotope distribution and reactivity of amino acid fragments for deuterium- and tritium-labeled peptides [^{2}H]DALG and [^{3}H]DALG demonstrate good correlation. Evenly deuterium- and tritium-labeled peptides [^{2}H]DALG and [^{3}H]DALG may be used for the determination of its peptidase biodegradation paths in vivo and in vitro.

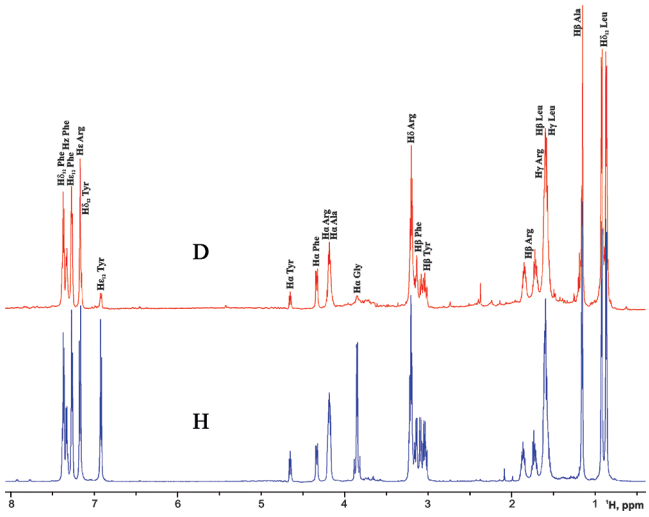

Figure 5. Comparison of one-dimensional ^{1}H NMR spectra of ^{2}H-labeled (top) and unlabeled (bottom) DALG peptides at 293 K, ^2H_2O, pH 5.0.

Table 1. Distribution of ^{2}H-Labeling in DALG Peptide with 10.2 Deuterium Atoms

residue	% D-labeling (±3)
Gly C_αH2	96
Tyr C_εH2	91
Tyr C_δH2	76
Tyr C_βH2	54
Tyr C_αH	23
Ala C_βH3	27
Ala C_αH	78
Phe C_εH2	34
Phe C_δH2	23
Phe C_ζH	24
Phe C_βH2	14
Phe C_αH	8
Leu	<3
Arg	<3

The HSCIE reaction is applicable to a range of organic compounds of different classes.[13] Deuterium-labeled [^{2}H]-melatonin and [^{2}H]histamine were obtained by the HSCIE reaction at 190 °C. The average incorporations of deuterium into the evenly deuterium-labeled [^{2}H]melatonin and [^{2}H]-histamine are 10.1 and 5.5 atoms, respectively. Under electrospray conditions, a positive monovalent molecular ion of 233 Da was formed from melatonin. The distribution of isotopomers in [^{2}H]melatonin is shown in Figure 6. Neither unreacted starting compounds nor deuterated molecules up to [$^2H_5$]melatonin were detected. The mean calculated rate of exhaustive hydrogen substitution for deuterium was 72%. Isotopomers from [$^2H_8$]melatonin to [$^2H_{12}$]melatonin constitute about 87% of the total amount of labeled molecules. The degree of substitution of all C−H bonds in [^{2}H]histamine was 92% (Figure 7). Isotopomers [$^2H_5$]histamine and [$^2H_6$]-histamine constitute about 92% of the total amount of labeled molecules. The isotope label was shown to be distributed throughout the ligand molecules. These deuterium-labeled biogenic amines can be used as internal standard for quantitative MS analysis. Tritium-labeled [^{3}H]melatonin and [^{3}H]histamine were obtained at 190 °C with a specific

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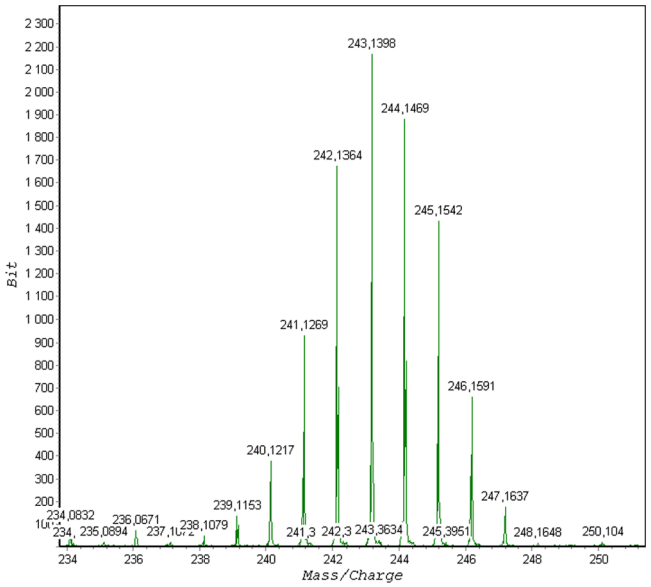

Figure 6. Solid-state isotope exchange reaction with D_2 in melatonin. MS analysis of [G-^{2}H]melatonin with average incorporation of 10.1 deuterium atoms. Degree of substitution of all C−H bonds is 72%.

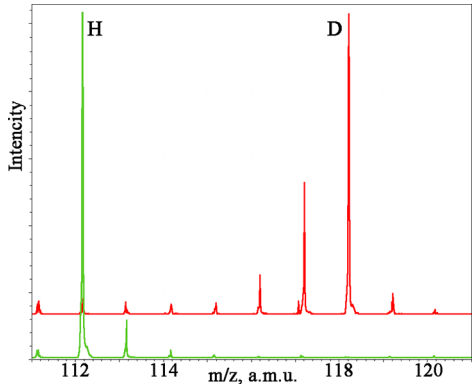

Figure 7. Solid-state isotope exchange reaction with D_2 in histamine. MS analysis of [G-^{2}H]histamine with average incorporation of 5.5 deuterium atoms. Degree of substitution of all C−H bonds is 92%.

radioactivity of 170 and 95 Ci/mmol, respectively. These tritium-labeled biogenic amines may be used for the radio-receptor analysis.

Deuterium-labeled histamine was used to study isotopic effects in electronic spectra of organometallic compounds. The isotopic effect in electronic excitations in organometallic molecules was theoretically analyzed for the first time. This phenomenon is concerned with zero-point energies (ZPEs) of the ground and excited states of the isotope-substituted organometallic molecules. To account for this effect, quantum chemical calculations of the geometric and electronic structures, frequencies of normal vibrations, and transition energies have been performed using the restricted Hartree−Fock method, density functional theory, and configuration interaction method. The results of these studies are presented in Table 2 and Figures 8−10. The calculations were performed with full optimization of the geometry. The second column of Table 2 shows the values of the electron excitation energy obtained using the configuration interaction software CIS/LanL2DZ. The third column shows the values of the total energy and zero-point energies. These values were calculated for the ground state by means of DFT B3LYP/LanL2DZ and for the excited states using the configuration interaction method. The total energy with the zero-point energy and the energy shifts due to isotopic substitution of hydrogen by deuterium are presented in the third column. Our results imply that in the UV spectrum of the histamine complex [7,9,24,26-D_4]Cu(him)$_2$Cl the energy absorption band is shifted to the lower energies on 0.02 eV (460 cal), and for the histamine complex [7,9,11,12,14,15,24,26,28,29,31,32-D_{12}]Cu(him)$_2$C1 it is shifted to the lower energies on 0.08 eV (1840 cal) (Figure 8, Table 2). There is a long-wavelength shift in the UV spectrum.

In the spectrum of the histamine complex [7,9,24,26-D_4]Pd(him)$_2$Cl$_2$, the first energy absorption band of the UV spectrum is shifted to lower energies on 0.02 eV (460 cal), and for the histamine complex [7,9,11,12,14,15,24,26,28,29,31,32-D_{12}]Pd(him)$_2$C1$_2$, it is shifted to lower energies on 0.07 eV (1610 cal) (Figure 9, Table 2). The second absorption band is also shifted due to the replacement of hydrogen with deuterium to the red part of the spectrum on 0.2 eV (460 cal). Thus, the manifestation of the isotope effect in the UV spectrum of the palladium−histamine complex is the same as for the copper−histamine complex. In the UV spectrum of the imidazole complexes [9,11,12,18,20,21-D_6]Zn(Im)$_2$Cl$_2$, the first energy absorption band is shifted to higher energy (short-wavelength shift) on 0.01 eV (230 cal) (Figure 10, Table 2). The second absorption band is shifted due to the substitution of hydrogen with deuterium to the longer wavelengths on 0.18 eV (4140 cal), and the third absorption band is shifted on 0.19 eV (4370 cal) to the longer wavelengths.

CONCLUSIONS

Solid-state catalytic isotope exchange of hydrogen under the action of spillover hydrogen in organic compounds applied on a nonorganic carrier has been studied. Activation energies of hydrogen isotope exchange for tritium and deuterium in glycine and α-aminoisobutyric acid were measured experimentally and calculated using a quantum chemical approach. The values of activation energies for tritium−hydrogen and deuterium−hydrogen exchange in each amino acid are very close, and hydrogen isotopes react at virtually the same rate. It was shown that for the studied solid-state reaction with deuterium and tritium the kinetic isotopic effect is equal to 1.2−1.4 which is several times smaller than the kinetic isotopic effect in hydrogen transfer reactions in the liquid phase. Quantum chemical calculations of the kinetic isotopic effects with use of the one-center mechanism correlate with experimental data.

Using solid-state catalytic isotope exchange reaction, deuterium-labeled melatonin and histamine with the 72−92% degree of substitution for all C−H bonds and the deuterium-labeled hexapeptide dalargin containing 14 deuterium atoms were produced. It was shown that the isotope label was distributed over the whole peptide molecule, and this peptide may be used as an internal standard for quantitative MS analysis and for the determination of its peptidase biodegradation paths in vivo and in vitro.

The isotopic effect for electron excitations in organometallic molecules was analyzed theoretically for the first time. For histamine complexes [D_{12}]Pd(him)$_2$Cl$_2$ and [D_{12}]Cu(him)$_2$CL it was shown that the isotopic effect for UV spectra is 1600 and 1800 cal, respectively. It was shown for imidazole complex [D_6]Zn(Im)$_2$Cl$_2$ that the isotopic effect in the UV spectrum is 4370 cal. The origin of this phenomenon is attributed to the

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Table 2. Energetic Properties of Histamine and Imidazole Complexes $Cu(him)_2Cl$, $Pd(him)_2Cl_2$, and $Zn(Im)_2Cl_2$

complex	$E_1 - E_0$ (eV, nm)	E' (au), ΔE (eV)
$Cu(him)_2Cl$	-	−931.169557, -
$[7,9,24,26\text{-}D_4]Cu(him)_2Cl$	-	−931.182614, 0.36 eV
$[7,9,11,12,14,15,24,26,28,29,31,32\text{-}D_{12}]Cu(him)_2C1$	-	−931.209697, 1.09 eV
$Cu(him)_2Cl$	first excited state, 5.38, 230.53	−924.880617
$[7,9,24,26\text{-}D_4]Cu(him)_2Cl$	5.38, 230.53	−924.894702, 0.38 eV
$[7,9,11,12,14,15,24,26,28,29,31,32\text{-}D_{12}]Cu(him)_2C1$	5.38, 230.53	−924.923450, 1.17 eV
$Pd(him)_2Cl_2$	-	−876.757444, -
$[7,9,24,26\text{-}D_4]Pd(him)_2Cl_2$	-	−876.770640, 0.36 eV
$[7,9,11,12,14,15,24,26,28,29,31,32\text{-}D_{12}]Pd(him)_2C1_2$	-	−876.797839, 1.10 eV
$Pd(him)_2Cl_2$	first excited state, 2.0275 eV, 611.51 nm	−870.637387, -
$[7,9,24,26\text{-}D_4]Pd(him)_2Cl_2$	2.0275 eV, 611.51 nm	−870.651501, 0.38 eV
$[7,9,11,12,14,15,24,26,28,29,31,32\text{-}D_{12}]Pd(him)_2C1_2$	2.0275 eV, 611.51 nm	−870.680377, 1.17 eV
$Pd(him)_2Cl_2$	second excited state, 2.3453 eV, 528.64 nm	−870.625874,
$[7,9,24,26\text{-}D_4]Pd(him)_2Cl_2$	2.3453 eV, 528.64 nm	−870.639993, 0.38 eV
$Pd(him)_2Cl_2$	third excited state, 2.4459 eV, 506.90 nm	−870.622155
$Zn(Im)_2Cl_2$	-	−542.480839
$[9,11,12,18,20,21\text{-}D_6]Zn(Im)_2Cl_2$	-	−542.502013, 0.58 eV
$Zn(Im)_2Cl_2$	first excited state, 7.2375 eV, 171.31 nm	−542.225739, -
$[9,11,12,18,20,21\text{-}D_6]Zn(Im)_2Cl_2$	7.2375 eV, 171.31 nm	−542.246714, 0.57 eV
$Zn(Im)_2Cl_2$	second excited state, 7.2955 eV, 169.94 nm	−542.207743
$[9,11,12,18,20,21\text{-}D_6]Zn(Im)_2Cl_2$	7.2955 eV, 169.94 nm	−542.235856, 0.76 eV
$Zn(Im)_2Cl_2$	third excited state, 7.9639 eV, 155.68 nm	−542.197749
$[9,11,12,18,20,21\text{-}D_6]Zn(Im)_2Cl_2$	7.9639 eV, 155.68 nm	−542.225895, 0.77 eV

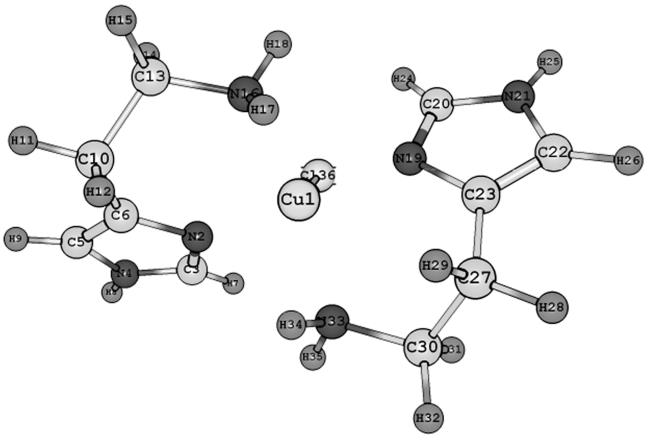

Figure 8. Histamine complex of Cu, $Cu(him)_2Cl$.

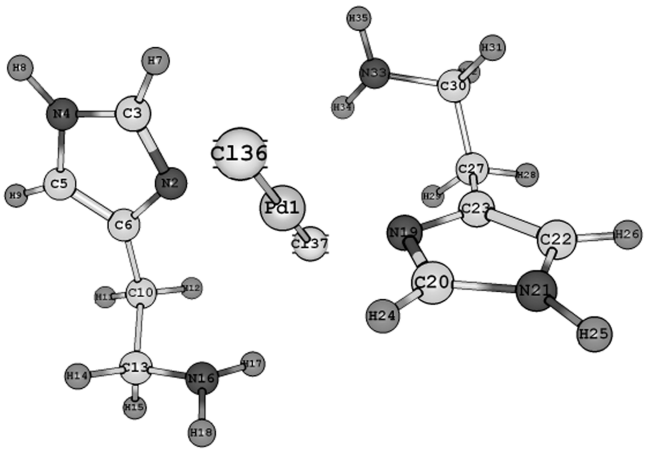

Figure 9. Histamine complex of Pd, $Pd(him)_2Cl_2$.

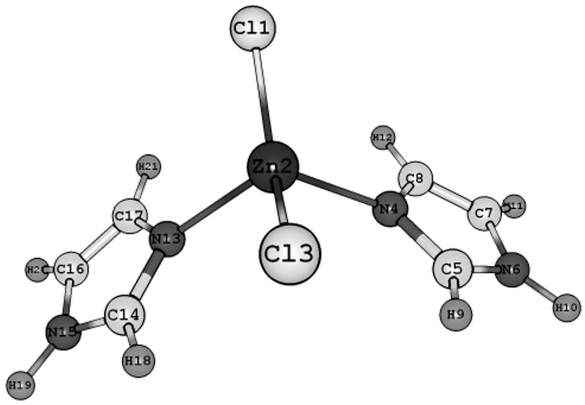

Figure 10. Imidazole complex of Zn, $Zn(Im)_2Cl_2$.

difference in the zero-point energy of the ground and excited states of the isotope-substituted molecule. The measurement of isotopic shifts for electronic transitions in organometallic compounds potentially can be utilized as a new informative method for the investigation of chemical bonds and complex formation.

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Notes

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