TRITIUM INCORPORATION IN α-AMINO ACIDS BY ISOTOPE EXCHANGE USING HIGH-TEMPERATURE SOLID-STATE CATALYSIS.

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SUMMARY

Solid-state catalytic reactions provide a new effective method for the synthesis of tritium-labelled biologically active compounds. We present the synthesis of tritium-labelled amino acids through high-temperature solid-state catalytic isotope exchange (HSCIE). Under HSCIE, isotope exchange with gaseous tritium was shown to proceed at all hydrogen atoms in the molecules of solid organic compounds, which opens the possibility of producing biologically active compounds uniformly labelled with tritium at high molar activity. The configuration is retained upon the hydrogen atom substitution at asymmetrical carbon atoms under HSCIE conditions, allowing the synthesis of uniformly-labelled amino acids in the optically active form.

Key words: Isotope exchange, Tritium labelling

Introduction

Catalytic hydrogenolysis by gaseous tritium is the key reaction for the preparative production of labelled organic compounds. This reaction is usually carried out in water or in organic solvents in the presence of applied catalysts of the platinum group [1]. The hydrogenolysis of multiple bonds (carbon-carbon, carbon-nitrogen, carbon-oxygen) and of hydrogen-halogen

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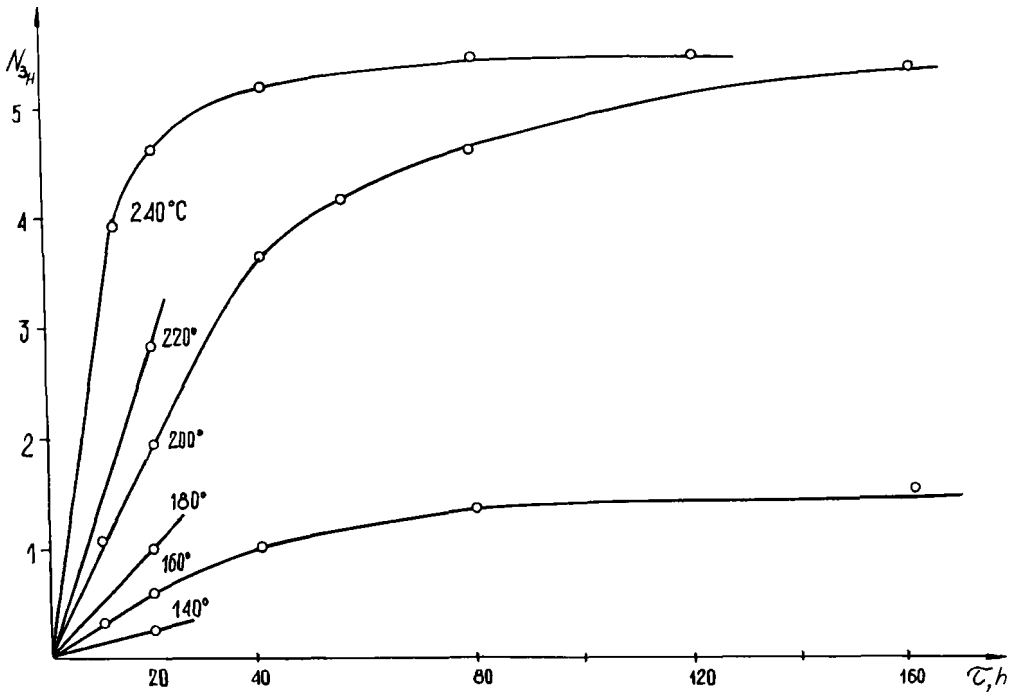
bonds can also be accomplished in the solid state without solvent. These catalytic reactions proceed at elevated temperatures, and we used them for the synthesis of tritium-labelled compounds. Solid-state hydrogenolysis was first used for the synthesis of tritium-labelled components of nucleic acids through solid-state catalytic dehalogenation [2]. The isotope exchange reaction was found to proceed intensively under solid-state hydrogenolysis conditions at elevated temperatures. In our opinion, high-temperature solid-state catalytic isotope exchange (HSCIE) reactions are of special interest for the synthesis of tritium-labelled biologically active compounds. In the presence of a highly dispersed platinum metal, hydrogen atoms of a solid organic compound exchange with gaseous tritium to form an isotope-substituted compound. Raising temperature increases the rate of isotope exchange and the degree of hydrogen-tritium substitution. At elevated temperatures, all hydrogen atoms of the organic compound molecule enter the HSCIE reaction, which allows uniformly tritium-labelled compounds to be obtained. The isotope exchange between the catalyst-activated tritium and the solid-state organic compound hydrogen proceeds with a high retention of hydrogen configuration when carbon atoms are asymmetric, making it possible to obtain tritium-labelled amino acids with a high degree of tritium-hydrogen substitution in the optically active form [3].

Results and Discussion

The HSCIE method was effectively used for the synthesis of amino acids and peptides uniformly labelled with tritium [4,5]. We believe it is important to study the distribution of isotopic label in relation to the labelled compound structure, the reactive ability of the hydrogen atoms present at different carbon atoms, and HSCIE conditions. The exchange ability of hydrogen atoms under HSCIE conditions was found to be markedly different from that registered in the liquid-state isotope exchange reaction. In the

310-410°K temperature range, isotopic exchange usually proceeds selectively at certain positions of the biologically active molecule. ^3H NMR spectroscopy has shown that the following hydrogen atoms are the most reactive in HSCIE at the 2-nd carbon in methionine, at the 3-rd carbon in alanine, at methyl carbons in valine, at the 5-th carbon in hydroxyproline, at the second carbon atom of the imidazole ring in histidine. The selectivity of isotopic label incorporation is 70% or higher. At a temperature of 450°K and more, isotope exchange proceeds between all hydrogen atoms of the solid organic compound and the gaseous state hydrogen isotopes. In HSCIE, the isotope exchange reaction reaches a level that can only be accounted for by the hydrogen-tritium ratio in the gaseous and solid states. This has been the first recorded experimental demonstration of the isotopic equilibrium of the qaseous phase and the hydrogen atoms in the solid state organic molecul. The kinetics of isotopic equilibration of gaseous tritium and hydrogen atoms of the L-valine molecule was examined in the 413-513°K temperature range in the presence of an applied palladium catalyst (Fig.1). Tritium-labelled valine with a 440 TBq/mol specific activity is produced at 413°K during 1 hour, and 96% of the label is incorporated at the methyl carbon atoms according to ^3H NMR data. Under similar reaction conditions valine a specific activity of 1100 TBq/mol is produced during 48 hours.

At temperatures of 450°K and higher, the isotopic label is uniformly incorporated into the valine molecule, and isotopic equilibrium is achieved. The HSCIE activation energy (13 kcal/mol) was determined from the initial linear regions of the isotopic exchange kinetics in the 433-513°K temperature range. This value is in good agreement with the activation energy of hydrogen spillover on platinum metals [6]. This indicates that spillover hydrogen formation could be the kinetically decisive stage of the HSCIE reaction.



1. Dependence of tritium incorporation in the valine molecule on the temperature and duration of the HSCIE reaction

Uniformly labelled L-valine with specific activity 5330 TBq/mol was obtained by HSCIE at 493°K in a 20 minutes' reaction time. According to ^3H NMR data, tritium distribution in different positions of the valine molecule is as follows: $\alpha:\beta:\gamma$ = 1:1:6, which corresponds to the stoichiometrical hydrogen content at the carbon atoms in these positions. The mathematical model of HSCIE based on equiprobable hydrogen exchange in different positions of the molecule describes well the distribution of labelled compound molecules according to the degree of isotopic substitution and the kinetics of isotopic exchange. The distribution of labelling in the amino acid according to the degree of tritium substitution for hydrogen at different carbon atoms was determined by high-resolution ^3H NMR spectroscopy without suppressing the interaction of protium and tritium nuclei [7,8]. Data are cited for tritium distribution in alanine synthesized at 493 K with a specific

activity 2070 TBq/mol . According to ^3H NMR data, 8 possible isotopic forms are present in this sample that differ in the tritium substitution for hydrogen. The mixture can be represented as I + II, where I is $CH_n{}^3H_{3-n}C^3HNH_2CO_2H$ with n=0,1,2,3 - a,b,c,d, respectively: II is $CH_n{}^3H_{3-n}CHNH_2CO_2H$ with n=0,1,2,3 - e,f,g,h, respectively. The results are presented in Table 1. The same table shows data for tritium distribution in a molecule of alanine obtained by calculations based on the mathematical model described above. Table 1 makes it clear that the tritium distribution in L-[2,3-^3H]-alanine synthesized by HSCIE with gaseous tritium is quite well described by the mathematical model based on equiprobable exchange of hydrogen atoms in the molecule. The amount of tritium incorporated at the 2-nd and 3-rd positions of

Table 1

Tritium distribution along the [2,3-^3H]-alanine molecule determined from ^3H NMR spectra and calculated from the mathematical model

	Content of the isotope-substituted form,%							
	a	b	c	d	e	f	g	h
^3H NMR	3	15	22	5	5	17	23	7
Calculation	5	17	18	6	6	19	20	7

the molecule is in a 1:3 ratio, corresponding to the content of hydrogen atoms in these positions.

The HSCIE reaction makes it possible to synthesize amino acids uniformly labelled with tritium with a high degree of hydrogen substitution (Table 2). Some of the amino acids shown in the table could be synthesized by traditional methods of catalytic hydrogenolysis in solution. The use of HSCIE makes it possible to achieve higher specific activities for [2-^3H] glycine, L-[2,3-^3H] alanine, L-[2,3-^3H] serine, while the traditional method does not

Table 2

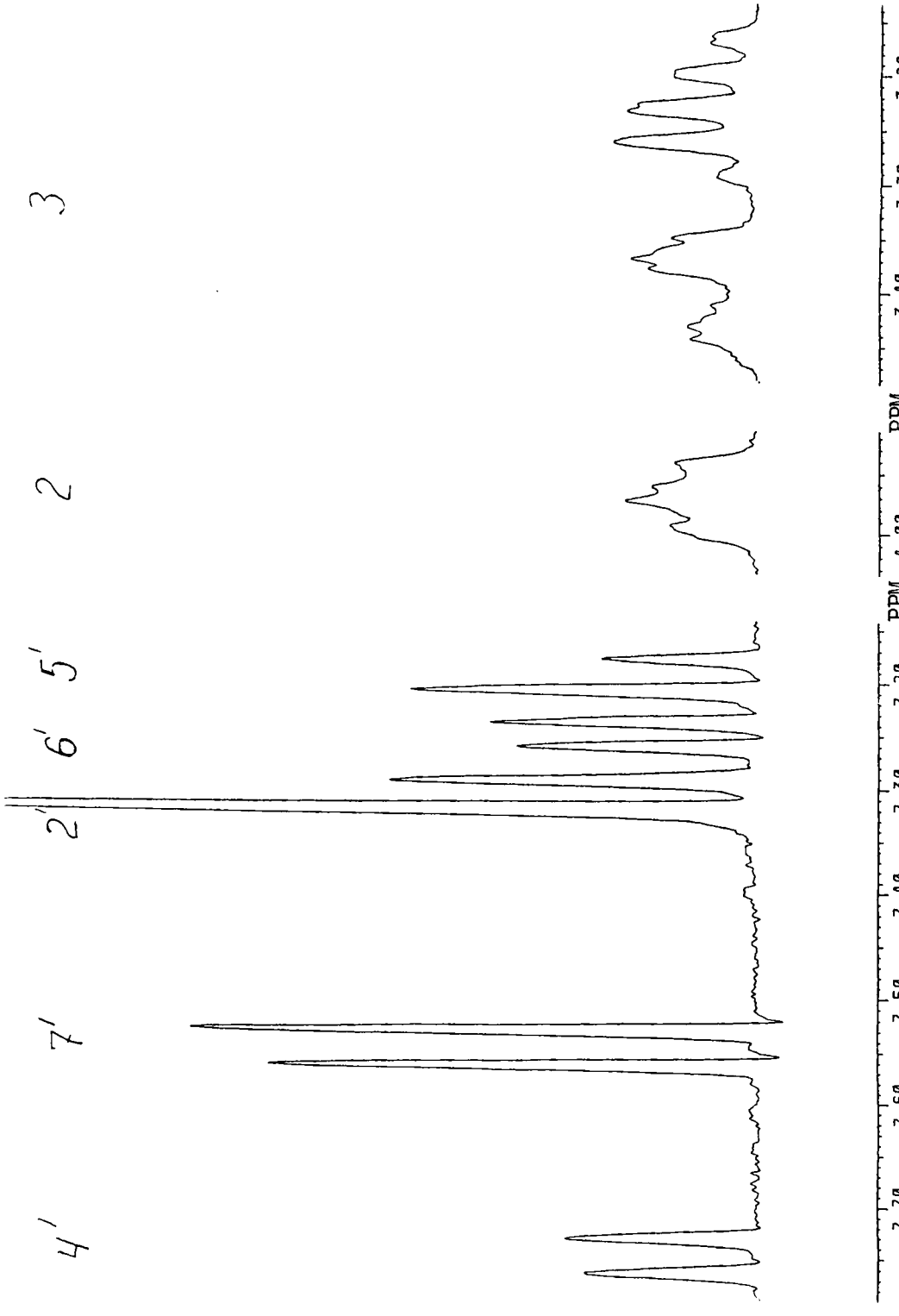
HSCIE of Amino Acids With Gaseous Tritium

Amino acid	Molar activity TBq/mol	Optical purity, %	Yield %
[2-^{3}H]-Glycine	1800	-	90
L-[2,3-^{3}H]-Serine	1400	75	50
L-[2,3-^{3}H]-Alanine	3200	82	65
L-[2,3,4,4'-^{3}H]-Valine	6500	94	85
L-[2,3,4,5,5'-^{3}H]-Leucine	7900	85	70
L-[2,3,4,4',5-^{3}H]-Isoleucine	8100	88	75
L-[2,3,2',5'-^{3}H]-Histidine	4500	50	20
L-[2,3,2',4',5',6',7'-^{3}H]-Tryptophane	3600	70	30

work at all for [2,3,4,4-^{3}H] valine, [2,3,4,5,5'-^{3}H] leucine, [2,3,4,4',5-^{3}H] isoleucine, [2,3,2',5'-^{3}H] histidine, [2,3,2',4',5',6',7'-^{3}H] tryptophane. Another advantage of HSCIE is that highly-labelled amino acids are produced with 80-90% optical purity.

The amount and distribution of isotopic label in amino acids obtained in solid-state and liquid-state catalytic reactions of isotopic exchange are essentially different. According to ^{3}H NMR data, isotopic exchange with gaseous tritium in solution in the presence of heterogeneous catalysts produces [G-^{3}H]-tryptophane with the isotopic label distributed only over the heteroaromatic portion of the molecule [9].

By ^{3}H NMR spectroscopy tritium distribution was determined in L-[2,3,2',4',5',6',7'-^{3}H]-tryptophane (Fig.2, Table 3). HSCIE ensures the production of [^{3}H]-tryptophane with approximately equal distribution of the isotopic label in the aliphatic and heteroaromatic portions of the molecule. The configuration of the asymmetric α-carbon atom is retained to a high degree.



2. ^3H NMR spectrum of [2,3,2',4',5',6',7'-^3H]-tryptophane

Table 3

^{3}H NMR spectrum of [^{3}H]-tryptophane

Position	Chemical shift ppm	Activity quota %	Degree of substitution %
2	3.95	12	41
3	3.35	28	47
	3.25		
2′	7.32	17	57
4′	7.75	6	20
5′	7.22	11	37
6′	7.28	11	37
7′	7.55	14	47

We have therefore found a new effective method for the synthesis of biologically active compounds labelled with hydrogen isotopes, based on high-temperature solid-state catalytic isotopic exchange and leading to the isotopic equilibration of hydrogen atoms in the solid and liquid phases. On reaching isotopic equilibrium in HSCIE, the isotopic label is uniformly distributed over the amino acid molecule regardless of the exchangability of the hydrogen atoms. The specific activity of the product is usually much higher than in conventional methods.

Experimental

1. Synthesis of L-[2,3,4,4′-^{3}H]-valive

1 mg of L-valine, 0.5 ml of water and 10 mg of 10 % palladium-on-charcoal were placed into a 10 ml glass vial. The suspension was frosen in liquid nitrogen as the vial was rotating. Water was removed by lyophilic drying in vacuum. The reaction mixture was vacuumized, then tritium was introduced to a pressure of 250 torr. The vial was heated to a temperature of 473 °K and kept at this

temperature during an hour. The vial was then cooled, tritium was removed, the vial was washed with hydrogen.

Tritium-labelled valine was isolated from the reaction mixture by treating it with 5 ml of 1 M NH_4OH containing 20 % ethanol. The catalyst was filtered out, and filtrate was evaporated to dryness at a lower pressure. Labile tritium was removed by evaporating 10 ml portions twice with 20 % aqueous ethanol under reduced pressure.

The chromatographic purification was conducted on a cationite Amberlite CG 50 (III) filling with copper ions. The degree of filling of the sorbent with copper ions was 70 % . The sorbent was transferred to a 140 mm x 8 mm column. The eluent was a 0.2 M ammonium hydroxide. Traces of copper ions are removed from the eluent by passing the latter through a chromatographic column of 15 mm x 10 mm size packed with a complex-forming Dowex A-1. The fraction of valine was collected according to the readings of UV-detector from 22 to 27 ml. Valine was isolated by a chromatography on a cation exchanger. Aminex Q-150-S sulfocationic exchanger in the H^+ form was placed in a 50 mm x 8 mm column and the amino acid fraction collected was passed through it. The sorbent was washed with water, and the amino acid was desorbed by 1.0 M aqueous ammonia. The eluent was evaporated, valine was dissolved in 50 % aqueous ethanol to a radiactive concentration of 37 GBq/l.

The analysis for chemical purity was effected by means of thin-layer chromatography on "Silufol" in a system isopropanol-aceton-ammonia (15 : 9 : 9). The mobility of valine is 0.45 . The radiochemical purity is 98 % . There are obtained 320 μg [2,3,4,4′-^3H]-valine with specific activity 4800 TBq/mol and with the chemical yield 32 % . To analyze and prepare tritium-labelled optically active amino acids, we used high performance ligand-exchange chromatography on modified silica gel sorbents contaning L-hydroxyproline groups [10]. The sorbent was packed in a 250 mm x

4 mm column. Solution containing 70 % 0.01 M ammonium acetate, 1.0 x 10^{-4} M copper acetate , pH 4.5 and 30 % methanol was used as eluent. The sorbent particle size was 5 μm. L-Valine was eluted from 18 to 22 ml. The optical purity amino acid was aqual to 80 %.

2. Synthesis of isotope-substituted amino acids.

In a 10 ml glass vial, a solid mixture comprising a non-organic carrier (barium sulphate, calcium carbonate, aluminium oxide, charcoal), a platinum group catalyst metal (rhodium, palladium, platinum) and 1 mg of L-amino acid reacts with gaseous tritium or deuterium under a pressure of 200-400 torr at a temperature of 373-513°K during 20-200 min. Isotope-substituted amino acid is isolated from the reaction mixture with 4-6 ml of 1.0 M aqueous ammonia containing 20% ethanol. Labile tritium is removed by evaporating 10 ml portions twice with 20% aqueous ethanol under reduced pressure. Amino acid is isolated by chromatography on Aminex 150-Q sulphocationic exchanger in the H^+ form and ligand exchange chromatography on carboxylic cationic exchanger Amberlite CG 50 (III) filled with copper ions (II). To isolate optically active labelled amino acid, ligand exchange chromatography was used on sorbents with L-phenylalanine and L-hydroxyproline groupings [10,11,12].

3. ^3H NMR and ^1H NMR spectroscopy

^3H NMR and ^1H NMR spectra of amino acid solutions in ^2H_2O were obtained on a AC 250 Bruker NMR spectrometer equipped with a $^1H/^3H$ 5-mm dual probe with operating frequencies of 266.8 and 250 MHz respectively. Chemical shifts of tritium signal in completely tritium-substituted components are assumed to be equal to corresponding proton shifts [13]. Multiplet signals were modeled

with the help of the standard Panic program by Bruker. Measurements were taken by placing 50-100 mCi of tritium labelled amino acid dissolved in 0.5 ml of ^2H_2O in the vial.

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