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Short Research Article

Solid state isotopic exchange of hydrogen in proteins and peptides[†]

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Introduction

Data have been summarized on the production of evenly hydrogen labelled proteins and peptides with the use of the high-temperature solid-state catalytic isotopic exchange (HSCIE) reaction.[1] The HSCIE reaction proceeds at an elevated temperature in a solid mixture consisting of an organic substance applied onto an inorganic support, highly dispersed metal of the platinum group, and gaseous tritium or deuterium. It was possible to substitute deuterium or tritium for hydrogen almost completely in a number of amino acids.[2,3] The HSCIE reaction remains up to now the only experimental opportunity to obtain peptides ultimately labelled by tritium. The high degree of tritium substitution for protium at HSCIE can be achieved provided that amino acids, biogenic amines, peptides, carbohydrates, nucleosides, or heterocyclic acids have been preliminarily deposited onto inert inorganic supports.[4,5] Hydrogen atoms bound to the surface atoms of platinum metals can migrate to the inorganic support (e.g. alumina, barium sulphate, etc.). Such active atoms were given the name of spillover hydrogen (SH).[6] The HSCIE reaction in amino acids preliminarily applied on an inert inorganic support occurs at the acidic catalytic centres formed in the solid mixture under the action of SH.[7]

The fact that the isotope exchange in amino acids and peptides at 150–200°C proceeds with a high degree of chirality retention[5,8] is an important distinctive feature of the HSCIE reaction. The mechanism of the HSCIE reaction can be represented as synchronous one-centre substitution at the saturated carbon atom, characterized by the formation of pentacoordinated carbon and a three-centre bond with involvement of the incoming and outgoing hydrogen atoms in the transition state.[9] The virtually complete absence of racemization in HSCIE makes this reaction a valuable preparative method. A number of tritium-labelled proteins and peptides with completely retained biological activity have been obtained using HSCIE.[10] The molar radioactivities of these peptides were many times higher than those of labelled preparations obtained by traditional methods, which is a particularly important advantage for studying specific binding. The evenly tritium labelled peptides obtained with the use of the HSCIE reaction were employed for studying the receptor binding to opioid and nicotinic acetylcholine receptors.

Results and discussion

Catalytic reaction of solid protein and spillover tritium (ST), based on HSCIE, was studied. Using hemoglobin and its α- and β-chain polypeptides, the effect of the solid state composition, temperature and HSCIE reaction duration on the chemical yield and the molar radioactivity of the tritium-labelled polypeptides formed was studied. The reaction conditions of the HSCIE reaction, that allow both high incorporation of

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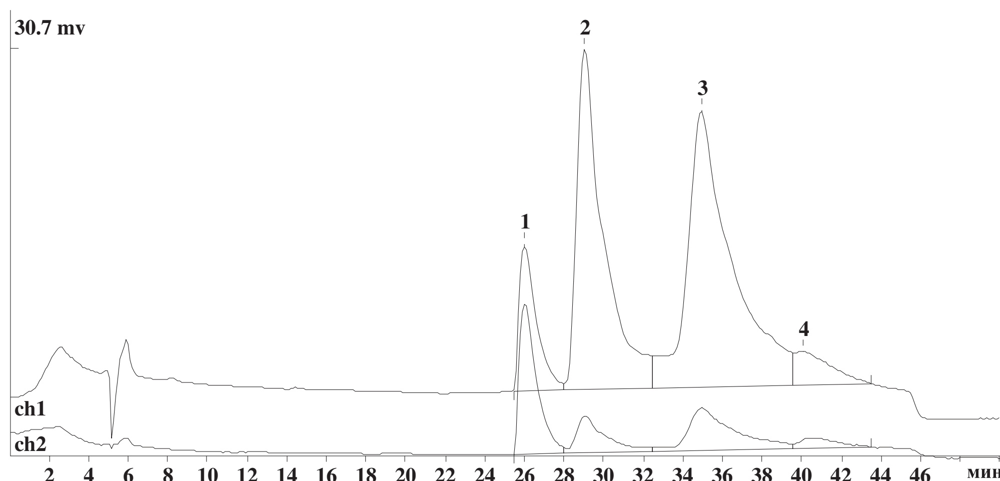

Figure 1 Chromatographic resolution of tritium labelled hemoglobin. A MN column C_8 300A, 8 × 250 mm, a gradient elution with acetonitrile in 0.1% TFA; Detection at (ch1) 220 nm and (ch2) 280 nm; 1-hem; 2-α-chain; 3-β-chain.

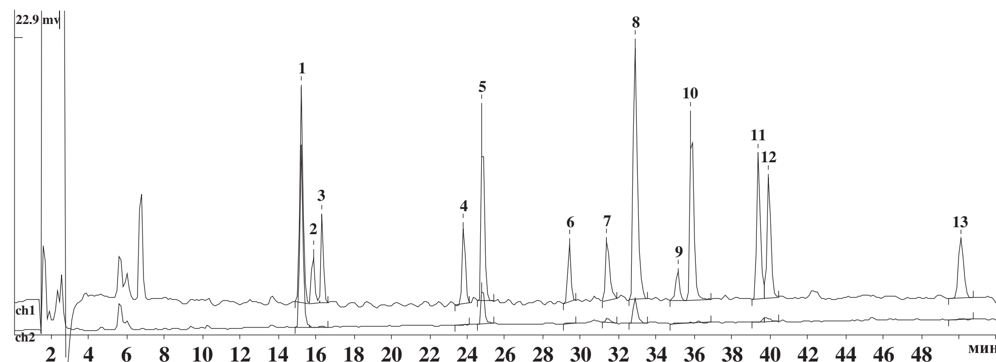

Figure 2 The trypsinolysis of α-chain of [G-^{3}H]hemoglobin: A Nova-Pak column C_8 4 μm, 3.9 × 150 mm, a gradient elution with acetonitrile/methanol in 0.1% TFA; Detection at (ch1) 210 nm and (ch2) 280 nm; **1**–AAWGK$_{12–16}$; **2**–VAAALTK$_{62–68}$; **3**–VLSAADKGNVK$_{1–11}$; **4**–VGGHAAEYGAEALER$_{93–99}$; **5**–VGGHAAEYGAEALER$_{17–31}$; **6**–LRVDPVNFK$_{91–99}$; **7**–TYFPHFDLSHGSAQVKGHGAK$_{41–61}$; **8**–TYFPHFDLSHGSAQVK$_{41–56}$; **9**–FLANVSTVLTSK$_{128–139}$; **10**–MFLSFPTTK$_{32–40}$; **11**–AVEHLDDLPGALSELSDLHAHK$_{69–90}$; **12**–AVEHLDDLPGALSELSDLHAHKLR$_{69–90}$; **13**–LLSHSLLVTLASHLPSDFTPAVHASLDK$_{100–127}$.

the isotope label and protein retention at 100–120°C, were established. Chromatographic resolution of tritium labelled α- and β-chain polypeptides from tritium labelled hemoglobin is shown in Figure 1. The specific radioactivity of labelled α- and β-chains was 140 and 150 Ci/mmol, respectively.

The fermentative hydrolysis conditions of tritium labelled polypeptides with consecutive use of 6 M guanidine hydrochloride and TPSC-treated bovine trypsin were selected for the analysis of the labelled compounds. Analyses of peptides formed during trypsinolysis were performed using MALDI mass spectrometry on an Ultraflex Tof/Tof instrument and an Agilent LC/MS instrument with an ESI (electric spray) source (Figure 2 and 3).

Using the generated peptide map of hemoglobin allowed data on tritium distribution in [G-^{3}H]hemoglobin was obtained. Tritium label was present in all peptides formed after the fermentative hydrolysis. The effect of the three-dimensional structure of hemoglobin on the reactivity of its peptide fragments in HSCIE was obtained. The significant differences in radioactivity distribution in α-chain polypeptide in the free form and in hemoglobin complex show clearly that the HSCIE reaction can be used to generate information about the three-dimensional structure of protein complexes. As the result of studying the ST-hemoglobin reaction, the HSCIE conditions were used for preparative production of unique tritium labelled proteins (KD 10–180), with the isotopic label in all peptide fragments and specific

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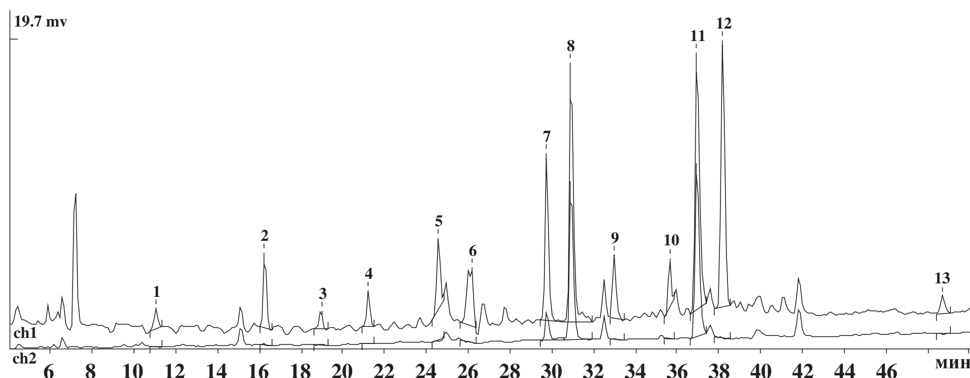

Figure 3 The trypsinolysis of β-chain [G-^{3}H]hemoglobin: The conditions of chromatography as on Figure 1; **1**–NFGK$_{116–119}$; **2**–MLTAEEK$_{1–7}$; **3**–HLDDLK$_{76–81}$; **4**–VDEVGGEALGR$_{19–29}$; **5**–VKVDEVGGEALGR$_{17–29}$; **6**–VLDSFSNGMK$_{66–75}$ +KVLDSFSNGMK$_{65–75}$; **7**–VVAGVANALAHR$_{132–145}$; **8**–AAVTAFWGK$_{8–16}$; **9**–EFTPVLQADFQK$_{120–131}$; **10**–NFGKEFTPVLQADFQK$_{116–131}$; **11**–FFESFG DLSTADAVMNNPK$_{40–58}$; **12**–LLVVYPWTQR$_{30–39}$; **13**–LLGNVLVVVLAR$_{104–115}$.

Table 1 The solid-state catalytic isotope exchange of hydrogen in ligands of receptors by the high-temperature solid-state catalytic isotope exchange (HSCIE)

No	Tritium-labeled ligand	Receptor	Molar radioactivity (Ci/mmol)
1	SCH 23390	D1 Dopamine receptor	60
2	Spiperone	D2 Dopamine receptor	120
3	7-OH-DPAT	D3 Dopamine receptor	120
4	MK-801	NMDA Glutamate receptor	210
5	Conotoxin M II	Nicotinic acetylcholine receptor	28

Table 2 Deuterium distribution in [G-^{2}H]dalargine with average incorporation of 4.4 (I) and 5.9 (II) deuterium atoms, respectively

	Deuterium distribution (%)														
N_D	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	0.8	1.3	6	16	30	29	14	3	0.6						
II	0.8	0.3	1	3	11	26	30	16	5	2	1.7	0.9	0.9	0.7	0.6

radioactivity 30–300 Ci/mmol. Complete retention of their biological activity was observed.

The HSCIE reaction was used for the production of labelled peptide toxins and selective ligands for receptors (Table 1). The specific radioactivity in some cases are higher than for commercially available products.

The HSCIE reaction makes it possible to produce uniformly deuterium labelled amino acids.[3] The opioid 6-member peptide dalargine with both deuterium and tritium label was produced with high isotope substitution using the HSCIE reaction. The tritium distribution in [G-^{3}H]dalargine was analysed with ^{3}H-NMR.[9] [G-^{2}H]dalargine was analysed with MS. Deuterium labelled dalargine has been produced, with average incorporation of 4.4 and 5.9 deuterium atoms. The isotopomeric compositions of these deuterium labelled peptides are shown (Table 2, Figure 4). Deuterium labelled peptide can be used for the creation of sensitive procedures for *in vivo* and *in vitro* quantitative assessment with MS.

To solve the task of quantitative analysis in proteomics, the employment of evenly deuterium labelled proteins produced by the reaction of high temperature solid-state catalytic isotope exchange (HSCIE) with gaseous deuterium has been suggested. Introduction of specified amounts of these deuterium labelled proteins to biological objects prior to isolation, separation and trypsinolysis will make it possible to collect, with the help of chromatography–mass spectrometry, quantitative information about the presence of proteins and polypeptides under study in the tissues.

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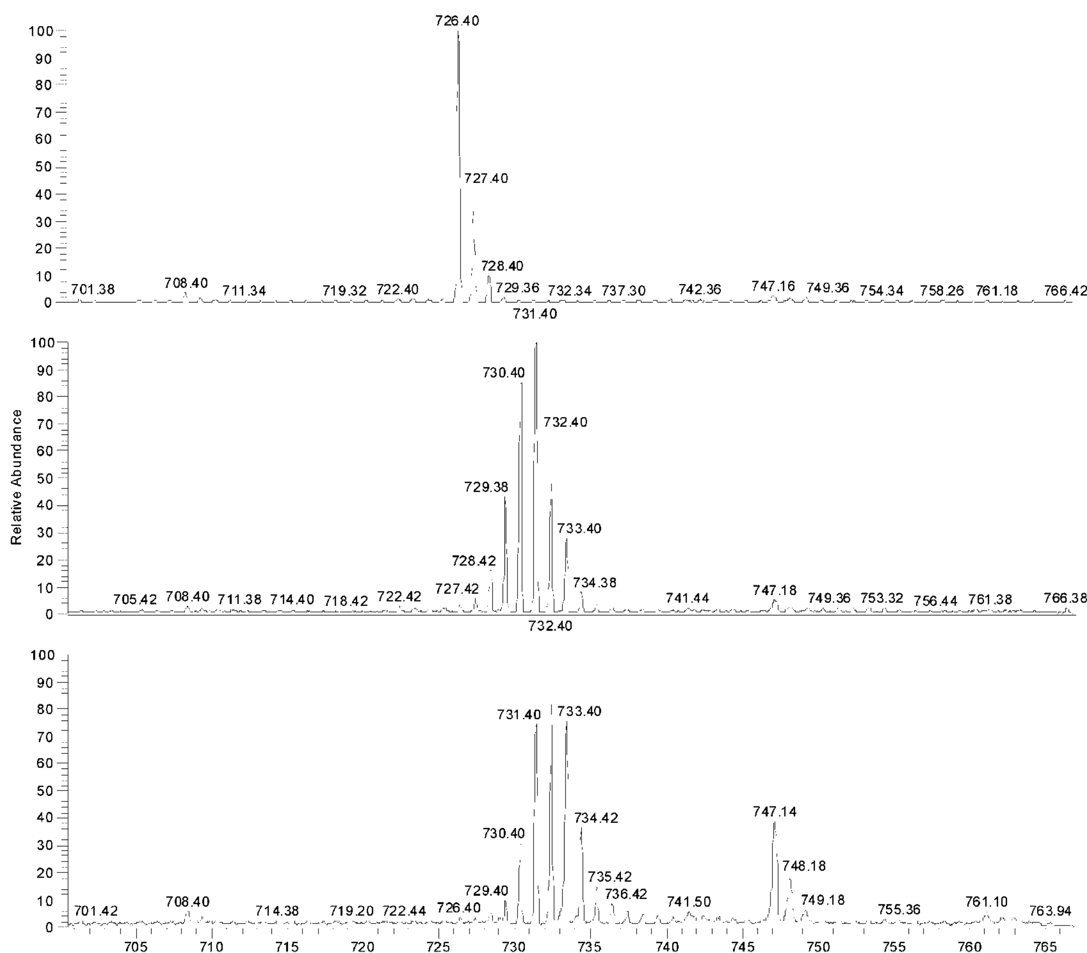

Figure 4 MS analysis with ion trap of [G-^{1}H]dalargine (a) and [G-^{2}H]dalargine (b), (c) with average incorporation of 4.4 and 5.9 deuterium atoms, respectively.

Conclusion

The HSCIE reaction can be used as for the production of tritium labelled proteins and peptides as for the investigation of spatial interactions in protein complexes. These products have high specific radioactivity and completely retain their biological activity. Deuterium labelled proteins and peptides can be used to facilitate the task of quantitative MS analysis.

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