Tritium-Labeled 5-Oxo-Pro-Arg-Pro
V. P. Shevchenko, I. Yu. Nagaev*, L. A. Andreeva, and Academician N. F. Myasoedov
Received October 4, 2016

Abstract—Conditions of tritium introduction into 5-oxo-Pro-Arg-Pro have been developed. Labelled 5-oxo-Pro-Arg-Pro has been obtained in 75% yield and molar radioactivity of 60 Ci/mmol. Tritium distribution over amino acid residues in the peptide has been determined. 5-Oxo-Pro : Arg : Pro ratio in the labelled peptide has been found to be 1 : 5.2 : 2.8. Nonterminal amino acid has been found to contain the major portion of tritium, about 35 Ci/mmol; i.e., arginine includes more than half of the label. Such a feature can be explained by the effect of readily protonated guanidine group of arginine. The influence of charged guanidine group of arginine seems to increase the shift of electron density in the neighboring C–H bonds of arginine and proton mobility, thus increasing protium–tritium exchange at these carbon atoms.

DOI: 10.1134/S0012500817040061

Tripeptide 5-oxo-Pro-Arg-Pro shows hypoglycemic effect in animal organism even on the background of such a pathology as metabolic syndrome, decreases thrombocyte aggregation, restores the normal values of total cholesterol and lipid profile.

The aim of this work is to synthesize tritium-labeled 5-oxo-Pro-Arg-Pro with molar radioactivity not less than 50 Ci/mmol. The labeled compound with such characteristics is necessary for pharmacokinetic investigation and studying the mechanism of physiological activity of 5-oxo-Pro-Arg-Pro. In this work, we pay a large attention to the distribution of the label between amino acids, because pharmacokinetic investigation requires information on the label content in resulting metabolites.

The character of tritium distribution in the molecule of organic compound is dependent on a number of factors. For example, irregular label distribution is observed upon introduction of tritium into peptides formed from aliphatic amino acids [1, 2] (Table 1).

The table shows that the introduction of tritium into Gly(I) and Gly(II) in the Gly-Gly-Val peptide differs by more than 5 times. Tritium label upon insertion into the same amino acid residue is introduced preferably into terminal amino acids. If both residues are terminal, amino acid residue at the N terminus of peptide contains a larger tritium amount. Thus, if peptide comprises amino acids where the mobility of protons bound to carbon atoms is close (for example, all aliphatic amino acids), the revealed feature will be observed. These conclusions drawn in earlier works did not take into account that the efficiency of isotope exchange is mainly determined by the ability of a compound applied to the carrier surface to solvate hydrogen isotope cations and electrons. Therefore, certain combinations of amino acid residues in peptide may cause tritium insertion much larger than into peptides where formation of ion pairs (3H+, e) solvated in the substance is hindered.

It is known that electron can overcome the dielectric layer due to tunnel effects [3–5]; i.e., electrons formed on the active centers of the catalyst can pass from one phase onto another due to similar effects. The emergence of negatively charged electrons on the carrier due to tunneling facilitates the subsequent transition of positively charged species of hydrogen isotopes onto the carrier. This effect enables one to explain the possibility of hydrogen spillover on carriers incapable of producing reversible redox reactions. Otherwise, as calculations show, hydrogen spillover can proceed only over the surfaces of transition metal oxides that can be reduced with hydrogen [6].

However, the most important feature is that the presented concept can explain the possibility of hydro-

Table 1. Dependence of label distribution on peptide nature (150°C) [2]

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Molar radioactivity, Ci/mmol</th>
<th>Yield, %</th>
<th>Val</th>
<th>Gly(I)</th>
<th>Gly(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val-Gly-Gly</td>
<td>69.9</td>
<td>45</td>
<td>72</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Gly-Val-Gly</td>
<td>38.1</td>
<td>30</td>
<td>13</td>
<td>59</td>
<td>28</td>
</tr>
<tr>
<td>Gly-Gly-Val</td>
<td>47.0</td>
<td>32</td>
<td>6</td>
<td>79</td>
<td>15</td>
</tr>
</tbody>
</table>
gen spillover from the metal catalyst onto the carrier or from one carrier to another, as well as the formation of activated tritium species solvated in the substance. The isotope exchange efficiency and tritium distribution within molecule fragments will depend not only on hydrogen spillover on the carrier surface but also on the probability of ion pairs \((^{3}H^+, \delta)\) forming in the substance, which ultimately determines the probability of formation of transition states necessary for isotope exchange of protium for tritium (Scheme 1) [7–10].

If one of amino acid residues in peptide is readily protonated by tritium cations to form positive charge, then the ion pair \((^{3}H^+, \delta)\) distribution in substance will also change. One can expect that the tritium distribution over the amino acid residues in such a peptide will be markedly different from the tritium distribution in peptides containing no such amino acids. The effect related to charge emergence upon amino acid protonation by tritium cations was revealed upon tritium introduction into alanine when 96% label was included into methyl group and upon tritium introduction into arginine when 44% label was bound to the fifth carbon atom neighboring to the guanidine moiety [1, 11]. The increase in proton mobility upon charge appearance on the nitrogen atom is associated with the ability of \(\sigma\) electrons to shift to the reaction zone in such cases. This phenomenon is named hyperconjugation [12]. The hyperconjugation of \(\sigma\) electrons of C–H bonds is the most important form of hyperconjugation. It was proved experimentally that the electron pair of C–H bonds is much less localized than the electrons of C–C bonds and can shift to the reaction zone like lone electron pairs. This bond becomes weaker and proton mobility increases when the electron pair of a C–H bond is shifted.

The important role of hyperconjugation in isotope exchange can be confirmed by the tritium distribution in 5-oxo-Pro-Arg-Pro, which contains nonterminal arginine, whose guanidine moiety is readily protonated to form a charged group (Scheme 2).

If the presented arguments are sound, the tritium content in nonterminal arginine should be larger than in the terminal amino acids.

At the first stage of the work, we determined the temperature dependence of tritium introduction into this peptide (Table 2). Reaction mixtures were analyzed by HPLC on a Milikhrom-A02 chromatograph (Institute of Chromatography EcoNova, Russia) using a ProntoSIL-120-5-C_{18} AQ DB-2003 column (BISCHOFF Analysetechnik, Germany, 2 × 75 mm, particle size 5 μm), in methanol–buffer gradient (0.2 M LiClO_{4} and 0.005 M HClO_{4}, pH 2.24), methanol from 1 to 30% over 12.5 min, elution rate 0.2 mL/min; methanol from 1 to 80% over 12.5 min, elution rate 0.15 mL/min, retention time of 5-oxo-Pro-Arg-Pro is 5.11 min.

Scheme 1. The types of transition states proposed in literature sources.

Scheme 2. Introduction of tritium into arginine fragment of 5-oxo-Pro-Arg-Pro (Z is the cluster of activated tritium species solvated in the substance pool).