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Short Research Article

Synthesis of selectively labeled fatty acids and tritium labeled acyl-CoA and CoA†

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Introduction

CoA and acyl-CoAs of high specific activity were needed in studies of a low-activity enzyme system in brain.[1] The current work is devoted to tritium labeling of different acyl-CoA at acyl or CoA fragment.

In order to introduce tritium into the unsaturated long-chain acyl part, the desired [^{3}H]fatty acid is usually prepared by selective hydrogenation of its acetylenic precursor. However, in some cases simpler ways can be used namely, the selective hydrogenation of the often easily available polyunsaturated fatty acids.

Thus, [5,6-^{3}H]8-*cis*,11-*cis*,14-*cis*-eicosatrienoic and [6,7-^{3}H]linoleic (9-*cis*,12-*cis*-octadecadienoic) acids were synthesized by selective hydrogenation with tritium gas of solutions of arachidonic and γ-linolenic (6-*cis*,9-*cis*,12-*cis*-octadecatrienoic) acids, respectively.

Results and discussion

As shown in Figure 1, the radioactive yields of [5,6-^{3}H]8,11,14-eicosatrienoic and [6,7-^{3}H]linoleic acids were 15 and 27%, respectively.

[5,6-^{3}H]8,11,14-eicosatrienoic and [6,7-^{3}H]linoleic acids were isolated with the yield of 12 and 22%, respectively, by a combination of several HPLC methods. The radiochemical purity was confirmed by RP-HPLC and Ag^+-TLC and was found to be about 99%.

Condensation of fatty acids with CoA was performed by the chloroanhydride method.[1] Since the hydrolysis of fatty acid chloroanhydride in aqueous solution is very fast, it was first dissolved in THF and then treated with solution of coenzyme A in buffer. A special attention should be paid to the lability of coenzyme A itself. The condensation yielded [5,6-^{3}H] eicosatrienoyl-CoA and [6,7-^{3}H]linoleoyl-CoA were formed with specific activities of 27 and 22 Ci/mmol, respectively.

The direct labeling of CoA was achieved by isotope exchange with tritiated water. Several methods of exchange with tritiated water are known.[2] The best results were obtained using the following method: a mixture of PdO and catalyst was treated with tritium gas at 70°C for 10–15 min. The resulting tritiated water and activated catalyst[3] was frozen with liquid nitrogen, the ampoule was evacuated and the filled with argon. After injection of a mixture of triethylamine-dioxane, the ampoule was sealed and the exchange reaction was carried out at 150–200°C. The improved isotopic exchange in this case can be explained via a back spillover process that leads to protonated water clusters [$^3H^+(^3H_2O)_n$] (where n is number of water molecules in the cluster).[3–5]

Quantum-chemical calculations show a considerable increase in energy of proton elimination with water

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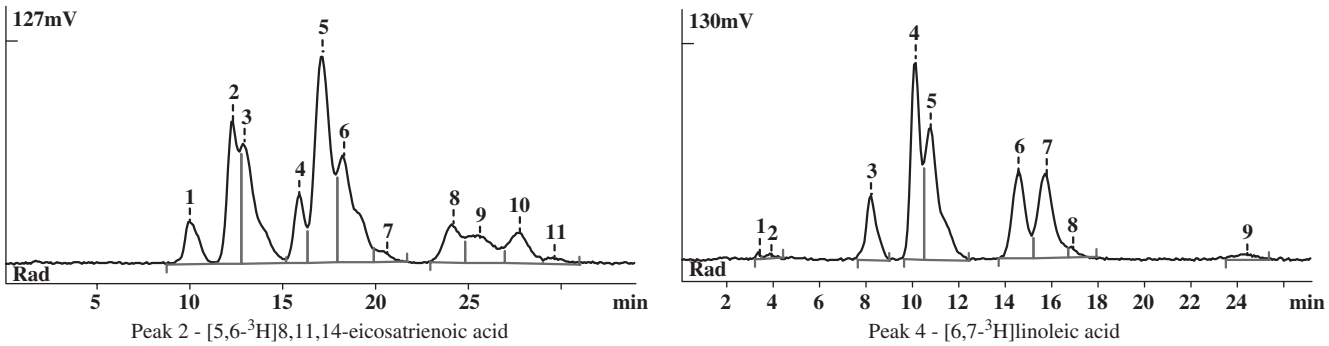

Peak 2 - [5,6-^{3}H]8,11,14-eicosatrienoic acid

Peak 4 - [6,7-^{3}H]linoleic acid

Figure 1 Selective hydrogenation of arachidonic acid (left) and γ-linolenic acid (right) with tritium gas (Lindlar catalyst, solvent–dioxane). HPLC conditions: radioactivity trace, Kromasil 100C_{18} 6.0 μm 4.0 × 150 mm, methanol–water–TFA (90:10:0.1), 1.0 ml/min.

cluster growth from 1 to 3 molecules ($n = 1-163.1$, $n = 2-196.6$, n=3–220.7 kcal/mol).[6] So, minimizing the tritiated water content in the solution to a reasonable value and careful drying of solvents, catalyst and substrate are necessary not only to prevent the isotopic dilution,[7] but also to decrease the effective size of water clusters on the surface of the catalyst thus increasing the efficiency of the isotopic exchange process. Finally, the specific activity of the resulting compound depends upon the competition between the tritiated water and the substrate molecules on the active centers, and in some cases optimization of the substrate-catalyst-tritiated water ratio is necessary.

This method was applied to prepare tritium labeled coenzyme A and acetyl coenzyme A with yields of 32 and 45%, and specific activities of 3.8 and 9.2 Ci/mmol, respectively.

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REFERENCES

1. Bishop JE, Hajra AK. *Anal Biochem* 1980; **106**: 344–350.
2. Shevchenko VP, Nagaev IYU. *Russ J Bioorg Chem* 1999; **25**(7): 427–441.
3. Rosanov VV, Krylov OV. *Russ Chem Rev* 1997; **66**(2): 107.
4. Shevchenko VP, Nagaev IYU, Myasoedov NF. *Radiochemistry* 2005; **47**(4): 403–409.
5. Shevchenko VP, Nagaev IYU, Shevchenko KV, Myasoedov NF. Synthesis of tritium labelled biologically active compounds for research of actual problems in biology and medicine. In *Isotopes in Medicine*, (vol. 2), Baranov VY (ed.). PhysMatLit: Moscow, 2005; 484–538.
6. Borisov YA, Zolotarev YA. *Russ J Phys Chem* 2002; **76**(4): 635.
7. Shevchenko VP, Nagaev IYU, Myasoedov NF. *Radiochemistry* 1999; **41**(1): 85–88.