A Micro-scale Synthesis of (2-^{14}C)- and (methyl-^{14}C)-5-Methyl-2′-deoxycytidine from Radioactive Thymidine Analogues

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Modification of DNA-cytosine by a 5-methyl group is thought to be an important mechanism which regulates the expression of eukaryotic genes.[1] This modification takes place after semiconservative replication.[2] There is very little evidence, if any, that 5-methyl-2′-deoxycytidine (5MedCyd) could be naturally incorporated into mammalian DNA in semiconservative replication. In order to clarify the possibility of incorporating 5MedCyd pharmacologically into human leukemic cells the synthesis of (2-^{14}C)- and (methyl-^{14}C)-5MedCyd has been performed starting from commercially available ^{14}C-thymidine (Thd).

Experimental. (2-^{14}C)-5MedCyd and (methyl-^{14}C)-5MedCyd were prepared from formamide and (2-^{14}C)-Thd or (methyl-^{14}C)-Thd (The Radiochemical Centre, Amersham, England), respectively, essentially according to the procedure of Vorbrüggen and coworkers.[3] Formamide (10 μl, 0.25 mmol), (^{14}C)-Thd (7.4 MBq) and hexamethyldisilazane (100 μl, 0.48 mmol) were heated in a glass ampoule at 140 °C for 76 h. The reaction mixture was then refluxed with absolute methanol (0.8 ml, 20 mmol) for 3 h. The reaction products were rigorously purified using TLC plates. The first purification step was performed on cellulose plates with butanol–water (86:14). The 5MedCyd spots were localized under appropriate UV-light, scraped from the plate and extracted carefully with water. The cellulose particles were centrifuged (9000×*g*, 2 min) and the supernatant was mixed with two volumes of methanol and evaporated to dryness using a nitrogen gas flow. The reaction product was then redissolved in 50 % methanol and chromatographed on cellulose plates using butanol–water–methanol–ammonia (60:20:20:1). The 5MedCyd spots were scraped off, extracted and handled as described above. The final product was dissolved in 20 mM potassium phosphate buffer, pH 7.4, and stored in 2 % ethanol at –20 °C until used. The distribution of radioactivity at both purification steps was analyzed by cutting the chromatography plates and counting the radioactivity using a Wallac scintillation spectrophotometer. The UV-spectrum of the reaction products was recorded with a Varian Cary 118C spectrophotometer.

Results and discussion. The R_f-values of the radioactive derivatives were identical to those of the authentic external markers. The conversion of (^{14}C)Thd to (^{14}C)5MedCyd was performed in the same way independent of starting material. The mother compound and the product counted for more than 80 % of the radioactivity recovered.

The UV-spectra of the purified products and non-radioactive 5MedCyd corresponded. The overall yield of both compounds was 25 %, respectively. The calculated specific activities compared well with the specific activities given for the mother compounds by the manufacturer: (2-^{14}C)-derivatives, 1.8 GBq/mmol (5MedCyd) *vs.* 2.0 GBq/mmol (Thd) and (methyl-^{14}C)-derivatives, 2.0 GBq/mmol (5MedCyd) *vs.* 2.2 GBq/mmol (Thd).

1. Hattman, S. In Boyer, P. D., Ed., *The Enzymes, Vol. XIV, Nucleic Acids, Part A,* Academic, New York 1981, p. 517.
2. Bird, A. P. *J. Mol. Biol. 118* (1978) 49.
3. Vorbrüggen, H., Krolikiewicz, K. and Niedballa, U. *Justus Liebigs Ann. Chem.* (1975) 988.

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