SYNTHESIS AND QUALITY CONTROL OF ^{125}I- AND ^{14}C-LABELLED 5-IODO-2'-DEOXYCYTIDINE TRIPHOSPHATE

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SUMMARY

A method is described for a no-carrier-added synthesis and purification of doubly-labelled [5-^{125}I, U-^{14}C]-iododeoxycytidine triphosphate. Iodination of ^{14}C-dCTP was achieved with a radiochemical yield of 80%. Separation and purification of the reaction product were accomplished by reversed-phase high performance liquid chromatography (RP-HPLC).

Key words: No-carrier-added doubly-labelled [5-^{125}I, U-^{14}C]-IdCTP, RP-HPLC.

INTRODUCTION

As direct precursors of DNA, deoxyribonucleoside 5'-triphosphates (dNTPs) have become important substrates in nucleic acid chemistry and molecular biology. Radioactively-labelled dNTPs have become more and more essential for DNA hybridization techniques and recombinant DNA studies. Many of these are already commercially-available as singly-labelled compounds with various β^--emitting isotopes. Research on the chemical and biological effects of the radioactive decay of iodine-125 at a molecular level which is being carried out in this institute required doubly-labelled DNA constituents. As a continuation of previous

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work on the chemical consequences of iodine-125 decay in 5-iodouracil,[1] we have examined techniques for synthesizing doubly-labelled IdCTP in order to study the type, extent and distribution of damage in DNA caused by the Auger effect following the EC-decay of iodine-125 (for a review cf. 2). In particular, we wished to find out whether the complete fragmentation of the pyrimidine ring which had been observed in iodouracil also occurs in DNA. Several procedures have already been published on the iodination of dCTP.[3-6] These are either inappropriate for carrier-free radioiodination because of the use of molecular iodine,[3] because they apply time-consuming purification procedures[5,6] or because they result in rapidly-deteriorating products.[5]
The procedure presented in this paper leads to preparations which are not only of high radiochemical yield, but are also free of buffering agents.

Methods and material

Iodine-125 was purchased from New England Nuclear as no-carrier-added $Na^{125}I$ in dilute NaOH solution. [U-^{14}C]-dCTP, obtained from the Radiochemical Centre Amersham, was purified thoroughly before use. Two purification steps: a) reversed-phase chromatography (RP 18), using water as eluent and b) ion-exchange chromatography (Aminex A25), using $(NH_4)_2CO_3$-solution as eluent,[7] were necessary to remove interfering impurities. Inactive dCTP was obtained from Boehringer Mannheim, Aminex A25 ion-exchange material was from Bio Rad, Munich. All other reagents were of analytical grade from Merck, Darmstadt.

Separation and purification of the labelled products were performed by means of high performance liquid chromatography on an apparatus equipped with a Latek injection valve and a UV-absorbance detector from Waters Associates (Model 440).

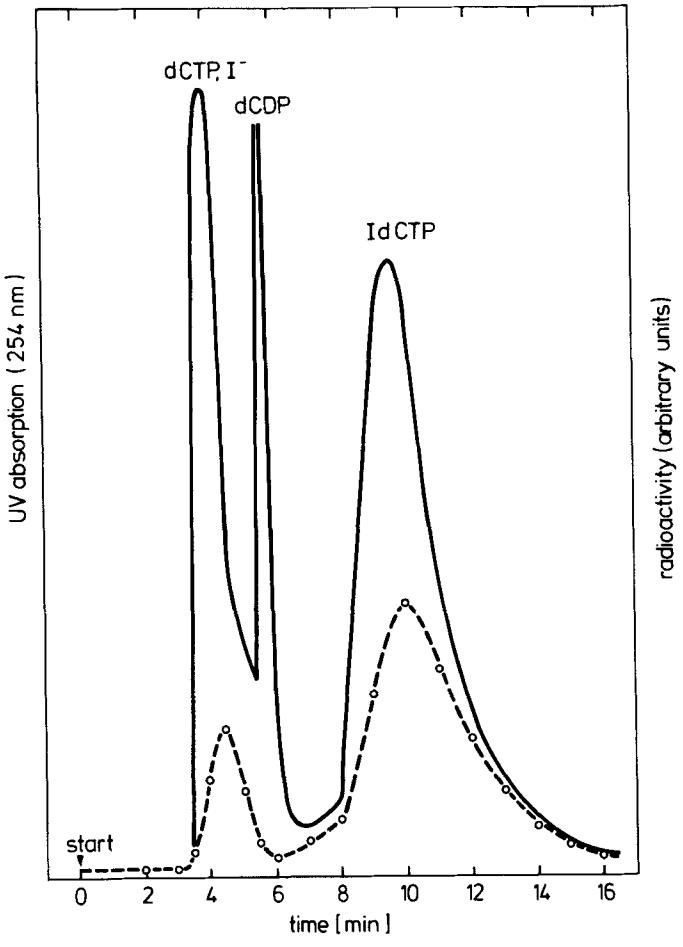

Fig. 1 UV_{254}-absorption (solid line) and radioactivity profile (dashed line) following HPLC separation of the iodination reaction mixture containing dCTP (conditions see text).

Iodination procedure and purification

The procedure based on the iodination reaction of Commerford for nucleic acids,[8] using Tl^{3+}-ions as oxidizing agent. The optimized reaction started from 1.1 MBq (31 µCi) of purified [U-^{14}C]-dCTP (~90 nmol) in 400 µl 0.3 M acetic acid - 0.05 M sodium acetate (pH 3.8). 3.5 GBq (95 mCi) carrier-free $Na^{125}I$ and finally 100 µl Tl(III)acetate (1 mg/ml acetate buffer) were added at room temperature. The mixture was heated to 60 °C in a sealed reactivial. After 90 minutes, the reaction was stopped by the addition of 20 µl $Na_2S_2O_5$ (10 mg/ml). The sample was injected onto an HPLC column (250 x 4 mm; 10 µm LiChrosorb RP 18) and eluted with 0.05 M ammonium phosphate buffer, pH 3.2. Figure 1 shows a typical separation profile. Aliquots of the eluate fractions were measured discontinuously on an Auto-Gamma-Scintillation spectrometer (Packard 5375). The pooled IdCTP peak is evaporated to dryness. The residual ammonium phosphate is removed by a second chromatographic step (column: 250 x 4 mm, 10 µm LiChrosorb RP 18) using triply-distilled water as eluent. The overall product yield is 70-80 %.

RESULTS AND DISCUSSION

Before performing the double-labelling procedure, the reaction conditions were optimized. Figure 2 shows the radiochemical yield as function of pH value. The curve clearly shows a sharp maximum at pH 4 which drops steeply at both more acidic and more basic pH values. As the optimum pH range of the reaction is rather narrow, it is important for the success of the radiochemical synthesis to know the concentration of NaOH in the

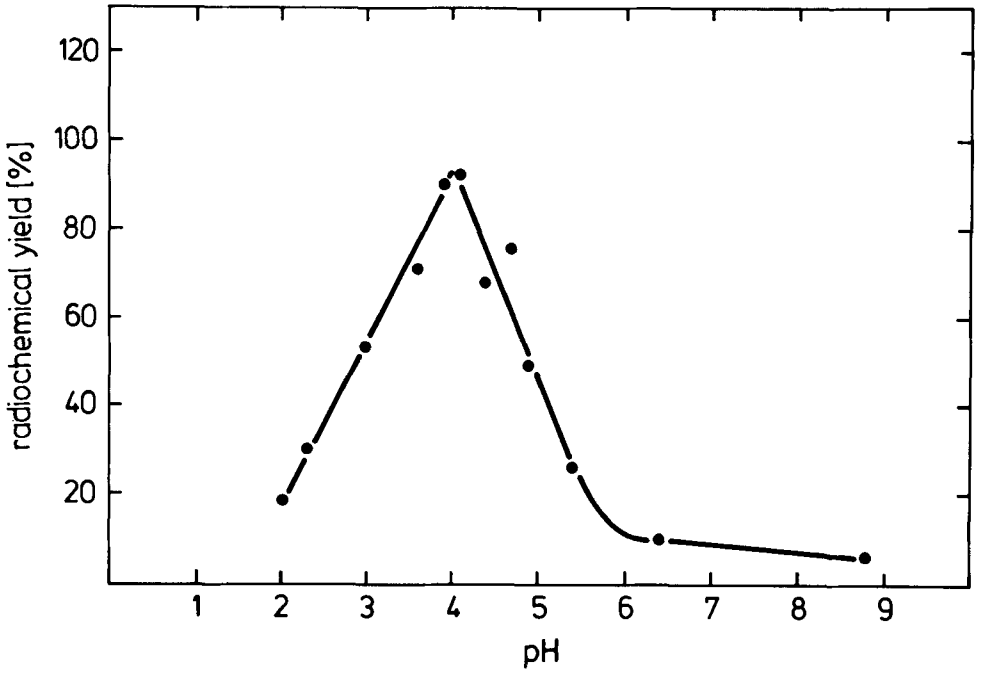

Fig. 2 Variation of yield as function of pH. 200 nmol dCTP, 120 nmol NaI, 500 nmol Tl(III)acetate in 1000 µl 0.35 M acetate buffer of varying pH; reaction time: 120 min, T = 60 °C.

$Na^{125}I$ solution used and to apply a buffer with sufficient buffering capacity.

The influence of Tl(III) acetate concentration on the yield of the iodination reaction at pH 4 is demonstrated in Fig. 3. No iodination takes place without the presence of oxidizing agent. Increasing the molarity of Tl(III)acetate to that of NaI results in a linear increase of the iodination product up to 56%. The curve then flattens, although it still rises with a slower

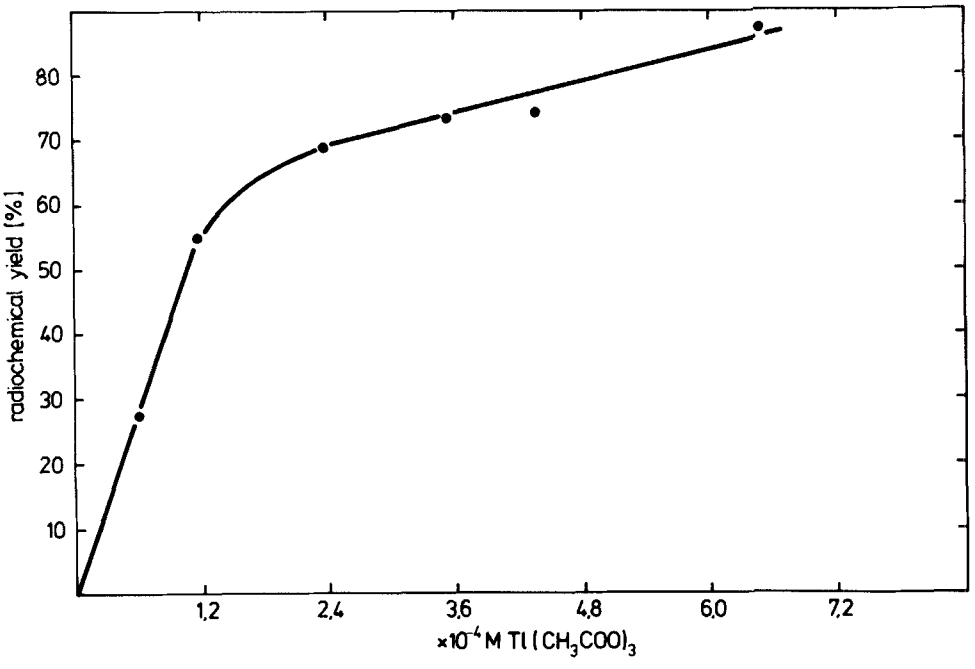

Fig. 3 Variation of iodination yield as function of the concentration of oxidizing agent. Other conditions: 200 nmol dCTP, 120 nmol NaI in 0.3 M acetic acid - 0.05 M sodium acetate, total volume: 1 ml, reaction time: 120 min, T = 60 °C, pH 4.

slope. The course of the curve shows that even a large excess of Tl^{3+} ions does not lead to interfering side reactions. The further slow increase is probably caused by the removal of traces of disturbing impurities by oxidation.

The dependence of the relative (i.e. radiochemical) yield on the NaI:dCTP ratio is plotted in the upper curve of Fig. 4. It is clear from the graph, that for a fixed amount of dCTP the

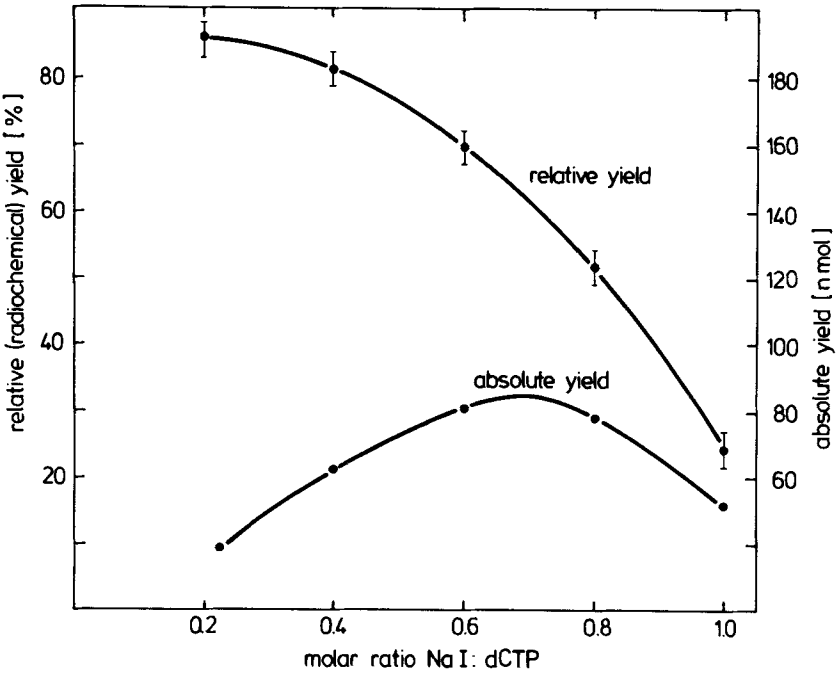

Fig. 4 Dependence of relative and absolute yield on NaI:dCTP ratio. Other conditions: 200 nmol dCTP in (0.3 M acetate buffer pH 4) Tl(III)-acetate concentration 6 times the iodide concentration but not less than 200 nmol. Total volume: 1 ml, reaction time: 120 min, T = 60 °C.

yield decreases with increasing amount of NaI. For double-labelling one has to optimize the reaction conditions for both radioactive reactants. This is shown in the lower curve of Fig. 4, in which the best results of double-labelling are achieved with NaI:dCTP ratios of 0.6-0.7.

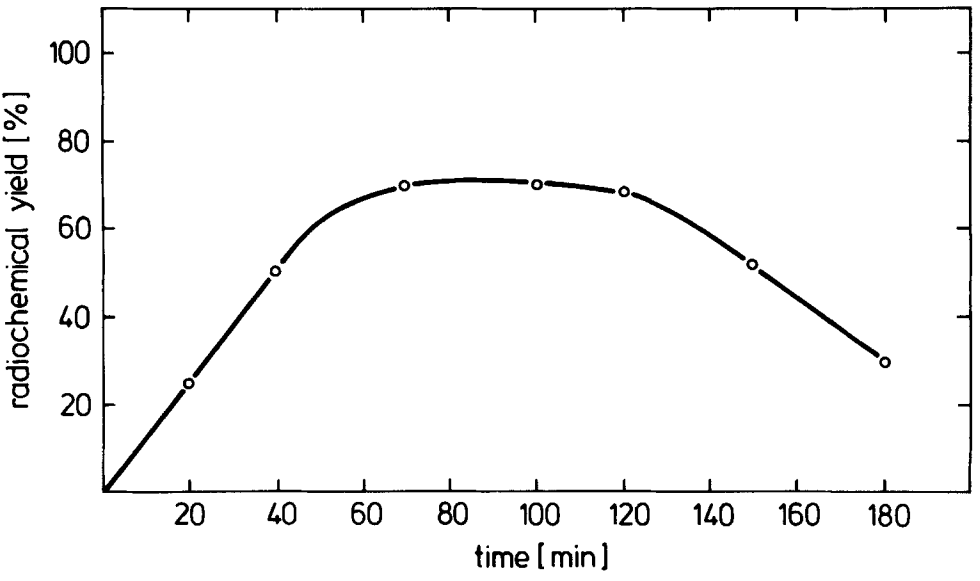

Fig. 5 Time dependence of iodination yields of dCTP. Other conditions: 200 nmol dCTP, 120 nmol NaI, 600 nmol (Tl(III)acetate in 1000 µl 0.3 M acetic acid - 0.05 M sodium acetate, T = 60 °C.

Variation of reaction time showed (Fig. 5) that a minimum of 70 minutes is needed to obtain maximal yield at 60 °C reaction temperature. After this time, the yield remains constant for reaction times of about one hour. Then it tends to decrease rapidly (at least at high Tl^{3+} concentrations). Side reactions such as oxidation of the aromatic ring seem to become more significant with longer reaction times.

Termination of the reaction with $Na_2S_2O_5$ is indispensable when

working with radioactive iodine. However, it should be mentioned that prolonged action of this reducing agent on the reaction mixture leads to considerable deiodination and other side reactions.

The procedure described in this work has proven to be suitable for rapid preparation and subsequent purification of doubly-labelled [U-^{14}C, 5-^{125}I]-IdCTP. Material prepared in this manner was free of starting material and mono- or diphosphorylated nucleosides. As a substrate for DNA synthesis the product was successfully incorporated in vitro into plasmid DNA.

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REFERENCES

1. Deutzmann, R. and Stöcklin, G. - Radiation Research 87: 24-36 (1981)
2. Halpern, A. and Stöcklin, G. - Radiat. Environ. Biophys. 14: 167-183 (1977)
3. Chang, P.K. and Welch, A.D.J. - J. Med. Chem. 6: 428 (1963)
4. Springl, M., von der Haar, F., Schlimme, C., Sternbach, H. and Cramer, F. - Eur. J. Biochem. 25: 262 (1972)
5. Scherberg, N.H. and Refetoff, S. - Biochim. Biophys. Acta 340: 446 (1974)
6. Bhalla, R.B., Geraci, D., Modak, M.J., Preusky, W. and Marcus, S.L. - Biochem. Biophys. Res. Comm. 72: 513 (1976)
7. Linz, U. - in preparation
8. Commerford, S.L. - Biochem. 10: 1993 (1971)