# AN IMPROVED METHOD FOR SYNTHESIS AND PURIFICATION OF $^{125}I$ OR $^{131}I$ LABELED CARRIER-FREE 5-IODO-2'-DEOXYURIDINE

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SUMMARY

Consistently high yields (up to 95%) of carrier-free 5-iodo-2'-deoxyuridine-$^{125}I$ (or $^{131}I$) can be obtained by iodination of 2'-deoxyuridine at varying concentrations of radioactive NaI (1-10 mCi), as long as the molarity of deoxyuridine in the reaction mixture is kept at or above $10^{-2}$ M. At lower deoxyuridine molarities the reaction yield is significantly reduced. Several different purification procedures (Biogel P-2 400 mesh or Sephadex G-10 column chromatography, paper chromatography with water-saturated ethyl acetate or water-saturated butanol) are described. The two column chromatography systems yield iododeoxyuridine of > 95% chemical and radiochemical purity. The two paper chromatography systems are somewhat more involved, but yield $^{125}I$ or $^{131}I$ labeled 5-iodo-2'-deoxyuridine of > 99% chemical and radiochemical purity.

Key Words: 5-iodo-2'-deoxyuridine-$^{125}I$ (or $^{131}I$), halogenated pyrimidine, synthesis, purification

## INTRODUCTION

Radioactive 5-iodo-2'-deoxyuridine (IUdR), an analogue of thymidine (1,2), is widely used for labeling the DNA of proliferating cells (3,4,5), for monitoring proliferation, migration, and death of normal and neoplastic cells (3,6), and for evaluating the effectiveness of various chemotherapeutic or radiotherapeutic procedures in the treatment of experimental tumors (7). IUdR is preferred for such studies because, unlike $^{3}H$ or $^{14}C$ labeled DNA

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precursors, it can be labeled with gamma or X-ray emitting radionuclides such as $^{125}I$ or $^{131}I$ ($^{125}$IUdR, $^{131}$IUdR). This obviates the need for time-consuming digestion and solubilization of labeled cells and permits assays of intact tissues or whole animals. Another advantage of IUdR is the fact that following death and metabolic breakdown of labeled cells the rate of IUdR reutilization is much lower than is the case with $^{3}H$ or $^{14}C$ labeled thymidine (8).

In addition to the research applications outlined above, $^{125}$IUdR is currently being considered as a possible radiotherapeutic agent in clinical cancer therapy. Reports from various laboratories indicate that $^{125}$IUdR incorporation into the DNA leads to highly selective irradiation of the nucleus of proliferating cells (9,10,11). This produces pronounced biological damage because the genetic material in the cell nucleus constitutes the most radiosensitive target within the cell (12). Based on these findings the International Atomic Energy Agency in Vienna is currently initiating a coordinated research program designed to evaluate the potential of $^{125}$IUdR in human cancer therapy (13).

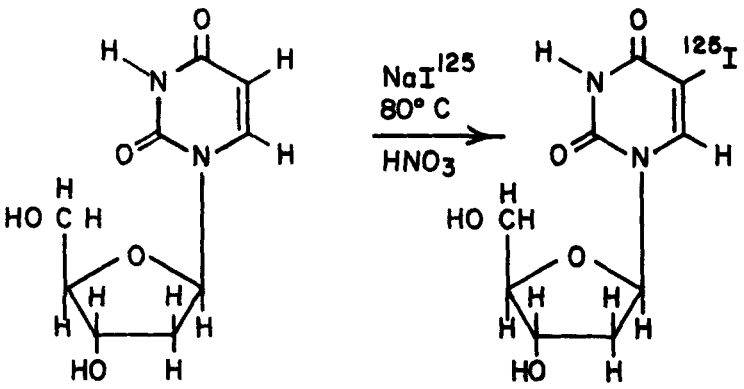
A major area of concern in many of these applications is the potential distortion of experimental data resulting from the presence of large quantities of nonradioactive IUdR ($^{127}$IUdR) in commercial samples of radioactive IUdR. Recent reports indicate that mammalian tissue culture cells can be damaged by IUdR concentrations as low as $10^{-7}$ M (9). The use of carrier-free IUdR would reduce or eliminate such pharmacological side-effects by minimizing thymidine substitution in the DNA. Carrier-free IUdR also would increase the labeling efficiency, because competitive inhibition resulting from the presence of cold IUdR would be eliminated.

The theoretical maximum specific activity of carrier-free IUdR is 2,200 Ci/mmol for $^{125}$IUdR and 16,100 Ci/mmol for $^{131}$IUdR. Unfortunately, $^{125}$IUdR is not supplied in carrier-free form and

$^{131}$IUdR is no longer commercially available. To avoid these difficulties and the ever-increasing price of commercially produced $^{125}$IUdR, we have developed a simple, inexpensive procedure for synthesizing and purifying carrier-free radioactive IUdR.

## MATERIALS AND METHODS

Synthesis procedure: The synthesis of IUdR was carried out basically as described by Prusoff (1) and Hughes, et al. (3), with slight modifications to obtain the high specific activity and high yield desired. The detailed procedure was as follows: 10 mCi of carrier-free Na$^{125}$I (or Na$^{131}$I with a specific activity of 2,200 Ci/mmol; New England Nuclear Corporation) and 22.8 mg of deoxyuridine (UdR, Sigma Chemical) were dissolved in 0.1 ml of distilled deionized $H_2O$. An equal volume of 2 N $HNO_3$ was added. The reaction mixture (total volume 0.2 ml) was then placed in a closed vial and heated for 30 min in an 80°C water bath. After heating, the solution was neutralized with conc. $NH_4OH$. The whole procedure was carried out in a hood to protect the worker from volatile radioactivity.




Purification techniques: Separation of the reaction mixture and purification of $^{125}$IUdR (or $^{131}$IUdR) was accomplished by two different procedures:

(a) Gel-filtration column chromatography: The reaction mixture was layered on a 120 cm column (1 cm ID) of Biogel P-2 400 mesh (BioRad Laboratories) or Sephadex G-10 (Pharmacia Fine Chemicals). The eluant was 0.1 M sodium citrate (pH 4.5). As can be seen from the elution pattern shown in Fig. 1, this procedure provided adequate separation of IUdR from the unreacted NaI and UdR. The radiochemical purity of IUdR obtained in this manner was 95% or higher. This degree of purity proved adequate for most biological experiments carried out in our laboratory. For some biological applications the presence of 0.1 M sodium citrate was undesirable. To eliminate this, the IUdR fractions were evaporated to .5 ml, layered on a desalting column of Biogel P-2 or Sephadex G-10, and eluted with distilled $H_2O$.

(b) Paper chromatography: This method of separation was somewhat more involved, but yielded IUdR of significantly higher purity. The initial reaction mixture, after cooling and neutralization, was evaporated to 0.1 ml and spotted along a 10 x 20 cm strip of cellulose chromatography paper (Eastman Chromagram Sheet). Although a number of solvent systems provided successful separation, the two preferred ones were $H_2O$-saturated ethyl acetate and $H_2O$-saturated 1-butanol (for Rf values see Table I). These solvent

TABLE I. Rf VALUES ON CELLULOSE

| | Solvents | | |
|---|---|---|---|
| | I | II | III |
| IUdR | .53 | .75 | .65 |
| UdR | .07 | .88 | .44 |
| NaI | 1.00 | .92 | .95 |

Solvent: I - ethyl acetate saturated with $H_2O$; II - 0.05 M sodium formate pH 3.5; III - 1-butanol saturated with $H_2O$.

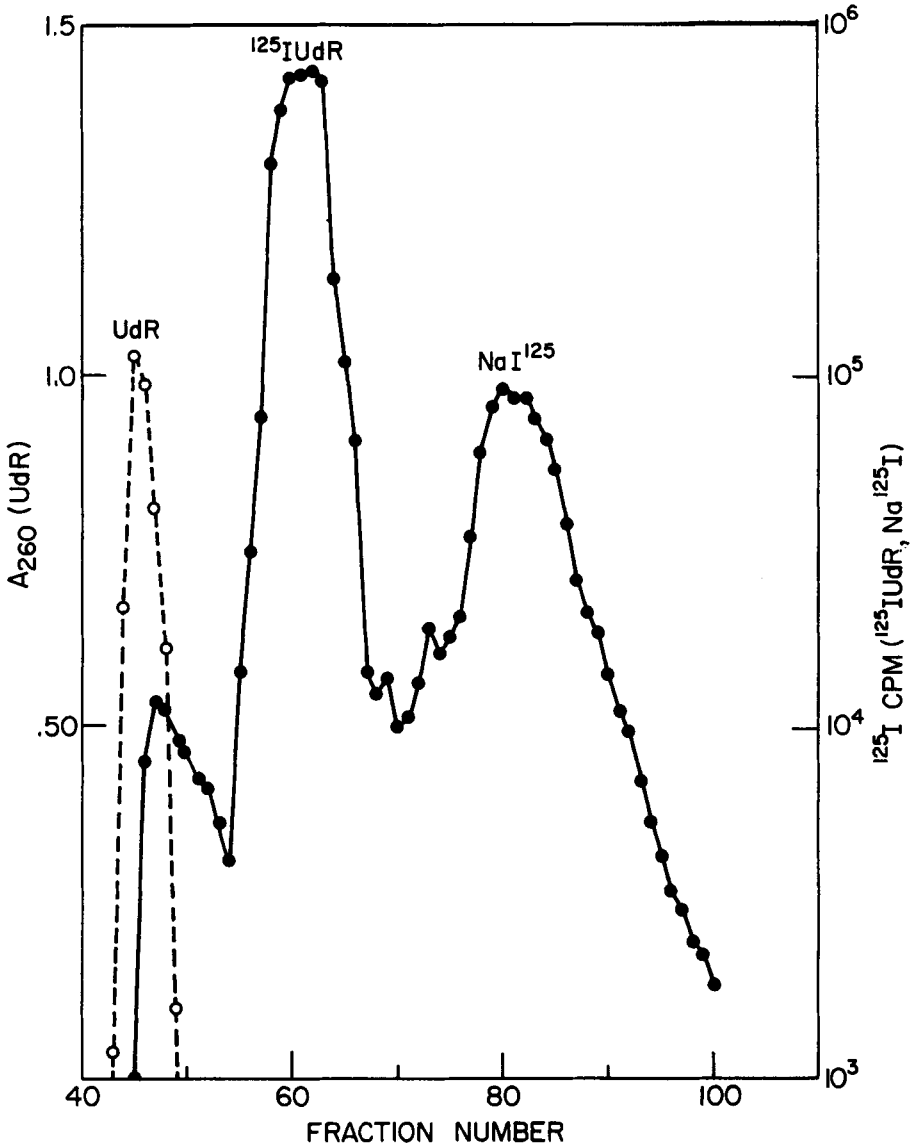



Fig. 1. Elution profile of the IUdR reaction mixture on Biogel P-2 400 column.

systems have the advantage of being volatile and the added convenience of a relatively fast developing time. After development, the chromatogram was dried and the strip containing the radioactive IUdR was cut out. The IUdR was eluted off the strip by descending chromatography using distilled $H_2O$ as the eluant.

## RESULTS AND DISCUSSION

Radiochemical yield. Using the synthesis and purification techniques described above, 90-95% of the radioactive iodine was routinely recovered in the form of radioactive IUdR. Reducing the amount of radioactive NaI from 10 mCi to 1 mCi did not significantly change the yield, but reducing the concentration of deoxyuridine in the reaction mixture drastically reduced the radiochemical yield (Table II).

TABLE II. EFFECT OF 2'-DEOXYURIDINE CONCENTRATION ON IUdR YIELD.

| UdR molarity | Reaction yield* | UdR molarity | Reaction yield* |
|---|---|---|---|
| $10^0$ | 95% | $10^{-4}$ | 48% |
| $10^{-1}$ | 87% | $10^{-5}$ | 38% |
| $10^{-2}$ | 74% | $10^{-6}$ | 25% |
| $10^{-3}$ | 64% | $10^{-7}$ | 14% |

*Reaction yield expressed as percent of radioactive iodine.

Radiochemical purity. Unincorporated radioactive iodine was easily and completely separated from radioactive IUdR by column chromatography or paper chromatography. Complete removal of unreacted UdR proved more difficult. Control experiments using nonradioactive IUdR mixed with 2'-deoxyuridine-$^{14}C$ (New England Nuclear Corporation) indicated that in Biogel P-2 columns about

0.001-0.01% of the 2'-deoxyuridine-$^{14}C$ tailed into the IUdR peak. The presence of such small amounts of UdR did not affect in vivo incorporation of radioactive IUdR, but it did adversely affect incorporation in certain tissue culture experiments (14).

The problem of UdR contamination in tissue culture experiments was alleviated by repeated column chromatography or by ascending paper chromatography. Both the ethyl acetate system and the butanol system gave complete separation between IUdR and UdR. Control experiments with mixtures of 2'-deoxyuridine-$^{14}C$ and cold IUdR revealed no detectable traces of $^{14}C$ activity in the IUdR region, i.e., the maximum UdR concentration in the IUdR spot had to be less than $10^{-12}$ moles. This degree of chemical and radiochemical purity proved sufficient for the most stringent chemical and biological applications.

## ACKNOWLEDGEMENTS

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