EFFECT OF REACTION TEMPERATURE ON ENZYME CONVERSION OF PYRIMIDINE BASES TO NUCLEOSIDES AND 2´- DEOXYNUCLEOSIDES

J. Filip, L. Přitasil, Z. Nejedlý, J. Veselý[+] and A. Čihák[+]

Institute for Research, Production and Uses of Radioisotopes,
Prague 7, Czechoslovakia

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SUMMARY

Conversions of uracil to uridine and of uracil and thymine to the corresponding 2´-deoxyribonucleosides catalyzed by the cell-free extract of Escherichia coli B take place both at 37°C and 2°C. The yield of nucleosides formed during the short-term incubation was higher at 37°C while during long-term incubation it was higher at 2°C with the implication that the degradation of ribose- and 2´-deoxyribose-1-phosphates in reaction mixtures kept at lower temperature is less considerable. The amount of 2´-deoxyribonucleosides formed at 2°C was higher than that of ribonucleosides.

[+]/Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

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INTRODUCTION

The high specific activity of various components of nucleic acids is indispensable especially for the experimental studies in the field of modern biochemistry and molecular biology. Consequently, different methods of enzymatic synthesis of deoxyribonucleosides labelled in the base moiety[1-4] have been worked out not only for the natural precursors but also for their derivatives[4,5]. Using bases as starting compounds for the synthesis of deoxyribonucleosides two synthetic reactions have been proposed, namely, the catalytic transformation using trans-N-deoxyribosylase[3,6-8] or nucleoside phosphorylase with low substrate specifity[9]. A second enzyme was isolated from a number of sources[1,9] including E. coli B[10,11].

In the present work we have investigated the synthesis of ^{14}C labelled 2-deoxy-and ribonucleosides in relation to the duration of the incubation period and temperature. As a source of the enzyme unpurified cell-free extract from E. coli B was used. It has been established that both types of compounds are formed also at $2^{\circ}C$ at a very considerable yield.

MATERIALS AND METHODS

Chemicals. Thymine-[2-^{14}C] /50 /uCi/umol/ and uracil-[2-^{14}C] /50 /uCi/umol/ were prepared in the Institute for Research, Production and Uses of Radioisotopes, Prague. 2´-Deoxyuridine, thymidine, α-D-ribose-1-phosphate and 2´-deoxy-α-D-ribose-1-phosphate, both di-/cyclohexylammonium/ salts, were obtained from Calbiochem, Luzern.

Preparation of E. coli cell-free extract. Cultivation of E. coli B was carried out at 37°C for 16 h in a volume of 4 litres or a synthetic medium containing glucose[12]. For the inoculation 24 hour-old E. coli B culture /100 ml/ was used. Bacteria were centrifuged, washed with cold 0,9% NaCl, suspended in 40 ml of 50 mM Tris-HCl buffer /pH 7,5/, and sonicated /70 sec, 2°C, 1,5 kc/. The sonicate was centrifuged /100 000 g, 2 hours, 2°C/ and the supernatant fraction, stored at -20°C for 7-10 days without detectable decrease of activity, was used as a source of enzymes.

Incubation and analysis of reaction mixture. Incubations were carried out at 37°C, 20°C or 2°C, in 100 mM Tris-HCl buffer, pH 7,4, in the presence of the appropriate substrate /4 /umol/ and 2-deoxyribose- or ribose-1-phosphates /12 /umol/ in a total volume 1 ml. Aliquots of the mixture withdrawn at various time intervals were analyzed by chromatography on Whatman paper No. 1 in a solvent system composed of isobutyric acid-ammonium hydroxide-water /44:1:22/. Radioactive compounds on chromatograms were located according to position of standards, cut out and their radioactivity was measured with a Packard Liquid Scintillation counter.

RESULTS AND DISCUSSION

The ability of unpurified cell-free extract prepared from E. coli to metabolize uracil to uridine is shown in Figure 1. It is apparent that the enzyme extract containing ribose-1-phosphate transforms uracil very effectively not only during incubation at 37°C but also at lower temperatures, and even at 2°C. The figure shows the course of reaction in relation to the amount of added enzyme preparation.

The transformation of uracil to 2-deoxyuridine in the cell-free extract supplemented with 2-deoxyribose-1-phosphate is even more pronounced than the conversion of uracil to uridine /Figure 2/. Uracil was transformed to its deoxyribonucleoside at 37°C and 20°C almost completely; at 2°C the conversion amountal to 75 percent after the period of 20 min.

The data presented in Figure 3 show the course of thymidine synthesis from thymine and 2-deoxyribose-1-phosphate carried out under similar experimental conditions. The transformation of thymine to 2-deoxyribonucleoside is almost complete; also in this instance the reaction is effective at 2°C.

The comparison of the synthesis of uridine, 2-deoxyuridine and thymidine during long-term incubation period using low concentrations of the enzyme is shown in Figure 4. It is apparent that 2-deoxyribonucleosides are formed from bases at higher rates than uridine from uracil. Furthermore the long-term incubation at 2°C is more advantageous for the total nucleoside synthesis. The reasons for this phenomenon are the lower degradation of

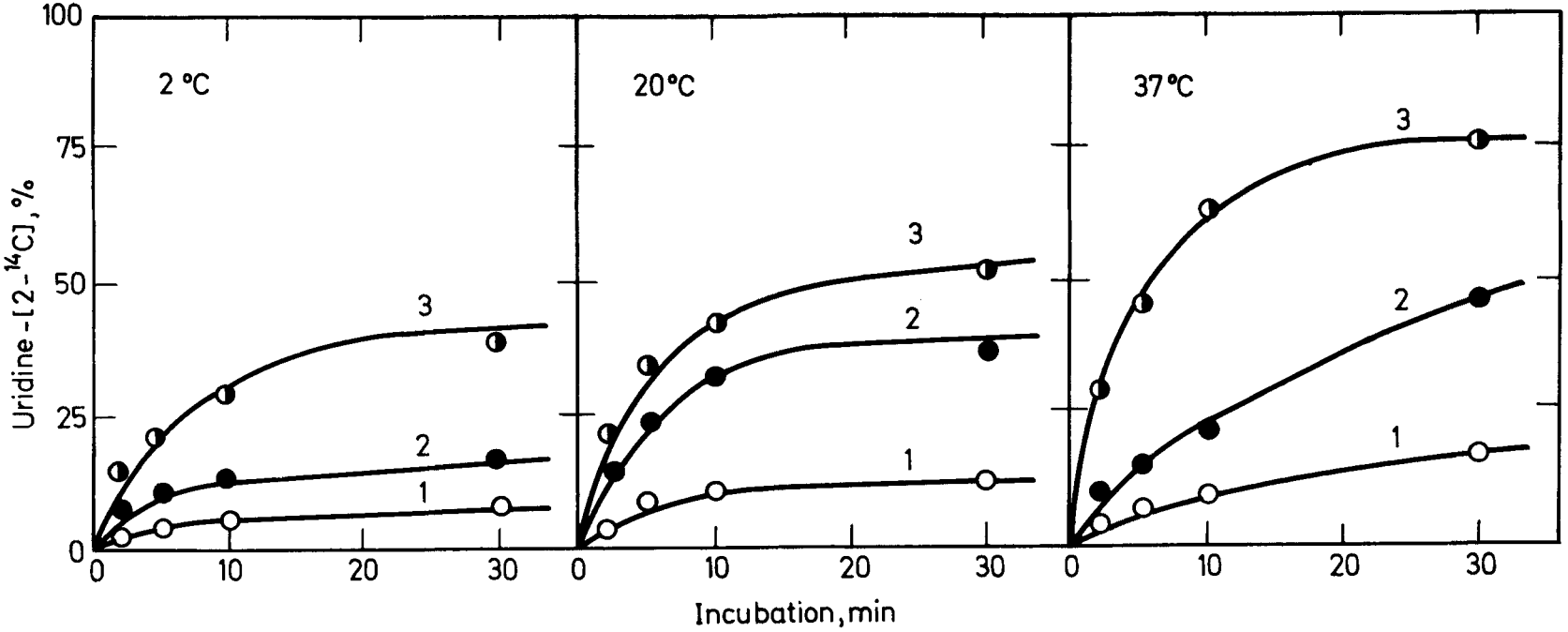

Figure 1. Synthesis of Uridine-[2-^{14}C] from Uracil- 2-^{14}C and Ribose-1-phosphate in Relation to the Amount of Enzyme Extract and Reaction Temperature

1, 0.03 ml; 2, 0.1 ml and 3, 0.5 ml of an enzyme extract in 1.0 ml of incubation mixture containing 4 mM uracil- 2-^{14}C , 12 mM ribose-1-phosphate and 100 mM Tris-HCl buffer /pH 7.5/.

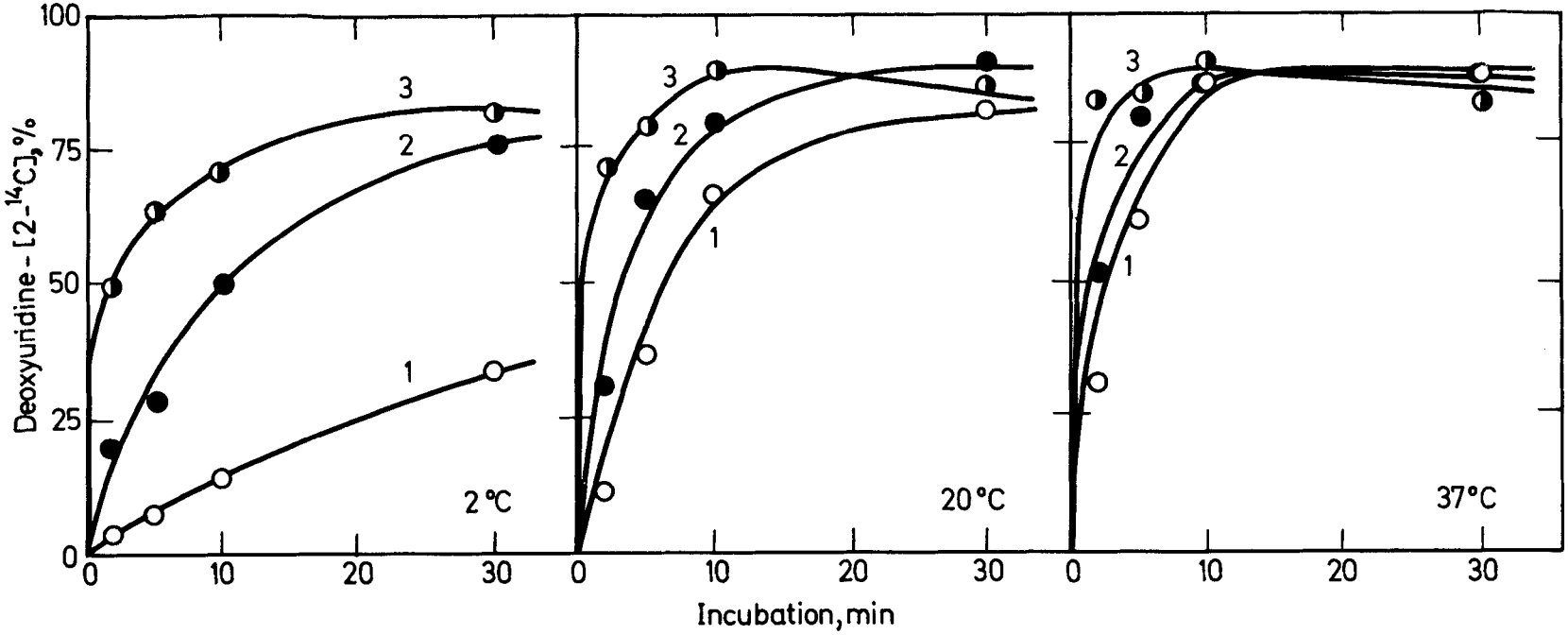

Figure 2. Synthesis of 2-Deoxyuridine-[2-^{14}C] from Uracil-[2-^{14}C] and 2-Deoxyribose-1-phosphate in Relation to the Amount of Enzyme Extract and Reaction Temperature

1, 0.03 ml; 2, 0.1 ml and 0.5 ml of an enzyme extract in 1.0 ml of incubation mixture containing 4 mM uracil-[2-^{14}C], 12 mM 2-deoxyribose-1-phosphate and 100 mM Tris-HCl buffer /pH 7.5/.

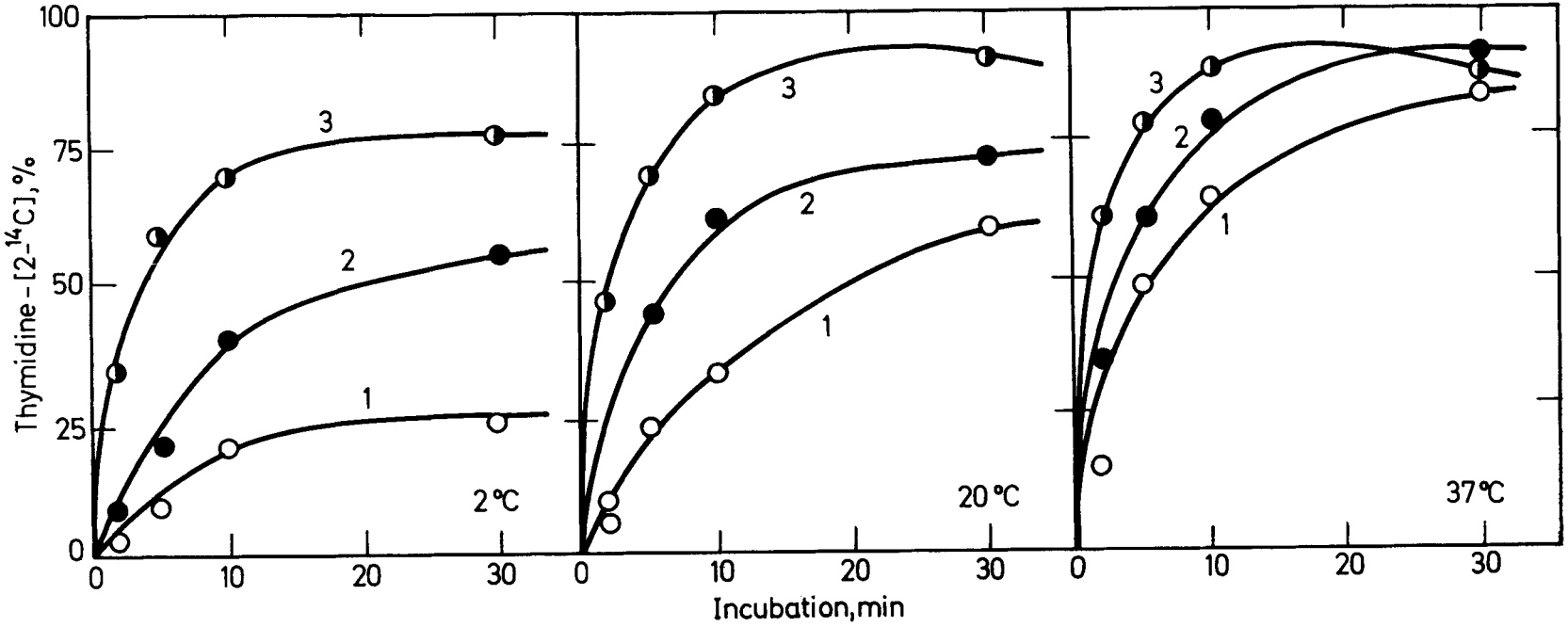

Figure 3. Synthesis of Thymidine-[2-^{14}C] from Thymine-[2-^{14}C] and 2-Deoxyribose-1-phosphate in Relation to the Amount of Enzyme Extract and Reaction Temperature

1, 0.03 ml; 2, 0.1 ml and 0.5 ml of an enzyme extract in 1.0 ml of incubation mixture containing 4 mM thymine-[2-^{14}C], 12 mM 2-deoxyribose-1-phosphate and 100 mM Tris-HCl buffer /pH 7.5/.

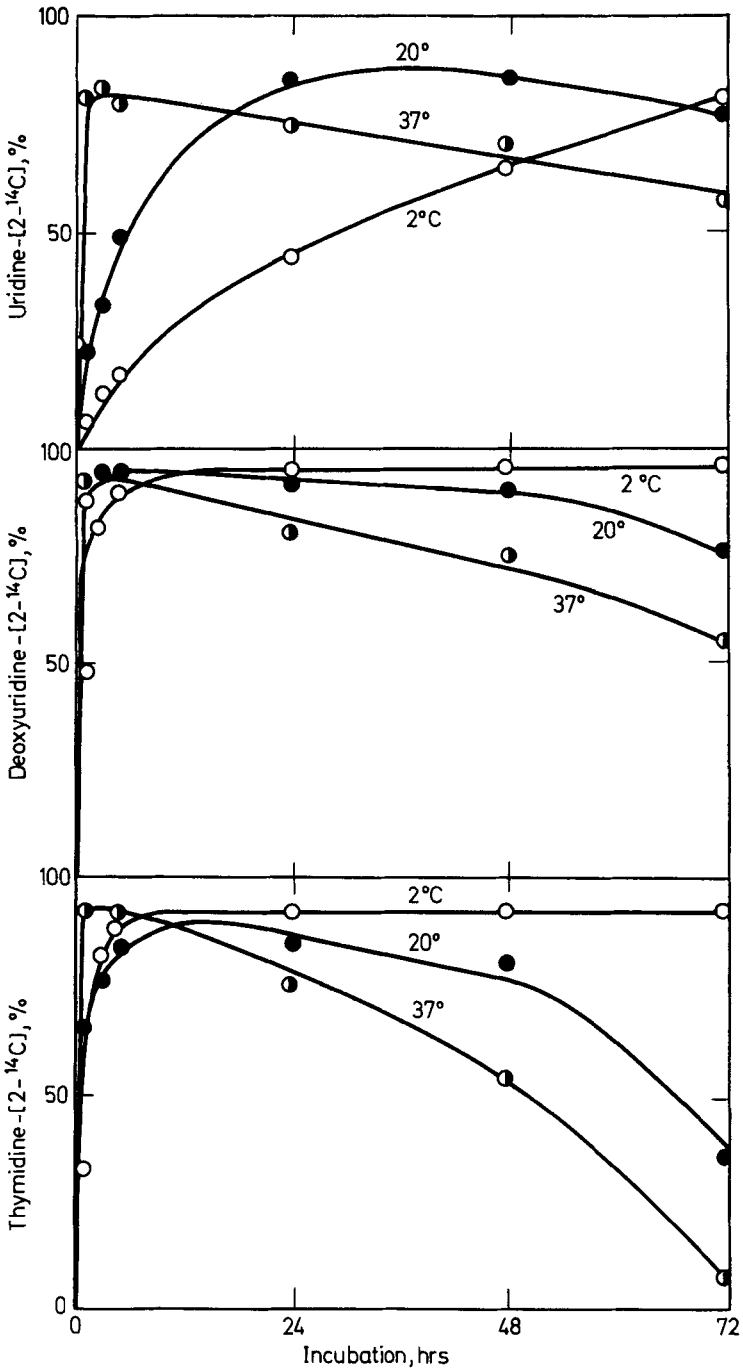

Figure 4. Synthesis of [^{14}C] -Labelled Uridine, 2-Deoxyuridine and Thymidine during Long-Term Incubation at Different Temperatures

ribose- and 2-deoxyribose-1-phosphates added to the incubation mixtures and the diminshed degradation of newly synthesized nucleosides.

The described highly effective conversion of pyrimidine bases to 2-deoxyribonucleosides during the long-term incubation period at 2°C can be utilized for the preparative synthesis of these compounds on an industrial scale using lower amounts of the enzyme preparation and less considerable concentrations of 2-deoxyribose-1-phosphate.

Incubation was carried out in 1.0 ml of 50 mM Tris-HCl buffer /pH 7.5/ using 4 mM [^{14}C]-labelled substrates, 12 mM sugar donors and 0.03 ml of an enzyme extract.

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