RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE FROM Escherichia coli : A NEW APPROACH TO THE PROBLEM OF STEREOSPECIFICITY

Serge David and Claudine Rouzeau*, Laboratoire de Chimie des Composés Biologiques, Bât. 420, Université de Paris-Sud, 91-Orsay, (France).

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Starting with [2'-^{3}H]-uridine the nucleoside [2'-^{3}H]CMP has been prepared which, in the presence of a partially purified system of E. coli, was phosphorylated and reduced to [2'-^{3}H]dCDP. This compound was successively transformed into dCMP, 2'-déoxycytidine, 2'-deoxyuridine, 3'-O-mesyl-5'-O-trityl-2'-deoxyuridine 5, 2,3'-anhydro-1-(2'-deoxy-5'-O-trityl-β-D-lyxosyl) uracil 6, 1-(3'-O-mesyl-5'-O-trityl-2'-deoxy-β-D-lyxosyl)uracil 9, and ultimately into 1(5'-O-trityl-2',3'-dideoxy-2'-ene-β-D-glycero-pentofuranosyl) uracil 7. The constancy of the specific activity in the trans eliminations 6 → 7 and 9 → 7, indicates that ^{3}H is cis to the leaving group, and that retention of configuration occurs in this enzymatic reduction.

The stereospecificity of the reduction of CDP to dCDP by the Escherichia coli B[1] system, of ATP to dATP by the Lactobacillus leichannii[2] system, and also of CTP to dCTP by the same organism[3], has been examined in each case in a similar manner ; the reaction is performed in ^2H_2O and the position of the ^2H, introduced on C-2', is deduced from the reduced nucleoside's NMR spectra. Because of the absence of deuterated model compounds, the conclusions rest on a theoretical approach which largely employs the Karplus equation[4], a relationship which, when applied to furanoses, has raised many problems. As a result in some cases [1,3] the conclusions have been reported as being tentative. In addition, the above method requires a relatively large quantity of product, and the large scale preparation of a very pure enzymatic system.

The method described here permits the localization of the reduced pyrimidine nucleoside's ^{3}H by chemical degradation. This could easily be applied to the enzymatic examination of a tumor from a single rat. With the E. coli system this method rigorously confirms the previously proposed orientation.

The reduction of 1-(3,5-di-O-trityl-β-D-erythro-furanosyl-2-ulose)uracil by means of sodium borotritide yielded, after detritylation and paper

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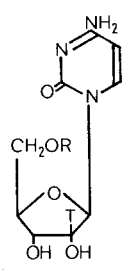
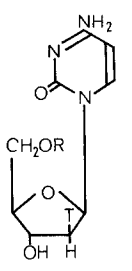
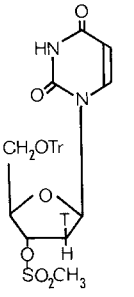
chromatography separation (butanol-ethanol-water, 40:11:19), [2'-^{3}H] uridine[5,6]. This compound was converted to [2'-^{3}H] cytidine 1 by a series of known reactions[7] adapted to the scale of 40 mmoles : the thiation was performed on the crude acetylation product (after evaporation of the acetic anhydride and pyridine) with P_2S_5 (10 mg) in pyridine (total volume : 0.5 ml), and the 2',3',5'-tri-O-acetyl-4-thiouridine was isolated by thin layer silica gel chromatography, R_f 0.58 (benzene-ethyl acetate, 1:1) (22 mmole). Further treatment with ammoniacal methanol for 48 hr at 100° yielded [2'-^{3}H] cytidine, 1 (100%), which was then phosphorylated according to Darlix et.al.[8]. After removal of the protective groups the crude product was chromatographed on a 0.8 x 10 cm colum of Dowex-1, formate. The unreacted [2'-^{3}H] cytidine was eluted with water, then a second elution with 0.01 M HCOOH gave two well-separated bands, first the [2'-^{3}H]CMP 2 (10 mmoles) and then a radioactive impurity which had to be eliminated because its zone of elution was the same as dCMP.



1 R = H
2 R = PO_3H_2



3 R = PO_3H_2
4 R = H



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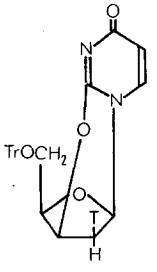

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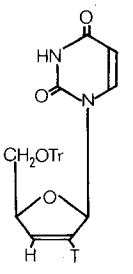

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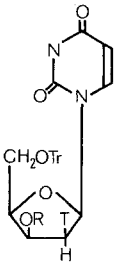

8 R = OH
9 R = SO_2CH_3

T = ^{3}H
Tr = $C(C_6H_5)_3$

The fractions A and B described by Reichard[9] were prepared using the strain E. coli K-12 (Laboratoire de Chimie Microbienne du CNRS, 13 Marseille, France). Fraction A contained the kinases which transform CMP to CDP, the authentic

substrate of the ribonucleoside diphosphate reductase. The incubation of the precursor [2'-^{3}H]CMP (0.05 µmole, 1 µC) and the isolation of the reduced product 3 (1%) were done by exactly following the standard method[9]. After hydrolysis of the [2'-^{3}H]dCMP with alkaline phosphatase, the [2'-^{3}H]-dexoxycytidine 4 was purified by paper chromatography employing Reichard's borate system[10]. Inactive deoxycytidine chlorohydrate was added to active deoxycytidine (40 mg ; 0.15 mmole) which on deamination with HNO_2 gave [2'-^{3}H]-uridine, which was treated by a series of reactions described by Horwitz et. al.[11] The methyl sulfonate 5 was transformed into an anhydro-nucleoside 6 which, in the presence of $(CH_3)_3COK$, undergoes an alimination to "uridinene" 7. This reaction was difficult to control on a small scale and a large fraction was hydrolyzed to the D-threo derivative 8. The "uridinene" 7 has also been obtained by elimination[11] of the methyl sulfonate 9. At each step the products were isolated by thin layer silica gel chromatography (chloroform-ethyl acetate, 1:1; ethanol-benzene, 3:7), systems which completely separated the products from the starting material. Table 1 indicates that the ^{3}H was not eliminated during the reactions 6 → 7 and 9 → 7. If one accepts the general principle of trans elimination, this implies that the ^{3}H is cis to the leaving group and that there has been a retention of configuration in the enzymatic reduction.

TABLE 1 : CHEMICAL DEGRADATION OF [2'-^{3}H]dCMP

Compound	Quantity (µmole)	Sp. radioactivity Counts /min./µmole
5		126
9	25	137
7, from 6	1,4	126
7, from 9	6,2	150

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