Synthesis of 5-Hydroxymethylcytidine

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The DNA of *E. Coli* T even bacteriophages e.g. T_2, T_4 and T_6 contains, apart from normal deoxycytidine a fair proportion of 5-hydroxymethyldeoxycytidine.[1] Furthermore, the 5-hydroxymethyl group in the deoxycytidine can be either mono or diglucosylated[2,3]. The precise biological function of these modifications in the DNA of these bacteriophages is not known. In order to elucidate their biological role 5-hydroxymethyldeoxycytidine and its analogs were required in sufficient quantities. Condensation of mercury salt of protected hydroxymethylcytosine with blocked ribofuranosyl chloride has been reported[4] in the literature to yield 5-hydroxymethyldeoxycytidine, but the poor yields and the number of steps involved does not make this procedure convenient to use.

The direct hydroxymethylation using formaldehyde attracted our attention, firstly due to its simplicity and secondly it is reported that uridine and isopropylidene uridine can be hydroxymethylated by this procedure in reasonable yields.[5,6] 5-Hydroxymethyldeoxycytidine monophosphate has also been prepared by this procedure in low yields, but hydroxymethylation of cytidine or deoxycytidine has not been reported in the literature. The present communication describes a one step synthesis of 5-hydroxymethylcytidine by base catalysed hydroxymethylation of cytidine using paraformaldehyde.

Discussion

The reaction of cytidine 1 with paraformaldehyde and 0.5 N KOH was carried out in a sealed tube at 90°C for 48 hours. The viscous reaction product on paper chromatography in solvents A, B and C revealed, apart from starting material, the formation of two new products. The reaction mixture from a scaled up preparation was separated by preparative paper chromatography using Whatman 3 MM sheets in solvent A and the products were eluted with water. Low temperature evaporation of aqueous solutions afforded pure products which were homogeneous on chromatography in solvents A, B and C. The compound possessing higher mobility (R_f 0.15, solvent A) as compared to cytidine (R_f 0.13, solvent A) was identified as uridine3 (14%), by comparison of its R_f and U.V. spectrum with that of authentic uridine. The formation of uridine in this reaction can be readily accounted due to deamination of cytidine under alkaline condition. The product possessing lower mobility (R_f 0.08, solvent A) as compared to cytidine was obtained in 17.3% yield and was subsequently identified as 5-hydroxymethylcytidine 2

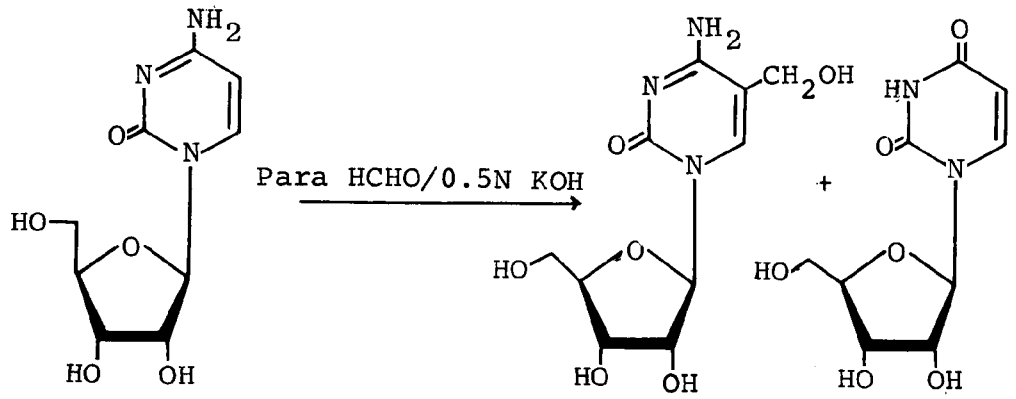
The lower R_f values of 5-hydroxymethylcytidine as compared to cytidine in solvents A, B and C and the U.V. spectral shift at acidic and alkaline pH are comparable with those reported earlier for 5-hydroxymethyldeoxycytidine-5'-monophosphate[7]. Further proof of the identity of the product is provided by its n.m.r. and mass spectra. The n.m.r. spectrum in D_2O exhibited, instead of doublet, a singlet at δ=7.91 for the H-6 of the cytosine base indicating substitution at 5-position. The ribose anomeric proton (H-1'), as expected exhibited a doublet at δ=5.81 with a small coupling constant, J = 2Hz.

As is common with underivatised nucleosides, the mass spectrum does not show the molecular ion but the fragments corresponding to base and sugar at m/e 145 and m/e 133 are present. The general fragmentation pattern of the base corresponds to that of the 5-hydroxymethylcytosine[7] and fragments corresponding to m/e 125, 111, 97, 85, 81 and 67 can be clearly identified.

Experimental

Cytidine was purchased from E. Merck, Darmstadt, and was checked by paper chromatography before use. Paper chromatography was performed in a descending manner using all glass apparatus in the following solvent systems:

A) n-Butyl alcohol:water (86:14 v/v)
B) n-Propyl alcohol:conc. NH_4OH:water



1 2 3

(55:10:35 v/v)

C) iso-Propyl alcohol : conc NH_4OH: 1M Boric acid (70:10:20 v/v)

The nucleosides were detected by an ordinary U.V. lamp. The n.m.r. spectrum was determined on JOEL-PMX-60 and mass spectrum on varian-MAT-112 spectrometer.

5-Hydroxymethylcytidine 2: Cytidine 1 (400 mg), paraformaldehyde (4.0 gm) and a solution of potassium hydroxide (0.5 N 2.5 ml) were heated in a sealed tube at 90°C for 48 hours. The viscous reaction mixture was diluted with water (2 ml) and filtered. The filtrate on paper chromatography showed the presence of two new products and the starting material.

The filtrate (4 ml) was chromatographed on 4 sheets of Whatman 3MM paper using solvent A. The bands corresponding to products were excised and eluted with distilled water. The products possessing R_f 0.15 in solvent A was identified as uridine 3 (57 mg, 14%) by its U.V. spectrum and co-chromatography with the authentic uridine. The product possessing R_f 0.08 in solvent A was obtained as an amorphous solid, and was identified as 5-hydroxymethylcytidine 2 (69 mg, 17%), R_f solvent A = 0.08; solvent B = 0.48; solvent C = 0.25. The Ultra violet spectrum: λ max at pH 1.0 = 285nm; ε 61, 111; O.D. 250/260 = 0.53; 280/260 = 2.35; λmax at pH 14.0=276 nm ε=43,888; O.D. 250/260=1.0; 280/260=1.19. H^1 n.m.r. (D_2O), H-6, δ7.91 (singlet), H-1′ (ribose) δ5.81 (doublet) (J=2H_z). Mass spectrum, m/e 145 (base), m/e 133 (ribose) and m/e 125, m/e 111, m/e 97, m/e 85, m/e 81 and m/e 67.

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