Metabolism of Pyrimidine Bases and Nucleosides in *Neisseria meningitidis*

SIDSEL JYSSUM* AND KAARE JYSSUM

*Kaptein W. Wilhelmsen og Frues Bakteriologiske Institut, University of Oslo, Rikshospitalet, Oslo, Norway*

Received for publication 5 January 1979

In *Neisseria meningitidis*, uridine, deoxyuridine, cytosine, cytidine, or deoxyctydine could not be used by uracil-requiring mutants as pyrimidine sources. Consistent with these findings, only 5-fluorouracil of the different fluoropyrimidine bases and nucleosides showed any inhibitory effect on the growth of four prototrophic strains of *N. meningitidis*. Likewise, only radioactive uracil was readily incorporated into nucleic acids, whereas uptake of radioactive uridine, cytosine, or cytidine could not be demonstrated. Uracil was converted to uridine 5’-monophosphate by uracil phosphoribosyltransferase, whereas enzyme activities for conversion of cytosine or any of the nucleosides were not detectable in meningococcal extracts.

The metabolism of thymine and thymidine in *Neisseria meningitidis* has been investigated (8, 9, 15). Thymine and thymidine are not utilized for specific incorporation into DNA (9). Consistent with this, enzyme activities corresponding to thymidine phosphorylase (EC 2.4.2.4), nucleoside deoxyribosyltransferase (EC 2.4.2.6), or thymidine kinase (EC 2.7.1.75) are not present in extracts from *N. meningitidis* (8).

In enteric bacteria, UMP is synthesized de novo, whereas cytidine (Cyd) nucleotides are formed by the amination of UTP to CTP (24). Prototrophic strains of *N. meningitidis* can be adapted to growth on synthetic media containing glucose as the only source of carbon, and ammonia as the only source of nitrogen (5), indicating that the microbe can synthesize UMP de novo.

In this paper the utilization of exogenous uracil (Ura), uridine (Urd), 2’-deoxyuridine (dUrd), cytosine (Cyt), Cyd, and 2’-deoxycytidine (dCyd) has been examined.

**MATERIALS AND METHODS**

**Bacterial strains.** Meningococcal strains were the wild-type strains M1 of serogroup B, M5 of group C, M6 of a subgroup within group B (5), and Ma-1 of group A (4). Uracil-requiring mutants were isolated from the auxotrophic strain M1-8 his arg as described below. A 5-fluorouracil (FUra)-resistant spontaneous mutant, M1 Fur(R), was isolated from M1. Control experiments were performed with a strain of *Escherichia coli* K-12 as before (8).

**Media and growth conditions.** The media and the growth conditions were as previously described (6, 11, 15, 19).

**Chemicals.** [2-14C]Ura, 59 mCi/mmole; [2-14C]Urd, 55 mCi/mmole; [2-14C]dUrd, 59 mCi/mmole; [2-14C]Cyt, 61 mCi/mmole; [U-14C]Cyd, 485 mCi/mmole; and [U-14C]Cyd, 467 mCi/mmole, were all supplied by the Radiochemical Centre, Amersham, England.

5-Fluorocytosine (FCyt), 5-fluorocytidine (FCyd), and 5-fluorouridine (FUrd) were gifts from Hoffmann-La Roche & Co., Basel, Switzerland. FCyt, which was found to contain about 0.1% FUra, was chromatographically purified before use (12) with 86% 1-butanol as solvent (2).

*N-methyl-N'-nitro-N-nitroso guanidine* (nitroso guanidine) was from Koch-Light Ltd., Bucks, England. It was handled as previously described (7). Other fine chemicals were from Koch-Light or from Sigma Chemical Co., St. Louis, Mo.

**Mutagenesis.** Mutagenesis with nitroso guanidine and assay were as described before (7). Detection of Ura mutants was accomplished by using the velvet replica technique of Lederberg and Lederberg (18).

**Incorporation of radioactive bases and nucleosides into nucleic acids.** The culture in medium KC (15) was supplemented at the start of the experiment with 10 μg of [2-14C]Ura, [2-14C]Urd, [2-14C]Cyt, or [U-14C]Cyd per ml. Specific activity was 0.1 μCi/μg. Samples of 0.2 ml were withdrawn and incorporation of radioactivity was determined by collecting material insoluble in 5% trichloroacetic acid on Whatman GF/A glass fiber paper. The filters were counted in a liquid scintillation counter. For incorporation of radioactivity into DNA, the cells were subjected to hydrolysis in 0.35 N KOH at 37°C for 17 h. DNA was precipitated with trichloroacetic acid and counted as described above.

**Preparation of crude extracts.** The cells were grown on blood agar plates or exponentially in medium KC (15), and handled as before (8, 10). Extracts were prepared in buffer systems as described by Beck et al. (1). The meningococcal extracts were used immediately. Protein was determined by the method of Lowry et al. (20).
Enzyme assays. (i) Cyt deaminase (EC 3.5.4.1) and (ii) Cyd (dCyd) deaminase (EC 3.5.4.5) were assayed as described by Beck et al. (1). (iii) Uridine phosphorylase (EC 2.4.2.3) was assayed by a possible arsenolysis of uridine by the method of Beck et al. (1) or by a possible phosphorylization of uridine by the method of Hammer-Jespersen et al. (3). (iv) Urd (Cyd) kinase (EC 2.7.1.48) was assayed as described by Beck et al. (1). The reaction was stopped, and the protein was precipitated as described before for thymidine kinase (10). After centrifugation, 10 or 20 µl of the supernatants was spotted to cellulose thin-layer plates (Eastman Chromagram with fluorescent uridine indicator, 20 by 20 cm) and developed in 86% 1-butanol (2) for about 3.5 h (17 cm). Any phosphorylated products formed will remain at the application point. The actual areas were cut out and counted as described above. (v) dUrd and dCyd kinases were assayed essentially as described by Okazaki and Kornberg for thymidine kinase (25). Time of incubation was 60 min at 37°C. The rest of the assay was as described above for Urd kinase. (vi) Ura phosphoryltransferase (EC 2.4.2.9) was assayed by the method of Beck et al. (1). The assay mixture minus [14C]Ura was preincubated for 5 min at 37°C (22). At appropriate times, the reaction mixture was processed as described for Urd kinase.

Calculation of enzyme activities. When enzyme activity was present, the crude extract was diluted with the actual buffer (1) to give linearity over a period of 10 or 15 min. When a N. meningitidis extract showed no actual enzyme activity, undiluted extract was used. Specific activity is expressed as nanomoles of substrate utilized per minute per milligram of protein.

RESULTS

Isolation and growth of Ura mutants. Three Ura-requiring mutants were isolated from the amino acid-requiring strain M1-8 arg his after treatment with nitrosoguanidine. The mutants were tested on medium A (6) supplemented with the amino acids and one of the following pyrimidine compounds (19): Ura, Urd, dUrd, Cyt, Cyd, or dCyd. All three mutants were able to utilize uracil only.

Growth in the presence of fluoropyrimidines. The growth of N. meningitidis in medium KC (15) was inhibited by FUrA concentrations exceeding 0.1 µM, and 5 µM completely blocked the growth. No inhibition was observed in the presence of 1 mM FUrD, 1 mM FCyd, or 0.18 mM FCyt. The inhibitory effect of FUrA on the growth of N. meningitidis was, as expected, abolished only by the addition of Ura. When 50 µM Ura was present, there was no inhibition by 5 µM FUrA. Addition of 1 mM Urd, dUrd, or Cyd, 0.5 mM Cyt, or 0.22 mM dCyd had no effect. When used in the same concentrations all the fluoropyrimidines reduced the growth of E. coli in the minimal medium (11). In this microbe, the growth inhibition observed in the presence of 5 µM FUrA was abolished by the different pyrimidine bases and nucleosides (24).

Labeling of nucleic acids from radioactive precursors. Figure 1 shows the incorporation of radioactive Ura into RNA and DNA of the wild-type strain M1 of N. meningitidis. When radioactive Urd, Cyt, or Cyd was used in the same concentration (10 µg/ml of culture), no significant incorporation into nucleic acids could be demonstrated.

Enzyme activities. Crude extracts from N. meningitidis strains belonging to serogroup A, B, or C showed no activities corresponding to the enzymes Cyt deaminase, Cyd (dCyd) deaminase, Urd (Cyd) kinase, Urd phosphorylase, a dCyd kinase, or a dUrd kinase. dUrd, which serves as substrate for both thymidine kinase and thymidine phosphorylase in enteric bacteria (24), could hardly be expected to be metabolized by N. meningitidis in view of the previous results already mentioned (8).

All assays were performed with dialyzed as well as undialyzed meningococcal extracts. In the Urd (Cyd) kinase assay, both GTP (1) and ATP were used as possible phosphate donors, but no activity could be found. In contrast, extracts from E. coli always showed activities corresponding to the above-mentioned enzymes (24) in the techniques used (1, 25). E. coli is reported not to have a dCyd kinase (16).

Activity corresponding to the enzyme Ura phosphoryltransferase was readily demonstrated in crude extracts from the meningococcal strains M1, M5, M6, and Ma-1 (Table 1). The FUra-resistant strain M1 Fur(R) revealed, on the contrary, no significant activity (Table 1). Like in E. coli (22), the enzyme activity in extracts from N. meningitidis was greatly increased by preincubation of the reaction mixture with GTP (1 mM) for 5 min, before adding radioactive Ura to start the reaction (Table 1). Dialysis of the extracts for 22 h reduced the activity considerably, but in meningococcal extracts it could not be restored to 100% by the preincubation procedure (Table 1) (22).

DISCUSSION

The metabolism of the pyrimidine bases and nucleosides in the enteric bacteria E. coli and Salmonella typhimurium is well established (1, 24). Exogenous Ura, Urd, dUrd, Cyt, Cyd, dCyd, thymine, and thymidine are metabolized by these organisms. But the metabolism of these compounds shows a great diversity in different microorganisms (10, 14, 21, 26, 27). N. meningitidis has been shown to belong to the bacteria having a rather restricted metabolism of pyrimidine bases and nucleosides (10, 14, 26). This
knowledge is of importance in classification, and it is essential when selecting radioactive compounds for the labeling of nucleic acids (11, 13, 17, 26), as well as when attempting to isolate mutants blocked in steps of the salvage pathways.

The failure of *N. meningitidis* to utilize Cyt or any of the nucleosides Urd, dUrd, Cyd, and dCyd seems to be explained by its lack of appropriate metabolic enzymes. It cannot be ascribed to impermeability for these compounds, as found in some mutants of *E. coli* with altered nucleo-
side transport character (23).

The inability to demonstrate kinases in the meningococcal extracts for phosphorylation of the nucleosides and deoxynucleosides seems not to be due to 5'-nucleotidase or phosphatase activity. Crude extracts from *N. meningitidis* strain M1 have been shown not to dephosphorylate the pyrimidine nucleotide dTMP (S. Jyssum, unpublished data) or the purine nucleotides AMP (12), IMP, or GMP (13).

From the known pathways of pyrimidines in other microorganisms (24) and because *N. meningitidis* is capable of forming the pyrimidine nucleotides de novo (5), the results presented here suggest that *N. meningitidis* can synthesize pyrimidine nucleotides as outlined in Fig. 2 for incorporation into RNA and DNA.

**LITERATURE CITED**


