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

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In Neisseria meningitidis, uridine, deoxyuridine, cytosine, cytidine, or deoxy-
cytidine could not be used by uracil-requiring mutants as pyrimidine sources.
Consistent with these findings, only 5-fluorouracil of the different fluoropyrimi-
dine 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), nucleo-
side 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 contain-
ing 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 ur-
cacil (Ura), uridine (Urd), 2'-deoxyuridine
(dUrd), cytosine (Cyt), Cyt, and 2'-deoxycytidi-
(dCyd) has been examined.

MATERIALS AND METHODS

Bacterial strains. Meningococcal strains were the
wild-type strains M1 of serogroup B, M6 of group C,
M6 of a subgroup within group B (5), and Ma-1 of
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 Esche-
richia 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/mm; [2-14C]Urd,
55 mCi/mm; [2-14C]dUrd, 59 mCi/mm; [2-14C]Cyt
sulfate, 61 mCi/mm; [U-14C]Cyd, 485 mCi/mm; and [U-14C]Cyd, 467 mCi/mm, were all supplied by
the Radiochemical Centre, Amersham, England.

5-Fluorouracil (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 chromatog-
raphically purified before use (12) with 86% 1-butanol
as solvent (2).

N-methyl-N'-nitro-N-nitrosoguanidine (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 Chemi-
cal Co., St. Louis, Mo.

Mutagenesis. Mutagenesis with nitrosoguanidine
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 nucleo-
sides 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) Cyt (dCyd) deaminase (EC 3.5.4.5) were assayed as described by Beck et al. (1). (iii) Uridine phosphoribosyltransferase (EC 2.4.2.3) was assayed by a possible arsenolysis of uridine by the method of Hammer-Jepsersen et al. (8). (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 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 (22). Time of incubation was 60 min at 37°C. The rest of the assay was as described above for Urd kinase. (vi) Ura phosphoribosyltransferase (EC 2.4.2.9) was assayed by the method of Beck et al. (1). The assay mixture minus [3H]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 mitrosoguanidine. 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 inhibited 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 phosphoribosyltransferase, and dCyd kinase. A Urd kinase and a dUrd kinase. dUrd, which serves as substrate for both thymidine kinase and thymidine phosphoribosylase 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 phosphoribosyltransferase 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 thyidine 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
Fig. 1. Incorporation of [\(^{14}\)C]Ura into RNA and DNA in N. meningitidis strain M1 grown in medium KC. At the indicated absorbances, 0.2-ml samples were taken for measurement of trichloroacetic acid-insoluble radioactivity (●), and for alkali-stable, trichloroacetic acid-insoluble radioactivity (○), as described in the text.

Table 1. Ura phosphoribosyltransferase activities in crude extracts from strain M1 and strain M1 Fur(R) of N. meningitidis

<table>
<thead>
<tr>
<th>Strain</th>
<th>State of extract</th>
<th>Protein (mg/assay)</th>
<th>Time of incubation (min)</th>
<th>[(^{14})C]Ura converted (nmol)</th>
<th>Sp act[^a^]</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Not dialyzed, preincubated with GTP</td>
<td>0.019</td>
<td>5</td>
<td>2.06</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>Not dialyzed, preincubated without GTP</td>
<td>0.019</td>
<td>5</td>
<td>0.52</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Dialyzed 22 h, preincubated with GTP</td>
<td>0.016</td>
<td>5</td>
<td>0.45</td>
<td>5.6</td>
</tr>
<tr>
<td>M1 Fur(R)</td>
<td>Not dialyzed, preincubated with GTP</td>
<td>0.11</td>
<td>60</td>
<td>&lt;0.06</td>
<td>&lt;0.009</td>
</tr>
</tbody>
</table>

[^a^] Similar activities were also measured in assays with the strains M5, M6, and Ma-1.
[^b^] The Ura phosphoribosyltransferase assay was as described by Beck et al. (1). Extracts were made from cells grown on blood agar plates.
[^c^] Specific activity is expressed as nanomoles of substrate utilized per minute per milligram of protein.

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. Jysum, 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


