SECTION H, MICROBIOLOGY

Nucleic Acids from Uracil-requiring Escherichia coli Grown in the Presence of 2-Thiouracil

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2-Thiouracil is a structural analog of uracil and has been reported to be incorporated into nucleic acids (Carbon et al., 1965). It has also been found that 2-thiouracil will partially support the growth of uracil-requiring mutants of *Escherichia coli* (Bauernfeind, 1963). The present investigation describes analytical studies carried out on nucleic acids isolated from a uracil-requiring *E. coli* which utilized 2-thiouracil as a source of pyrimidines.

EXPERIMENTAL

Uracil-requiring *E. coli* was grown without aeration in minimal salt medium containing 14.0 g K_2HPO_4, 6.0 g KH_2PO_4, 2.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4$, 10 g glucose, and either 50 mg uracil or 50 mg 2-thiouracil dissolved in 1000 ml water.

Absorption spectra were determined with a Cary Model 15 ultraviolet recording spectrophotometer. Thin layer chromatography was carried out with apparatus from Brinkman Instruments, Inc. using cellulose MN 300 and MN Silica Gel G-HR/UV adsorbents. 2-Thiouridine was synthesized using a published procedure (Brown et al., 1958). 2-Thiouracil was purchased from Nutritional Biochemicals (Cleveland, Ohio) and 2-thiouracil-S^{35} was synthesized (Wheeler and Liddle, 1908) using thiourea-S^{35} obtained from New England Nuclear (Boston, Mass.). Other biochemicals used in these experiments were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Nucleic acids were isolated from *E. coli* harvested by centrifugation, washed, and disrupted by blending for 3 minutes at 15 C with 2.5% (w/v) sodium lauryl sulfate in a 0.14 *M* NaCl and 0.01 *M* sodium citrate solution. The nucleic acids were precipitated by adding two volumes of ethanol. DNA was extracted with 1.4 *M* NaCl and precipitated again with ethanol (Lehman, 1960). RNA was extracted with phenol (Kirby, 1956). Nucleosides were obtained from the DNA by treatment with DNase followed by hydrolysis with lyophilized snake venom *(Crotalus adamanteus)*. Nucleosides were obtained from RNA by hydrolysis with snake venom. Nucleases, protein, and unhydrolyzed nucleic acids were removed by precipitation with 1% perchloric acid at 0 C. Insoluble potassium perchlorate was removed after neutralization of the perchloric acid at 0 C with *N* KOH, and the nucleosides which remained were collected upon lyophilization of the solution. All traces of the salt were removed from the nucleosides by absorbing them on activated charcoal, collecting the charcoal by centrifugation, and eluting the nucleosides with 95% ethanol : 0.75 *M* NH_4OH (1 : 1).

RESULTS

The average yield of uracil-requiring *E. coli* cells grown in the presence of 2-thiouracil was 0.213 g per 1000 ml, which is 14% of the yield obtained when uracil replaced 2-thiouracil in the minimal salt medium. Approximately 28 mg of nucleosides were recovered per g dry weight of cells. The nucleoside recovery represents 16% of the reported nucleoside content of *E. coli* cells (Roberts et al., 1963).

Nucleosides recovered from the nucleic acids were separated by thin layer chromatography using cellulose as the adsorbent and 5.5 *N* HCl (70 ml) in 2-propanol (130 ml) as the solvent. RNA nucleosides obtained from uracil-requiring *E. coli* grown in the presence of 2-thiouracil contained six distinct ultraviolet-absorbing components. The six components were further purified by chromatography. Purified compounds were identified by thin-layer chromatography in three solvents and by ultraviolet spectrophotometry. The compounds were numbered 1 to 6 with compound 1 being the ultraviolet-absorbing band closest to the origin and compound 6 the band furthest from the origin after 2-propanol-HCl chromatography. RNA-band 1 was identified as guanosine, RNA-band 2 as adenosine, RNA-band 3 as cytidine, and RNA-band 4 as uridine. RNA-bands 5 and 6 could not be identified. The chromatographic properties of bands 5 and 6 were different from those of nucleoside or nucleotide derivatives of the five bases usually found in nucleic acids and were different from 2-thiouracil, 2-thiocytosine, 2-thiothymine, or 2-thiouridine. The ultraviolet spectra of RNA-bands 5 and 6 were not characteristic of any of the standard nucleic acid derivatives or thiopyrimidines. The ultraviolet spectra had no distinct peaks, and the absorbance gradually increased as the wavelength was decreased from 340 $m\mu$ to 200 $m\mu$. Further purification by thin-layer chromatography did not seem to change these unusual ultraviolet spectra. The ultraviolet absorbancy of bands 5 and 6 each represented about 5% of the total ultraviolet absorbancy at 260 $m\mu$ in the RNA-nucleoside mixture. When uracil-requiring *E. coli* cells were grown in the presence of 2-thiouracil-S^{35} and the nucleosides isolated as previously described, bands 5 and 6 contained over 90% of the radioactivity (Table I).

DNA nucleosides were analyzed in a manner similar to the RNA nucleosides. When the nucleoside mixture was separated by thin-layer chromatography, six ultraviolet-absorbing bands were found and purified,

TABLE I. DISTRIBUTION OF RADIOACTIVITY IN NUCLEOSIDE FRACTIONS ISOLATED FROM URACIL-REQUIRING *E. coli* CELLS GROWN IN THE PRESENCE OF 2-THIOURACIL-S^{35}.

Fraction	Identification	Counts per Minute	% Total Radioactivity
RNA-band 1	guanosine	10	3.7
RNA-band 2	adenosine	3	1.1
RNA-band 3	cytosine	8	3.0
RNA-band 4	uridine	5	1.9
RNA-band 5	unknown	58	21.8
RNA-band 6	unknown	184	68.5
DNA-band 1	deoxyguanosine	1.5	1.8
DNA-band 2	deoxyadenosine	0	0
DNA-band 3	deoxycytosine	1.0	1.2
DNA-band 4	unknown	0.5	0.5
DNA-band 5	thymidine	4.5	5.5
DNA-band 6	unknown	73.5	91.0

as described for RNA nucleosides. DNA-band 1 was identified by thin-layer chromatography and ultraviolet absorption spectra as deoxyguanosine, DNA-band 2 as deoxyadenosine, DNA-band 3 as deoxycytidine, and DNA-band 5 as thymidine. DNA-bands 4 and 6 were not identified. Ultraviolet spectra of DNA-band 4 had three sharp peaks located at wavelengths 250 $m\mu$, 255 $m\mu$, and 261 $m\mu$ in acid solution (pH 2.0 to 5.0) and four peaks at wavelengths 245 $m\mu$, 250 $m\mu$, 256 $m\mu$, and 264 $m\mu$ in alkaline solution (pH 7.0 - 10.0). There was also a sharp hypsochromic effect when the pH was increased from 5 to 7, indicating that a functional group was being titrated at about pH 6. The ultraviolet spectra and chromatographic properties of DNA-band 6 were different from any of the standard nucleic acid derivatives. DNA-band 6 produced no characteristic ultraviolet absorption spectra, and the spectra were similar to those of RNA-band 6, showing a gradual increase in absorption as the wavelength was decreased from 340 $m\mu$ to 200 $m\mu$. Absorbancy at wavelengths 260 $m\mu$ of DNA-band 6 represented about 5% of the absorbancy at wavelength 260 $m\mu$ of the nucleoside mixture. Based on thin layer chromatography RNA-band 6 and DNA-band 6 appear to be different compounds. Separation of DNA nucleosides obtained from uracil-requiring *E. coli* grown in 2-thiouracil-S^{35} showed that only DNA-band 6 had significant amounts of radioactivity with 91% of the total radioactivity recovered located in this band (Table I). DNA-band 4 has not been identified but this compound has also been recovered from nucleosides of uracil-requiring *E. coli* grown in the presence of uracil and it is presumed not to be a thiopyrimidine.

Discussion and Conclusions

Uridine, cytidine, deoxycytidine, and thymidine have been found in the nucleic acids of uracil-requiring *E. coli* utilizing commercial 2-thiouracil as the pyrimidine source. Since uracil is the precursor of these nucleosides, the 2-thiouracil must be contaminated with uracil or the organism can convert some 2-thiouracil to uracil. 2-Thiouracil recrystallized from water produced a smaller amount of growth than the commercial 2-thiouracil; therefore, contamination of the commercial 2-thiouracil with uracil seems to be the best explanation for the growth of uracil-requiring *E. coli* in 2-thiouracil.

The occurrence of three unidentified radioactive nucleoside components in the nucleic acids from uracil-requiring *E. coli* grown in the presence of 2-thiouracil-S^{35} indicates that thiopyrimidines are present in the nucleic acids. None of the unidentified components has a spectrum or chromatographic properties similar to the synthesized 2-thiouridine standard or properties reported for 2-thiouridine. This finding cannot be explained at the present time.

Work is now in progress to identify each of the three unknown compounds. It is hoped that identification of the unknowns will allow a clearer understanding of the extent to which 2-thiouracil can be utilized in pathways of pyrimidine metabolism.

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