Preliminary Notes

Uridine nucleotides containing α,ε-diaminopimelic acid from *Escherichia coli*

PARK initially reported[1] the accumulation of amino acid containing nucleotides in penicillin-treated *Staphylococcus aureus*. Subsequent work[2,3] has shown that the structure of one of these nucleotides is UDP-GNAc-lactyl-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala. Nucleotides lacking one or more of these amino acids ("incomplete peptides") have also been isolated from this organism.

A similar nucleotide containing α,ε-diaminopimelic acid in place of lysine was isolated from a DAP-requiring mutant of *Escherichia coli*[4]. This nucleotide has tentatively been assigned the structure UDP-GNAc-lactyl-L-Ala-D-Glu-meso-DAP-D-Ala-D-Ala. It is believed that the peptide is linked to the carboxyl group of the lactic acid.

The present communication reports the isolation and partial characterization of four uridine nucleotides from *E. coli* K235. Two of these nucleotides contain DAP but differ from one another and from the nucleotide described above in the number of alanine residues in the peptide portion of the molecule. The other two nucleotides probably represent "incomplete" nucleotides similar to those reported from *Staph. aureus*.

Nucleotides were extracted from 100 g of acetone-dried *E. coli* with 3 l 80% ethanol. After removal of ethanol by evaporation *in vacuo*, they were chromatographed on Dowex-1 resin, chloride form, by batchwise elution with LiCl as indicated in Fig. 1a.

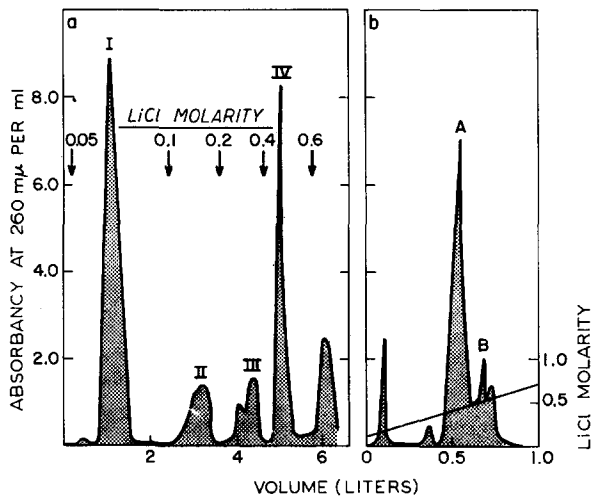

Fig. 1a. Chromatogram of nucleotides from *E. coli* K235.
Fig. 1b. Chromatogram of Peak IV (Fig. 1a) after rechromatography with a linear LiCl gradient.

Abbreviations: UDP, uridine diphosphate; GNAc, N-acetylglucosamine; DAP, diaminopimelic acid; DPN^+, diphosphopyridine nucleotide.

Peak I is DPN^+, II is cytidine 5'-monophospho-N-acetylneuraminic acid[5], III is a mixture of nucleoside mono- and diphosphates, and IV contains the four uridine nucleotides described below.

All four of the nucleotides described below contain the 3-O-lactyl ether of N-acetylglucosamine (N-acetylmuramic acid) as indicated by acid hydrolysis followed by paper chromatography, and by the absorption spectra exhibited in the hexosamine method[6].

Peak IV was concentrated to a syrup and the nucleotides precipitated as lithium salts by the addition of 1 vol. methanol and 20 vol. acetone. The precipitate was dissolved in water and rechromatographed on Dowex-1 chloride resin by means of a linear gradient of LiCl (Fig. 1b). Peaks A and B (Fig. 1b) gave a positive reaction for N-acetylhexosamines after mild acid hydrolysis (0.01 *N* HCl, 100° for 17.5 min), but only Peak A yielded a positive ninhydrin reaction (before hydrolysis).

The material in Peak A was freed from excess LiCl as described above and separated into two nucleotide components (R_{UMP}: *a*, 0.43 and *b*, 0.29) by descending chromatography on Whatman 3 MM paper in ethanol–1.0 *M* ammonium acetate, pH 7.5 (7:3) for 48 h. On the paper chromatograms, *a* and *b* gave a positive ninhydrin reaction which coincided exactly with the ultraviolet-quenching spot. The ultraviolet adsorption spectra of *a* and *b* proved identical to that of uridine at pH 7.0 and 11. Both nucleotides proved homogeneous by paper chromatography in four different solvent systems (acidic, neutral, and alkaline) and by paper electrophoresis at pH's 5.0, 7.0, and 9.0. Incubation of *a* and *b* with purified snake-venom phosphodiesterase followed by paper chromatography in isobutyric acid–NH_4OH–water (57:4:39) yielded in each case a single ninhydrin-positive spot (R_{UMP}: *(a)*, 0.83; *(b)*, 0.60) and a single ultraviolet-quenching spot identical in R_F to UMP. The ninhydrin-positive material released from *a* and *b* by phosphodiesterase was eluted from the paper and an aliquot hydrolyzed with 0.01 *N* HCl at 100° for 17.5 min. For each mole of uridine originally present in *a* or *b*, 1.07 moles of acid-labile P and 1.14 moles of N-acetylhexosamine-positive material were released.

Strong acid hydrolysis (6 *N* HCl, 100° for 15 h) released three amino acids from both nucleotides. They have been identified as alanine, glutamic acid, and meso- or DD-DAP (the LL isomer is easily separated from the meso or DD isomer of DAP[7]) by paper chromatography in four solvent systems and by paper electrophoresis at pH's 3.7, 7.0 and 9.0.

Quantitative determination of amino acids was performed as follows. An aliquot of hydrolyzed nucleotide *a* or *b* was spotted on Whatman 3 MM paper and subjected to a current of 63 V/cm of paper for 20 min in 1 % borate buffer, pH 9.0. Known concentrations of authentic samples of these amino acids were spotted on either side of the experimental material so that both standards and unknowns would be treated in exactly the same manner. After electrophoresis, the paper was dried, dipped in 0.05 % ninhydrin in acetone–glacial acetic acid (96:4), and heated at 60° until the spots were faintly visible. These conditions completely separate alanine, glutamic acid and α,ε-diaminopimelic acid. The ninhydrin-positive spots were cut out and eluted with 1 ml of the citrate–$SnCl_2$ buffer used in preparing the ninhydrin reagent[8]. An aliquot of this material was mixed with an equal volume of 4 % ninhydrin in methyl cellosolve and the reaction completed as described[8]. The analytical results are summarized in Table I.

TABLE I

ANALYSIS OF THE FOUR URIDINE NUCLEOTIDES ISOLATED FROM *E. coli* K235

Nucleotide	Total μmoles isolated	Moles/mole uridine			Moles/mole glutamic acid*		
		Base	Total P	N-acetyl-muramic acid	Al	Glu	DAP
a	20	1.0	2.1	1.1	1.9	1.0	1.2**
b	24	1.0	2.1	1.1	0.9	1.0	1.3**
c	8	1.0	—***	1.0	0.0	0.0	0.0
d	2	1.0	—***	1.0	0.9	1.0	0.0

* Approx. 1.0 mole of glutamic acid was present per mole of uridine. Owing to the small amounts of material analyzed and the many manipulations required for this determination the results varied but never more than 20% from showing a 1:1 ratio.

** These values are high owing to incomplete separation from residual muramic acid during paper electrophoresis.

*** Not dermined directly.

Peak B (Fig. 1b) was separated into two nucleotide components (R_{UMP}: *c*, 1.9 and *d*, 0.71) with the ethanol–ammonium acetate solvent as described for Peak A. Nucleotides *c* and *d* yielded positive reactions for N-acetylhexosamine[9] on the paper chromatograms* which coincided with the ultraviolet-quenching spots. The ninhydrin reactions were negative. Each contained uridine and two moles of phosphate per mole of base as determined by the phosphodiesterase treatment described above. Mild acid hydrolysis of *c* resulted in the liberation of a single sugar component which proved identical to the 3-O-lactic acid ether of N-acetylglucosamine (N-acetylmuramic acid) by paper chromatography and paper electrophoresis. Strong acid hydrolysis of *c* and *d* revealed the presence of equimolar concentrations of alanine and glutamic acid in *d*; no amino acids were liberated from *c*. The analytical data obtained with *c* and *d* are presented in Table I.

From these data, the following partial structures can be assigned to these nucleotides:

UDP–GNAc–lactyl- { (Ala, Glu, DAP, Ala) *a*; (Ala, Glu, DAP) *b*; none *c*; (Ala, Glu) *d* }

The sequence and configuration of the amino acids in the peptide portion of the molecules have not been determined but may be the same as in the DAP-containing nucleotide reported by STROMINGER and co-workers. No evidence was obtained for more than two moles of alanine per mole of glutamic acid in any of these nucleotides and it may well be that nucleotide *a* represents the "complete" nucleotide in this strain of *E. coli*.

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* The chromatograms were first dipped in 5% trichloroacetic acid in ethanol and heated at 100° for 10 min to hydrolyze the acetylhexosamine linkage to the nucleotide.

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Observations on the rate of DNA synthesis in *Lactobacillus leichmanii*

WACKER *et al.*[1] have reported that cultures of *Lactobacillus leichmanii* show a 5-fold increase in DNA per unit dry mass when grown with vitamin B_{12} rather than with deoxycytidine as supplement. The possibility that this observation may affect the conclusions of DINNING *et al.*[2] with respect to the role of vitamin B_{12} in [^{14}C]-formate incorporation into the DNA thymine of *L. leichmanii* prompted us to investigate the effect of vitamin B_{12} and deoxycytidine on the growth rate and DNA synthetic rate in *L. leichmanii* 313 as part of a wider study of the mechanism of DNA thymine biosynthesis.

Under the conditions described in the legend to Figs. 1 and 2, cultures grew with identical mean generation times to the same final populations as judged by turbidimetric or dry-cell-mass measurements. Fig. 1 shows the rate of DNA synthesis in comparable cultures. It may be seen that the rate of DNA synthesis in cultures grown in the presence of deoxycytidine is greater than that of cultures grown with vitamin B_{12} supplements. These differences are particularly marked when the results are expressed in terms of changes in DNA per unit dry mass of cells (Fig. 2). The mechanism of the apparent promotion of DNA synthesis in cells by deoxycytidine is under investigation.

Differences in DNA per unit dry mass of cells grown in the presence of vitamin B_{12} or deoxycytidine comparable to those reported by WACKER *et al.*[1] have not been found under the conditions used in our experiments. The results obtained, however, do show that difficulties may well arise in the interpretation of data on the incorporation of labelled substrates into the DNA of *L. leichmanii*. Cultures grown in the presence of vitamin B_{12} or deoxycytidine, while otherwise apparently comparable, differ considerably in their rate of DNA synthesis. Accordingly, the significance of the apparent effect of vitamin B_{12} on the incorporation of a labelled precursor into DNA would be obscure if the precursor were metabolised simultaneously by other pathways at rates dependent on total cell mass.

The significance of these observations in relation to the possible role of vitamin B_{12} in formate and formaldehyde metabolism in *L. leichmanii* is under investigation.

Abbreviation: DNA, deoxyribonucleic acid.