Isolation of thymidine diphosphate rhamnose and a novel thymidine diphosphate sugar compound from *Escherichia coli* strain B

Previous papers from this laboratory[1-4] presented evidence for the occurrence of new thymidine compounds in the bacteria, *Lactobacillus acidophilus* and *Escherichia coli*. The compound in *L. acidophilus* has recently been isolated and identified as thymidine diphosphate rhamnose (TDP-rhamnose)[4]. More recently the presence of three unidentified TDP-sugar compounds in a diaminopimelic acid-requiring mutant of *E. coli* has been shown by STROMINGER AND SCOTT[5]. The present paper reports the isolation of two TDP-sugar compounds from *E. coli* strain B. One of these compounds is identified as TDP-rhamnose.

If the growing cells of *E. coli* B were exposed to [^{3}H]thymidine, the labeled deoxyriboside was incorporated very rapidly into acid-soluble compounds and DNA, so that even after a few seconds exposure, the isotopic label was found in both the acid-soluble and DNA fractions. In one experiment cells exposed to [^{3}H]thymidine (3.0 μC/mμmole) for about 3 sec at 30° were chilled quickly to 0°, precipitated and washed. An acid-soluble extract was prepared from these cells and chromatographed on a Dowex 1 column with the formic acid system[6]. Besides the fractions of thymidine, TMP, TDP and TTP, a fraction eluted between TMP and TDP contained a large portion (44 %) of radioactivity.

For isolation of the compounds, the corresponding fraction was prepared as above in a large-scale experiment (performed with 19 l culture exposed to [^{3}H]-thymidine for about 10 sec) and subjected to paper chromatography in isobutyric acid-1 *N* NH_4OH (10:6). The paper chromatography resulted in separation of five ultraviolet-absorbing components. On examination of each component after elution from the paper, two major components (R_{TMP}, 0.66 and R_{TMP}, 0.86) were found to be radioactive and have the spectra of thymidine. Both contained two moles phosphate/mole thymidine and showed a reducing activity[7] equivalent to 1 mole rhamnose after mild hydrolysis with 0.01 *N* HCl at 100° for 10 min (Table I, TDP-X_1 representing the slower compound; TDP-X_2 the faster one). The microbiological assay after snake-venom treatment[1] of these compounds proved the presence of deoxyriboside in amounts expected from the ultraviolet absorption.

The cysteine-H_2SO_4 reaction for 6-deoxyhexoses (methylpentoses)[8] was positive with TDP-X_1 and gave approximately 1 mole 6-deoxyhexose/mole thymidine. The mobilities of TDP-X_1 and TDP-rhamnose isolated from *L. acidophilus* were identical

TABLE I

ANALYTICAL DATA OF TDP-X_1 (= TDP-RHAMNOSE) AND TDP-X_2

	Thymidine μmoles	*Total P* μmoles	*Reducing value** μmoles	*6-Deoxyhexose* μmoles
TDP-X_1	1.00	2.04	0.99	0.92
TDP-X_2	1.00	2.00	1.00	0.18

* Expressed as rhamnose equivalent. The reducing value of rhamnose was about half that of glucose on a molar base.

Abbreviations: TMP, TDP, TTP, thymidine mono-, di- and triphosphate; DNA, deoxyribonucleic acid.

in isobutyric acid-NH_4OH as well as in ethanol-ammonium acetate. Furthermore, in four solvent systems tested, the R_F value of X_1 agreed with that of authentic rhamnose but differed from that of fucose (Table II). It leaves little doubt that TDP-X_1 is identical with TDP-rhamnose which was first isolated from *L. acidophilus*[4*].

X_2 was chromatographed in pyridine–butanol–water (4:6:3) and located by $AgNO_3$ spraying. The spot could hardly be detected with aniline hydrogen phthalate which made visible X_1 as well as authentic rhamnose and fucose. The R_F value of X_2 was as high as that of rhamnose, suggesting that it might be a deoxyhexose.

TABLE II

R_F VALUES OF X_1 AND OF AUTHENTIC RHAMNOSE AND FUCOSE

Solvents	X_1	*Rhamnose*	*Fucose*
Butanol–acetic acid–water (4 : 1 : 5)	0.38	0.38	0.32
Butanol–ethanol–water (104:62:34)	0.48	0.48	0.42
Phenol saturated with water	0.49	0.49	0.56
Ethyl acetate–pyridine–water (8:2:1)	0.45	0.45	0.32

Diphenylamine[9], thiobarbituric acid[10] and *p*-nitrophenylhydrazine[11] reactions for 2-deoxysugars were negative. It failed to react with *p*-nitrophenylhydrazine even after periodate treatment, indicating that it is neither 3-deoxyhexose nor 3,6-dideoxyhexose[12]. In the cysteine-H_2SO_4 test for 6-deoxyhexoses, X_2 produced a reaction product with an absorption maximum at 400 mμ as did authentic rhamnose or fucose, but its extinction coefficient was as low as 20 % of that of these 6-deoxyhexoses. This fact might suggest that X_2 is somehow related to 6-deoxyhexose. The anthrone reaction[9] was positive, while all the following reactions[9] were negative: orcinol for pentoses and hexoses, cysteine-carbazole for ketosugars and trioses, carbazole-H_2SO_4 for uronic acids and Elson-Morgan for amino sugars.

Comparing the present results with those of STROMINGER AND SCOTT[5] it appears that none of the three TDP-sugars isolated by them is identical with either TDP-rhamnose or with TDP-X_2 reported here.

In the present experiment 8.5 μmoles TDP-rhamnose and 8.4 μmoles TDP-X_2 were obtained from 19 l culture. The specific activity of TDP-X_2 (31,720 counts/min/μmole) was much higher than that of TDP-rhamnose (9,480 counts/min/μmole). This fact may suggest more active turnover of the former within the cell.

Active incorporation of thymidine into the fraction containing TDP-rhamnose and TDP-X_2 is also observed in *E. coli* B 15 min after T2-phage infection[13]. Further studies are being made of the chemical nature and metabolic role of these TDP-sugar compounds.

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* However the possibility has not strictly been excluded that the sugar moiety of both compounds might be a stereoisomer of rhamnose.

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The presence of basic proteins in microsomes

The treatment of microsomes from rat liver with the detergents "Lubrol W" and sodium perfluoro-octanoate has revealed the presence of several protein fractions[1] into each of which amino acids are found to become incorporated to a different extent. Since the structure and composition of these fractions may throw light on their behaviour, in the course of the work the content of amino acids in the protein has been determined in several preparations of microsomes and their fractions. The N-terminal groups of the protein of some samples were also determined. Some amino acid analyses of the protein of whole microsomes[2] and of ribonucleoprotein particles[3] have previously been reported. SIMKIN AND WORK[4] gave the analyses of two fractions prepared by extraction of whole microsomes from guinea-pig liver with salt, which did not differ greatly from the whole microsome.

In the present work the protein of the microsomes of the Lubrol and PFO pellets were prepared as previously described[1]. The HCl extract was obtained by treating the PFO pellet with 0.2 *N* HCl for 16 h at 4°. The residue was re-extracted for 6 h,and the combined extracts dialysed and freeze-dried. The total amino acid analyses of the proteins were carried out by the FDNB method of LEVY[5] and FRAENKEL-CONRAT, HARRIS AND LEVY[6] with the modifications described by PHILLIPS AND JOHNS[7]. The N-terminal groups were determined according to the technique of PHILLIPS[8].

Table I gives the results of complete amino acid analyses of samples from three different preparations. The proportions of various N-terminal groups of some of the samples can be seen in Table II. The results show that the total protein of the microsomes is not predominantly basic since the sum of the acidic amino acids is greater than that of the basic. However, there is some tendency for the content of basic amino acids to increase in the Lubrol and PFO pellets. The HCl extract of the PFO pellet shows a marked increase of the basic residues indicating that there are among the microsomal proteins some which are markedly basic. The total percentage of basic amino acids in this fraction is 27.8, which is higher than that normally found in histones.

Abbreviations: PFO, perfluoro-octate; FDNB, fluorodinitrobenzene.