STUDIES OF DEOXYRIBONUCLEIC ACID SYNTHESIS AND CELL GROWTH IN THE DEOXYRIBOSIDE-REQUIRING BACTERIA, *LACTOBACILLUS ACIDOPHILUS*

III. IDENTIFICATION OF THYMIDINE DIPHOSPHATE RHAMNOSE

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SUMMARY

A new deoxynucleotide, thymidine diphosphate rhamnose, was isolated from *Lactobacillus acidophilus* R-26 which had been subjected to a treatment which induced an accumulation of deoxyribosidic compounds. The results presented indicate that in the nucleotide, thymidine-5′-monophosphate is linked to rhamnose-1-phosphate through a pyrophosphate bond. The possible metabolic function of the nucleotide is discussed.

INTRODUCTION

In previous papers[1,2], we have presented evidence for the occurrence of a new thymidine nucleotide in the acid-soluble extract of *Lactobacillus acidophilus* R-26. It has also been reported that the same nucleotide accumulates markedly when DNA synthesis is induced in the resting cells of this micro-organism by replenishment with thymidine after depletion of uracil and deoxyriboside[3]. The nucleotide has now been isolated from these cells and identified as thymidine diphosphate rhamnose (TDP-rhamnose).

Preliminary reports of this work have been published[4,5].

MATERIALS AND METHODS

The micro-organism and culture medium

L. acidophilus strain R-26 was grown in the synthetic medium, the composition of which has been given previously[3]. The procedures for the depletion and replenishment were shown in the legend of Fig. 2. The cells were harvested by centrifugation in the cold, washed in saline and frozen with CO_2–ethanol.

Abbreviations: DNA, deoxyribonucleic acid; TMP, TDP and TTP, thymidine 5′-mono-, di- and triphosphate; UDP, uridine 5′-diphosphate; GDP, guanosine 5′-diphosphate; CDP, cytidine 5′-diphosphate; AMP, adenosine 5′-monophosphate; DPN, diphosphopyridine nucleotide; PCA, perchloric acid; TCA, trichloroacetic acid.

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Preparation of the acid-soluble extract

The frozen cells were thawed, extracted with ice-cold 0.5 *N* PCA and washed twice with 0.2 *N* PCA. The original extract and the washings were combined, neutralized with KOH and concentrated by lyophilization, the precipitate of $KClO_4$ being removed by centrifugation.

Ion-exchange chromatography

The acid-soluble extract was added onto a column of Dowex-1-formate (X 10, 200–400 mesh), 1.25 × 16 cm, and fractionated as below by using a modification of the extended gradient-elution chromatographic method with the "formic acid system"[6].

After washing the column with 35 ml of water, elution was carried out in the cold by introducing successively the following eluents into a mixing flask initially filled with 500 ml of water: 100 ml of 1 *N* formic acid; 500 ml of 4 *N* formic acid; 500 ml of 0.2 *M* ammonium formate in 4 *N* formic acid; 300 ml of 0.4 *M* ammonium formate in 4 *N* formic acid; 650 ml of 1 *M* ammonium formate in 4 *N* formic acid. The flow rate was 15–25 ml/h, and samples containing 5 ml effluent were collected. The extinction at 260 mμ and 280 mμ was measured for each tube in a Beckman DU spectrophotometer. Effluent fractions corresponding to the peaks or portions of peaks were pooled and lyophilized to remove formic acid and ammonium formate.

Chromatography of the phosphodiesterase digest of the isolated nucleotide was performed with a smaller column (0.6 × 17 cm) and a 100 ml mixing flask.

Paper chromatography

Descending paper chromatography was carried out on Whatman No. 1 paper in the following solvents: Isobutyric acid–1 *N* NH_4OH (10:6) (modified from KREBS AND HEMS[7]); butanol saturated with water[8]; butanol–acetic acid–water (4:1:5)[8]; butanol–ethanol–water (104:62:34)[9]; phenol saturated with water[8]; ethyl acetate–pyridine–water (8:2:1)[10]; ethyl acetate–acetic acid–water (3:1:3)[11]; butanol–pyridine–water (6:4:3)[12].

Nucleotides were located on the chromatograms by means of u.v. photography with a filter (253.7 mμ) of the Scientific Research Institute*. For preparative purposes, the nucleotide was chromatographed as a band and eluted from the paper with water.

Sugars were detected by spraying with aniline–hydrogen phthalate reagent[13]. For the isolation of sugar liberated on acid hydrolysis from the nucleotide, the hydrolyzed nucleotide was run in butanol–ethanol–water as a band. After chromatography a guide strip was cut out from the paper and sprayed with aniline–hydrogen phthalate. The sugar was located by reference to this guide strip and eluted with water.

Paper electrophoresis

The paper electrophoresis of sugars was carried out with the apparatus described by MARKHAM AND SMITH[14]. Samples were run in 0.05 *M* borate buffer, pH 8.6[15], on Whatman No. 1 for 1.5 h at a potential gradient of about 30 Volts/cm. Sugars were made visible by spraying with aniline–hydrogen phthalate acidified with acetic acid.

* Kindly supplied by Dr. E. IWASE.

Charcoal treatment of the nucleotide

The nucleotide sample eluted from the paper chromatogram was further purified by charcoal treatment. Norit A which had been successively washed with 50 % ethanol containing 1 % conc. ammonia, 0.1 *N* HCl and water was added to the nucleotide solution adjusted to pH about 5. After vigorous stirring for 30 min the charcoal was precipitated by centrifugation. It was then re-suspended in cold 2 *N* formic acid, centrifuged and washed with water until the washings were neutral[16]. The nucleotide was eluted from the charcoal with cold 50 % ethanol containing 0.7 % conc. ammonia. The ammoniacal solution of nucleotide obtained was evaporated *in vacuo* and taken up in a small volume of water. In some cases the solution of the nucleotide in 0.1 *N* HCl was passed through a small column of Dowex-50-H^+ to ensure complete removal of ammonium ions.

Analyses

Phosphorus determination was carried out according to MARTIN AND DOTY[17]. Total phosphorus was determined after digestion with PCA. Determination of labile phosphorus was carried out by a 10 min hydrolysis in 1 *N* HCl at 100°. Nitrogen was determined according to LEVY AND PALMER[18]. Reducing sugar was estimated by the method of PARK AND JOHNSON[19]; methylpentose (6-deoxyhexose) by the method of DISCHE AND SHETTLES[20].

Deoxyriboside was assayed microbiologically after treatment of the sample with snake venom[1].

Biochemicals

Thymidine and 2′,3′-cytidylic acid were purchased from Schwarz Laboratories, Inc.; TMP from the California Foundation for Biochemical Research; L-rhamnose from Wako Pure Chemical Industries, Ltd.; L-fucose from Bios Laboratories, Inc. TDP and TTP were obtained through the courtesy of Professor A. KORNBERG, Stanford University. Purified snake (*Agkistrodon blomhoffii*) venom phosphodiesterase and bull semen 5′-nucleotidase were generously provided by Dr. S. IWANAGA, Kyoto University. Crude snake venom, *Agkistrodon blomhoffii* was obtained from Bunkyu Do Co. (Tokyo).

RESULTS

Isolation of the nucleotide

In Fig. 1 the ion-exchange chromatogram of an acid-soluble extract prepared from a growing culture in the complete medium was shown, together with an example of the behavior of authentic TMP, TDP and TTP in the same system. The major portion of the deoxyribosidic compounds in the original extract was recovered in a fraction eluted in tubes No. 180–200. It is evident that the behavior of the chief deoxyribosidic compound in the extract, which was later identified as TDP-rhamnose, was distinct from any known thymidine nucleotide.

As reported in an earlier paper[3], DNA synthesis can be induced in resting cells by adding thymidine to the bacteria previously starved of uracil and deoxyriboside. Under these conditions a marked accumulation of acid-soluble deoxyribosidic compounds precedes the active synthesis of DNA. Acid-soluble extracts were prepared

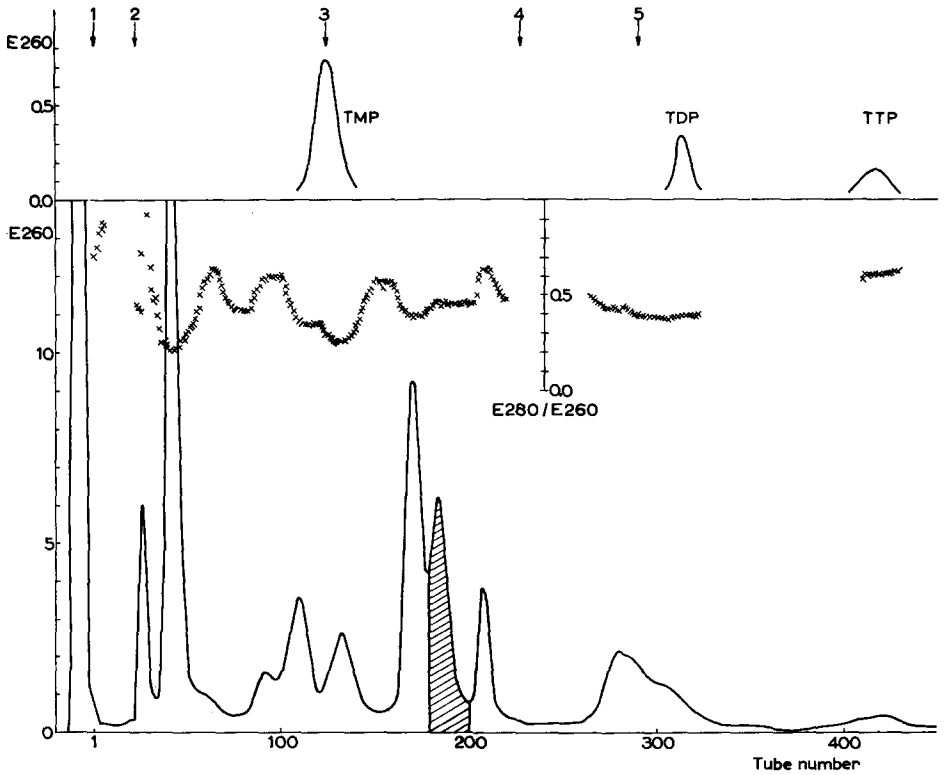


Fig. 1. Ion-exchange chromatogram of the acid-soluble extract of normally grown cells. The shaded area indicates the fraction containing chief deoxyribosidic compound (TDP-rhamnose). In the upper portion of the figure the behavior of authentic thymidine nucleotides is shown. The extract was prepared from 35 l culture grown in a complete medium containing thymidine and added onto a column of Dowex-1-formate (X 10, 200–400 mesh), 1.25 × 16 cm. Extended gradient elution was carried out by introducing the following eluents at the indicated points into the reservoir which was connected to a 500 ml mixing flask initially filled with water: (1) 100 ml of 1 *N* formic acid; (2) 500 ml of 4 *N* formic acid; (3) 500 ml of 0.2 *M* ammonium formate in 4 *N* formic acid; (4) 300 ml of 0.4 *M* ammonium formate in 4 *N* formic acid; (5) 650 ml of 1 *M* ammonium formate in 4 *N* formic acid.

from the cells subjected to such treatments and chromatographed on Dowex-1 columns (*cf.* Figs. 2a and 2b).

A large amount of deoxyribosidic compound (55–75 % of the total deoxyribosidic compounds in the extract) was eluted in approximately the same region (tubes No. 186–214 in Expt. 1; No. 181–204 in Expt. 2) as in the chromatogram of the extract of normal cells. However, it will be noticed that the ratio, E280/E260 of this fraction was much higher than that of the corresponding fraction of the extract from normal cells (0.45–0.47 in the normal cells; 0.55–0.60 in the treated cells). As this fraction contained a uridine nucleotide (E280/E260 = 0.38) in addition to the thymidine nucleotide under consideration (E280/E260 = 0.73), the higher value of the ratio E280/E260 of the fraction indicated the relatively high proportion of the thymidine nucleotide.

The crude fraction so prepared was subjected, after removal of formic acid and ammonium formate by lyophilization, to paper chromatography in isobutyric acid–

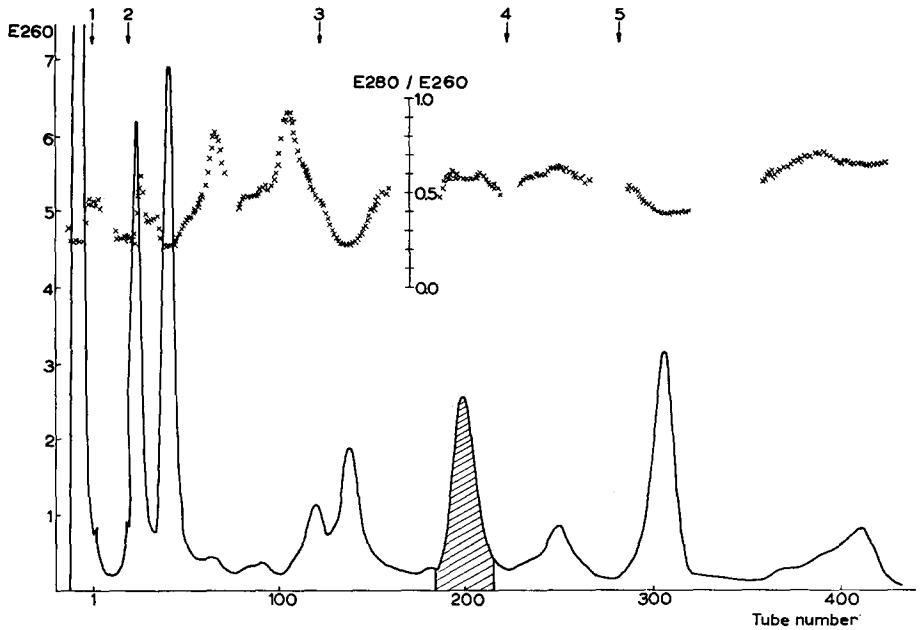


Fig. 2a.

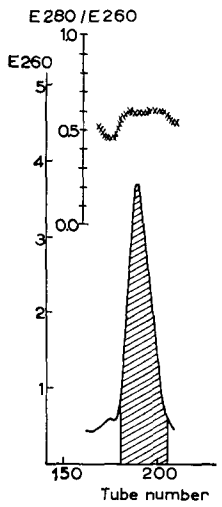


Fig. 2b.

Fig. 2a and 2b. Ion-exchange chromatogram of the acid-soluble extract of the cells subjected to treatment inducing the accumulation of deoxyribosidic compounds (a: Expt. 1; b: Expt. 2). The shaded area indicates the fraction containing chief deoxyribosidic compound (TDP-rhamnose). Cells grown overnight in the complete medium were washed and re-suspended at 0.1 the original cell concentration in 15 l (Expt. 1) or 18 l (Expt. 2) of the medium deficient in uracil and deoxyriboside. After 5.5 h incubation in this medium at 37°, cells were supplied with excess (40 μmoles/l) thymidine, and incubated further for 30 min. The acid-soluble extract was prepared from these cells and chromatographed in the same manner as in Fig. 1.

NH_4OH. Two u.v.-absorbing components (R_F = 0.35 and 0.20) were separated on the chromatogram (Fig. 3). On examination of each component after elution from the paper, the fast component was found to have the spectrum of thymidine and to show deoxyribosidic growth activity after venom treatment[1], while the slow component having the spectrum of uridine was microbiologically inactive.

The fast component was isolated by large scale chromatography, adsorbed on Norit A and eluted from the charcoal with ammoniacal ethanol–water. The ammoniacal solution obtained was evaporated *in vacuo*.

TABLE I

SPECTRAL PROPERTIES OF TDP-RHAMNOSE

Substance	λ_{max}		λ_{min}		E_{250}/E_{260}		E_{280}/E_{260}	
	0.1 N HCL (mμ)	0.1 N NaOH (mμ)	0.1 N HCl (mμ)	0.1 N NaOH (mμ)	0.1 N HCl	0.1 N NaOH	0.1 N HCl	0.1 N NaOH
TDP-rhamnose	267	266	234–235	246	0.65	0.71	0.73	0.68
TMP	267	267	235	245–246	0.63	0.72	0.73	0.68
Thymidine	267	267	235	245	0.63	0.72	0.73	0.68

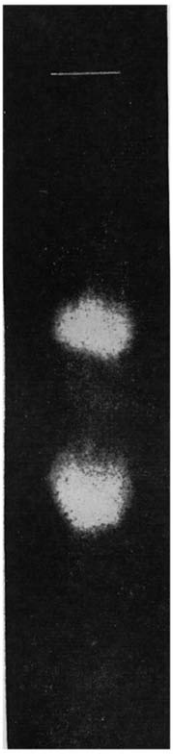


TABLE II

ANALYTICAL DATA FOR TDP-RHAMNOSE

Substance	μmoles
Thymidine*	1.00
Deoxyriboside**	0.99
Total phosphorus	2.05
Labile phosphorus	0.91
Nitrogen	2.05
Reducing value***	0.99
Methylpentose	0.98

* Calculated from the absorbance at 260 mμ in 0.1 *N* HCl by assuming a value of 8,400 for molar extinction coefficient.
** Assayed microbiologically after snake venom digestion.
*** Expressed as rhamnose equivalent.

Fig. 3. Paper chromatogram of the crude fraction of TDP-rhamnose. The fast component is TDP-rhamnose. Solvent: *iso*-butyric acid–1 *N* NH_4OH (10:6).

By these procedures, 7–10 μmoles of the nucleotide were prepared, starting from 15 l culture.

Identification of the nucleotide

General properties: The u.v. absorption spectrum of the isolated nucleotide was in agreement with the spectrum of TMP and thymidine both in acidic and basic solutions (Fig. 4; Table I). Microbiological assay after venom treatment[1] proved the presence of deoxyriboside in the amount expected from u.v. absorption (Table II).

The nucleotide contained two moles organic phosphate per mole thymidine and upon treatment with 1 *N* HCl at 100° for 10 min liberated one mole phosphate in the inorganic form (Table II).

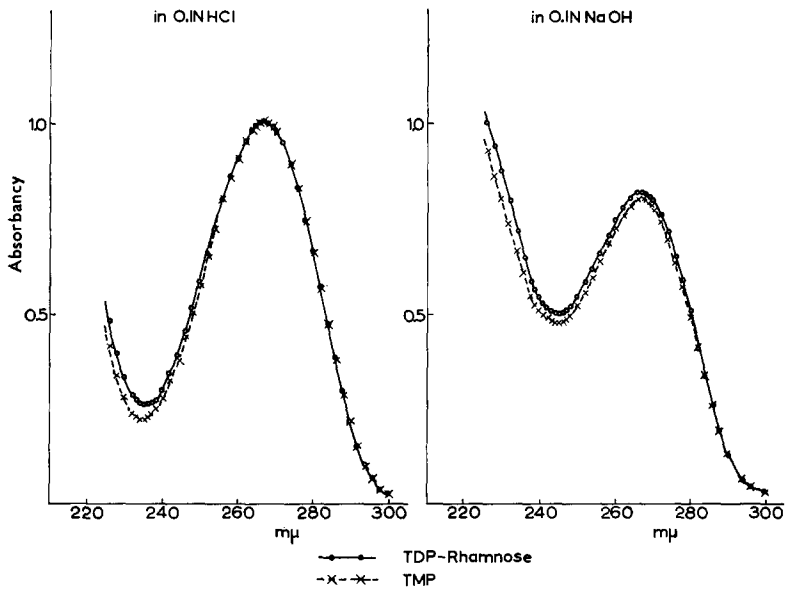


Fig. 4. u.v.-absorption spectra of TDP-rhamnose as compared with the spectra of TMP.

Nitrogen analysis of the sample, which was passed through a column of Dowex-50 for complete removal of contaminating ammonium ions, gave 2 atoms of nitrogen per mole, indicating that it did not contain nitrogen in excess of that accounted for by the thymine moiety.

Mild hydrolysis in 0.01 N HCl at 100° for 10 min yielded a reducing group measurable by ferricyanide reduction[19].

Identification of rhamnose: Various color reactions for sugars were carried out on the hydrolysate of the nucleotide. The following reactions[21] were negative: diphenylamine for 2-deoxysugars; orcinol for pentoses and hexoses; cysteine–carbazole–

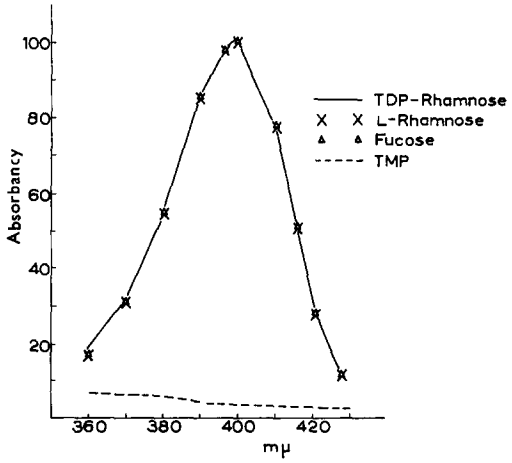


Fig. 5. Absorption spectrum of the reaction product formed in the cysteine–H_2SO_4 reaction for methylpentoses.

H_2SO_4 for ketoses and trioses; carbazole–H_2SO_4 for uronic acids; Elson–Morgan for amino sugars.

The only positive result was obtained with the cysteine–H_2SO_4 reaction for methylpentoses (6-deoxyhexoses)[20]. The nucleotide preparation as well as authentic rhamnose and fucose formed a reaction product with an absorption maximum at 400 mμ. The whole absorption curve of the reaction product formed by the nucleotide was in complete agreement with that of the authentic methylpentoses (Fig. 5). The

TABLE III

R_F VALUES OF THE SUGAR COMPONENT LIBERATED BY MILD ACID HYDROLYSIS OF TDP-RHAMNOSE AND AUTHENTIC METHYLPENTOSES

TDP-rhamnose was hydrolyzed in 0.01 *N* HCl at 100° for 10 min. The sugar in the hydrolysate was isolated by large scale chromatography in ethanol-butanol-water and then subjected to a second paper chromatography using 7 different solvent systems.

Solvents	*Sugar liberated from TDP-rhamnose*	L-*Rhamnose*	L-*Fucose*
Butanol saturated with water	0.24	0.24	0.18
Butanol–acetic acid–water (4:1:5)	0.30	0.31	0.24
Butanol–ethanol–water (104:62:34)	0.49	0.49	0.43
Phenol saturated with water	0.56	0.56	0.64
Ethyl acetate–acetic acid–water (3:1:3)	0.34	0.34	0.28
Ethyl acetate–pyridine–water (8:2:1)	0.34	0.34	0.22
Butanol–pyridine–water (6:4:3)	0.55	0.55	0.46

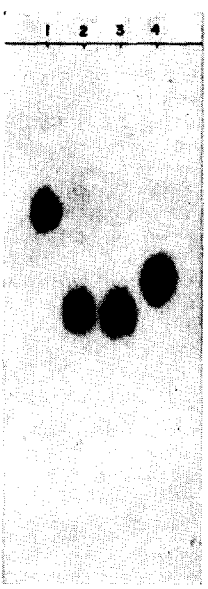


Fig. 6a.

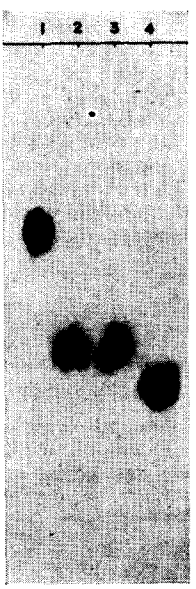


Fig. 6b.

Fig. 6(a) and (b). Paper chromatograms showing that the sugar liberated from the nucleotide (TDP-rhamnose) is identical with rhamnose. (a): Butanol–ethanol–water (104:62:34); (b): Phenol saturated with water. (1) D-glucose; (2) sugar component liberated from TDP-rhamnose; (3) L-rhamnose; (4) L-fucose.

stability of the reaction product to dilution with water[20] was also consistent with the conclusion that the reacting substance in the nucleotide is methylpentose. The amount of methylpentose per mole thymidine was approximately 1 when calculated using rhamnose or fucose as the standard. As shown in Fig. 5, TMP failed almost completely to form a colored product under the conditions of this reaction.

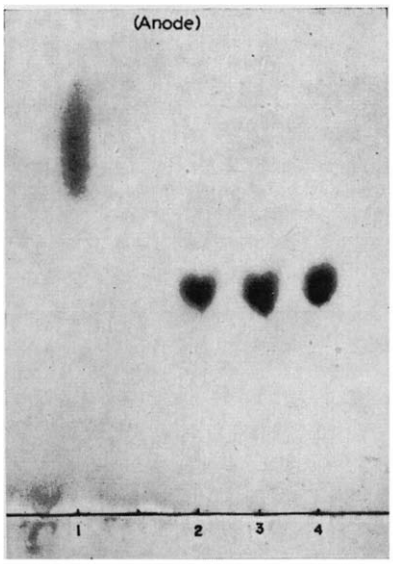


Fig. 7. Paper electrophoresis showing that the sugar liberated from the nucleotide (TDP-rhamnose) is identical with rhamnose. TDP-rhamnose was hydrolyzed in 0.01 *N* HCl at 100° for 10 min. Samples were run in 0.05 *M* borate buffer, pH 8.6 at a potential gradient of about 30 Volts/cm for 1.5 h. (1) L-fucose ($R_{gluc.} = 0.65$); (2) Hydrolysate ($R_{gluc.} = 0.40$); (3) hydrolysate + L-rhamnose ($R_{gluc.} = 0.40$); (4) L-rhamnose ($R_{gluc.} = 0.41$).

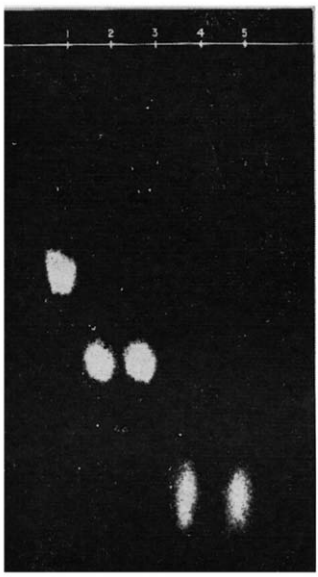


Fig. 8. Paper chromatography in isobutyric acid–1 *N* NH_4OH (10:6) of: (1) TDP liberated from TDP-rhamnose by hydrolysis in 0.01 *N* HCl at 100° for 10 min; (2) TMP liberated from TDP-rhamnose by hydrolysis in 1 *N* HCl at 100° for 10 min; (3) authentic TMP; (4) thymidine obtained by 5′-nucleotidase digestion of sample 2; (5) thymidine obtained by 5′-nucleotidase digestion of sample 3. 5′-nucleotidase digestion was carried out by incubating 0.11 μmole nucleotide with 0.1 μmole $MgCl_2$ and 4.4 units[22] bull semen 5′-nucleotidase at pH 8–9 at 37° for 30 min.

TABLE IV

IDENTIFICATION OF TMP LIBERATED FROM TDP-RHAMNOSE BY ACID HYDROLYSIS

TDP-rhamnose was hydrolyzed in 1 *N* HCl at 100° for 10 min. The TMP liberated was isolated by paper chromatography in isobutyric acid–NH_4OH. For 5′-nucleotidase digestion, 0.11–0.15 μmole nucleotide was incubated with 20 μmoles glycine buffer, pH 8.5, 0.1 μmole $MgCl_2$ and 4.4 units[22] bull semen 5′-nucleotidase in a total volume of 0.9 ml at 37° for 30 min.

Samples	*Total P (μmoles per μmole nucleoside)*	*P split by 5′-nucleotidase (μmoles per μmole nucleoside)*
TMP liberated from TDP-rhamnose	1.02	0.95
Authentic TMP	1.04	0.96
2′,3′-Cytidylic acid		0.00

The sugar was isolated from the acid hydrolysate by large scale chromatography in butanol–ethanol–water, and run with authentic sugars in 7 different solvent systems. As is evident from Table III and Fig. 6 the mobility of the sugar in the hydrolysate was identical with the mobility of rhamnose in all 7 solvents but distinct from that of fucose.

Its electrophoretic mobility in borate buffer pH 8.6[15] also agreed with that of rhamnose (Fig. 7).

These results thus provide sufficient evidence that the reducing residue of the isolated nucleotide is rhamnose.

The molar ratio of the reducing value to thymidine was 1 when the sugar was calculated as rhamnose, lending additional support to the above conclusion. The reducing value of rhamnose was about half that of glucose on a molar base.

Evidence for the attachment of phosphate at the 5′-position of thymidine: Upon hydrolysis of the nucleotide preparation in 1 *N* HCl at 100° for 10 min, a component with the composition and chromatographic mobility of TMP was liberated quantitatively. Treatment with bull semen 5′-nucleotidase converted this component to thymidine with liberation of phosphate (Fig. 8; Table IV). It is evident therefore that one of the phosphates in the compound is attached at the 5′ position of thymidine.

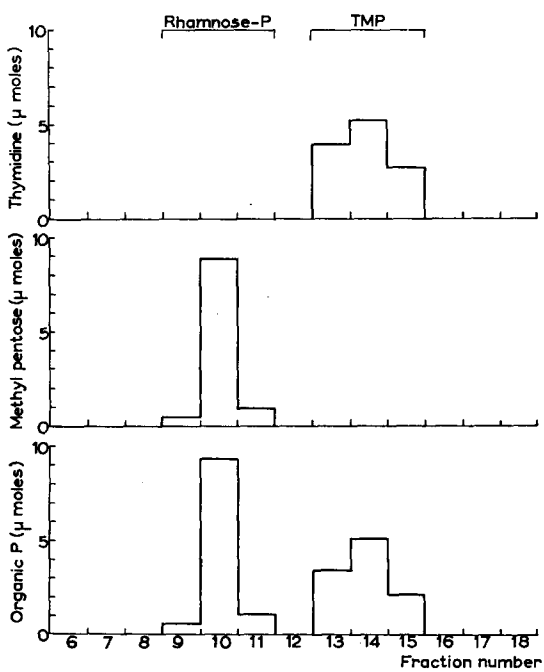
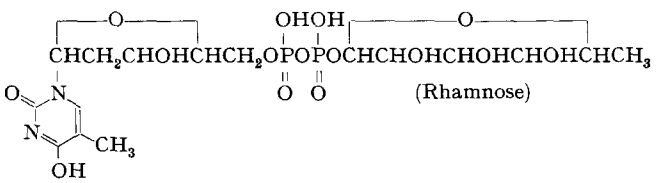


Fig. 9. Ion-exchange chromatography of phosphodiesterase digest of TDP-rhamnose. 2.25 μmoles TDP-rhamnose were incubated with 8 μmoles $MgCl_2$ and 0.04 unit[23] snake venom phosphodiesterase in a total volume of 4 ml at 37° for 30 min. During incubation the pH of the reaction mixture was maintained between 8.0–9.0 by occasional addition of NaOH. The reaction was stopped by adding TCA. After deproteinization and removal of TCA with ether, an aliquot of the sample was applied onto a column of Dowex-1-formate (X 10, 200–400 mesh), 0.6 × 17 cm. Gradient elution was carried out by introducing successively 50 ml of 1 *N* formic acid and 190 ml of 4 *N* formic acid into the mixing flask initially filled with 100 ml of water. 4.4 ml samples were collected at intervals of about 20 min. Analyses were made on each fraction after removal of formic acid by lyophilization. The amount of thymidine was calculated from extinction at 260 mμ.

Evidence for the pyrophosphate linkage between TMP and rhamnose-1-phosphate: The nucleotide was digested with purified snake venom phosphodiesterase which also possessed a strong nucleotide pyrophosphatase activity (tested by using DPN as substrate). Two phosphate-containing products were separated on Dowex-1 column (Fig. 9). One component possessed equimolar amounts of thymidine and phosphate and was eluted at the position corresponding to TMP, while the other component eluted just before the former, contained equimolar amounts of methylpentose and phosphate. The latter substance showed reducing activity only after liberation of phosphate by acid hydrolysis, indicating that the phosphate is substituted on the 1-position of the methylpentose. Thus it appeared that equimolar amounts of TMP and rhamnose-1-phosphate were released by the enzyme, probably by nucleotide pyrophosphatase activity. This fact provides evidence that in the compound, TMP is linked to rhamnose-1-phosphate through a pyrophosphate bond.

This conclusion is further supported by the fact that the hydrolysis with 0.01 *N* HCl at 100° for 10 min resulted in the liberation of TDP (with a smaller amount of TMP) as well as the formation of reducing group. TDP was characterized by analysis (thymidine/phosphate/reducing value = 1.00/2.00/0.00) and by conversion on hydrolysis with 1 *N* HCl at 100° to TMP, which was in turn identified as indicated in the previous section.

Conclusion: It is concluded that the nucleotide studied has the following structure:



DISCUSSION

A great number of acid anhydrides of ribonucleotides, in which the terminal phosphate or pyrophosphate of ribonucleoside triphosphate is replaced by some other group, have been isolated from various biological sources (*cf.* ref. 24). Some of these are UDP-sugars (*e.g.* UDP-glucose; UDP-N-acetylglucosamine), GDP-sugars (*e.g.* GDP-mannose), CDP-alcohols (*e.g.* CDP-ethanolamine) and acyl-AMP (*e.g.* amino acyl-AMP). Extensive studies have revealed that the major function of this type of compounds is in the activation of various groups for synthetic reactions.

Two acid anhydrides of deoxynucleotide were previously isolated: deoxy-CDP-choline from sea urchin eggs[25] and rat hepatoma[26], and deoxy-CDP-ethanolamine from calf thymus[27] and rat hepatoma[26]. An enzymic study[28] has shown that these compounds could be formed and utilized for the synthesis of lecithine or of phosphatidylethanolamine by the action of the same enzymes that catalyze the metabolism of the corresponding ribonucleotides. No result has been reported so far to assign a special metabolic role to these deoxynucleotide anhydrides.

The results presented in this paper leave little doubt that the nucleotide isolated from *L. acidophilus* is TDP-rhamnose. This is the first example of the isolation and identification of an acid anhydride of thymidine nucleotide.

After the publication of the preliminary report of the present work[4], STROMINGER AND SCOTT[29] have independently reported the isolation of three unidentified TDP-sugar compounds (TDP-X, TDP-Y and TDP-Z) from a diaminopimelic acid-requiring mutant of *Escherichia coli*. Two TDP-sugar compounds (TDP-X_1 and TDP-X_2) have also been isolated in our laboratory from *E. coli* strain B (Hershey), one of them (TDP-X_1) being identified as TDP-rhamnose[30]. The properties of all four unidentified sugars (STROMINGER's X, Y and Z and our X_2) suggested that they might also be deoxysugars.

The metabolic functions of TDP-rhamnose and other TDP-sugar compounds call for special attention because of their possible relation to the function and metabolism of DNA.

By analogy with the metabolism of acid anhydrides of ribonucleotides such as UDP- and GDP-sugars, TDP-sugar compounds may well be considered to function as means of activation of sugars for synthesis of bacterial polysaccharides. In this connection it should be emphasized that rhamnose and other deoxysugars are known as constituents of antigenic polysaccharides of bacteria; *e.g.* rhamnose in capsular polysaccharides of type II *pneumococcus*[31], rhamnose and colitose in O antigen of *E. coli*[32,33], and paratose, abequose and tyvelose in specific polysaccharides of group A, B and C *Salmonellae*[34].

On the other hand some observations we have made[1,2,35] suggest that TDP-rhamnose and other TDP-sugar compounds may play some role in DNA synthesis. It has been shown, for example, that when *L. acidophilus* which has been labeled with ^3H by exposure to a medium containing ^3H-thymidine was incubated in a non-labeled medium, the labeled thymidine moiety of TDP-rhamnose was transferred to DNA[2]. Active incorporation of labeled thymidine into the TDP-sugar fraction was also observed in T2-phage infected *E. coli* in the phase of active synthesis of phage DNA as well as in the normally growing bacteria[35]. Although these observations by no means give evidence for the direct participation of these compounds in DNA synthesis, it appears worth-while to explore this possibility further. It might be also conceivable that TDP-sugar compounds are the common intermediate of DNA and polysaccharide synthesis.

SMITH, GALLOWAY AND MILLS[36] have recently reported the isolation of UDP-rhamnose from type II *pneumococcus*. The relation between TDP-rhamnose and UDP-rhamnose remains to be investigated.

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