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Ribosyl and Deoxyribosyl Transfer by Bacterial Enzyme Systems

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The enzymatic transfer of ribose and deoxyribose residues in pyrimidine nucleosides to purines was catalyzed by cell-free extracts of various bacteria. Almost all the strains belonging to *Enterobacteriaceae* were capable of catalyzing the transfer reactions. The transfer activities were also detected among some bacterial strains of other families: *Pseudomonadaceae*, *Corynebacteriaceae*, *Micrococcaceae*, *Bacteriaceae*, and *Bacillaceae*. The rates of the transfer reactions were greatly enhanced in the presence of phosphate ion, and the participation of nucleoside phosphorylases in the reactions was suggested. Uridine phosphorylase, thymidine phosphorylase, and purine nucleoside phosphorylase were purified from cell-free extract of *Aerobacter aerogenes* IFO 3321. The ribosyl transfer from uridine to hypoxanthine was found to be catalyzed by the coupled reactions of uridine and purine nucleoside phosphorylases and the deoxyribosyl transfer from thymidine to hypoxanthine by the coupled reactions of thymidine and purine nucleoside phosphorylases.

Several investigations have appeared on the nucleoside-*N*-glycosyl transfer reactions. In deoxyribonucleoside-requiring lactobacilli, MacNutt (13) first demonstrated a *trans-N*-glycosidase (*trans-N*-deoxyribosylase) which catalyzes the direct transfer of the deoxyribosyl group in purine or pyrimidine deoxyribonucleosides to other bases. Since then, enzymological studies on this enzyme have been carried out by a number of workers (2, 3, 8, 9, 15, 23). In contrast to the direct transfer of deoxyribose residues by *trans-N-deoxyribosylase* of lactobacilli, the participation of nucleoside phosphorylases in the deoxyribosyl-transfer reaction by extracts of *Escherichia coli* has been suggested by Manson and Lampen (14), Lampen (11), Zimmerman and Seidenberg (27, 28), and C. E. Hoffmann (Federation Proc. **11**:231, 1952). Recently, Abrams et al. (1) reported that the deoxyribosyl-transfer activity was associated with nucleoside phosphorylase activity, though the rate of the exchange reaction was independent of the presence of phosphate ion.

There have been a few reports on the ribosyl-transfer reactions. Ott and Werkman (17-19) demonstrated that the transfer of ribose from inosine to adenine yielding adenosine was brought about by the coupled reaction of the purine nucleoside phosphorylase of *E. coli*. A similar reaction was observed with cell-free extract of *Aerobacter cloacae* (26). As to the ribosyl-transfer from pyrimidine nucleosides to purines, Koch (10) pointed out an enzyme which catalyzes the transfer of ribose between purine and pyrimidine nucleosides. However, the mechanism of the reaction has remained unclear.

In the previous paper (6), we worked out a preparation method of purine ribo- and deoxyribonucleosides from pyrimidine nucleosides and purine bases by the use of bacterial enzyme systems catalyzing the *trans-N*-glycosylation reactions. Recently, Sakai et al. (24, 25) reported on a similar reaction.

In this paper, we shall show the distribution among microorganisms of the enzymes catalyzing the ribosyl- and deoxyribosyl-transfer reactions from pyrimidine nucleosides to purines. Other characteristics of the bacterial transfer enzyme systems were also examined to determine whether the transfer reactions are brought about by direct transfer or by the coupled reactions of nucleoside phosphorylases. Furthermore, uridine, thymidine, and purine nucleoside phosphorylases were purified from cell-free extract of *A. aerogenes* and the mechanisms of the transfer reactions were studied by using these purified enzymes.

MATERIALS AND METHODS

Reagents. Hypoxanthine (Zellstofffabrik, Waldhof, Germany), cytidine, uridine (Nutritional Biochemicals Corp., Cleveland, Ohio), inosine (Katayam Chemical Co., Osaka, Japan), and thymidine (Calbiochem, Los Angeles, Calif.) were obtained from commercial sources. Deoxycytidine and deoxyuridine were prepared from codfish deoxyribonucleic acid by degradation with phosphodiesterases of *Strep-*

tomyces aureus (16), followed by dephosphorylation with a phosphomonoesterase of *Phytophthora infestans* IFO 4872. Ribose-1-phosphate was prepared from uridine by use of a uridine phosphorylase of *Pseudomonas putrefaciens* IFO 3910, and its cyclohexylamine salt was prepared according to the method of Plesner and Klenow (21).

Microorganisms. Strains studied in these experiments were supplied by the Institute for Fermentation, Osaka, Japan.

Culture. Bacterial strains were cultured in DX-medium consisting of 2% dextrin (Wako Pure Chemicals, Osaka), 1% Beef Extract (Wako Pure Chemicals), 1% Polypeptone (Daigo Eiyo, Osaka), 0.1% K_2HPO_4 and 0.5% NaCl (*p*H 7.2). Nutrient agar slants containing 1% glucose were used for the maintenance of stock cultures.

An Erlenmeyer flask (200 ml) containing 40 ml of DX-medium was inoculated with a loopful of freshly grown cells and was incubated on a rotary shaker at 28 C for 44 hr. After centrifugation, the cells from 80-ml samples of culture broth were washed in 0.8% NaCl and were centrifuged at 10,000 $\times$ *g* for 15 min. The cell paste was suspended in 30 ml of water.

Extracts. The suspension of washed cells was treated for 10 min in an ice water-cooled Sakuma SV 505 (10-kc) sonic oscillator at 100 w and was then centrifuged at 18,500 $\times$ *g* for 30 min. The supernatant fluid was designated "crude extract." Protein concentration of this solution was usually 3 to 10 mg/ml. The crude extract was dialyzed against 0.01 M tris(hydroxymethyl)-aminomethane (Tris) buffer (*p*H 7.5) for 20 hr at 4 C, and the dialyzed solution was designated "dialyzed crude extract."

Assays. The standard assay system for the ribosyl-transfer reaction contained 50 μmoles of Tris buffer (*p*H 7.5), 1 μmole of hypoxanthine, 2.5 μmoles of uridine, 5 μmoles of potassium phosphate buffer (*p*H 7.5), and 0.1 ml of enzyme preparation in a total volume of 0.5 ml. In the screening tests, the crude extract was used as the enzyme preparation, and the reaction was carried out at 37 C for 1 hr. For examination of the effect of phosphate on the transfer reaction, the dialyzed crude extract was diluted to give a protein concentration of 1 mg/ml and was used as the enzyme preparation. In this case, the reaction was carried out in the presence and absence of phosphate at 37 C for 30 min.

For the deoxyribosyl-transfer reaction from thymidine to hypoxanthine, thymidine was used in place of uridine in the above-described systems.

Inosine formation from hypoxanthine and ribose-1-phosphate was examined in a reaction system containing 1 μmole of hypoxanthine, 1.25 μmoles of cyclohexylamine salt of ribose-1-phosphate, 50 μmoles of Tris buffer (*p*H 7.5), and 0.1 ml of the dialyzed crude extract (1 mg of protein per ml) in a total volume of 0.5 ml. The mixture was incubated for 30 min at 37 C.

The above-described reactions were stopped by heating the reaction mixtures for 3 min in a boiling water bath. After centrifugation, the amount of formed inosine or deoxyinosine in the supernatant fluid was measured by Kalckar's enzymatic method (7).

Degradation of uridine and thymidine was tested as follows. The reaction mixture containing 50 μmoles of potassium phosphate buffer (*p*H 7.5), 1 μmole of uridine or thymidine, and 0.1 ml of the dialyzed crude extract (1 mg of protein per ml) in a total volume of 0.3 ml was incubated for 30 min at 37 C. Then, the mixture was cooled to 0 C, and 0.4 ml of 2 N NaOH was added immediately. The volume was filled to 4 ml with distilled water. The rates of degradation of uridine and thymidine were estimated by measuring increases of optical densities at 290 and 300 mμ in 0.2 N NaOH. These increases correspond to 4.76×10^3 at 290 mμ and 4.31×10^3 at 300 mμ, resulting from the complete conversion of uridine to uracil and thymidine to thymine, respectively.

Nucleoside phosphorylases were assayed as follows. The reaction mixture for uridine phosphorylase assay contained 40 μmoles of sodium phosphate buffer (*p*H 7.5), 20 μmoles of sodium arsenate buffer (*p*H 7.5), 2 μmoles of uridine, and less than 0.02 unit of enzyme in a total volume of 0.5 ml. The mixture was incubated for 15 min at 37 C, and the reaction was stopped by the addition of 0.5 ml of 2 N NaOH.

For the assay of thymidine phosphorylase, 4 μmoles of thymidine was used in place of uridine. The amount of enzyme used in the reaction system was less than 0.04 unit. The rate of conversion of uridine and thymidine to their corresponding bases was determined spectrophotometrically.

Purine nucleoside phosphorylase was assayed in a reaction mixture containing 120 μmoles of sodium phosphate buffer (*p*H 7.5), 1.5 μmoles of inosine, 130 units of Step III fraction of milk xanthine oxidase (5), and less than 0.015 unit of enzyme in a total volume of 3.2 ml. One unit of xanthine oxidase causes an increase in optical density of 0.001/min in the above reaction mixture, which contains hypoxanthine in place of inosine. After the reaction mixture was incubated for 15 min at 37 C, the reaction was terminated by heating in a boiling water bath for 3 min. Inosine degradation was estimated by measuring the increase of optical density at 290 mμ.

In nucleoside phosphorylase assays, 1 unit of the enzyme was defined as the amount causing the degradation of 1 μmole of substrate per min in the above-mentioned assay systems. Specific activity was defined as units of enzyme per milligram of protein. Under the conditions of assay, the rate of the degradation of each substrate was proportional to the amount of enzyme.

Protein was measured by the method of Lowry et al. (12) or by the absorbancy at 280 mμ.

RESULTS

Distribution of the transfer activity. The results of the screening tests are shown in Table 1. Bacteria are listed according to *Bergey's Manual for Determinative Bacteriology*, 6th ed., 1948.

The ribosyl transfer from uridine to hypoxanthine was detected in the extracts of all strains of *Enterobacteriaceae*. Most of them were able to transfer deoxyribose residue in thymidine to hypoxanthine also. A few strains of *Pseudo-*

TABLE 1. *Distribution of transfer activity among bacteria*[a]

Bacteria	Ribosyl transfer	Deoxyribosyl transfer
Pseudomonadaceae		
Pseudomonas aeruginosa	−	−
P. aureofaciens	−	−
P. chlororaphis	−	−
P. fluorescens	−	−
P. fragi	−	−
P. graveolens	−	−
P. indoloxidans	−	−
P. iodinum	−	−
P. marginalis	−	−
P. mildenbergii	−	−
P. ovalis	−	−
P. riboflavina	−	±
P. striafaciens	−	−
P. putrefaciens	+	±
P. syncyanea	−	−
P. synxantha	−	−
P. vendrelli	−	−
Aeromonas hydrophila	+	+
Protaminobacter alboflavus	±	±
Azotobacteriaceae		
Azotobacter agilis	−	−
Micrococcaceae		
Micrococcus caseolyticus	−	−
M. flavus	−	−
M. subflavus	−	−
M. freudenreichii	−	−
M. lysodeikticus	−	−
M. luteus	−	−
M. roseus	−	−
M. rubens	−	−
M. ureae	−	−
M. varians	−	−
Sarcina aurantiaca	−	±
S. lutea	+	+
S. marginata	−	−
S. variabilis	−	−
Corynebacteriaceae		
Corynebacterium equi	−	−
C. sepedonicum	+	+
Achromobacteriaceae		
Achromobacter liquidum	−	−
Flavobacterium aquatile	−	−
F. arborescens	−	−
F. esteroaromaticum	−	−
F. flavescens	−	−
F. suaveolens	−	±
Enterobacteriaceae		
Escherichia coli	+	+
E. coli var. *communior*	+	+
Aerobacter aerogenes	+	+
Erwinia aroideae	+	+
E. carotovora	+	+
Serratia marcescens	+	+ and −[b]
S. plymuthicum	+	−
S. indica	+	+
Proteus vulgaris	+	+
P. mirabilis	+	+
P. morganii	+	+
P. rettgeri	+	+
Salmonella typhimurium	+	Not examined
S. enteritidis	+	+
Bacteriaceae		
Bacterium cadaveris	+	+
B. mycoides	−	−
B. orleanense	−	−
Cellulomonas cellacea	−	−
C. flavigena	−	−
C. gelida	−	−
Bacillaceae		
Bacillus brevis	+	+
B. cereus	−	−
B. cereus var. *mycoides*	−	−
B. firmus	−	−
B. licheniformis	−	−
B. megaterium	−	−
B. polymyxa	−	−
B. pumilus	−	−
B. roseus	−	−
B. sphaericus	+	+
B. subtilis	−	−
B. subtilis var. *niger*	−	−

[a] Conversion rate of purine base to the nucleoside was above 25% (+), between 5 and 25% (±), and below 5% (−). Some of the experiments were carried out with adenine as acceptor substrate, and the amount of adenosine formed was enzymatically assayed (6).

[b] Different results were obtained with separate strains of this species.

monadaceae, *Micrococcaceae*, *Corynebacteriaceae*, *Bacteriaceae*, and *Bacillaceae* showed transfer activity. Other microorganisms, including yeasts, molds, and *Streptomyces*, did not have the transfer activity.

Types of transfer reactions. The nucleoside-*N*-glycosyl transfer reaction is catalyzed either by direct transfer or by the coupled reactions of nucleoside phosphorylases. These two reaction types can be distinguished by the effect of phosphate on the reaction; i.e., the reaction of phosphorylase type is dependent on the presence of

phosphate, whereas that of another type is independent.

The effect of phosphate on the ribosyl- and deoxyribosyl-transfer reactions by various bacterial extracts is indicated in Table 2. All of the transfer reactions were stimulated to a great extent in the presence of phosphate ion. Therefore, nucleoside phosphorylases seem likely to be involved in these reactions.

If the glycosyl transfer from pyrimidine nucleosides to purines is catalyzed by the coupled reactions of nucleoside phosphorylases, bacterial extracts having the transfer activity should phosphorolyze pyrimidine nucleosides and should form inosine (deoxyinosine) from hypoxanthine and ribose-1-phosphate (deoxyribose-1-phosphate). Since purine nucleoside phosphorylase shows no specificity in regard to ribo- and deoxyribonucleosides (4), we have examined the latter reaction using ribose-1-phosphate only.

The extracts possessing the ribosyl-transfer activity phosphorolyzed uridine and those possessing the deoxyribosyl-transfer activity phosphorolyzed thymidine (Table 2). Both extracts catalyzed inosine formation from hypoxanthine and ribose-1-phosphate. The transfer rates seem to be determined either by the pyrimidine nucleoside phosphorolyzing activity or by inosine-forming activity from hypoxanthine and ribose-1-phosphate. The extracts of *E. coli* K-12, *E. coli* var. *communior*, *A. aerogenes*, *Erwinia aroideae*, *Serratia marcescens*, *P. putrefaciens*, and *Corynebacterium sepedonicum* which had strong ribosyl-transfer activity were able to phosphorolyze uridine and to form inosine from ribose-1-phosphate. On the other hand, *Bacillus subtilis* and *B. licheniformis*, which showed little or no ribosyl-transfer activity, could not degrade uridine. The ribosyl-transfer rate in *Sarcina lutea* seemed to be limited by uridine degrading activity, and that in *Proteus vulgaris*, *Salmonella enteritidis*, *Aeromonas hydrophila*, and *Bacterium cadaveris*, by the activity to form inosine from hypoxanthine and ribose-1-phosphate. A similar relationship was observed between the deoxyribosyl-transfer rate and the thymidine-phosphorolyzing or the inosine-forming rate.

The degradation of pyrimidine nucleosides was examined in Tris buffer (*p*H 7.5) instead of phosphate buffer (*p*H 7.5). The degradation in Tris buffer was negligible in most cases. Therefore, uridine and thymidine might be cleaved phosphorolytically. *S. marcescens* and *B. cada-*

TABLE 2. *Ribosyl and deoxyribosyl transfer by bacterial enzyme systems in the presence and absence of phosphate and the relationship of the transfer activities to nucleoside phosphorylase activities*[a]

Enzyme preparation	Ribosyl transfer (formation of inosine from uridine and hypoxanthine)		Deoxyribosyl transfer (formation of deoxyinosine from thymidine and hypoxanthine)		Degradation of		Formation of inosine from hypoxanthine and ribose-1-phosphate
	Phosphate absent	Phosphate present	Phosphate absent	Phosphate present	Uridine (formation of uracil)[b]	Thymidine (formation of thymine)[b]	
Escherichia coli K-12 IFO 3208	Trace	3.78	0	6.38	8.21	5.96	4.31
E. coli var. *communior* IFO 3552	0	3.03	0.59	8.03	4.36	7.28	7.16
Aerobacter aerogenes IFO 3321	Trace	2.24	0.65	7.61	4.15	6.92	4.20
Erwinia aroideae IFO 3830	0	5.00	0.02	3.61	8.10	4.30	6.25
Proteus vulgaris IFO 3167	0	0.74	0	1.08	6.14	2.54	1.11
Salmonella enteritidis IFO 3313	0	0.32	0	0.44	2.68	0.94	1.09
Serratia marcescens IFO 3047	0	2.05	0	0.13	6.15[c]	0.53	2.83
Pseudomonas putrefaciens IFO 3908	0	3.09	0.07	0.94	7.32	1.17	4.05
Aeromonas hydrophila IFO 3820	0	0.55	0	0.44	2.69	4.74	0.74
Bacillus brevis IFO 3864	0	0.55	0	0.44	2.54	4.65	0.92
Bacterium cadaveris IFO 3731	0	1.50	0.15	2.72	6.27[c]	3.37	1.44
Corynebacterium sepedonicum IFO 3306	0	2.20	0.76	7.35	4.32	6.05	4.96
Sarcina lutea IFO 3232	0	0.58	0	2.17	0.56	2.47	8.16
Bacillus subtilis IFO 3010	0	Trace	0	Trace	0.06	0.23	2.68
B. licheniformis ATCC 9945a	0	Trace	0	0	0.02	0.05	0.81

[a] See text for experimental details. Results expressed in micromoles per milligram of protein per 30 min.

[b] Degradation in the absence of phosphate was negligible in most cases.

[c] Rate of uridine degradation in the absence of phosphate was subtracted.

veris were the exceptions. They degraded uridine to some extent in the absence of phosphate, but, even with these strains, uridine degradation was greatly enhanced by phosphate ion.

Verification of participation of nucleoside phosphorylases. To prove the involvement of nucleoside phosphorylases in the transfer reactions, uridine phosphorylase, thymidine phosphorylase, and purine nucleoside phosphorylase were purified from cells of *A. aerogenes* IFO 3321. Purification procedures are illustrated in Fig. 1.

Uridine phosphorylase was precipitated at *p*H 4.8 as described by Razzell and Khorana (22) for the enzyme of *E. coli*. After thymidine phosphorylase was inactivated by heat treatment, purine nucleoside phosphorylase was removed by ion-exchange chromatography (Fig. 2). This enzyme, similar to uridine phosphorylase of *E. coli* (20), phosphorolyzed uridine exclusively, whereas cytidine, deoxyuridine, deoxycytidine, and thymidine were not cleaved by the enzyme. Optimal *p*H was 7.6, and apparent K_m values for uridine, phosphate, and arsenate were 1.0×10^{-3}, 1.0×10^{-2}, and 2.0×10^{-2} M, respectively.

Most of the uridine phosphorylase and purine nucleoside phosphorylase was precipitated by adjusting to *p*H 4.8 and 4.6, respectively. Thymidine phosphorylase was in the supernatant fluid after precipitation at *p*H 4.6 and was concentrated and freed from other phosphorylases by ammonium sulfate fractionation at the lower saturation levels. This enzyme phosphorolyzed thymidine and deoxyuridine, but not uridine,

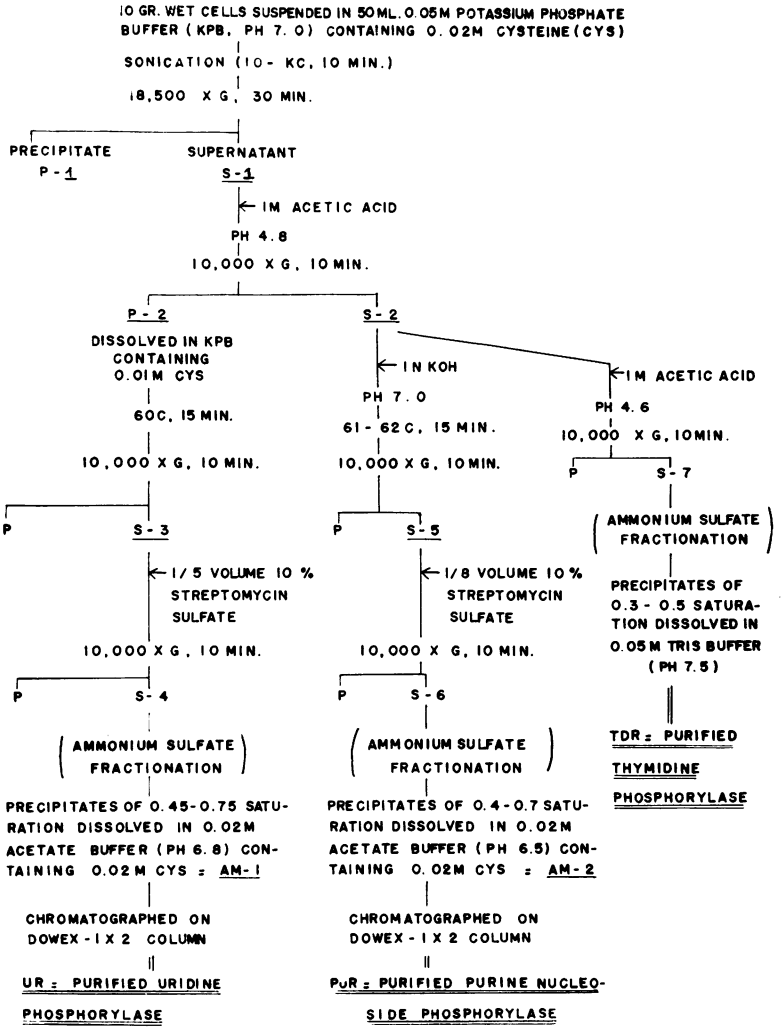

FIG. 1. *Purification procedures of nucleoside phosphorylases.*

cytidine, and deoxycytidine. Optimal *p*H was 7.2, and apparent K_m values for thymidine, phosphate, and arsenate were 4.0×10^{-3}, 5.3×10^{-3}, and 4.0×10^{-3} M, respectively.

Uridine phosphorylase was precipitated at *p*H 4.8, and thymidine phosphorylase was inactivated by heat treatment. Purine nucleoside phosphorylase was also purified by ion-exchange chromatography (Fig. 3). Studies on the properties of this enzyme are in progress.

Chromatographic purification procedures depicted in Fig. 2 and 3 were originally described for the purification of thymidine phosphorylase of *E. coli* (22). The same procedure has been applied for the purification of uridine and purine nucleoside phosphorylases. Thymidine phosphorylase could be separated from uridine phosphorylase, but could not be separated from purine nucleoside phosphorylase by this chromatographic technique.

Summary of the purification processes is shown in Table 3. Three purified nucleoside phosphorylases were mixed or used separately in the ribosyl-transfer and the deoxyribosyl-transfer reactions. The former reaction was catalyzed only by the coexistence of uridine and purine

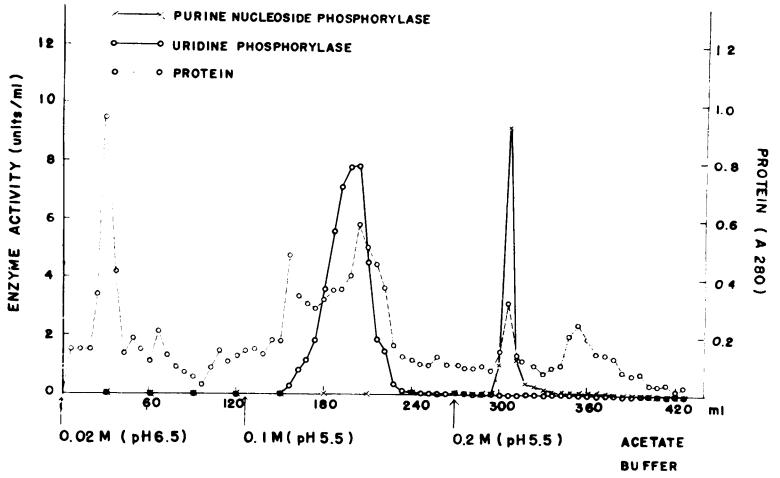

FIG. 2. *Chromatographic purification of uridine phosphorylase. An 8.8-ml sample of fraction AM-1 (see Fig. 1) containing 108 mg of protein was dialyzed against 0.02 M acetate buffer (pH 6.5) containing 0.02 M cysteine and was then loaded onto a 2 × 18 cm column of Dowex-1 × 2, 200- to 400-mesh, equilibrated with the same buffer. Buffers used for elution contained 0.02 M cysteine. Flow rate was 15 ml/hr.*

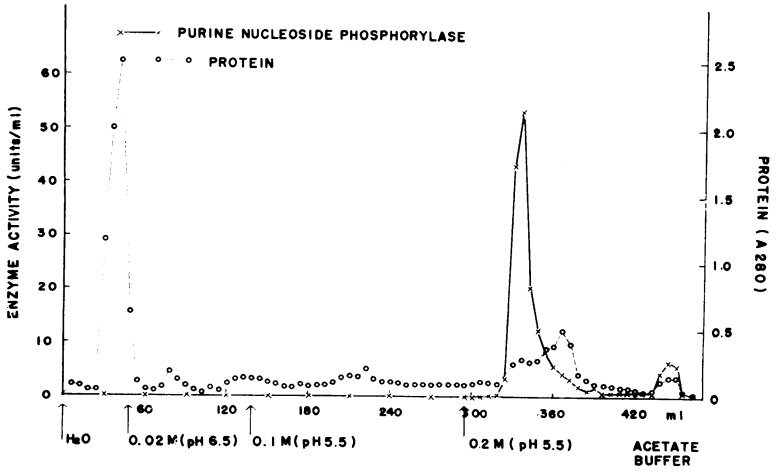

FIG. 3. *Chromatographic purification of purine nucleoside phosphorylase. A 7-ml sample of fraction AM-2 (see Fig. 1) containing 115 mg of protein was dialyzed and chromatographed as described in Fig. 2.*

nucleoside phosphorylases, and the latter by the coexistence of thymidine and purine nucleoside phosphorylases (Table 4). No single enzyme was able to catalyze the transfer reactions.

The crude extract which possessed the three phosphorylases catalyzed both the ribosyl- and deoxyribosyl-transfer reactions.

DISCUSSION

Until recently, the ribosyl- and deoxyribosyl-transfer reactions from pyrimidine nucleosides to purines were investigated only with a few bacterial strains of *E. coli* and *Lactobacillus*. In this report, such transfer reactions were studied with cell-free extracts of various aerobically grown bacteria.

The ribosyl- and deoxyribosyl-transfer reactions by all the bacterial strains tested in these experiments were considerably enhanced in the presence of phosphate ion. This fact may reflect the participation of nucleoside phosphorylases in the reactions. This presumption is further supported by measurement of the activities to phosphorolyze pyrimidine nucleosides and to

TABLE 3. *Summary of purification of nucleoside phosphorylases*[a]

Fraction	Volume (ml)	Protein (mg/ml)	Uridine phosphorylase		Thymidine phosphorylase		Purine nucleoside phosphorylase	
			Specific activity (units/mg of protein)	Recovery (%)	Specific activity (units/mg of protein)	Recovery (%)	Specific activity (units/mg of protein)	Recovery (%)
S-1	250	13.5	0.34	100	1.28	100	2.67	100
P-2	100	14.6	0.60	75.9	0.17	5.6	0.31	5.0
S-3	93.6	6.15	1.42	70.8	0	0	0.62	4.0
AM-1	18.1	12.3	3.48	67.3	0	0	1.50	3.8
UR	55.1	1.34	5.67	36.3	0	0	0	0
S-1	250	12.5	0.25	100	1.52	100	3.38	100
S-2	273	6.43	0.03	7.1	1.74	65.0	4.99	82.9
S-5	265	4.45	0.02	3.4	0	0	6.65	74.4
Am-2	26.6	16.4	0.06	3.4	0	0	21.6	89.6
PUR	66.0	0.23	0	0	0	0	199.9	28.8
S-1	80.0	20.0	0.18	100	1.43	100	2.27	100
TDR	10.0	8.5	0.05	1.5	4.07	15.1	0	0

[a] See Fig. 1 for purification procedures and fractions. Each nucleoside phosphorylase was purified from separate extracts.

TABLE 4. *Ribosyl and deoxyribosyl transfer with purified nucleoside phosphorylases*[a]

Enzyme no.	Enzyme	Activity (units per ml)[b]			Ribosyl transfer (formation of inosine)[c]				Deoxyribosyl transfer (formation of deoxyinosine)[c]			
		UR	TDR	PUR	0 min[d]	15 min	30 min	60 min	0 min	15 min	30 min	60 min
1	Crude extract	0.18	1.43	2.27	0	172	306	612	0	794	896	905
2	Purified UR	0.81	0	0	0	0	0	0	0	0	0	0
3	Purified Tur	0.01	1.15	0	0	0	0	0	0	0	0	0
4	Purified Pur	0	0	2.03	0	0	0	0	0	0	0	0
4′	Purified Pur	0	0	0.43	0	0	0	0	0	0	0	0
5	2 + 4′	0.81	0	0.43	0	653	828	850	0	0	0	0
6	3 + 4	0.01	1.15	2.03	0	13	39	86	0	789	884	919

[a] The reaction mixture containing 1 μmole of hypoxanthine, 4 μmoles of uridine or thymidine, 2.5 μmoles of potassium phosphate buffer (*p*H 7.5), 100 μmoles of Tris buffer (*p*H 7.5), and 0.2 ml of the enzyme in a total volume of 1 ml was incubated at 37 C. The activity of each nucleoside phosphorylase is indicated in the table.

[b] Abbreviations: UR = uridine phosphorylase; TDR = thymidine phosphorylase; and Pur = purine nucleoside phosphorylase.

[c] Expressed in millimicromoles per milliliter of reaction mixture.

[d] Refers to reaction period.

form inosine from hypoxanthine and ribose-1-phosphate. The transfer reactions were catalyzed by the extracts possessing both activities.

The participation of nucleoside phosphorylases in the transfer reactions was verified more decisively by separation and recombination of pyrimidine nucleoside phosphorylases and purine nucleoside phosphorylase of *A. aerogenes*.

The ribosyl transfer between purine nucleosides by the coupled reaction of purine nucleoside phosphorylase of *E. coli* was demonstrated by Ott and Werkman (17–19). In contrast to their reaction in which one enzyme seems to be involved, the transfer of ribose between purine and pyrimidine nucleosides was proved to be catalyzed by the coupling of two separate enzymes, i.e., uridine phosphorylase and purine nucleoside phosphorylase. Recently, Sakai et al. (25) recognized a similar reaction in cell-free extract of *C. sepedonicum*.

Though the involvement of nucleoside phosphorylases in the deoxyribosyl-transfer reaction from pyrimidine deoxyribonucleosides to purines and vice versa in *E. coli* was suggested by Manson and Lampen (14) and C. E. Hoffmann (Federation Proc. **11**:231, 1952), no detailed studies on the enzyme systems have appeared. Our present experiments have made it clear that thymidine phosphorylase and purine nucleoside phosphorylase acted jointly in the deoxyribosyl-transfer reaction.

In addition, as might be expected from the study of substrate specificity, separate pyrimidine nucleoside phosphorylases took part in the ribosyl- and deoxyribosyl-transfer reactions.

As to purine nucleoside phosphorylase, we have made no effort to separate it into ribonucleoside-specific and deoxyribonucleoside-specific portions. The properties of bacterial purine nucleoside phosphorylases remain to be investigated.

Purine and pyrimidine nucleotides are synthesized separately under rigid controls by their respective end products. Both purine and pyrimidine compounds are largely used for the biosynthesis of nucleic acids. However, little is known about the metabolic connection between these two groups of compounds. It is possible that the activities to transfer ribose and deoxyribose residues in pyrimidine nucleosides to purines play some role in keeping the metabolite balance between purine and pyrimidine nucleosides or nucleotides.

It was interesting that the ribosyl- and deoxyribosyl-transfer activities were detected very commonly among strains of *Enterobacteriaceae*, whereas the occurrence among strains of other families was rather restricted to a limited number of species. Though the biological meaning of the transfer reactions is not yet understood, the wide distribution of the transfer activities among enteric bacteria may suggest their nutritional contribution to this group of bacteria.

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