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Regulation of Purine Utilization in Bacteria

VI. Characterization of Hypoxanthine and Guanine Uptake into Isolated Membrane Vesicles from *Salmonella typhimurium*

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Uptake of hypoxanthine and guanine into isolated membrane vesicles of *Salmonella typhimurium* TR119 was stimulated by 5'-phosphoribosyl-1'-pyrophosphate (PRPP). For strain *proAB47*, a mutant that lacks guanine phosphoribosyltransferase, PRPP stimulated uptake of hypoxanthine into membrane vesicles. No PRPP-stimulated uptake of guanine was observed. For strain TR119, guanosine 5'-monophosphate and inosine 5'-monophosphate accumulated intravesicularly when guanine and hypoxanthine, respectively, were used with PRPP as transport substrates. For strain *proAB47*, IMP accumulated intravesicularly with hypoxanthine and PRPP as transport substrates. For strain TR119, hypoxanthine also accumulated when PRPP was absent. This free hypoxanthine uptake was completely inhibited by *N*-ethylmaleimide, but the PRPP-stimulated uptake of hypoxanthine was inhibited only 20% by *N*-ethylmaleimide. Hypoxanthine and guanine phosphoribosyltransferase activity paralleled uptake activity in both strains. But, when *proAB47* vesicles were sonically treated to release the enzymes, a three- to sixfold activation of phosphoribosyltransferase molecules occurred. Since *proAB47* vesicles lack the guanine phosphoribosyltransferase gene product and since hypoxanthine effectively competes out the phosphoribosylation of guanine by *proAB47* vesicles, it was postulated that the hypoxanthine phosphoribosyltransferase gains specificity for both guanine and hypoxanthine when released from the membrane. A group translocation as the major mechanism for the uptake of guanine and hypoxanthine was proposed.

Three basic mechanisms of substrate transport have been identified in bacteria. the facilitated diffusion of glycine exemplifies a non-energized, non-concentrative carrier-mediated mechanism (17, 18). The concentrative uptake of lactose, proline, and other amino acids in enteric bacteria, coupled to a lactic dehydrogenase-mediated energized membrane state (1, 19, 20, 27), represents a second transport mechanism. In both of these systems, the substrate without alteration traverses the membrane.

The third type of transport reaction, in which covalent change of the substrate occurs during transport, is referred to as group translocation. This mechanism is exemplified by the phosphoenolpyruvate-phosphotransferase system which converts a variety of monosaccharides to their sugar phosphates during the permeation process (22, 23, 24), and by the adenine phosphoribosyl-transferase system which, in the presence of 5'-phosphoribosyl-1'-pyrophosphate (PRPP), converts external adenine into adenosine 5'-monophosphate (AMP) during the transport process (3, 13, 14). These systems have been studied in *Bacillus subtilis* and *Escherichia coli*. In addition, the lactose permeation mechanism in *Staphylococcus aureus* (30, 31) is similar to these last two systems in that a vectorial phosphorylation of lactose occurs across the membrane.

The development of appropriate techniques for the isolation of transport systems on isolated membrane vesicles devoid of intracellular metabolic enzymes has greatly facilitated the impressive progress made in bacterial transport in the last decade. Thus, the isolated membrane vesicle as a transport-competent subcellular system has contributed major evidence for the above concepts. One of these studies, the adenine phosphoribosyltransferase system (13, 14), bears direct relationship to the work described in this report.

In *E. coli*, adenine uptake is mediated by membrane-localized adenine phosphoribosyl-

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transferase which results in the intravesicular accumulation of AMP by a PRPP-dependent transport process (13, 14). The enzyme is largely released from the periplasmic space by osmotic shock (14). The kinetics and regulatory parameters of the soluble enzyme purified to homogeneity (12), of the enzyme on isolated membrane vesicles (13), and of the enzyme in the intact cell (14) are virtually identical.

Initial work on the guanine and hypoxanthine phosphoribosyltransferase system (14) utilized *E. coli* and characterized uptake of hypoxanthine and guanine into starved, resting cells and into osmotically shocked cells (14). The initial rate of uptake of these purines by starved cells was stimulated at least 50-fold by PRPP. Althouh PRPP dependence for hypoxanthine uptake was sigmoidal rather than hyperbolic, it showed saturation kinetics. Further, most of the phosphoribosyltransferase activities for both guanine and hypoxanthine were released upon osmotic shock, localizing them at or near the membrane surface. The shocked cells were capable of hypoxanthine uptake comparable only to the amount of hypoxanthine phosphoribosyltransferase remaining associated with the cells (14). Other work compared the vesicle uptake of purines to purine nucleosides (10). This work gave evidence that nucleosides were first processed at the membrane surface by purine nucleoside phosphorylase, resulting in intravesicular ribose 1′-phosphate (10; R. L. Rader and J. Hochstadt, submitted for publication). The purine base remained at the membrane surface and could interact with a phosphoribosyltransferase and PRPP, resulting in the accumulation of intravesicular nucleoside monophosphate (10). Thus, the kinetics, inhibition, and mechanism of uptake for the purine moiety of nucleosides or free purine bases appeared to be the same. Recently, this mechanism of uptake for adenosine into *E. coli* whole cells has been independently confirmed by Yagil and Beacham (32).

In previous work (11), we described the role that the hypoxanthine and guanine uptake systems may have in amino acid control of growth in bacteria. This role is related to the response of these phosphoribosyltranferases and vesicle uptake systems to guanosine tetraphosphate (ppGpp) (11). More recently, this laboratory has described the purification and characterization of the 6-OH purine phosphoribosyltransferase from *E. coli* K-12 (8). The activity for forming nucleotides from guanine, hypoxanthine, and xanthine was derepressed by growth on purines and amethopterin, and this derepression was coordinate for the three activities (8). Several subfractions of enzyme activity were obtained, but each fraction had more than a single substrate specificity. This has also been shown in *E. coli B* (21, 26) and *E. coli* W3110 (J. A. Holden, P. D. Harriman, and J. D. Wall, submitted for publication).

In *Salmonella*, mutants are available that are unable to take up either hypoxanthine (5) or guanine (6). This report describes the utilization of hypoxanthine and guanine by isolated membrane vesicles of *Salmonella typhimurium* TR119 and the utilization of hypoxanthine by strain *proAB47* (6), a mutant unable to utilize guanine. This report also describes the phosphoribosyltransferase activity for intact membrane vesicles and after release from membrane vesicles by sonic treatment.

(A preliminary report of this work was presented by L. Jackman, and J. Hochstadt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K160, p. 173.)

MATERIALS AND METHODS

Chemicals. Chemicals were obtained from standard commercial sources or as describd (12). PRPP was purchased from Sigma Chemical Co. as the tetrasodium salt. Purity was checked as described previously (12). Aminopterin was purchased from Nutritional Biochemicals Corp.

Bacteria. *S. typhimurium* TR119 *(aroP505 gal-501 ilvA405 proA46 purC7 purI509 rha-416 str)* was obtained from Janice Chou and Robert G. Martin. Strain TR119 exhibits both guanine and hypoxanthine phosphoribosyltransferase. The proline deletion mutant, *proAB47* (derived from *S. typhimurium* LT-2), was obtained from Mark Levinthal and Joseph S. Gots. Strain *proAB47* cannot utilize guanine and does not exhibit the *gpt* gene product (6).

Media. Cells were grown on PAT medium as described previously (12) with the exception that thymine was used at 10^{-4} M. Aminopterin was used in place of amethopterin in most experiments.

Culture and storage of cells. Cells were grown until late log or early stationary phase in 1 liter of medium per 2-liter flasks at 37 C with rotary gyration at 150 rpm. Cells were then harvested in the cold by centrifugation and either used immediately or stored overnight in an ice bath prior to membrane vesicle preparation. Cells grown to be used for sonic extracts were disrupted immediately upon harvest or frozen in liquid N_2 and stored at −20 C overnight or at −79 C for longer times.

Preparation of spheroplasts and membrane vesicles. The procedure for vesicle preparation, unless otherwise noted in the text, was an ethylenediaminetetraacetate (EDTA)-lysozyme method described previously (9) under the section for *Salmonella* strains. The only modification was that the 0.1 M potassium phosphate buffer was used at pH 7.4 throughout the entire vesicle preparation and storage. Vesicles were stored at −79 C.

Preparation of sonic extracts. Disruption of membrane vesicles and whole cells by sonic oscillation was performed using a Heat Systems Ultrason-

ics Inc. Sonifier (model 185 W) at maximum output. Whole cells were disrupted for six 30-s periods in ice with intermittent cooling. Membrane vesicles were disrupted for 30 to 90 s in ice with cooling every 30 s. Debris was removed by centrifugation.

Uptake studies. Two methods were used to determine the uptake of guanine and hypoxanthine. In method A (filter assay), uptake was measured as described previously (9, 13). Between 300 to 500 μg of vesicle protein was used per assay, and potassium phosphate buffer (pH 7.4) was always 0.05 M. Radioactively labeled substrates were kept at 33 C prior to addition to initiate the reaction. Uptake was performed at 33 C. Portions (2 ml) of 0.5 M NaCl at 33 C were used to terminate the reactions. The reaction mixtures were then rapidly filtered through 0.45-μm nitrocellulose filters and washed with 1 ml of 0.5 M NaCl. The tubes were then rinsed with 1 ml of the NaCl, and the contents were again filtered through the same nitrocellulose filters and washed with 3 ml of the NaCl. This washing procedure gave results low in background for hypoxanthine and guanine. As indicated in the figures, zero time controls were subtracted from experimental values. These controls contain the reaction mixture diluted with 2 ml of 0.5 M NaCl before label was added.

Alternatively, in some experiments uptake was measured by method B (pellet assay). In this method reactions were performed in tubes (6 by 50 mm) containing 300 to 500 μg of vesicle protein, 0.05 M potassium phosphate buffer (pH 7.4), 2 mM magnesium acetate, 1 mM PRPP, and radioactively labeled hypoxanthine or guanine at concentrations indicated in the footnotes of the tables. Before addition of the labeled substrate, the reaction mixture was preincubated for 5 min at 33 C unless otherwise noted in the figure legends. Reactions were incubated for various times at 33 C and terminated by immersing quickly in an ice bath for a few seconds and then centrifugating at 41,500 × *g* at 2 C for 1 to 2 min. The supernatant fluids were carefully aspirated and put in an ice bath, and the walls of the tubes were wiped dry with pipe cleaners. The pellets were immediately resuspended in 10 μl of water and boiled for 2 to 5 min. Samples of supernatant and pellet were chromatographed and radioactivity was determined as described below.

In all experiments PRPP magnesium acetate and all effectors were added prior to membrane addition unless otherwise noted. This allows for the possibility of shocking the effectors into the vesicles (8).

Chromatographic separation of purines, nucleosides, and nucleotides. Chromatography was performed as previously described (8, 12) using thin-layer cellulose sheets (Eastman Chromagram). An additional solvent convenient for separation of the compounds under study in this report was Solvent 9 (Schwartz-Mann), which contains water-saturated ammonium sulfate, 0.1 M potassium phosphate buffer (pH 6.0), and isopropanol in a proportion of 79:19:2. The purine derivatives corresponding to standards are visualized under shortwave ultraviolet light and cut out, and radioactivity is determined by liquid scintillation using toluene-based scintillation fluid. Cellulose thin-layer sheets were ruled in channels 2.0 by 10 cm for Solvent 1 (12) and 2.0 by 20 cm for Solvent 9. Samples were always applied to the chromatograms with unlabeled hypoxanthine, inosine, or inosine 5'-monophosphate (IMP) or with guanine, guanosine, and guanosine 5'-monophosphate (GMP) to faciliate visualization under shortwave ultraviolet light. For most experiments, Liquifluor (New England Nuclear) was used as the scintillating agent (227 ml/8 pints [about 3.8 liters] of toluene).

Assay of guanine and hypoxanthine phosphoribosyltransferase activity. Assay conditions were described previously (12, 13). Briefly, this method involves incubation of the [^{14}C]purine with enzyme fractions in the presence of PRPP and magnesium ions. The reaction is terminated by addition of potassium EDTA, by boiling, or by cooling in an ice bath. Conversion of purine to nucleotide or nucleoside is monitored by separation of the purines from nucleotide or nucleoside on cellulose thin-layer chromatography sheets as described above. When whole membrane phosphoribosyltransferase activity was measured, the reaction was terminated by boiling for 3 to 10 min.

Deviations from previously published procedures (12) were as follows. The tetrasodium salt PRPP was used exclusively. Reactions were always initiated by addition of the labeled substrate. Controls contained no PRPP or magnesium acetate.

Using an ammonium sulfate fraction (42 to 49%) of the phosphoribosyltransferase from *S. typhimurium* TR119, activity is a linear function of time for at least 1 h and directly proportional to the enzyme concentration. In addition, up to 80% of the labeled substrate can be converted during the reaction without affecting linearity.

Determination of intravesicular volume. Intravesicular volume of TR119 membrane vesicles was determined as described previously (13). By this method the intravesicular volume of TR119 membrane vesicles prepared as described above is 1.94 μl/mg of vesicle protein. This is only 40% of the intravesicular volume determined for *E. coli* K-12 membrane vesicles prepared by a different procedure (13).

Protein determination. Protein concentration was determined by the method of Lowry et al. (25) using bovine serum albumin as a standard.

RESULTS

Guanine and hypoxanthine uptake into TR119 and *proAB47* membrane vesicles and guanine and hypoxanthine phosphoribosyltransferase activity. As measured by method A (filter assay), the uptake of [8-^{14}C]guanine and [8-^{14}C]hypoxanthine as a function of time into isolated membrane vesicles of strain TR119 and *proAB47* is shown in Fig. 1A and 1B, respectively. In TR119, uptake of both guanine and hypoxanthine was stimulated by PRPP, both initial rate and maximal accumulation. However, in strain *proAB47*, only the uptake of hypoxanthine was stimulated by PRPP. No

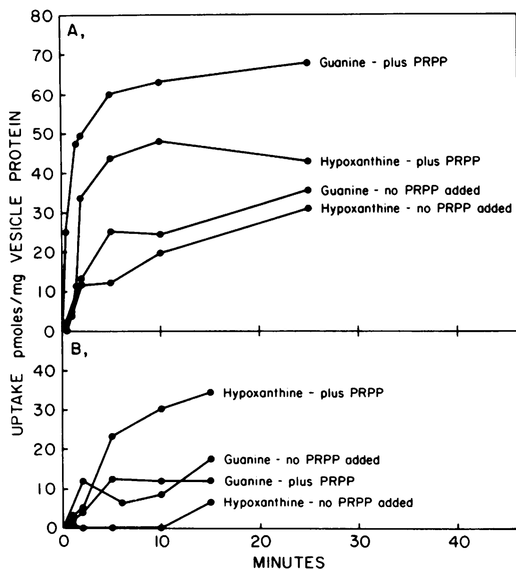

FIG. 1. *Uptake of guanine and hypoxanthine into TR119 and proAB47 isolated membrane vesicles. For TR119, reaction mixtures contained, in 100 μl, 0.35 mg of membrane vesicle protein, 2 mM magnesium acetate, and either 40 μM [8-^{14}C]hypoxanthine (62 mCi/mmol) or 80 μM [8-^{14}C]guanine sulfate (56 mCi/mmol). For proAB47, reaction mixtures contained, in a total 150-μl volume, 0.52 mg of membrane vesicle protein, 2 mM magnesium acetate, and either 40 μM [8-^{14}C]hypoxanthine (62 mCi/mmol) or 54 μM [8-^{14}C]guanine (56 mCi/mmol). For each substrate, one series contained in addition 1 mM PRPP. Zero time control values were subtracted from experimental values. Termination, filtration, drying, and counting were as described in Materials and Methods. A, TR119; B, proAB47.*

PRPP-stimulated uptake of guanine was observed in *proAB47* vesicles.

When uptake of guanine and hypoxanthine was measured by method B (pellet assay), intravesicular and extravesicular (supernatant) products could be identified and concentrations of these products could be calculated. For strain TR119 in the presence of PRPP, the predominant intravesicular products were GMP and IMP with guanine and hypoxanthine as substrates, respectively (Table 1). Without added PRPP, GMP and IMP were again found intravesicularly, but were usually less than 10% of the amount formed with PRPP. This indicates some endogenous stores of PRPP or the ability to form it in membrane vesicles. As observed with AMP in the adenine system (13), GMP and IMP were formed at a faster rate extravesicularly than they accumulated intravesicularly. Thus, the low intravesicular volume of *Salmonella* membrane vesicles relative to that of *E. coli* vesicles suggests the presence of unsealed membrane fragments that might transport but would allow mixing of products with the extravesicular medium. These products would be indistinguishable from products formed without traversing the membrane (nonvectorial phosphorylation) (16). The possibility that GMP and IMP are formed extravesicularly and transported intact is unlikely because [8-^{14}C]GMP was not taken up by strain TR119. Very low, but detectable, levels of [8-^{14}C]IMP were taken up; but this uptake was not inhibited by *N*-ethylmaleimide. Furthermore, IMP concentration curves obey linear, not hyperbolic, kinetics consistent with passive diffusion.

In strain *proAB47*, which lacks the guanine phosphoribosyltransferase gene product, GMP was not found intravesicularly when guanine was the substrate with or without PRPP. With hypoxanthine and PRPP as substrates, the predominate intravesicular product was IMP (Table 2). Very little IMP was found intravesicularly without PRPP. [8-^{14}C]IMP was not taken up significantly.

In strain TR119, a 12-fold intravesicular accumulation of GMP and a 6-fold intravesicular accumulation of IMP with respect to extravesicular space was observed in strain TR119 with guanine and hypoxanthine, respectively, and PRPP as substrates. In strain *proAB47*, a 9-fold intravesicular accumulation of IMP with respect to extravesicular space was observed.

In strain TR119 small amounts of guanine, when guanine was the substrate, were found within the vesicles. Also, when hypoxanthine was the substrate, small amounts of hypoxanthine were found intravesicularly, but only when PRPP was not added. To identify the possibility of a mechanism for guanine and hypoxanthine uptake not dependent upon PRPP, the effect of *N*-ethylmaleimide upon uptake of guanine and hypoxanthine with and without PRPP was tested. The results are shown in Fig. 2. Uptake of hypoxanthine in the presence of PRPP was inhibited by only 20%, but uptake of hypoxanthine without PRPP was almost completely inhibited by *N*-ethylmaleimide. Therefore, two mechanisms of hypoxanthine uptake may have been present. The major mechanism was dependent upon PRPP whereas a minor mechanism was not. That hypoxanthine had been transported inside first and subsequently converted to IMP was unlikely, since the uptake of hypoxanthine alone was almost totally inhibited by *N*-ethylmaleimide, whereas in the presence of PRPP uptake was still 80% functional. This would seem to rule out free base transport as the first step followed by phospho-

TABLE 1. *Distribution of radioactive components after incubation of [8-^{14}C]hypoxanthine and [8-^{14}C]guanine with TR119 membrane vesicles*[a]

With guanine as substrate

Addition of PRPP	Incubation time (min)	Concn (μM)					
		In supernatant[b]			In membrane vesicles[c]		
		Guanine	Guanosine	GMP	Guanine	Guanosine	GMP
Plus PRPP	2	25.2	3.6	10.7	8.4	58.7	124.8
	5	17.0	4.0	22.4	0	61.2	250.7
	10	4.5	3.4	34.1	0	33.9	335.8
No PRPP	2	42.4	3.7	0.134	0	56.2	3.5
	5	44.0	4.6	0.158	16.9	64.6	7.7
	10	42.4	5.4	0.35	57.1	75.9	8.7

With hypoxanthine as substrate

Addition of PRPP	Incubation time (min)	Concn (μM)					
		In supernatant[b]			In membrane vesicles[c]		
		Hypoxanthine	Inosine	IMP	Hypoxanthine	Inosine	IMP
Plus PRPP	2	5.02	1.1	17.2	0	19.6	106
	5	2.25	1.4	21.3	0	39.8	364.5
	15	0.362	1.1	24.4	0	10.0	168.1
No PRPP	2	20.2	0.81	27.1	0	14.8	27.1
	5	31.8	1.3	22.0	0.705	21.8	22.6
	15	29.7	1.63	30.8	2.07	21.8	28.9

[a] Reaction mixtures (100 μl) contained either 40 μM [8-^{14}C]hypoxanthine (62 mCi/mmol) or 80 μM [8-^{14}C]guanine (56 mCi/mmol), 0.35 mg of membrane vesicle protein, and other substances as described in Materials and Methods. Reaction mixtures were incubated at 33 C for the indicated times; termination and determination of distribution of radiolabel are described in Materials and Methods. Experimental values were corrected for a control for each series containing membranes denatured by boiling prior to addition of labeled substrate. Total radioactivity values were corrected for volumes. Concentrations were calculated directly from the total radioactivity values after determination of intravesicular volume.

[b] Extravesicular space.

[c] Intravesicular space.

ribosyltransferase-mediated conversion as the second step; neither step could proceed if the first step was blocked. Free hypoxanthine was not detected intravesicularly in the presence of PRPP because, possibly, some free hypoxanthine may have been phosphoribosylated. This is not inconsistent with group translocation; even though extravesicular glucose is the preferred substrate for intravesicular glucose 6′-phosphate production in the phosphoenolpyruvate-phosphotransferase system, intravesicular glucose also leads to glucose 6′-phosphate production (15).

Using method B in the presence of 10 mM *N*-ethylmaleimide, IMP was found intravesicularly when 40 μM hypoxanthine and 1 mM PRPP were added. The total radioactivity recovered intravesicularly represented 75% of that recovered when *N*-ethylmaleimide was not added (data not shown).

Guanine uptake with or without PRPP was not significantly inhibited by *N*-ethylmaleimide (Fig. 2B).

As shown in Table 1, some guanosine and inosine were found intravesicularly. This indicates the presence of a purine nucleoside phosphorylase or possibly a nucleotidase. Ribose 1′-phosphate does not stimulate uptake of either guanine or hypoxanthine. Membranes show purine nucleoside phosphorylase activity for both guanine and hypoxanthine with added ribose 1′-phsphate, and also show nucleotidase activity.

In strain *proAB47*, free hypoxanthine was not found intravesicularly with or without PRPP when [8-^{14}C]hypoxanthine was the substrate, but some free guanine was found intravesicularly (Table 2) when [8-^{14}C]guanine was the substrate without PRPP. Inosine and guanosine were also found. Again the effect of *N*-ethylmaleimide on hypoxanthine and guanine uptake was tested. The results (Fig. 3) indicate

TABLE 2. *Distribution of radioactive components after incubation of [8-^{14}C]hypoxanthine and [8-^{14}C]guanine with proAB47* membrane vesicles[a]

With guanine as substrate

Addition of PRPP	Incubation time (min)	Concn (μM) In supernatant[b] Guanine	Guanosine	GMP	In membrane vesicles[c] Guanine	Guanosine	GMP
Plus PRPP	0.5	159	0.78	0.05	0	15.1	0.11
	1	154	1.45	0.05	0	24.8	0.48
	2	148	1.43	0.103	0	17.7	0.32
No PRPP	0.5	155	0.833	0.003	51	12.0	0.38
	1	150	1.04	0.011	52.2	12.4	0.09
	2	160	1.53	0.029	172.1	15.3	0.16

With hypoxanthine as substrate

Addition of PRPP	Incubation time (min)	Concn (μM) In supernatant[b] Hypoxanthine	Inosine	IMP	In membrane vesicles[c] Hypoxanthine	Inosine	IMP
Plus PRPP	1	65	0.39	0.52	0	12.9	5.0
	2	65	0.55	1.17	0	15.3	10.3
	5	64	0.91	2.7	76.6	17.9	22.0
No PRPP	1	63	0.39	0.02	0	14.3	.87
	2	65	0.5	0.03	0	13.6	.92
	5	65	0.82	0.006	0	14.0	.92

[a] Reaction mixtures (150 μl) contained either 80 μM [8-^{14}C]hypoxanthine (62 mCi/mmol) or 200 μM [8-^{14}C]guanine (56 mCi/mmol) and 0.52 mg of vesicle protein. Experimental conditions, determination of total radioactivity, and calculation of concentrations were the same as for Table 1.

[b] Extravesicular space.

[c] Intravesicular space.

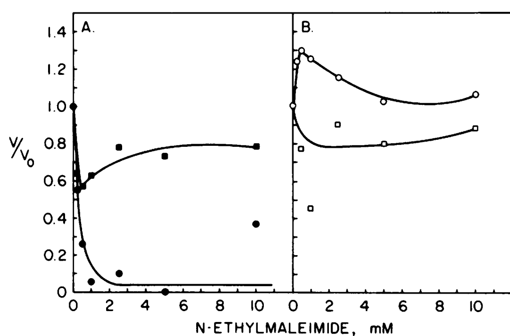

FIG. 2. *Effect of N-ethylmaleimide upon hypoxanthine and guanine uptake into TR119 membrane vesicles. Uptake was measured exactly as in Fig. 1 except that [8-^{14}C]hypoxanthine (58 mCi/mmol) and 0.51 mg of membrane vesicle protein were used per reaction mixture. The indicated concentrations of N-ethylmaleimide were added to each reaction mixture prior to preincubation. Reactions were incubated for 2 min with hypoxanthine as substrate or for 30 s with guanine as substrate. A zero time control for each concentration of N-ethylmaleimide was substracted from each corresponding experimental value. Values are plotted as the fraction of activity observed in the presence of N-ethylmaleimide (V) as compared to the activity observed in an experimental sample without N-ethylmaleimide (V_0). A, Hypoxanthine uptake with PRPP added (■); hypoxanthine uptake without PRPP added (●). B, Guanine uptake with PRPP added (□); guanine uptake without PRPP added (○).*

that hypoxanthine uptake without PRPP is inhibited more significantly than in the presence of PRPP. A possible explanation for this is that a precursor to endogeneous PRPP, rather than PRPP itself, is stored in the vesicles, and PRPP formation is sensitive to *N*-ethylmaleimide. The partial inhibition of uptake of hypoxanthine using exogenous PRPP would, therefore, be due to reduction of endogenous PRPP stores. This explanation is valid only if endogenous PRPP is utilized in the transport process. This *N*-ethylmaleimide inhibition would be similar to the role of NaF in preventing phosphoenolpyruvate formation necessary for transport in the

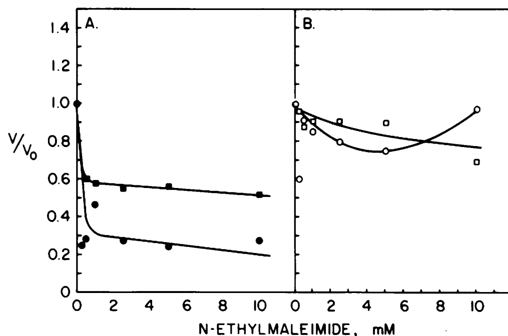

FIG. 3. *Effect of N-ethylmaleimide upon hypoxanthine and guanine uptake into proAB47 membrane vesicles. Uptake was measured as described in Fig. 2 except that 0.53 mg of vesicle protein was used for each reaction mixture. Reaction mixtures were incubated for 2 min. Values are plotted exactly as in Fig. 2. A, Hypoxanthine uptake with PRPP added (■); hypoxanthine uptake without PRPP added (●). B, Guanine uptake with PRPP added (□); guanine uptake without PRPP added (○).*

phosphoenolpyruvate-phosphotransferase system (15).

The inosine and guanosine detected in *proAB47* vesicles (Table 2) may have been due to a phosphorylase reaction subsequent to free base uptake. Inosine or guanosine may have also been formed by a nucleotidase, since these membranes exhibit nucleotidase activity.

Guanine and hypoxanthine phosphoribosyltransferases catalyze the following reactions: guanine + PRPP → GMP + PP_i; hypoxanthine + PRPP → IMP + PP_i.

Isolated membrane vesicles of TR119 exhibited both guanine and hypoxanthine phosphoribosyltransferase activity; *proAB47* membrane vesicles exhibited hypoxanthine phosphoribosyltransferase activity and low but detectable levels of guanine phosphoribosyltransferase activity (Fig. 4A). Upon release of the phosphoribosyltransferase enzyme from the membranes by sonic disruption (Fig. 4B), the guanine phosphoribosyltransferae activity in strain *proAB47* was higher than intact membrane phosphoribosyltransferase activity. In both strains the specific activity of the hypoxanthine and guanine phosphoribosyltransferase released from the membrane was higher than for membrane vesicle phosphoribosyltransferase activity. Thus, membrane enzyme specificity was as strict as uptake specificity in strains TR119 and *proAB47*. Membrane phosphoribosyltransferase activity correlated with uptake activity in both strains. Phosphoribosyltransferase activity after release from the membrane included specificities not observed

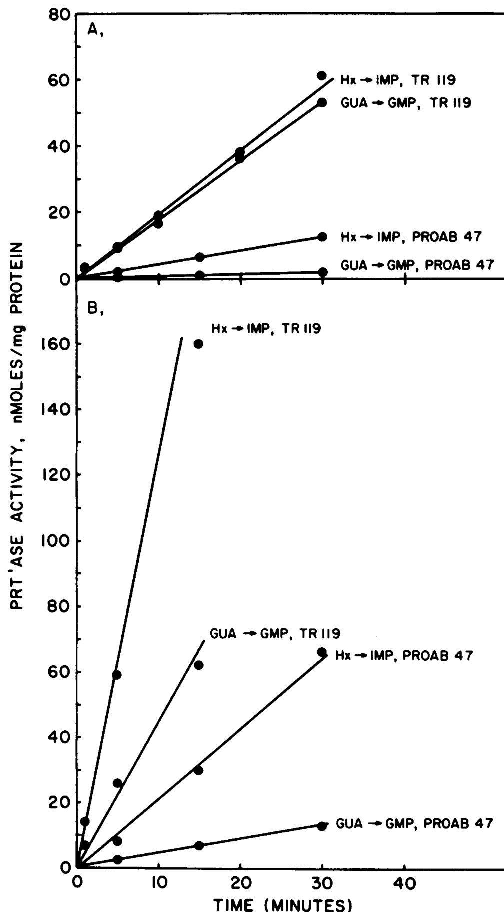

FIG. 4. *Phosphoribosyltransferase activity of membrane and membrane-released phosphoribosyltransferase from TR119 and proAB47. For untreated membrane vesicles, phosphoribosyltransferase activity was measured in reaction mixtures, 50 μl, containing 30 to 40 μg of vesicle protein, 80 mM Tris-hydrochloride (pH 7.8), 2 mM PRPP, 4 mM magnesium acetate, and either 200 μM [8-^{14}C]hypoxanthine (62 mCi/mmol) or [8-^{14}C]guanine (56 mCi/mmol). Reaction mixtures were incubated at 37 C for the indicated times. For phosphoribosyltransferase activity released from the membranes, membranes were first subjected to sonic disruption, and debris was centrifuged away prior to enzyme assay at 33,500 × g for 10 min. Reaction mixtures were identical to the above, except 5 to 10 μg of the post-centrifugation supernatant protein was used per reaction mixture. A zero time control was subtracted from each experimental value. A, Untreated membrane vesicle enzyme activity; B, enzyme activity released from membrane.*

when the enzyme was intact on the membrane.

Effect of guanine, hypoxanthine, and PRPP on uptake and phosphoribosyltransferase activity in strain TR119 and *proAB47*. Ta-

ble 3 is a summary of experiments testing the effect of guanine, hypoxanthine, and PRPP concentrations upon uptake and phosphoribosyltransferase activity in both strains TR119 and *proAB47*. The K_m values reported in Table 3 were extrapolated from double reciprocal plots of rates versus substrate concentration.

In all cases, K_m values for uptake were higher than K_m values for phosphoribosyltransferase activity of whole membrane vesicles. The K_m for guanine for phosphoribosyltransferase activity of *proAB47* whole membrane vesicles was 80 times higher than the K_m for guanine for phosphoribosyltransferase activity of TR119 whole membrane vesicles. This suggests there is no guanine phosphoribosyltransferase gene product in strain *proAB47*, and phosphoribosylation of guanine represents cross specificity of the hypoxanthine phosphoribosyltransferase. Upon release of the phosphoribosyltransferase from *proAB47* vesicles by sonic treatment, the K_m for guanine for phosphoribosyltransferase activity was lower than the K_m for guanine for phosphoribosyltransferase activity of *proAB47* whole membrane vesicles.

Regulation by 5'-nucleotides. Uptake of hypoxanthine in the presence of PRPP into TR119 vesicles was inhibited by 5'-nucleotides (Fig. 5A). Whole membrane phosphoribosyltransferase activity was also inhibited by 5'-nucleotides as shown in Fig. 5B. Uptake of guanine into TR119 vesicles was inhibited by 5'-nucleotides (Fig. 6A). Guanine phosphoribosyltransferase activity of TR119 whole membrane vesicles was also inhibited (Fig. 6B).

Like TR119, hypoxanthine uptake into *proAB47* vesicles was inhibited by 5'-nucleotides (Fig. 7A). Guanosine 5'-triphosphate (GTP) and especially IMP activated the apparent uptake (Fig. 7A). Although the reason for GTP activation is not understood, the apparent IMP activation may be due to an exchange reaction similar to that observed for the adenine system in *E. coli* (13). Figure 7B shows that *proAB47* whole membrane hypoxanthine phosphoribosyltransferase activity was inhibited by 5'-nucleotides.

Recovery of phosphoribosyltransferase activity after release from the membrane. Table 4 shows data for release of phosphoribosyltransferase activity after sonic disruption of TR119 and *proAB47* membrane vesicles. About 90% of the guanine and hypoxanthine phosphoribosyltransferase activity from TR119 membrane vesicles could be released by sonic disruption. Only 64% and 74%, respectively, were released from *proAB47* membrane vesicles. Table 4 also shows the percentage of guanine and hypoxan-

TABLE 3. *K_m values for uptake and phosphoribosyltransferase activity in TR119 and proAB47*

Determination	K_m values (μM)			
	Hypoxanthine[a]	PRPP[a] with hypoxanthine	Guanine[a]	PRPP[a] with guanine
TR119 uptake[b]	11	90	50	48
TR119 whole membrane phosphoribosyltransferase[c]	7.0	29	2.7	26
TR119 enzyme released from membrane[d]	8.1	NT[e]	3.6	NT
TR119 whole cell extract[f]	18	39	4.5	48
proAB47 uptake[b]	22	131		
proAB47 whole membrane phosphoribosyltransferase[c]	7.4	50	220	16
proAB47 enzyme released from membrane[d]	6.3	NT	143	NT
proAB47 whole cell extract[f]	6.[illegible]	51	166	80

[a] Substrate.

[b] Uptake was measured as described in Fig. 1, except that several hypoxanthine and guanine concentrations ranging from 4 μM to 800 μM were used. Reaction mixtures were incubated for 2 min except for guanine in TR119 in which incubation was for 30 s (in the linear region for each). A zero time control for each concentration was subtracted from each corresponding experimental value. For determination of the Michaelis constant for PRPP, concentrations from 8 μM to 4,000 μM of PRPP were used. For TR119, hypoxanthine or guanine was used at 40 or 80 μM, respectively; for *proAB47*, hypoxanthine was used at 100 μM. Zero time control reactions were also performed as above.

[c] K_m values for guanine and hypoxanthine were obtained for whole membrane phosphoribosyltransferase activity using reaction mixtures containing 14 to 20 μg of membrane protein, 80 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.8), 4 mM magnesium acetate, 2 mM PRPP, and hypoxanthine or guanine as above. K_m values for PRPP were determined in identical reaction mixtures, except several concentrations of PRPP, as for uptake, were used. Reactions were incubated for 5 to 30 min at 37 C.

[d] K_m values for the substrates of the phosphoribosyltransferase released from membrane vesicles by sonic disruption were determined in the same manner as for the K_m values for whole membrane phosphoribosyltransferase activity except that samples of supernatant fluids of sonic extracts from whole membranes were used in place of whole membrane vesicles.

[e] NT, Not tested.

[f] K_m values for substrates of the phosphoribosyltransferase from whole cell extracts were determined using sonic extracts from whole cells of TR119 and *proAB47*. Whole cells were resuspended in 9 mM Tris-hydrochloride-0.2 mM $MgSO_4$ (pH 8.2) after harvesting, and then subjected to sonic disruption. Reaction mixtures and conditions were as described above for whole membrane phosphoribosyltransferase.

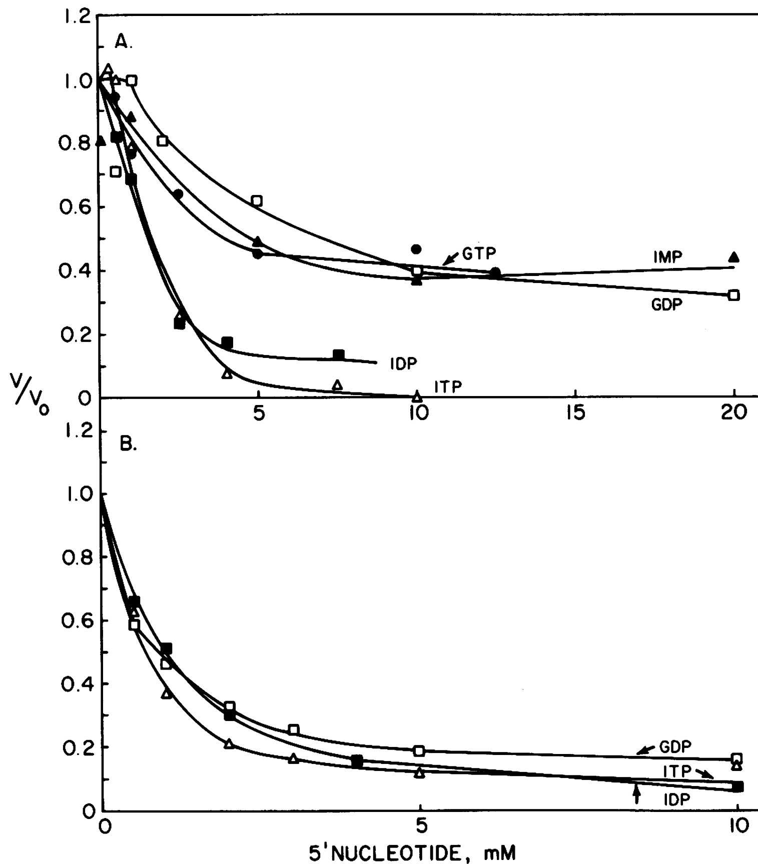

FIG. 5. *Effect of 5'-nucleotides on hypoxanthine uptake and membrane hypoxanthine phosphoribosyltransferase activity in TR119. Hypoxanthine uptake was measured as described in Fig. 1, except that between 0.28 and 0.36 mg of vesicle protein was used per reaction mixture, and the indicated concentrations of the specified 5'-nucleotide were added at the beginning of the preincubation period for 15 min at 33 C. Reactions were then initiated by addition of labeled substrate and incubations were allowed to proceed for 1.5 or 2 min at 33 C. Uptake is plotted as the fraction of activity observed in the presence of 5'-nucleotide (V) as compared to an identical sample without 5'-nucleotide (V_0). For membrane hypoxanthine phosphoribosyltransferase activity, reaction mixtures contained 80 mM Tris-hydrochloride (pH 7.8), 21 μg of vesicle protein, 0.8 mM magnesium acetate, 0.4 mM PRPP, 80 μM [8-^{14}C]hypoxanthine (62 mCi/mmol), and the indicated concentrations of 5'-nucleotide prior to preincubation. Reaction mixtures were preincubated for 5 min before initiation with labeled hypoxanthine, and then incubated for 10 min at 37 C. A zero time control without 5'-nucleotide was substracted from each corresponding experimental value. Activity is expressed as for uptake. A, Hypoxanthine uptake; B, membrane hypoxanthine phosphoribosyltransferase activity.*

thine phosphoribosyltransferase activity recovered soluble after sonic disruption of TR119 and *proAB47* membrane vesicles. In *proAB47*, 171% of the original guanine phosphoribosyltransferase activity was recovered soluble; 36% of the original guanine phosphoribosyltransferase activity was still associated with the membrane. Correction to 100% guanine phosphoribosyltransferase activity released gives a higher recovery value of 267%. Only 38% of the TR119 guanine phosphoribosyltransferase activity released was recovered soluble; 10% activity remained with the vesicles. Therefore, 58% of the guanine phosphoribosyltransferase activity was lost owing to the sonic treatment. Twenty-two percent of the TR119 hypoxanthine phosphoribosyltransferase activity was lost due to the sonic treatment. Similar results were obtained in subsequent experiments. Thus, a substantial portion of the enzyme molecules of TR119 were inactivated by sonic treatment. If it is further assumed that in strain *proAB47* (as in TR119) some enzyme molecules were inactivated by the sonic treatment (and are corrected for in our calculations), then a 3.4- to 6.3-fold activation of each enzyme molecule released in

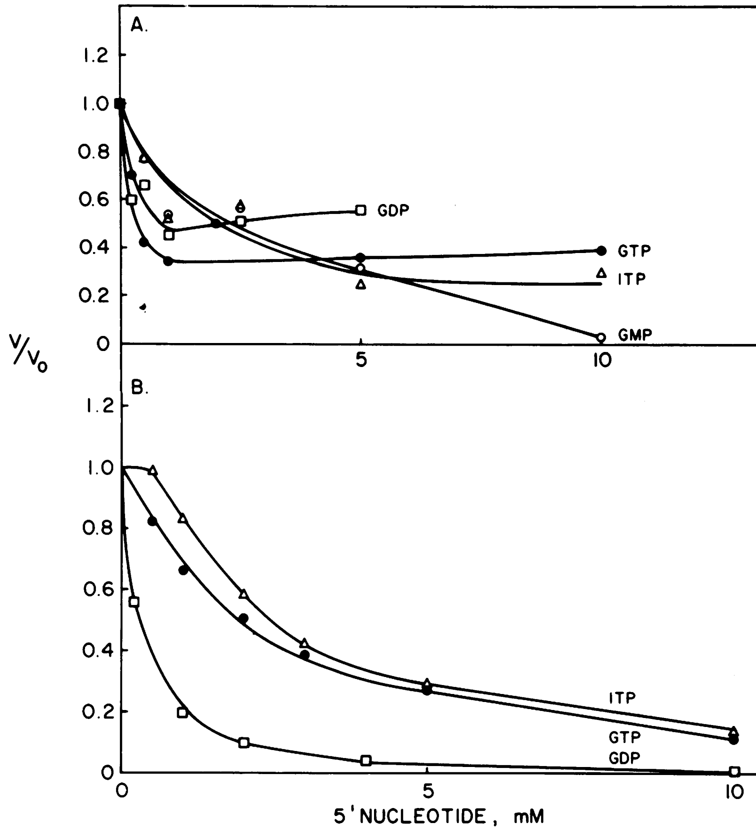

FIG. 6. *Effect of 5'-nucleotides on guanine uptake and membrane guanine phosphoribosyltransferase activity in TR119. Guanine uptake is measured and activity is expressed exactly as in Fig. 5, except that 40 μM [8-14C]guanine (56 mCi/mmol) and 0.51 mg of vesicle protein were used per reaction mixture. Reaction mixtures were preincubated for 10 min, initiated with labeled guanine, and incubated for 30 s. Membrane guanine phosphoribosyltransferase activity was determined and activity expressed as in Fig. 5, except that 40 μM [8-14C]guanine was used per reaction mixture. A, Guanine uptake; B, membrane guanine phosphoribosyltransferase activity.*

active form occurred in strain *proAB47*. The more conservative 3.4-fold activation relates to the stability of the TR119 hypoxanthine phosphoribosyltransferase gene product (which in strain *proAB47* is responsible for the observed guanine activity). The 6.3-fold activation estimate is calculated from the stability of the TR119 guanine phosphoribosyltransferase, and may not be relevant.

Of the 3.4-fold or greater increase in activity upon solubilization, there were two components. The first component may have been due to the K_m change that accompanies release from the membrane. In strain *proAB47*, the guanine phosphoribosyltransferase activity present exhibited a high K_m value (220 μM on the membrane, and 143 μM released from the membrane [Table 3]), and it was difficult to perform these experiments at saturating guanine concentrations. Thus, when assayed at 200 μM (as in this experiment), an enzyme exhibiting a K_m of 220 μM on the membrane and 143 μM after solubilization would appear to be activated approximately 1.5-fold upon solubilization. Since the activation measured was at least 3.4-fold, then a second component not due to substrate affinity also conributed to this activation. This second component, representing greater than twofold activation, may have resulted from increased substrate turnover number of enzyme molecules. This explanation is compatible with possibly greater free rotation and a new conformation of the enzyme in aqueous solution as compared to intact on the membrane. Similar activation upon solubilization has also been observed for a variety of other membrane enzymes (4, 28, 29; D. C. Quinlan and J. Hochstadt, J. Biol. Chem., in press; P. S. Rudland et al., J. Biol. Chem., in press). On the basis of three experiments, we concluded that the

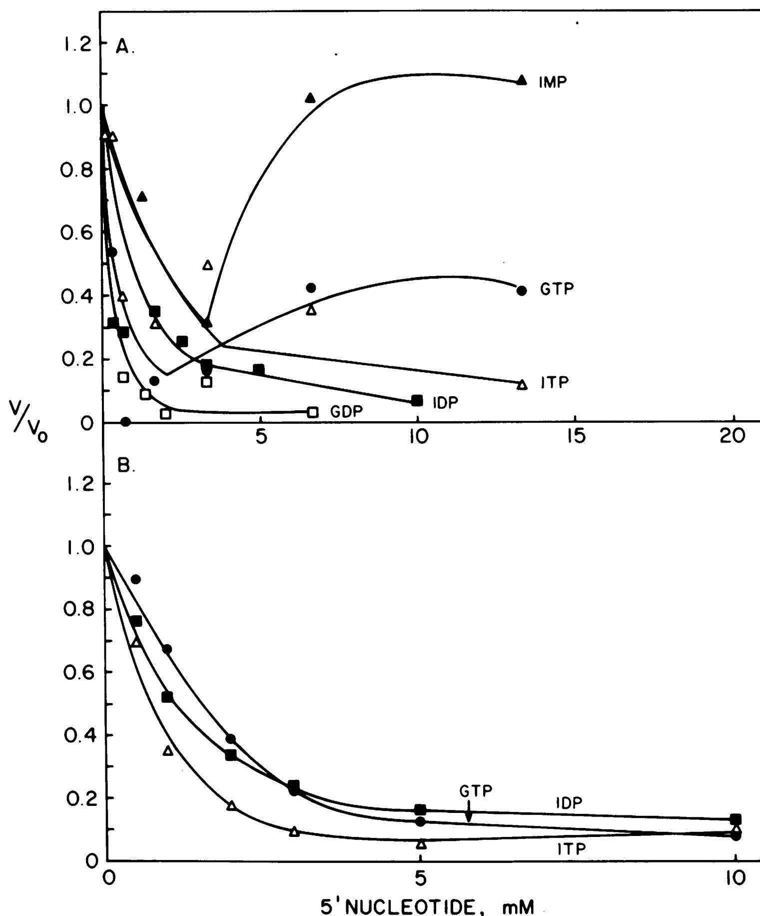

FIG. 7. *Effect of 5'-nucleotides on hypoxanthine uptake and membrane hypoxanthine phosphoribosyltransferase activity in proAB47. Uptake was measured as in Fig. 5, except that 0.52 mg of vesicle protein and 80 μM [8-^{14}C]hypoxanthine was used per reaction mixture. Reactions were incubated for 2 min at 33 C. Uptake is expressed as in Fig. 5. Membrane hypoxanthine phosphoribosyltransferase activity was determined and activity was expressed as in Fig. 5, except 150 μg of vesicle protein was used per reaction mixture. A, Hypoxanthine uptake; B, membrane hypoxanthine phosphoribosyltransferase activity.*

proAB47 hypoxanthine phosphoribosyltransferase exhibits significantly greater guanine phosphoribosyltransferase activity in solution than on the membrane. Since no comparable activation of the hypoxanthine activity occurred (although a small activation of about 25% was observed), we cannot attribute this activation to the general enzyme activation which was assumed for these other examples (4, 28, 29; Quinlan Hochstadt, J. Biol. Chem., in press; Rudland et al., J. Biol. Chem., in press), but rather to a change or broadening of substrate specificity that accompanies solubilization.

Effect of hypoxanthine on guanine phosphoribosyltransferase activity of whole membrane vesicles. Since *proAB47* is a deletion mutant (2, 6) (possibly including the *gpt* region), then any guanine phosphoribosyltransferase activity would represent dual specificity of the hypoxanthine phosphoribosyltransferase. Unlabeled hypoxanthine should effectively compete out any guanine phosphoribosyltransferase activity in *proB47* membrane vesicles. Hypoxanthine is a competitive inhibitor of guanine phosphoribosyltransferase activity of whole membranes from both strains TR119 and *proAB47* (Fig. 8). However, the K_i for hypoxanthine in strain *proAB47* is 57 μM, whereas the K_i for hypoxanthine in TR119 is 132 μM. Thus, hypoxanthine is a much better inhibitor of guanine phosphoribosyltransferase activity in strain *proAB47* than in TR119. Embedded in the membrane in situ, the phosphoribosyltrans-

TABLE 4. *Sonic release of guanine and hypoxanthine phosphoribosyltransferase from intact isolated membrane vesicles and recovery of phosphoribosyltransferase activity in the supernatant fluid after sonic disruption of isolated membrane vesicles*[a]

Determination	Phosphoribosyltransferase activity (%)			
	Remaining associated with membrane vesicles after sonic disruption relative to intact membrane vesicles[b]		Recovered in the soluble fraction after sonic disruption relative to intact membrane phosphoribosyltransferase activity[c]	
	TR119	*proAB47*	TR119	*proAB47*
Guanine phosphoribosyltransferase	10.0	35.6	38	171
Hypoxanthine phosphoribosyltransferase	10.6	25.6	70	97

[a] Isolated membrane vesicles prepared from TR119 and *proAB47* were subjected to sonic disruption and a 150-μl sample of the resultant material from each was centrifuged at 33,500 × *g* for 10 min. The pellets were resuspended to 150 μl. A sample of the original vesicles, the pellet, and the supernatant fluid was then assayed for phosphoribosyltransferase activity. Reaction mixtures contained 80 mM Tris-hydrochloride (pH 7.8), 4 mM magnesium acetate, 2 mM PRPP, either 0.2 mM [8-^{14}C]hypoxanthine (62 mCi/mmol) or [8-^{14}C]guanine (56 mCi/mmol), and 5 to 25 μl of either pellet, untreated membrane vesicles, or supernatant fluid after sonic disruption. Reaction mixtures were incubated for 5 min at 37 C. A zero time control was subtracted from each experimental value. Total activity for 5 μl of pellet, untreated intact membrane vesicles, or supernatant after sonic disruption was determined.

[b] Calculated as: 100 × [(pellet phosphoribosyltransferase activity after sonic treatment)/(untreated membrane vesicle phosphoribosyltransferase activity prior to sonic treatment)].

[c] Calculated as: 100 × [(sonically treated supernatant phosphoribosyltransferase activity after sonic treatment)/(untreated membrane vesicle phosphoribosyltransferase activity prior to sonic treatment)].

ferase of strain *proAB47* has strict substrate specificity. Upon release from the membrane, the phosphoribosyltransferase gains dual specificity.

DISCUSSION

Previous reports in this series (10, 12-14) have indicated a direct role for adenine phosphoribosyltransferase in the uptake of adenine by membrane vesicles from *E. coli*. We have provided evidence in this report which supports a group translocation mechanism for the uptake of guanine and hypoxanthine into membrane vesicles of *S. typhimurium*. In brief, the following findings support this type of mechanism. (i) Uptake of guanine and hypoxanthine into TR119 vesicles (both phosphoribosyltransferases intact) is stimulated by PRPP. (ii) Only hypoxanthine uptake into strain *proAB47* (no guanine phosphoribosyltransferase) is stimulated by PRPP. (iii) Most of the guanine and hypoxanthine taken up by TR119 and hypoxanthine taken up by strain *proAB47* accumulate intravesicularly as GMP and IMP, respectively. (iv) *N*-ethylmaleimide inhibition of free hypoxanthine uptake in TR119 is almost complete but has much less effect upon PRPP-stimulated uptake of hypoxanthine. Thus, uptake of

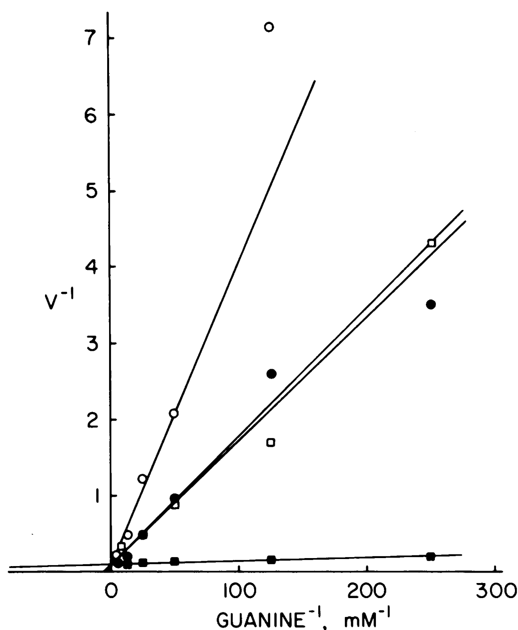

FIG. 8. *Effect of hypoxanthine on guanine concentration versus velocity for guanine phosphoribosyltransferase activity from TR119 and proAB47 whole membrane vesicles. First, competition curves for hypoxanthine were generated to determine what concentration of unlabeled hypoxanthine inhibited guanine phosphoribosyltransferase activity about 50%. For TR119, 50% inhibition occurs at 6 mM hypoxanthine. For proAB47, 50% inhibition occurs at 0.2 mM hypoxanthine. These concentrations were used to generate Lineweaver-Burk plots for guanine in TR119 and proAB47. Assay conditions for TR119 and proAB47 without hypoxanthine inhibitor are identical to Table 3 for whole membrane guanine phosphoribosyltransferase. Assay conditions for TR119 and proAB47 with hypoxanthine are identical to Table 3, except that unlabeled hypoxanthine at the above concentrations was added prior to initiation of reaction with labeled guanine. Symbols: □, TR119, unlabeled hypoxanthine at 6 mM; ■, TR119, control (no hypoxanthine); ○, proAB47, unlabeled hypoxanthine at 0.2 mM; ●, proAB47, control (no hypoxanthine).*

free base is not a prerequisite for IMP formation. (v) The GMP and IMP are concentrated intravesicularly several-fold with respect to extravesicular space. (vi) Phosphoribosyltransferase activity of the membrane vesicles correlates with uptake activity. That is, hypoxanthine and guanine phosphoribosyltransferase activity are found in TR119 membrane vesicles, but only hypoxanthine phosphoribosyltransferase activity is found in strain *proAB47*. (vii) Inhibition by 5'-nucleotides is similar for uptake and phosphoribosyltransferase activity. (viii) Substrate specificity alterations broadening the observable in situ specificities of the hypoxanthine phosphoribosyltransferase activity concomitant to solubilization from the membrane indicate that the enzyme resides on the membrane in situ and is not artificially adsorbed to the membranes during or after cell rupture. Therefore, artificial adsorption of a cytoplasmic enzyme resulting in group translocation that would not exist in situ is not probable.

The existence of separate forms of the guanine and hypoxanthine phosphoribosyltransferases is well established in *E. coli* (8, 21, 26), but in *Salmonella* there is less direct evidence. Genetic and biochemical evidence (2, 5, 6) suggest two forms of the enzyme in *Salmonella*. This report also gives evidence for two separate forms of the phosphoribosyltransferases in *Salmonella*. Uptake of guanine and hypoxanthine parallel phosphoribosyltransferase activity in the two strains studied (Fig. 1A and B). Guanine phosphoribosyltransferase activity is deleted in strain *proAB47*, yet cells still retain high levels of hypoxanthine phosphoribosyltransferase activity (Fig. 4A). Recently, Benson and Gots (2) separated the phosphoribosyltransferases of *S. typhimurium* LT-2 into two distinct components using an ecteola cellulose column. The hypoxanthine phosphoribosyltransferase peak (*hpt* gene product) also has guanine phosphoribosyltransferase activity and this correlates well with our finding that the phosphoribosyltransferase released from strain *proAB47* has dual specificity.

Our results are also relevant to the work of Zimmerman and Magasanik (33). The *Salmonella* 6-mercaptopurine-resistant mutants they describe are deficient in the uptake of guanine and hypoxanthine but still retain phosphoribosyltransferase activities in whole cell extracts. We also present evidence for a change in enzyme specificity upon release from the membrane (Fig. 4 and 8 and Table 4). The hypoxanthine phosphoribosyltransferase gains guanine phosphoribosyltransferase activity when released from *proAB47* membrane vesicles. This change in specificity can also be supported by the kinetic data of Table 3. The K_m for guanine phosphoribosyltransferase activity of *proAB47* membrane vesicles is 80 times higher than the K_m for guanine phosphoribosyltransferase activity of TR119 membrane vesicles. Also, the K_m for guanine for phosphoribosyltransferase activity decreased when released from *proAB47* membrane vesicles by sonic disruption. The membrane appears to constrict the specificity of the hypoxanthine phosphoribosyltransferase in strain *proAB47*. Moreover, the membrane restricts phosphoribosyltransferase in TR119 because upon release of the phosphoribosyltransferase from TR119 membranes an increase in total activity of the guanine and hypoxanthine phosphoribosyltransferases occurs (Table 4). The suppressor mutant of Benson and Gots, GP36, of *S. typhimurium* strain GP660 (*purAB-gpt* deletion, *purE*) lacks guanine phosphoribosyltransferase but retains the ability to grow on guanine as sole purine source (2). The ecteola cellulose elution profile of this mutant corresponds to the hypoxanthine phosphoribosyltransferase (*hpt* gene product) from the wild-type LT-2 elution profile. No guanine phosphoribosyltransferase (*gpt* gene product) is found in this mutant, but the hypoxanthine phosphoribosyltransferase has gained dual specificity for guanine. The mechanism for the reported suppression and the effect the mutation has on the hypoxanthine phosphoribosyltransferase to cause it to gain dual specificity is not known. The possibility that this change in specificity represents a change in membrane structure is being investigated (D. L. Hornick, C. E. Benson, and J. S. Gots, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K161, p. 173). The use of membrane vesicles isolated from the GP36 mutant and experiments characterizing enzyme and transport activity would be important in identifying the mechanism behind this change in specificity. The substrate specificities of the phosphoribosyltransferase in solution may not relate to the enzymes' specificities in situ on the membrane. If overlapping specificities are not found in situ as in strain *proAB47* (because there is virtually no guanine phosphoribosyltransferase activity in situ in the membrane), then possibly the best way to study such enzymes is in a hydrophobic environment. Studies using artificial liposomes made from single phospholipids (7) could be used to ascertain if specificity and kinetic parameters of membrane behavior are encoded in the primary structure of the enzyme and expression only requires transfer to a hydrophobic environment. If this is the case, then not only might the in situ type of enzyme activity be demonstrated in liposomes, but group translocation of

the substrates into the phospholipid vesicles might also be achieved.

Finally, an increasing number of reports describe the differences between enzymes on the membrane and in solution. These enzymes that are active both in aqueous and membrane environments may represent a significant portion of the constituent membrane enzymes. Examples of these are as follows.

The membrane enzyme adenine phosphoribosyltransferase is capable of carrying out an exchange reaction between AMP and adenine when on the membrane, but does not carry out this reaction when solubilized (13). It is also less sensitive to inhibition by ATP when on the membrane than when removed from the membrane (13).

Membrane guanine and hypoxanthine phosphoribosyltransferase activity in *E. coli* is less sensitive to inhibition by ppGpp and nucleotides than is the soluble form (11).

Thymidine phosphorylase activity increases as much as 12-fold when released from the membrane; however, its *trans-N*-deoxyribosylase function (catalyzing $nucleoside_1 + base_2 \rightleftharpoons base_1 + nucleoside_2$) is considerably diminished upon release from the membrane (28).

Inosine phosphorylase from mouse fibroblast membrane vesicles is activated 30-fold upon solubilization of the membranes (Quinlan and Hochstadt, J. Biol. Chem., in press).

UDPG:polyglyceroteichoic acid glucosyl transferase from *B. subtilis* membrane has a distinct pH optimum and a fivefold difference in K_m for its acceptor when removed from the membrane (4).

Alkaline phosphatase from isolated fibroblast membranes is activated fivefold upon liberation by *p*-lipases, detergents, or other treatments (S. C. Hung, and G. Molenkyovych, submitted for publication).

Guanylate cyclase activity increases 10-fold upon solubilization of the membrane-associated enzyme (Rudland et al., J. Biol. Chem., in press).

Succinic dehydrogenase isolated from *M. lysodeikticus* membranes is activated fourfold upon solubilization of the membranes (29).

The above examples, the results of this report, and the extensive information documenting the adenine uptake process and the adenine phosphoribosyltransferase (12–14) indicate that an enzyme cannot be shown to be a cytoplasmic component solely because its activity is recovered in the non-particulate fraction upon cell rupture.

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