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Metabolism of RNA-Ribose by *Bdellovibrio bacteriovorus* During Intraperiplasmic Growth on *Escherichia coli*

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During intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J on *Escherichia coli* some 30 to 60% of the initial *E. coli* RNA-ribose disappeared as cell-associated orcinol-positive material. The levels of RNA-ribose in the suspending buffer after growth together with the RNA-ribose used for bdellovibrio DNA synthesis accounted for 50% or less of the missing RNA-ribose. With intraperiplasmic growth in the presence of added U-^{14}C-labeled CMP, GMP, or UMP, radioactivity was found both in the respired CO_2 and incorporated into the bdellovibrio cell components. The addition of exogenous unlabeled ribonucleotides markedly reduced the amounts of both the $^{14}CO_2$ and ^{14}C incorporated into the progeny bdellovibrios. During intraperiplasmic growth of *B. bacteriovorus* on [U-^{14}C]ribose-labeled *E. coli* BJ565, ca. 74% and ca. 19% of the initial ^{14}C was incorporated into the progeny bdellovibrios and respired CO_2, respectively. Under similar growth conditions, the addition of glutamate substantially reduced only the $^{14}CO_2$; however, added ribonucleotides reduced both the $^{14}CO_2$ and the ^{14}C incorporated into the progeny bdellovibrios. No similar effects were found with added ribose-5-phosphate. The distribution of ^{14}C in the major cell components was similar in progeny bdellovibrios whether obtained from growth on [U-^{14}C]ribose-labeled *E. coli* BJ565 or from *E. coli* plus added U-^{14}C-labeled ribonucleotides. After intraperiplasmic growth of *B. bacteriovorus* on [5,6-^{3}H-]uracil-[U-^{14}C]ribose-labeled *E. coli* BJ565 (normal or heat treated), the whole-cell $^{14}C/^{3}H$ ratio of the progeny bdellovibrios was some 50% greater and reflected the higher $^{14}C/^{3}H$ ratios found in the cell fractions. *B. bacteriovorus* and *E. coli* cell extracts both contained 5′-nucleotidase, uridine phosphorylase, purine phosphorylase, deoxyribose-5-phosphate aldolase, transketolase, thymidine phosphorylase, phosphodeoxyribomutase, and transaldolase enzyme activities. The latter three enzyme activities were either absent or very low in cell extracts prepared from heat-treated *E. coli* cells. It is concluded that during intraperiplasmic growth *B. bacteriovorus* degrades some 20 to 40% of the ribonucleotides derived from the initial *E. coli* RNA into the base and ribose-1-phosphate moieties. The ribose-1-phosphate is further metabolized by *B. bacteriovorus* both for energy production and for biosynthesis of non-nucleic acid cell material. In addition, the data indicate that during intraperiplasmic growth *B. bacteriovorus* can metabolize ribose only if this compound is available to it as the ribonucleoside monophosphate.

During intraperiplasmic growth of *Bdellovibrio bacteriovorus*, an orderly degradation of the substrate cell's nucleic acids takes place. The degraded nucleic acids, mainly in the form of nucleoside monophosphates, serve as the major precursors for the synthesis of the progeny bdellovibrio's homologous nucleic acids (11, 23, 32). In a previous study (11), it was found that after growth large amounts of nucleic acid bases were present in the suspending buffer, but the balancing amount of ribose was not detected. Recently, it was shown that starving bdellovibrio cell suspensions can use exogenously supplied ribonucleoside monophosphates as energy sources, but only the ribose moiety of these molecules was catabolized (10). In addition, it was shown that when exogenous U-^{14}C-labeled ribonucleoside monophosphates were added to cultures of intraperiplasmically growing bdellovibrios, radioactivity was detected in the respired carbon dioxide, and only about half of the radioactivity that was incorporated into the progeny bdellovibrios was associated with the nucleic acid cell fraction.

Taken together, the results of the previous studies suggest that some of the ribose carbon of the substrate cell RNA serves as a precursor for synthesis of non-nucleic acid bdellovibrio cell material as well as being catabolized by bdellovibrios for energy generation. The results of the present study clearly show that *B. bacteriovorus* metabolizes RNA-ribose, but not the RNA-base material. The RNA-ribose contributes to a small extent to the bdellovibrio energy production and is metabolized by known pentose phosphate pathways to provide cell intermediates for biosynthesis on non-nucleic acid bdellovibrio cell material.

MATERIALS AND METHODS

Organisms and growth procedures. *E. coli* ML35 (*lacI lacY*) was obtained from nutrient broth cultures grown at 30°C, and *B. bacteriovorus* 109J was grown on pregrown *E. coli* substrate cells in dilute nutrient broth (33, 35). The pentose-requiring *E. coli* strain BJ565 (F^- *tkt gnd trp tyr his str*^{r}), obtained from D. Fraenkel (18), was grown at 30°C on a glucose salts medium (12) supplemented with 0.2% (wt/vol) tryptone plus (μg/ml, final concentration): histidine, 10; tryptophan, 10; tyrosine, 10; sedoheptulose, 5; para-aminobenzoic acid, 2; thiamine-hydrochloride, 2; and D-ribose, 5. Radioactively labeled *E. coli* BJ565 was obtained by diluting overnight cultures in fresh media to a cell concentration of ca. 8×10^7 cells per ml. The diluted cultures were incubated for 1 h, the appropriate compound(s) for radioactive labeling was added, and incubation was continued for 2 to 3 h (about 6×10^8 cells per ml, final concentration). The compounds used for radioactive labeling included: [5,6-^{3}H]uracil, 0.2 to 0.3 μCi/ml, 10 μM; D-[*U*-^{14}C]ribose, 0.03 to 0.05 μCi/ml, 10 μM; or D-[*U*-^{14}C]glucose, 0.05 μCi/ml, 1 mM.

All bdellovibrio and *E. coli* cultures were harvested by centrifugation, and cell suspensions were made in 5 mM *N*-2-hydroxyethyl piperazine-N'-ethanesulfonic acid (pH 7.6) containing 0.1 mM $MgCl_2$ and 0.1 mM $CaCl_2$ (HM buffer) after two washings by centrifugation in this buffer. Cell numbers in these suspensions were standardized by optical density measurements (660 nm) using reference curves based on plaque counts for bdellovibrio or plate counts for *E. coli* (38). Cell suspensions were used immediately after preparation.

Heat treatment of cells. *E. coli* cells in HM buffer were heated (55°C for 40 min) by standard procedures as described previously (9). The heat-treated cells were then washed twice by centrifugation with HM buffer and used as substrate cells immediately after preparation.

Intraperiplasmic growth experiments. Generally, 2×10^9 to 5×10^9 *E. coli* cells per ml in HM buffer was used, with input ratios (*B. bacteriovorus* to *E. coli* cells) of 1.3 to 2.0 for single-cycle cultures and ca. 2.5×10^{-3} for multicycle cultures. In single-cycle cultures, a relatively synchronous bdellovibrio attack on the substrate cells occurred such that, within 45 min or less, all substrate cells were converted to bdelloplasts (substrate cell containing an intraperiplasmic bdellovibrio). Appropriate compounds were added to these cultures at 60 min, at which time essentially no substrate cell respiratory potential exists and increased bdellovibrio respiration begins (12, 33). Bdellovibrio development was completed with subsequent lysis of the bdelloplasts within 220 to 240 min at 30°C with these single-cycle cultures (14 to 16 h with multicycle cultures). Bdellovibrios and bdelloplasts were harvested from the culture by centrifugation at the end of or during growth. Cells were washed twice by centrifugation with HM buffer before analyses were done.

Fractionation of cells. Cells were fractionated into their major cell components by sequential extractions as described previously (11, 13). The lipids were hydrolyzed, and the nonpolar and polar portions were separated as follows. The lipid-containing fraction was dried under a stream of N_2; the dried residue was dissolved in 1 ml of 0.5 N KOH in methanol and incubated at 30°C for 30 min. After adding 1 ml of distilled water, the mixture was shaken with diethyl ether (2 ml) and centrifuged (5,000 × *g* at 20°C for 5 min), and the ether layer was removed. The ether extraction was repeated twice more, and the extracts were combined and taken to dryness under N_2. The residue cell fraction, mainly protein, was treated to isolate the peptidoglycan. The fraction was suspended in 0.25 ml of 1 M NaOH plus 4 ml of 4% (wt/vol) sodium dodecyl sulfate (SDS) in 10^{-2} M ethylenediaminetetraacetate (pH 7.0), and the mixture was incubated (85°C, 60 min). After cooling to room temperature, the mixture was centrifuged (100,000 × *g* at 20°C for 120 min), and the resulting pellet was dispersed in 2 ml of 10^{-2} M NaCl after four washings by centrifugation as above with this solution. For lysozyme treatment, 1 ml of the washed material was incubated (37°C for 30 min) with 20 μg of white lysozyme and then centrifuged as above to remove the particulate material. Chemical analyses of whole cells and cell fractions were made by standard procedures. Protein- and ninhydrin-positive material was measured by the methods of Lowry et al. (22) and Spies (37) using, respectively, bovine serum albumin (fraction V) and leucine as the standards. RNA and ribose were determined by the orcinol assay (34) using D-ribose as a standard and a factor of 4.90 to convert ribose values to RNA values. DNA and deoxyribose were determined by the diphenylamine assay (3) using D-deoxyribose as a standard and a factor of 2.44 to convert deoxyribose values to DNA values.

Thin-layer chromatography. The procedures outlined by Randerath and Randerath (29) using the solvent systems described previously (10, 11) were used for thin-layer chromatography of nucleic acid compounds. When necessary, samples were concentrated by lyophilization, and the dried residue was suspended in distilled water at one-tenth of the original sample volume.

Enzyme activities. Cell extracts were prepared by passage (three times) of cell suspensions (ca. 10^{11} cells per ml) made in HM buffer containing 10^{-3} M dithiothreitol (DTT) through a cold French pressure cell (20,000 lb/in^2 at 5°C). Large cell debris and unbroken cells were removed from the extract by centrifugation

(14,000 × *g* for 20 min at 5°C). When appropriate, the resultant extract was separated into particulate (pellet) and soluble (supernatant liquid) fractions by further centrifugation (100,000 × *g* for 120 min at 5°C). All dilutions of extracts were made using HM buffer containing 10^{-3} M DTT and 0.1 mg of bovine serum albumin (fraction V) per ml, but the metals were deleted from the HM buffer.

Enzyme activities in cell extracts were measured using the below-listed assay mixtures (1 to 1.5 ml, final volume) as modified from the procedures given in the reference(s) following the enzyme name. Purine phosphorylase (EC 3.5.3.5) (17): KH_2PO_4 (pH 7.1), 0.18 M; inosine, 2 mM; and xanthine oxidase, 24 IU/ml. Thymidine phosphorylase (EC 2.4.2.4) (29): $NaAsO_4$ (pH 7.5), 0.1 M; thymidine, 20 mM; and tris(hydroxy methyl)aminomethane (Tris, pH 7.5), 0.2 M. Uridine phosphorylase (EC 2.4.2.3) (30): same as for thymidine phosphorylase, but with uridine (20 mM) in place of thymidine. 5'-Nucleotidase (EC 3.1.3.5) (26): sodium acetate (pH 6.0), 0.1 M; $CaCl_2$, 20 mM; $CoCl_2$, 5 mM; bovine serum albumin, 100 μg/ml; and AMP, 5 mM. Deoxyribose-5-phosphate aldolase (EC 4.1.2.4) (14): triethanolamine (pH 7.8), 40 mM; ethylenediaminetetraacetate, 1 mM; fructose-1,6-bisphosphate aldolase, 50 μg/ml; fructose-1,6-bisphosphate, 5 mM; and acetaldehyde, 10 mM. Phosphodeoxyribomutase (EC 2.7.5) (1, 36): Tris, 68 mM; ethylenediaminetetraacetate, 10 μM; $MnCl_2$, 1 mM; NaF, 10 mM; DTT, 10 mM; glucose-1,6-bisphosphate, 2.5 μM; and ribose-1-phosphate or deoxyribose-1-phosphate, 1 mM. Transketolase (EC 2.2.1.1) (19): Tris-hydrochloride (pH 7.6), 50 mM; $MgCl_2$, 10 mM; ribose-5-phosphate, 0.5 mM; xylulose-5-phosphate, 0.5 mM; reduced nicotinamide adenine dinucleotide, 0.1 mM; and ca. 50 μg each of α-glycerol-phosphate dehydrogenase and triose phosphate isomerase. Transaldolase (EC 2.2.1.2) (2): fructose-6-phosphate, 0.5 mM; DL-glyceraldehyde, 0.5 mM; Tris-hydrochloride (pH 7.6), 50 mM; reduced nicotinamide adenine dinucleotide, 0.1 mM; and ca. 50 μg each of triose phosphate isomerase and α-glycerophosphate dehydrogenase. Phosphoketolase (5): K_2HPO_4 (pH 6.1), 50 mM; sodium succinate (pH 6.2), 20 mM; DTT, 0.1 mM; $MgCl_2$, 2 mM; thiamine pyrophosphate, 2 mM; and xylulose-*t*-phosphate, 4 mM.

Radioactivity and miscellany. All radioactivity measurements were made by scintillation counting using Aquasol II fluid (New England Nuclear Corp., Boston, Mass.). Permeation of ribose into bdelloplasts was determined by methods similar to those described for phosphate permeation (32). Measurements of oxygen consumption by cell suspensions were made using an oxygen electrode (12). All biochemicals and reagents used were reagent or biological grade, and enzymes were of the highest grade available.

RESULTS

Changes in DNA and RNA content during growth of *B. bacteriovorus* on *E. coli*. The initial and final distributions of DNA and RNA in cultures of bdellovibrios growing intraperiplasmically on *E. coli* were determined. The data (Table 1) show that some 30 to 60% of the initial *E. coli* RNA-ribose disappeared (i.e., was not assayable as orcinol-positive material) during single- and multicycle intraperiplasmic bdellovibrio growth. The results of numerous experiments indicated that the percentage of initial *E. coli* RNA-ribose that disappears during growth is quite variable but always is greatest with *E. coli* substrate cells that have a high RNA content (e.g., log-phase cells) or low DNA content (e.g., hydroxyurea-inhibited cells). Often the DNA content of the progeny bdellovibrios was greater than that present in the initial *E. coli* (Table 1). As shown previously, bdellovibrio DNA can be synthesized from ribonucleoside monophosphates formed from the degradation of substrate cell RNA (11). Even assuming that all of the excess DNA is formed in this manner, the use of RNA-ribose for DNA synthesis can account for only a small portion (8 to 18%) of the total missing RNA-ribose. Thus, on the average some 20 to 40% of the initial *E. coli* RNA-ribose disappears during intraperiplasmic bdellovibrio growth and is neither used for synthesis of progeny bdellovibrio nucleic acids nor found in the suspending buffer after growth (Table 1).

Use of exogenous nucleoside monophosphates during intraperiplasmic growth of *B. bacteriovorus* on *E. coli*. When exogenous purine nucleoside monophosphates were added to a culture of intraperiplasmically growing bdellovibrios, a rapid loss of ribose (as measured by the orcinol assay) from the suspending buffer occurred and radioactivity from U-^{14}C-labeled exogenous nucleotides was found in the evolved CO_2 and progeny bdellovibrios (Table 2). When the cultures were also supplemented with a mix-

TABLE 1. *Initial and final distribution of nucleic acid material with intraperiplasmic growth of B. bacteriovorus 109J on E. coli ML35*[a]

Material	Single-cycle culture		Multicycle culture	
	DNA	RNA	DNA	RNA
A. Initial *E. coli*	62	303	53	386
B. Initial *B. bacteriovorus*	44	55	—[b]	—
C. Progeny *B. bacteriovorus*	132	185	72	124
D. Change	+26	−173	+19	−262
E. In suspending buffer after lysis	—	29	—	42
F. Net change (D − E)	+26	−144	+19	−220

[a] Cell suspensions were used to set up single-cycle or multicycle cultures which initially contained 4×10^9 *E. coli* per ml and either 7×10^9 (single-cycle) or 10^6 (multicycle) *B. bacteriovorus* per ml. The data are expressed as micrograms of nucleic acid equivalents per milliliter of cell culture, assayed as diphenylamine-positive (DNA) or ribose-positive material (RNA).

[b] —, Below detectable levels.

ture of unlabeled ribonucleoside monophosphates, the radioactivity in the evolved CO_2 was reduced 50% or more and less radioactivity was incorporated into the progeny bdellovibrios. However, the addition of ribose or ribose-5-phosphate had essentially no effect on the radioactivity level in the evolved CO_2. This latter result cannot be explained on the basis of exclusion of ribose from the bdelloplasts, since the data from other experiments indicated that ribose can effectively permeate into bdelloplasts obtained from 60- to 90-min cultures.

The distribution of radioactivity incorporated into the progeny bdellovibrios from exogenous nucleotides was determined by fractionation of the cells into their major cell components. The results showed that only trace amounts of the whole-cell radioactivity were in the cell pools (data not shown) and only 50% or less was in the nucleic acids (Table 2). About 10 to 15% of the whole-cell radioactivity was found in the cell lipid fraction. When the dried lipid fraction was hydrolyzed with dilute aqueous base and subsequently extracted with ether to remove free fatty acids, the bulk of the radioactivity (75% or more) remained in the aqueous phase, which presumably retained the glycerol moieties of the cell lipids. Approximately one-half of the radioactivity in the progeny bdellovibrios was found in the final "residue" fraction (Table 2), which is primarily protein. The radioactivities in both the lipid and residue cell fractions were substantially reduced when unlabeled ribonucleoside monophosphates were added to the cultures, but similar reductions were not observed with the nucleic acid fractions.

Intraperiplasmic growth of *B. bacteriovorus* on [*U*-^{14}C]ribose-labeled *E. coli* BJ565. The above experiments with intraperiplasmic bdellovibrio growth in the presence of exogenously added ribonucleoside monophosphates could represent an abnormal growth situation since a large pool of nucleotides does not exist during normal growth (11, 24, 34). To overcome this difficulty, the pentose-requiring *E. coli* BJ565 was used as the substrate cell and was radioactively labeled by growth on a basal medium containing [*U*-^{14}C]ribose. Analysis of these cells showed that some 84 to 91% of the whole-cell radioactivity was in the nucleic acid fraction (see Control, Table 3). Thin-layer chromatography of the hydrolyzed nucleic acid fraction showed that less than 5% of the nucleic acid fraction radioactivity was coincident with the locations of nucleic acid bases on the chromatograms. When these cells were selectively fractionated to obtain separate RNA and DNA fractions, the RNA/DNA radioactivity ratio varied from 7.1 to 11.2. The RNA/DNA radioactivity ratio was consistent with the cellular

TABLE 2. *Incorporation and catabolism of exogenous U-^{14}C-labeled nucleotide monophosphates during intraperiplasmic growth of B. bacteriovorus 109J on E. coli ML35*[a]

Exogenous *U*-^{14}C-labeled nucleoside monophosphate	Addition to suspending buffer	% Initial radioactivity found at the end of growth					
		Suspending buffer	Evolved CO_2	Cell fractions			
				Whole cells	Nucleic acids	Lipids	Residue
CMP	None	58.2	22.3	16.6	6.1	1.9	8.2
	Ribonucleotides	80.4	8.1	9.7	6.0	0.7	2.9
	Ribose	54.9	22.9	19.4	6.0	2.9	10.9
	Ribose-5-PO_4	56.2	22.6	16.7	6.1	2.1	8.2
GMP	None	79.7	23.5	10.5	3.8	1.2	5.6
	Ribonucleotides	74.8	9.0	9.1	5.8	0.9	3.7
	Ribose	70.7	15.5	13.9	5.5	1.7	6.5
	Ribose-5-PO_4	72.3	15.0	11.9	4.7	1.3	5.8
UMP	None	68.9	16.5	15.1	6.6	1.4	7.0
	Ribonucleotides	81.0	7.6	10.1	5.7	0.7	3.8
	Ribose	62.2	18.0	20.7	8.5	2.3	10.0
	Ribose-5-PO_4	71.5	16.6	18.1	8.7	1.5	8.6

[a] Parallel cultures were set up, each containing 5×10^9 *E. coli* per ml and 9×10^9 *B. bacteriovorus* per ml, after mixing the two cell suspensions to initiate single-cycle growth. At 60 min after mixing, the *U*-^{14}C-labeled nucleoside monophosphate (10^{-4} M final concentration) and the indicated supplement (10^{-3} M final concentration) were added to the culture. The ribonucleoside monophosphate mixture contained all four ribonucleotides each at 2.5×10^{-4} M (final concentration). The distribution of radioactivities was determined after completion of the developmental cycle (lysis of the bdelloplasts). Initial radioactivities for CMP, GMP, and UMP were 74,200, 31,500, and 67,800 cpm/ml, respectively.

TABLE 3. *Effects of exogenous compounds on the distribution of radioactivity during intraperiplasmic growth of B. bacteriovorus 109J on [U-^{14}C]ribose-labeled E. coli BJ565*[a]

Culture	Additions[b] to culture	% Initial radioactivity in:						
		Suspending buffer	Evolved CO_2	Cell fractions				
				Whole cells	Pools	Lipids	Nucleic acids	Residue
Control[c]	None	2.1	0.5	94.3	0.6	1.0	83.7	9.4
1	None	9.3	18.9	73.6	2.3	9.4	44.5	17.1
2	Glutamate	10.6	13.6	72.7	2.2	9.6	44.6	14.0
3	Ribonucleotides	35.0	13.2	57.8	1.9	4.5	37.9	12.4
4	Ribose-5-PO_4	14.8	17.2	67.2	2.1	8.8	40.4	16.4

[a] Parallel cultures were set up, each initially containing 3.3×10^9 *B. bacteriovorus* per ml and 1.9×10^9 [*U*-^{14}C]ribose-labeled *E. coli* BJ565 per ml (46,700 cpm/ml).

[b] Added at 60 min (after mixing the cell suspensions) to the cultures to a final concentration of 10^{-3} M, except the ribonucleotide mixture, which contained all four ribonucleotides, each at 2.5×10^{-4} M (final concentration).

[c] Culture was cooled to 4°C immediately after mixing (0 min) of the cell suspensions. Radioactivity distribution in cells represents that in the initial *E. coli*.

RNA/DNA ratio as determined by colorimetric assays.

With intraperiplasmic growth of *B. bacteriovorus* on [*U*-^{14}C]ribose-labeled *E. coli* BJ565 as substrate cells, some 70% or more of the initial radioactivity was incorporated into the progeny bdellovibrios and approximately 20% was in the evolved CO_2 (Table 3). The remaining radioactivity was released into the suspending buffer and by thin-layer chromatographic analysis was mainly found to be associated with the nucleosides and nucleotides normally present (11) in the buffer after bdellovibrio growth. Less than half of the radioactivity in the input *E. coli* BJ565 cells (or 60.5% of the progeny bdellovibrio whole-cell radioactivity) was in the nucleic acid fraction of the progeny bdellovibrios. The progeny bdellovibrio lipid and protein residue fractions both contained greater levels of radioactivity than those present in the homologous cell fractions of the input *E. coli* BJ565. The distribution of radioactivity was similar in progeny bdellovibrios whether obtained from growth on [*U*-^{14}C]ribose-labeled *E. coli* BJ565 (Table 3) or from *E. coli* ML35 plus exogenous *U*-^{14}C-labeled ribonucleotides (Table 2).

A substantial reduction in radioactivity in the evolved CO_2, but not in the progeny bdellovibrios, occurred when glutamate, a major bdellovibrio energy substrate (12), was added to a culture of *B. bacteriovorus* growing intraperiplasmically on [*U*-^{14}C]ribose-labeled *E. coli* BJ565 (Table 3). On the other hand, the addition of a mixture of ribonucleoside monophosphates to the culture significantly reduced the radioactivity in both the CO_2 and progeny bdellovibrios. The addition of exogenous ribose-5-phosphate appeared to have little, if any, effect on the distribution of radioactivity with intraperiplasmic bdellovibrio growth on [*U*-^{14}C]ribose-labeled *E. coli* BJ565.

Intraperiplasmic growth of *B. bacteriovorus* on normal and heat-treated [*U*-^{14}C]ribose-[5,6-^{3}H]uracil-labeled *E. coli* BJ565. To measure the distribution of substrate cell RNA-ribose and RNA bases incorporated into progeny bdellovibrio cells, *B. bacteriovorus* was grown on either normal or heat-treated [*U*-^{14}C]ribose-[5,6-^{3}H]uracil-labeled *E. coli* BJ565. From previous studies (9), bdellovibrio growth on heat-treated cells appeared normal as determined by control experiments. The ^{14}C/^{3}H ratios observed with the whole cells and cell fractions of the initial normal or heat-treated *E. coli* BJ565 were quite similar (Table 4). After growth on normal substrate cells, the whole-cell ^{14}C/^{3}H ratio of the progeny bdellovibrios was some 50% larger than that of the initial normal *E. coli*. When these progeny bdellovibrios were fractionated into their major cell components, significantly greater ^{14}C/^{3}H ratios were found with all cell fractions except the RNA. The ^{14}C/^{3}H ratios of the bdellovibrio and *E. coli* RNA fractions were equal, as expected since the bdellovibrio RNA is derived completely from intact ribonucleotides generated from the substrate cell RNA (11). When bdellovibrios were grown on heat-treated substrate cells, the ^{14}C/^{3}H ratio patterns of the progeny bdellovibrio whole cells and cell fractions were similar to those observed with growth on normal cells (Table 4). All fractions from bdellovibrios grown on normal and heat-treated substrate cells were also assayed for their DNA, RNA, and protein content, and their ^{14}C-^{3}H specific activities (counts per minute per microgram) were calculated from these data. The ratios of the ^{14}C-^{3}H specific activities (data not shown) were very similar to those calculated

TABLE 4. *Distribution of ^{14}C and ^{3}H radioactivities and $^{14}C/^{3}H$ ratios with intraperiplasmic growth of B. bacteriovorus 109J on [U-^{14}C]ribose-[5,6-^{3}H]uracil-labeled E. coli BJ565*[a]

Material	Normal *E. coli* substrate cells						Heated *E. coli* substrate cells					
	Initial *E. coli*			*B. bacteriovorus*			Initial *E. coli*			*B. bacteriovorus*		
	^{14}C (%)	^{3}H (%)	Ratio: ^{14}C/^{3}H	^{14}C (%)	^{3}H (%)	Ratio: ^{14}C/^{3}H	^{14}C (%)	^{3}H (%)	Ratio: ^{14}C/^{3}H	^{14}C (%)	^{3}H (%)	Ratio: ^{14}C/^{3}H
Suspending buffer	ND[b]	ND	—[c]	18.2	56.5	0.32	ND	ND	—	14.9	54.3	0.27
Evolved CO_2	ND	ND	—	13.7	—	—	ND	ND	—	12.8	—	—
Cell fractions												
Whole cells	100	100	1.00	73.8	50.8	1.45	100	100	1.0	75.3	48.8	1.54
Lipids	6.7	1.7	3.94	15.6	3.0	5.20	7.3	1.8	4.05	15.7	3.0	5.23
RNA	74.6	98.0	0.76	28.6	36.8	0.77	75.7	99.0	0.76	31.9	37.1	0.86
DNA	7.2	3.8	1.89	13.2	2.3	5.74	7.4	4.2	1.76	14.3	2.6	5.50
Residue	13.2	2.3	5.74	22.0	4.5	4.89	14.3	2.6	5.50	19.6	4.5	4.35

[a] Parallel developmental cultures were set up, each initially containing (per ml) 2.6 × 10^9 *B. bacteriovorus* and 1.4 × 10^9 of either normal (^{14}C, 19,330 cpm; ^{3}H, 6,180 cpm) or heated (^{14}C, 16,750 cpm; ^{3}H, 5,370 cpm) [*U*-^{14}C]ribose-[5,6-^{3}H]uracil-labeled *E. coli* BJ565. The data are expressed as percentages of the initial radioactivity and ^{14}C/^{3}H ratios of these percentages. The data are averages from two replicate experiments.

[b] ND, Not determined.

[c] —, Not applicable.

by radioactivities alone (Table 4).

The residues remaining after fractionation of cells from the above experiments were treated by boiling in SDS, and the SDS-insoluble material was isolated (see Materials and Methods). The SDS-insoluble material and its associated ^{14}C-^{3}H radioactivities were completely solubilized after incubation with lysozyme, suggesting this material consisted mostly of peptidoglycan. The *E. coli* SDS-insoluble material contained 3 to 5% of the total radioactivity of the residue fraction and had ^{14}C/^{3}H ratios varying from 2.8 to 3.5. On the other hand, the SDS-insoluble material from progeny bdellovibrios contained 8 to 12% of the radioactivity in the residue fraction and had larger ^{14}C/^{3}H ratios varying from 5.7 to 8.0. The ratios were very similar for bdellovibrios grown on normal or heat-treated substrate cells.

The lipid fractions were subjected to alkaline hydrolysis followed by partition into aqueous and ether extracts. With all lipid fractions, the majority (80 to 90%) of the ^{3}H radioactivity was associated with the aqueous, glycerol-containing phase. However, the aqueous phase contained only 26 to 35% of the ^{14}C radioactivity with *E. coli* lipid fractions, but contained 75 to 90% of the ^{14}C radioactivity with bdellovibrio lipid fractions. In addition, the amounts and distribution of radioactivity in the lipid fractions were similar both in normal and heat-treated *E. coli* BJ565 and in progeny bdellovibrios grown on these two types of substrate cells.

Distribution and use of *E. coli* RNA-ribose during intraperiplasmic growth of *B. bacteriovorus*. The net amount of the initial substrate cell RNA-ribose that disappears during intraperiplasmic growth of *B. bacteriovorus* on normal or heat-treated [*U*-^{14}C]ribose-labeled *E. coli* BJ565 was calculated colorimetrically via the standard orcinol assay (Table 5, section A). The distribution of RNA-ribose carbon that occurred with the same experiments was calculated from the ^{14}C radioactivity distribution (Table 5, section B). Since the initial *E. coli* contained some ^{14}C radioactivity in non-RNA cell materials (Tables 3 to 5), which could contribute to the synthesis of bdellovibrio non-RNA cell material, the ^{14}C radioactivity in non-RNA bdellovibrio cell material was corrected by subtracting the ^{14}C radioactivity of the initial *E. coli* non-RNA cell material. This calculation probably represents an over-correction since it assumes a 100% conversion efficiency of ^{14}C in non-RNA *E. coli* cell material into non-RNA bdellovibrio cell material, but no practical way(s) is available to calculate this conversion efficiency. The amount in micrograms of RNA-ribose equivalents in these fractions was estimated by dividing the ^{14}C radioactivity in the non-RNA bdellovibrio cell material and in the evolved CO_2 by the specific activity of the initial *E. coli* RNA-ribose (Table 5, section D). Given assumptions made in these calculations, the data indicate that during bdellovibrio intraperiplasmic growth the colorimetrically determined loss of substrate cell RNA-ribose is matched by an equivalent appearance of RNA-ribose carbon in the evolved CO_2 and in non-RNA bdellovibrio cell material.

Enzyme activities in cell extracts. Ex-

TABLE 5. *RNA-ribose balance sheet for intraperiplasmic growth of B. bacteriovorus 109J on [U-^{14}C]ribose-labeled E. coli BJ565*[a]

Determination	μg/ml		cpm/ml		cpm/μg		μg/ml		Percent	
	N[b]	HT[c]	N	HT	N	HT	N	HT	N	HT
1A. RNA-ribose in initial *E. coli*	90.3	79.3								
2A. RNA-ribose in progeny *B. bacteriovorus*[d]	29.9	26.5								
3A. RNA-ribose in suspending buffer at lysis	13.4	14.5								
4A. RNA-ribose: net disappearance [1A − (2A + 3A)]	47.0	38.3								
1B. Progeny *B. bacteriovorus* cells			17,846	15,031						
2B. Progeny *B. bacteriovorus* RNA			6,770	4,674						
3B. Progeny *B. bacteriovorus* non-RNA cell material (1B − 2B)			11,076	10,357						
4B. Initial *E. coli* non-RNA cell material			5,157	4,658						
5B. Net increase in non-RNA cell material (3B − 4B)			5,917	5,699						
6B. Evolved CO_2			3,451	2,693						
1C. Specific activity of initial *E. coli* RNA-ribose					187	189				
1D. RNA-ribose in non-RNA cell material (5B/1C)							31.6	30.2		
2D. RNA-ribose in evolved CO_2 (6B/1C)							18.4	14.2		
3D. RNA-ribose total (1D + 2D)							50.0	44.4		
Percent recovery [(3D/4A) × 100]									106	116

[a] Parallel cultures were set up, each containing ca. 3.3×10^9 *B. bacteriovorus* per ml and ca. 2×10^9 [*U*-^{14}C]ribose-labeled *E. coli* BJ565 (47,000 cpm/ml). The data are an average of three replicate experiments.

[b] N, Normal *E. coli* substrate cells.

[c] HT, Heat-treated *E. coli* substrate cells.

[d] Corrected for the amount of RNA-ribose (22 μg/ml) present in the input *B. bacteriovorus*.

tracts prepared from cells of both *B. bacteriovorus* and normal *E. coli* BJ565 possessed enzyme activities associated with the degradation of ribonucleoside and deoxyribonucleoside monophosphates (Table 6). Phosphodeoxyribomutase, which converts deoxyribose-1-phosphate and ribose-1-phosphate to their respective pentose-5-phosphates, was present in these extracts. However, this activity and thymidine phosphorylase activity were not detected in extracts prepared from heat-treated *E. coli* BJ565 cells. These latter extracts also had lower levels of deoxyribose-5-phosphate aldolase and purine phosphorylase, but increased levels of 5′-nucleotidase. All extracts prepared from normal or heat-treated *E. coli* BJ565 cells contained low detectable levels of transketolase, since the requirement for pentose for growth of this mutant *E. coli* strain is supposedly based on the absence of both transketolase and gluconate-6-phosphate dehydrogenase in this species (19). Bdellovibrio extracts contained both transketolase and transaldolase activity (Table 6). It should be noted that the modified transketolase assay required the endogenous formation of xylulose-5-phosphate from ribose-5-phosphate; thus pentose isomerase and epimerase activities were also present in the bdellovibrio extracts.

TABLE 6. *Enzyme activities in cell extracts of B. bacteriovorus 109J and of normal or heat-treated E. coli BJ565*

Enzyme	Sp act[a]		
	B. bacteriovorus	Normal *E. coli*	Heat-treated *E. coli*
5′-Nucleotidase	1.60	0.70	2.44
Uridine phosphorylase	147.4	36.2	36.8
Thymidine phosphorylase	75.8	31.5	0.00
Purine phosphorylase	227.4	233.2	205.9
Phosphodeoxyribomutase	21.8	16.7	2.7
Deoxyribose-5-phosphate aldolase	1.1	0.6	0.3
Phosphoketolase	ND[b]	ND	ND
Transketolase	15.0 (8.9)[c]	6.4	2.9
Transaldolase	2.6	5.7	0.5

[a] Expressed as nanomoles of substrate change per minute per milligram of cell extract protein.

[b] ND, Not detected.

[c] Activity present with assay mixture minus xylulose-5-phosphate.

DISCUSSION

The data demonstrate that during intraperiplasmic growth of *B. bacteriovorus*, some 20 to 40% of the ribonucleotide monophosphates derived from the substrate cell RNA are metabolized in ways not leading to biosynthesis of nu-

cleic acids. A proposed general scheme for the metabolism of nucleoside monophosphates by *B. bacteriovorus* is shown in Fig. 1. Initially, the substrate cell nucleic acids are degraded by nucleases. Bdellovibrios are known to make extracellular nucleases (4), and it has been found that chloramphenicol inhibits degradation of substrate cell ribosomes (11). With the additional data on recent studies of substrate cell DNA degradation (R. A. Rosson, Ph.D. thesis, University of California, Los Angeles, 1978), it can be stated that these nucleases responsible for substrate cell nucleic acid degradation are primarily of bdellovibrio origin. The end products of this nuclease action include nucleoside monophosphates, which are taken up intact by bdellovibrios (32) by a nucleotide transport system(s) which has not yet been characterized. The majority of these intracellular nucleotides are then used by *Bdellovibrio* for synthesis of its nucleic acids. Concomitantly, since more ribonucleoside monophosphates are available than are needed for bdellovibrio nucleic acid biosynthesis, some ribonucleotides undergo catabolism. By the concerted actions of nucleotidases, phosphorylases, and phosphodeoxyribomutase (Fig. 1), which are present in bdellovibrios (Table 6), nucleotides can be converted to the pentose-1-phosphates and the free nucleic acid bases.

With intraperiplasmically growing bdellovibrios, the nucleic acid bases apparently are not catabolized further, but are released into the suspending buffer. This conclusion is supported by several lines of evidence: (i) during intraperiplasmic growth on [^{3}H]uracil-[^{14}C]ribose-labeled *E. coli*, there occurs a highly preferential

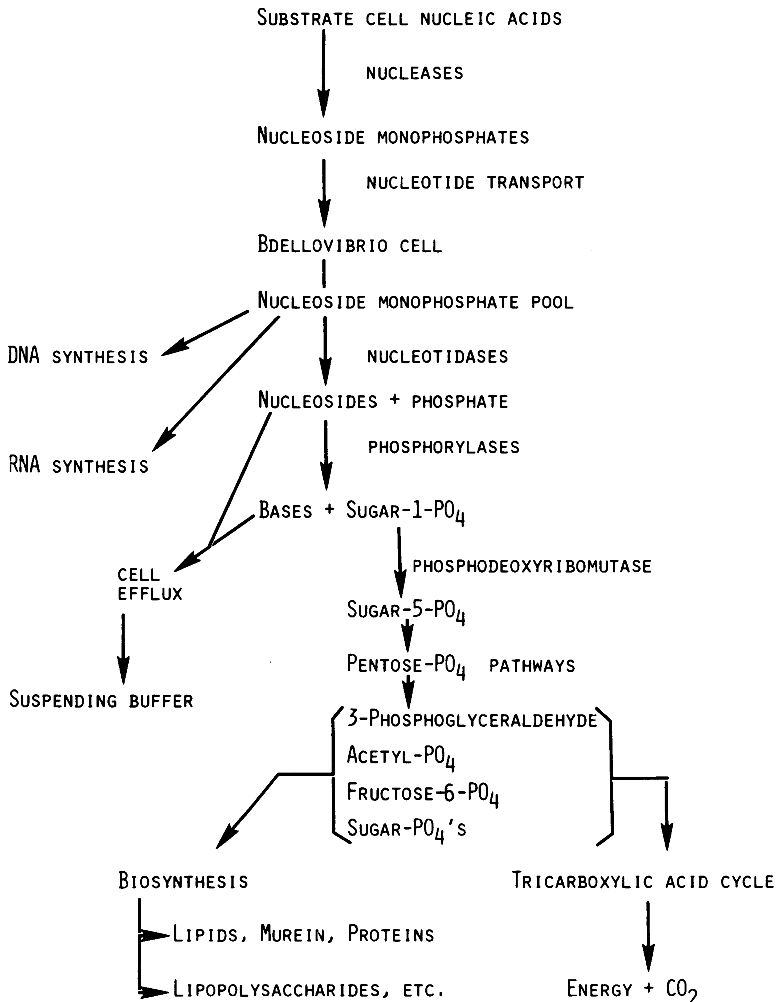

FIG. 1. *Proposed scheme for B. bacteriovorus 109J metabolism of RNA-derived ribose.*

flow of ribose moiety relative to base moiety, into non-nucleic acid bdellovibrio cell fractions (Table 4); (ii) with intraperiplasmic bdellovibrio growth on ^{14}C-labeled nucleic acid base-labeled *E. coli*, very little radioactivity is detected in the respired CO_2 and almost all of the radioactivity incorporated into the progeny bdellovibrios is found in the nucleic acid cell fraction (10, 11, 24); and (iii) when free bdellovibrio cell suspensions are incubated with ribonucleoside monophosphates, only the ribose portion of these compounds is catabolized, with equivalent levels of nucleic acid bases found in the suspending buffer after incubation (10).

On the other hand, the pentose-5-phosphates coming from nucleoside monophosphates, particularly ribose-5-phosphate, are extensively metabolized. Although the metabolism of deoxyribose-5-phosphate was not examined in detail, a net disappearance of deoxyribose (diphenylamine-positive material) during intraperiplasmic growth has been reported (24). As has been found for other bacteria (14, 15), deoxyribose-5-phosphate can be cleaved to 3-phosphoglyceraldehyde and acetaldehyde by deoxyribose-5-phosphate aldolase. A similar catabolism probably occurs with *B. bacteriovorus*, since this enzyme was detected in cell extracts (Table 6). Ribose-5-phosphate is metabolized further by bdellovibrios via the standard pentose-phosphate pathways. Thus, bdellovibrios are capable of forming important cellular intermediates such as 3-phosphoglyceraldehyde, fructose-6-phosphate, or erythrose-4-phosphate from the ribose-5-phosphate derived from the substrate cell RNA.

Analysis of the bdellovibrio lipid fraction after intraperiplasmic growth on [^{14}C]ribose-labeled *E. coli* BJ565 indicated that most of the lipid fraction radioactivity was retained in the glycerol-containing water phase. The data indicate that ribose carbon is not used by bdellovibrios for the synthesis of fatty acids. These findings are consistent with a previous study in which it was established that bdellovibrios derive most of their fatty acids per se from the substrate cell fatty acids (21). The precise pathway(s) in *B. bacteriovorus* for the total formation of the glycerol moieties of its lipids is not known. Our data show that bdellovibrios can take part of the 3-phosphoglyceraldehyde formed via pentose pathway interconversions of the RNA-ribose and convert it to glycerol-3-phosphate by triose phosphate and glycerol phosphate dehydrogenase enzyme activities. The pentose pathway interconversions can also yield fructose-6-phosphate, which is generally known to serve as a major precursor to *N*-acetylglucosamine and *N*-acetylmuramic acid. The levels and $^{14}C/^{3}H$ ratios of radioactivity detected in the peptidoglycan of the progeny bdellovibrios indicate that part of this bdellovibrio macromolecule can be derived in this manner from fructose-6-phosphate arising initially from the substrate cell RNA-ribose. Presently no definitive evidence exists, but it is plausible that some of the carbohydrates of the bdellovibrio lipopolysaccharide may also be formed from the phosphorylated cell carbohydrate intermediates generated by the interconversions of the ribose-5-phosphate in the pentose and hexose pathways.

Another major fate of the ribose carbon coming from the catabolism of ribonucleotides is its conversion to CO_2. On the average, some 14 to 18 μg of RNA-ribose or 5.2 to 7.2 μg of carbon is catabolized to CO_2 during intraperiplasmic bdellovibrio growth on ca. 2×10^9 *E. coli* (Table 5). Based on the results of a previous study (31), these *E. coli* would contain about 377 μg of carbon of which 12.5% or 47 μg of carbon would be respired to CO_2 for energy by bdellovibrios during intraperiplasmic growth. Therefore, the total contribution of RNA-ribose catabolism to bdellovibrio energy production would be 5.2/47 to 7.2/47, or 12 to 15%. These data plus the observation that exogenous glutamate effectively represses this catabolism as shown by the reduction of $^{14}CO_2$ with growth on [*U*-^{14}C]ribose-labeled *E. coli* (Table 3) indicate again that the major energy substrates used by bdellovibrio during intraperiplasmic growth are amino acids (12).

B. bacteriovorus clearly possesses the complete potential for degrading ribonucleoside monophosphates and metabolizing the ribose derived from these compounds. This conclusion is best supported by the presence of various enzyme activities in cell extracts (Table 6) and by the previous findings that only the ribose moiety of exogenous ribonucleoside monophosphates is catabolized to CO_2 by free bdellovibrio cell suspensions (10). It is well established that *E. coli* also possesses these enzymes (1, 6, 15, 16, 20, 23, 25, 28). Some of these *E. coli* enzymes may retain their enzymatic activities for varying times after bdellovibrio has entered the *E. coli* periplasmic space. The question then arises as to whether only bdellovibrio enzymes are involved in the observed catabolism of ribonucleotides during intraperiplasmic growth. The data obtained thus far strongly suggest that only bdellovibrio enzymes are involved in this ribonucleotide degradation. First, heat-treated *E. coli* BJ565 is devoid of thymidine phosphorylase and phosphodeoxyribomutase and has both low transaldolase and low deoxyribose-5-phosphate

aldolase activities (Table 4), yet no significant changes were observed in the distribution or degree of incorporation or radioactivities between bdellovibrio growth on normal or heat-treated *E. coli* BJ565 (Tables 4 and 5). Second, no appreciable differences in the amounts of respired $^{14}CO_2$ were noted between growth on [U-^{14}C]ribose-labeled *E. coli* and growth on unlabeled *E. coli* with exogenous U-^{14}C-labeled ribonucleotides, even though the ribonucleotides were added to the developmental culture at 60 min, at which time essentially no *E. coli* respiratory potential exists (33) and increased bdellovibrio respiration begins (12). Third, both *E. coli* and bdelloplasts but not bdellovibrios are permeable to ribose, yet exogenously added ribose (and ribose-5-phosphate) did not affect the overall metabolism of exogenous ribonucleoside monophosphates (Table 2) or of [^{14}C]ribose from [U-^{14}C]ribose-labeled *E. coli* (Table 3). Finally, bdellovibrios are permeable to nucleoside monophosphates (32), and exogenously added ribonucleotides significantly reduce the ribose metabolism in both of the situations given in the previous point.

From several standpoints, the bdellovibrio metabolism of RNA-ribose is a rather unusual type of bacterial metabolism. Although *B. bacteriovorus* has the enzymatic potential to metabolize ribose, whole cells do not take up ribose per se or use other exogenous sugars (10, 12). Bdellovibrio use of ribose requires that this compound be supplied in the form of ribonucleoside monophosphates which are taken up intact. As far as we are aware, the only other bacteria which might be capable of nucleotide transport are *Chlamydia* (7) and *Rickettsia* (39, 40), which are obligate intracellular parasites of animal cells. Finally and most importantly, the ribose metabolism of *B. bacteriovorus* may represent an evolutionarily acquired trait which reflects its unique mode of intraperiplasmic growth. The cytoplasm of most bacteria is nutritionally complete, but the availability of carbohydrates for bdellovibrio growth is minimal. Although carbohydrates are present in the cell wall of the gram-negative substrate cell, these sugars may not be available for bdellovibrio growth. It could be reasoned that these cell wall carbohydrates may be necessary for the integrity of the bdelloplast wall structure, which in turn is needed to maintain the bdellovibrio-created nutritional quality of the bdelloplast environment. Even if carbohydrates were available, it is questionable whether *B. bacteriovorus* could use them, since bdellovibrios do not take up glucose (12) and lack major catabolic enzymes such as glucokinase and fructose-1,6-bisphosphate aldolase (8). By using ribose derived from the substrate cell RNA, *B. bacteriovorus* overcomes the lack of carbohydrate availability in bacterial cytoplasm, fulfills the biosynthetic needs for phosphorylated carbohydrates with little energy expenditure, and maximizes utilization of the substrate cell RNA. This type of overall RNA-ribose metabolism is quite consistent with and no doubt contributes to the characteristically high energy efficiency that has been shown to occur with intraperiplasmic growth of *B. bacteriovorus* (31).

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