JOURNAL OF BACTERIOLOGY, Apr. 1984, p. 69–72
0021-9193/84/040069-04$02.00/0
Copyright © 1984, American Society for Microbiology
Vol. 158, No. 1

Conversion of D-Mannitol to D-Ribose: a Newly Discovered Pathway in *Escherichia coli*

H. ROSENBERG* AND C. M. HARDY

Department of Biochemistry, John Curtin School of Medical Research, Australian National University, Canberra City, A.C.T. 2601, Australia

Received 12 September 1983/Accepted 28 December 1983

A mutant (*mtlD*) strain of *Escherichia coli* unable to oxidize mannitol-1-phosphate to fructose-6-phosphate was used to study the fate of mannitol-1-phosphate. D-[1-^{14}C]mannitol entered the cells via the phosphotransferase system and was phosphorylated equally at carbon 1 or 6. The label disappeared gradually from the mannitol-1-phosphate pool, and some 60% of the ^{14}C was recovered in nucleic acids. Ribose was isolated from the purified RNA. The ^{14}C label distribution in the isolated ribose precluded a simple hexose-to-pentose conversion by elimination of one terminal carbon from mannitol-1-phosphate. The ^{14}C from mannitol-1-phosphate that did not enter macromolecules was found in CO_2 and in some organic, non-phosphorylated compounds that were not identified. We suggest that the de novo synthesis of mannitol-1-phosphate in *E. coli* may be a reaction specifically dedicated to the biosynthesis of ribose.

The de novo synthesis of mannitol-1-phosphate by *Escherichia coli* in the absence of mannitol was reported in 1962 by Helle and Klungsøyr (1). In the accompanying paper (5), we present evidence about the occurrence of mannitol-1-phosphate and its rapid turnover in *E. coli* grown aerobically with carbon sources other than mannitol. We found this compound both in wild-type strain cells and in *mtlD* mutant strains defective in the enzyme mannitol-1-phosphate dehydrogenase and unable to grow on mannitol (5). In the latter strains, an apparently undirectional reduction of fructose-6-phosphate by NADH, to produce mannitol-1-phosphate, was detected.

The finding raised two intriguing questions. Why is mannitol-1-phosphate produced de novo in substantial quantities? What is the ultimate fate of this compound? In this paper we present evidence that the carbon of mannitol-1-phosphate is transferred to pentose of nucleic acids and to CO_2.

MATERIALS AND METHODS

Most procedures and materials used were described in the accompanying paper (5).

Preparation of doubly labeled mannitol-1-phosphate. Growing cells of strain HR33 were prepared as described in the accompanying paper (5) and shaken at a density at 660 nm of 0.5 for 15 min with D-[1-^{14}C]mannitol (33 μM, 0.5 mCi/mmol) and $^{32}P_i$ (50 μM, 6.7 mCi/mmol). They were then collected and extracted with $HClO_4$, and the labeled mannitol-1-phosphate was purified as described in the accompanying paper (5).

Extraction of nucleic acids. We used strain HR33 (1.2 liters) shaken at an absorbance at 660 nm of 0.4 for 2 h at 37°C in the presence of 25 μM D-[1-^{14}C]mannitol (4 μCi). The cells were centrifuged, washed once with fresh medium, and disrupted by sonication. The RNA was extracted as described by Monier (3). The yield was about 1 mg. A batch of similarly prepared labeled cells was used to extract the DNA by the method of Saito and Miura (7).

* Corresponding author.

RNase and DNase were from Boehringer, Mannheim, West Germany. When selective cleavage of RNA by RNase was carried out, the enzyme was heated for 10 min at 80°C before use to remove traces of DNase activity.

RESULTS

^{32}P label distribution in intracellular mannitol-1-phosphate. We used strain HR33, lacking mannitol-1-phosphate dehydrogenase and constitutive in mannitol uptake, to load the cells rapidly with [^{14}C]mannitol-[1-^{32}P]phosphate, as described above. Since the mannitol molecule is symmetrical (apart from the label) it could be expected to be phosphorylated on entry randomly at either end, so that it would end up as [1,6-^{14}C]mannitol-[1-^{32}P]phosphate. However, for the interpretation of the results described below, the distribution of the label needed to be known unambiguously and was checked. In the experiment, the mannitol-1-phosphate was randomly cleaved with $NaIO_4$ under conditions where the C-O-P bond survived. A single passage through Dowex 1-HCO_3, followed by a water wash, removed any carbon fragments no longer attached to the phosphorus and representing the radioactivity of C_6. Subsequent elution with $KHCO_3$ removed the phosphate with any short fragments attached and containing the C_1. If phosphorylation occurred specifically at one end of the mannitol, the ^{14}C would be found exclusively in one of the two eluted fractions. We found (Table 1) that after chemical cleavage of the mannitol-1-phosphate about one half of the ^{14}C became detached from the phosphorus, the other half remaining with it, confirming that, during entry via the phosphotransferase system, the hexitol was phosphorylated randomly at either end.

Catabolism of mannitol-1-phosphate in *E. coli*. Growing cells of strain HR33, when presented with [1-^{14}C]mannitol rapidly equilibrated the label to the maximal value (about 90%) within 5 min. When a sample of the suspension was removed and treated with 2 mM unlabeled mannitol, the bulk of the label was exchanged out of the cells (Fig. 1). On the basis of our previous findings (5) and the known facts about the phosphotransferase system (6), this result shows that most of the label in the cell was present as mannitol-1-phosphate, its mannitol moiety freely exchanging with extra-

TABLE 1. Label distribution in the mannitol moiety of mannitol-1-phosphate derived from D-[1-^{14}C]mannitol[a]

Fraction	^{14}C (10^3 dpm)		^{14}C/^{32}P
	^{14}C	^{32}P	
Originally added label	28.4	95.0	0.30
Control sample			
Water wash	0	0	
1.0 M $KHCO_3$	29.3	94.7	0.31
Oxidized sample			
Water wash	13.8	0	
1 M $KHCO_3$	15.0	92.9	0.15
Total	28.8	92.9	

[a] Two samples of 20 μl of a 8.3 mM solution of doubly labeled mannitol-1-phosphate (see the text) were each mixed with 20 μl of 25 mM carrier mannitol-1-phosphate and 10 μl of 1.0 M $KHCO_3$. To one of the samples 20 μl of 0.1 M $NaIO_4$ was added, and both mixtures were held at room temperature for 15 min. Each mixture was then diluted to 0.5 ml with water and passed through a 2-ml bed of Dowex-1 × 8 (HCO_3 form) and washed through consecutively with 8 ml each of water and 1.0 M $KHCO_3$. Each eluate was made to 10.0 ml, and 1.5 ml samples were counted for ^{14}C and ^{32}P. All counts shown were calculated to the total reaction mixtures.

cellular mannitol. Sampling and exchange were repeated at intervals over a period of about 2 h, during which time the number of cells doubled. The amount of exchangeable ^{14}C within the cells declined to zero, and the total label within the cells fell to about 60% of that originally present. It was clear that the label was no longer in mannitol-1-phosphate, since it failed to exchange.

To check the nature of the material carrying the label, the suspension was centrifuged and the cells were fractionated (Table 2). The results indicate that close to 30% of the label

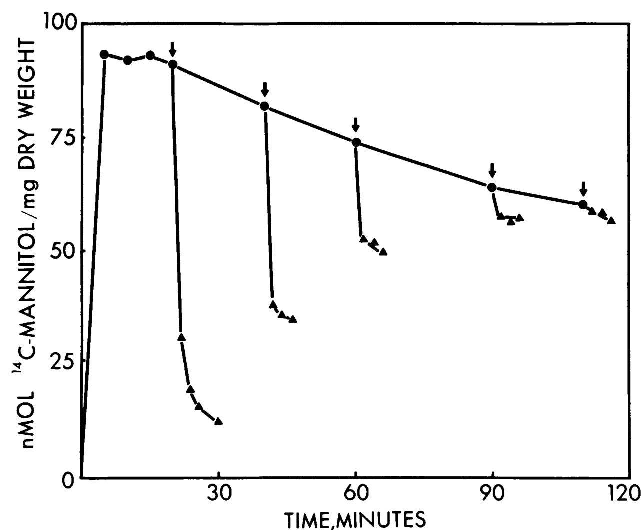

FIG. 1. Incorporation of ^{14}C from D-[1-^{14}C]mannitol into cellular components. Lactate-grown cells of strain HR33 (*mtlD*) were shaken at 37°C at a density at 660 nm of 0.33, with 20 μM D-[1-^{14}C]mannitol and with 10 mM lactate as the energy source. Samples (0.5 ml) were withdrawn (●), filtered, and counted as described in the accompanying paper (5). At each time shown by an arrow, a sample of 2.5 ml was withdrawn into a prewarmed flask containing 5 μl of 1 M unlabeled mannitol, and 0.5 ml of the suspension was withdrawn, filtered, and counted in the usual manner at the times shown (▲).

TABLE 2. Fractionation of the cells incubated with D-[1-^{14}C]mannitol[a]

Fraction	^{14}C (10^3 dpm)
Original cell suspension	1,264
Centrifuged supernatant plus wash with medium	348
Resuspended pellet in $HClO_4$	907
$HClO_4$ extract	29
Hot 1% acetic acid extract	25
Chloroform-methanol (1:1) (two extracts)	10.25
Remaining pellet (calculated)	843

[a] The suspension (60 ml) of cells, at the end of 120 min in an experiment similar to that described in Fig. 1, was centrifuged, and the cells were washed once with 20 ml of fresh medium. The pellet was extracted successively with 0.25 N $HClO_4$, 5 ml of 1% acetic acid at 100°C for 5 min, and twice with 5 ml chloroform-methanol (1:1, vol/vol). The ^{14}C content was assayed at each stage.

was in the suspending medium and that, of the label remaining in the pellet, the bulk was extracted neither by acid nor by solvents and was therefore in some polymeric compound. The pellet was extracted by the lithium diiodosalicylate-phenol method of Marchesi and Andrews (2). The isolated material, containing over 70% of the radioactivity originally present in the pellet, was found to contain also phosphorus and nitrogen. After further purification (2) the material was analyzed and was found to contain C, H, N, P, Na, and O in a molar ratio of 9.2:14.6:3.6:1:1:9.2, respectively, and the possibility of its being RNA suggested itself. A check of its spectrum showed an absorption minimum at 226 nm, a maximum at 255 nm, and a ratio of absorptions (260 nm/280 nm) of 2.08. The material became acid soluble after 17 h of incubation at 37°C in 1 N KOH and also after 40 min of incubation with RNase.

Isolation of ribose from the RNA of cells labeled with [1-^{14}C]mannitol. About 1 mg of labeled RNA, extracted from labeled cells as described above, was suspended in 10 ml of 150 mM NaCl, 15 mM sodium citrate (pH 7.0), and 1 mg of DNase-free RNase was added. The reaction at 37°C was monitored for 2 h, by which time the release of acid-soluble material, absorbing at 280 nm, had ceased. Ethanol (15 ml) was added, and the precipitated undegraded RNA fragments and protein were removed by centrifugation. The supernatant was taken to dryness under reduced pressure, the residue was dissolved in the SCC buffer (0.15 M NaCl plus 0.015 M sodium citrate), and the mixed nucleotides were converted to nucleosides by the method of Randerath and Randerath (4). Ribose was liberated from the mixture by treatment with Dowex 50-H^+ resin as described by Suhadolnik et al. (8) and separated from charged molecules by sequential treatment with Dowex 1-HCO_3^- and Dowex 50-H^+ resins. The nonionic material remaining was concentrated, and the ribose was purified by preparative chromatography on borate-impregnated silica plates developed with ethyl acetate-pyridine-water (7:2:1, vol/vol/vol). The eluted ribose was again treated as above to remove ions (borate, Na^+, and acetate) present and was taken to dryness. The material formed a glassy, noncrystalline film.

Label in the deoxyribose of DNA isolated from cells labeled with [^{14}C]mannitol. DNA from cells described above was isolated as described. Isolation of pure deoxyribose from this material presented difficulties due to degradation of the sugar. However, ^{14}C was present in the isolated DNA, the deoxynucleotides, and nucleosides (data not shown).

Distribution of radioactivity among the carbons of [^{14}C]ribose isolated from RNA. The label distribution in D-ribose is shown in Table 3. This represents a complex picture, considering that the mannitol-1-phosphate from which it was derived was labeled equally in positions 1 and 6.

Fate of the ^{14}C cleaved from mannitol. Of the total ^{14}C taken up by the cells initially, only about 60% was retained in the course of the 2-h incubation with lactate as the energy source (Fig. 1). This label was largely incorporated into the cell nucleic acids. When the cells were incubated in a closed vessel, some 14 to 18% of the total original label was trapped as CO_2 in an NaOH trap (data not shown). About 30% of the ^{14}C was found in the medium after 2 h. Fractionation of that material on Dowex 1-HCO_3 showed the presence of mannitol (15%) and mannitol-1-phosphate (85%). The remainder of the radioactivity (traces) was in non-phosphorylated compounds, which were slightly acidic since they were not eluted from the resin with water. Their nature was not investigated further. When cells of strain HR33 were grown on D-ribose as a source of carbon and were allowed to take up trace amounts of D-[1-^{14}C]mannitol under the conditions described in the legend to Fig. 1, with ribose present as the energy source, the amount of [^{14}C]CO_2 produced from mannitol rose to 30% of total label added (data not shown).

DISCUSSION

The demonstration (5) of de novo synthesis of mannitol-1-phosphate in *E. coli* growing on defined media devoid of mannitol raised the question concerning the ultimate fate of this compound. The present finding that about 40% of the ^{14}C label from mannitol-1-phosphate was found largely in the pentose of nucleic acid answered this question. It is tempting to suggest that the actual production of mannitol-1-phosphate may be dedicated to a pathway that leads to ribose- and deoxyribose-5-phosphates within the nucleic acid pools.

To the best of our knowledge, there are no known reactions from which such a pathway can be constructed without involving intermediates that may be channeled off to the gluconeogenic or oxidative pathways. This is a necessary proviso, since we have shown the conversion to take place in the *mtlD* mutant in which mannitol cannot serve as an energy source. The chirality of carbons 1, 2, and 3 in mannitol-1-phosphate is the same as that of carbons 5, 4, and 3, respectively, of ribulose-5-phosphate (Fig. 2). A hypothetical series of three reactions, which involves 2-oxo-6-phospho-D-gluconate, could lead to ribulose-5-phosphate and hence to ribose-5-phosphate.

The pathway shown in Fig. 2 requires the loss of one terminal carbon (C_6) of mannitol-1-phosphate, which we have shown (Table 1) to be approximately equally labeled at carbons 1 and 6. The other carbon (C_1) would be expected in the C_5 position of ribose. However, analytical data (Table 3) show a distribution of ^{14}C in the ribose that does not support a simple loss of terminal carbon, suggesting a far more complicated pathway. We are unable to suggest even a hypothetical set of reactions that could produce a labeling pattern shown in Table 3. One likely contributor to the redistribution of label is the possibility that the CO_2 liberated is recycled. The incorporation of label from D-[^{14}C]mannitol into cellular material can reach some 70% of the total label (Fig. 1), suggesting that there was no total loss of one of the terminal carbons. It is of interest, however, that only 50% of the total label remained in the cells under conditions where CO_2 was trapped. The appearance of some 50% of the label in C_4 of the ribose and some 20% in each C_1 and C_5 cannot be explained at present.

During the fractionation of $HClO_4$ extracts of cells that had taken up D-[1-^{14}C]mannitol for 15 to 20 min, a small peak of radioactivity was usually seen in the eluate from Dowex 1-HCO_3 just before the emergence of the mannitol-1-phosphate peak (5). This was identified as ribose-5-phosphate (data not shown), suggesting that this ester may be an intermediate in the proposed pathway.

One further point requires comment: some 10 to 15% of the total ^{14}C was found as [^{14}C]mannitol-1-phosphate in the medium after incubation of cells in the presence of D-[^{14}C]mannitol. In the accompanying paper (5) no mannitol-1-phosphate was detected in the medium. The difference is explained by the different timing of the two experiments—several minutes in the accompanying paper and 2 h in the present work. The long-term secretion of this phosphate ester may also be responsible for its reported finding by Helle and Klungsøyr (1).

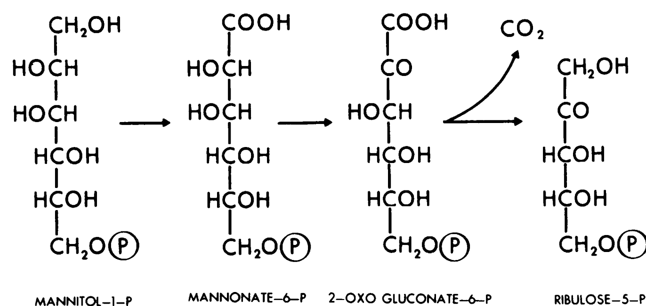

FIG. 2. Simplest (hypothetical) pathway from mannitol-1-phosphate to ribulose-5-phosphate.

TABLE 3. Distribution of ^{14}C in the carbon skeleton of ribose isolated from the RNA of cells incubated with D-[1-^{14}C]mannitol[a]

Carbon atom	Total radioactivity (%)	
	Assay 1	Assay 2
1	21.1	19.1
2	0.7	0.6
3	3.9	4.3
4	54.0	56.4
5	20.0	19.4

[a] The assays, described in detail by Williams et al. (9), were kindly carried out by J. F. Williams, Department of Biochemistry, Faculty of Science, Australian National University. The original ribose provided had a specific radioactivity of 460 dpm/mg.

ACKNOWLEDGMENTS

We thank J. F. Williams for the analysis of ^{14}C distribution in ribose, W. Armarego for advice on the preparation of ribose derivatives and on HIO_4 oxidation of mannitol-1-phosphate, and H. Gajardo for skilled technical assistance.

LITERATURE CITED

1. **Helle, K. B., and L. Klungsøyr.** 1962. Mannitol phosphate formation in *Escherichia coli* during glucose utilisation. Biochim. Biophys. Acta **65**:461–471.
2. **Marchesi, V. T., and E. P. Andrews.** 1971. Glycoproteins: isolation from cell membranes with lithium diiodosalicylate. Science **174**:1247–1248.
3. **Monier, R.** 1971. Preparation and properties of *Escherichia coli* 5S RNA, p. 618–628. *In* G. L. Cantoni and D. R. Davies (ed.),

Proceedings in nucleic acid research, vol. 2. Harper & Row, Publishers, New York.
4. **Randerath, K., and E. Randerath.** 1971. A tritium derivative method for base analysis of ribonucleotides and RNA, p. 796–812. *In* G. L. Cantoni and D. R. Davies (ed.), Proceedings in nucleic acid research, vol. 2. Harper & Row, Publishers, New York.
5. **Rosenberg, H., S. M. Pearce, C. M. Hardy, and P. A. Jacomb.** 1984. Rapid turnover of mannitol-1-phosphate in *Escherichia coli*. J. Bacteriol. **158**:69–72.
6. **Saier, M. H., Jr., B. U. Feucht, and W. K. Mora.** 1977. Sugar phosphate: sugar transphosphorylation and exchange group translocation catalysed by the enzyme II complex of the bacterial phosphoenolpyruvate: sugar phosphotransferase system. J. Biol. Chem. **252**:8899–8907.
7. **Saito, H., and K.-I. Miura.** 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. Biophys. Acta **72**:619–629.
8. **Suhadolnik, R. J., M. B. Lennon, T. Uematso, J. E. Monahan, and R. Baur.** 1977. Role of adenine ring and adenine ribose of nicotinamide adenine dinucleotide in binding and catalysis with alcohol, lactate, and glyceraldehyde-3-phosphate dehydrogenases. J. Biol. Chem. **252**:4125–4133.
9. **Williams, J. F., M. G. Clark, and P. F. Blackmore.** 1978. The fate of ^{14}C in glucose 6-phosphate synthesized from [1-^{14}C]ribose 5-phosphate by enzymes of rat liver. Biochem. J. **176**:241–256.