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Accumulation of Guanosine Tetraphosphate and Guanosine Pentaphosphate in *Myxococcus xanthus* During Starvation and Myxospore Formation

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Cultures of *Myxococcus xanthus* develop multicellular fruiting bodies when starved for carbon and nitrogen sources on an agar surface. Under these conditions of severe starvation, cultures rapidly accumulated a compound identified as guanosine tetraphosphate by chromatographic migration of the compound and of its major acid and alkali breakdown products. The accumulation of guanosine tetraphosphate was reduced in the presence of tetracycline, indicating that it may be synthesized by mechanisms similar to those of *Escherichia coli*. The guanosine tetraphosphate level was also reduced in starved cultures of a mutant unable to fruit normally, although it has not been determined whether the defect in guanosine tetraphosphate accumulation is responsible for the inability to fruit. Induction of spores by glycerol addition led to transient increases in both guanosine tetraphosphate and guanosine pentaphosphate at a stage following most cell shortening, but before spores had acquired full refractility.

Myxobacteria are gram-negative, rod-shaped procaryotes that show simple multicellular development (14). When vegetative myxobacteria encounter limiting nutrient, cells stop dividing and begin to aggregate. Later, within the aggregates, the vegetative rods convert into heat-resistant spores. Vegetative cells may be induced to form spores without prior aggregation if high levels of glycerol, dimethyl sulfoxide, or other compounds are added directly to growth medium (7). Glycerol-induced spores show some but not all of the properties of fruiting body spores (14).

Since the fruiting process is triggered by starvation, regulatory mechanisms known to respond to starvation may function in the control of fruiting. One such mechanism is mediated by increased levels of the guanosine polyphosphates guanosine tetraphosphate (Gp_4) and guanosine pentaphosphate (Gp_5), which accumulate in response to starvation for amino acids, carbon-energy sources, and other essential nutrients (3). Guanosine polyphosphates appear to mediate the "stringent" response, a broad readjustment of metabolism that includes inhibition of ribosome, peptidoglycan, and phospholipid biosynthesis (3, 6, 13), as well as stimulation of intracellular proteolysis and the expression of *his* and *lac* operons (10, 20, 23). In this paper we show that guanosine polyphosphate does indeed accumulate during myxobacterial development. The following paper (16) presents evidence that this accumulation may function in the initiation of fruiting.

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MATERIALS AND METHODS

Strains. The wild-type strain used in these studies is DK501, a yellow-phase single-colony isolate of *Myxococcus xanthus* FB originally obtained from M. Dworkin in 1973. Based on colony morphology, this strain appears closely related or identical to DK101 (12), which arose as a separate single colony isolated from the same source at about the same time.

DK510 and DK527 were selected after ethyl methane sulfonate mutagenesis for the ability to form colonies on F agar (17). Both DK510 and DK527 fail to aggregate or form spores under normal fruiting conditions. DK510 also fails to form glycerol-induced spores, and instead grows vegetatively in the presence of 0.75 M glycerol.

Media and reagents. Casitone medium (0.5%) consists of 0.5% Casitone (Difco), 10 mM Tris-hydrochloride, and 8 mM $MgSO_4$ (pH 7.6). Casitone medium (1%) differs only in the increased Casitone concentration. TM8 buffer is 10 mM Tris-hydrochloride–8 mM $MgSO_4$ (pH 7.6). TM8 agarose is TM8 buffer solidified with 0.8% agarose (Matheson, Coleman, and Bell Manufacturing Chemists). TPM buffer is TM8 buffer containing an additional 1 mM KPO_4.

$^{32}PO_4$ (carrier free) was supplied by New England Nuclear Corp. Although most batches of $^{32}PO_4$ were chromatographically homogeneous, there was occasional contamination of a batch by a radioactive compound with the chromatographic properties of pyrophosphate. Nucleotides used as standards were from ICN, Sigma, Calbiochem, and P-L Biochemicals. Tetracycline was from Calbiochem.

Bacterial growth and radioactive labeling.

Myxobacterial growth was monitored using a Klett-Summerson photoelectric colorimeter equipped with a red filter. One Klett unit corresponds to approximately 5×10^6 cells.

To label cells with ^{32}P, $^{32}PO_4$ was added at 50 to 200 μCi/ml final concentration to cultures growing exponentially at 33°C in 0.5% or 1% Casitone medium at least 2 h before sampling to ensure full labeling of nucleotide pools (C. Manoil, Ph.D. thesis, Stanford University, Stanford, Calif., 1978). The inorganic phosphate concentrations of different media were assayed by the colorimetric method of Ames and Dubin (1) and used to determine the $^{32}PO_4$ specific activity of each culture labelled. Casitone medium (0.5%) contains about 1 mM inorganic phosphate by this test, although there is some variation between batches of medium.

To impose carbon and nitrogen source starvation in liquid, $^{32}PO_4$-labeled exponentially growing cultures were centrifuged for 5 min at 6,500 × *g* at 4°C and suspended directly in an equal volume of either TPM buffer containing $^{32}PO_4$ at the same specific activity as the original culture, or TM8 buffer without additional $^{32}PO_4$. Cells were incubated with shaking at 33°C in these buffers, and the initial cell concentrations were determined by counting in a Petroff-Hausser chamber to correct for loss during centrifugation.

To impose carbon and nitrogen source starvation on a surface, $^{32}PO_4$-labeled DK501 cultures growing exponentially in 0.5% Casitone were centrifuged for 5 min at 6,500 × *g* at 4°C and the pellet was suspended in 1/25 volume of the 0.5% Casitone supernatant, at about 8×10^9 cells per ml. Ten-microliter droplets of the concentrated cell suspension were spotted onto nitrocellulose filters (Millipore, 0.45-μm pore size) on 30-ml TM8 agarose plates, which had been dried overnight at ambient temperature. Spots dried in approximately 15 min and were incubated at 33°C. Under these conditions, fruiting bodies containing refractile spores were visible after 72 h of incubation.

For glycerol-induced sporulation, 0.075 volume of 10 M glycerol was added to $^{32}PO_4$-labeled, exponential 0.5% Casitone cultures to give a final glycerol concentration of 0.75 M. Under these conditions, the same timing of morphological changes as described by Dworkin and Gibson (7) was routinely observed, with most cell shortening having occurred by 60 min after the addition of glycerol and most spores becoming optically refractile after 4 h.

Nucleotide extraction and thin-layer chromatography. The procedures used to extract and to separate nucleotides by chromatography are basically those of Cashel (2) and Gallant et al. (9). Nucleotides were acid-extracted directly from cell cultures by adding culture samples to 1/10 volume of 10 M formic acid on ice. Nucleotides were extracted from cell spots on nitrocellulose filters by immersing the filters in 1 M formic acid on ice. Extracts were stored frozen at −20°C.

Thin-layer chromatography was performed in polyethyleneimine-impregnated cellulose sheets (20 by 20 cm; Brinkman). The polyethyleneimine sheets were washed before use according to the following regimen: 5 min in 2 M formic acid adjusted to pH 2.2 with pyridine, followed by three washes of 5 min each in distilled water (22). For chromatography, the acid extracts were thawed and centrifuged for 5 min at 12,000 × *g*, with 10 to 30 μl of the supernatant spotted. As standards, 5 to 10 μg of unlabeled nucleotides was spotted with the unknowns.

For one-dimensional chromatography, the solvent used was 1.5 M KPO_4, pH 3.5 (5). For two-dimensional chromatography, three different solvent systems were used (4, 5, 9), as follows: (1) first dimension—4 M ammonium formate–0.5 M LiCl; second dimension—1.5 M KPO_4 (pH 3.5); (2) first dimension—2 M formic acid–1.5 M LiCl; second dimension—1.5 M KPO_4 (pH 3.5); (3) first dimension—3.3 M ammonium formate–4.2% boric acid (pH 7.0); second dimension—1.5 M KPO_4 (pH 3.5). Chromatograms were developed in the first dimension until the solvent front reached the end of the sheet; then sheets were dried and washed for 15 min with occasional agitation in absolute methanol (1 gallon [ca. 3.8 liters] total per 15 sheets). Each chromatogram was dried and then developed in the second dimension, again to the end of the sheet. The chromatograms were again allowed to dry, then marked with radioactive ink for identification and orientation, wrapped in plastic, and autoradiographed using Kodak XR-5 film with Kodak X-Omatic regular or Dupont Lightning Plus intensifying screens at −70°C.

The positions of standard nucleotides were determined using short-wavelength UV light. Compounds localized by autoradiography were cut out, and their radioactivity was determined in a toluene-based scintillation fluid (15.1 g of PPO [2,5-diphenyloxazole] and 0.38 g of dimethyl POPOP [1,4-bis-(5-phenyloxazolyl)benzene] per gallon) mixed 3:1 with Triton X-100. Areas of the chromatograms not showing radioactive spots near compounds of interest were cut out, and their radioactivity was used as background. The level of background radioactivity for Gp_4, such as in the chromatograms shown in Fig. 1, was typically 5 to 20 times lower than the radioactivity in the Gp_4 spot itself. Background radioactivity was subtracted from nucleotide spot radioactivity in the quantitation of nucleotide recoveries.

Isolation of Gp_4. $^{32}PO_4$-labeled Gp_4 was isolated from *M. xanthus* cultures by the method of Pao et al. (19). Exponential cultures of DK501 in 0.5% Casitone labeled with 250 μCi of $^{32}PO_4$ per ml were starved in Tm8 buffer for 30 min at 33°C, then centrifuged for 5 min at 6,500 × *g* at 4°C. The supernatants were discarded, and pellets were suspended in 1/10 volume of 1 M HCOOH. The extracts were frozen at −20°C, then thawed and centrifuged for 5 min at 10,000 × *g* to remove fast-sedimenting material. The supernatant was applied in a line along the bottom of a polyethyleneimine thin-layer plate and developed in one dimension with 1.5 M KPO_4 (pH 3.5). Autoradiograms of the resulting chromatograms were used together with unlabeled standards to localize Gp_4, which was cut out, placed in a small column, and washed with 3 ml of absolute methanol and 3 ml of 10 mM Tris-hydrochloride (pH 7.6), then eluted with three 3-ml fractions of 2 M triethylammonium bicarbonate (pH 8.0). The material eluted was lyophylized to a powder and taken up in water. Final recovery of Gp_4 was approximately 40%, based on the amount present in the initial extract determined by two-dimensional chromatography.

RESULTS

Detection of Gp_4 and Gp_5. Gp_4 and guanosine pentaphosphate (Gp_5) (together referred to as guanosine polyphosphates) were assayed by thin-layer chromatography after acid extraction of $^{32}PO_4$-labeled myxobacterial cultures (see Materials and Methods for details). Cultures were labeled for at least 2 h during exponential growth in 0.5% Casitone. Under these conditions, exogenous $^{32}PO_4$ rapidly enters nucleotides, so that after 2 h (about 0.4 generation time), the specific radioactivities of GTP and ATP have reached steady values (Manoil, thesis). One of two different two-dimensional solvent combinations was used in most of the experiments to be described, and sample chromatograms of these are shown in Fig. 1. Both solvent systems resolved Gp_4 and Gp_5 from other nucleotides present, although system 2 (Fig. 1B) resolved a greater number of other compounds than did system 1 (Fig. 1A). For example, ATP and GDP were resolved by using system 2 but not by using system 1. In both solvent systems, 2'-deoxyribonucleotides comigrated with their ribonucleotide analogs (e.g., dATP with rATP).

Starvation for carbon and nitrogen sources. *M. xanthus* is induced to form fruiting bodies if vegetative cells are starved for both carbon and nitrogen sources on buffered agar devoid of added nutrients (16). A similar starvation was imposed in liquid shaker culture (in which fruiting does not occur) by removing vegetative cells from 0.5% Casitone and resuspending them in nonnutrient buffer. Figure 2 shows that under these conditions, there is a rapid 10- to 20-fold increase in the level of radioactive Gp_4. Because the specific activity of ^{32}P in cellular nucleotide pools was constant at the time when starvation was imposed (note that the level of radioactive GTP was nearly unchanging), the recovery of radioactive Gp_4 reflected the total level of Gp_4. The Gp_4 concentration peaked at 30 to 60 min, followed by a gradual decline. No such increase was seen if cells were suspended in growth medium, 0.5% Casitone, rather than starvation buffer (Manoil, thesis). The level of Gp_4 reached a maximum at about 20% the level of GTP. In contrast to Gp_4, the level of Gp_5 did not increase perceptibly during carbon-nitrogen starvation. This is evident in the autoradiograms of Fig. 1, where no radioactivity corresponds to the position of standard Gp_5 (marked by outline). After 60 min of starvation, greater than 85% of the Gp_4, greater than 90% of the GTP, and greater than 95% of the ATP (and GDP) sedimented with cells and are therefore presumably intracellular (Manoil, thesis). Retention of nucleotide label in cells implies that little cell lysis occurs during the first hour of starvation.

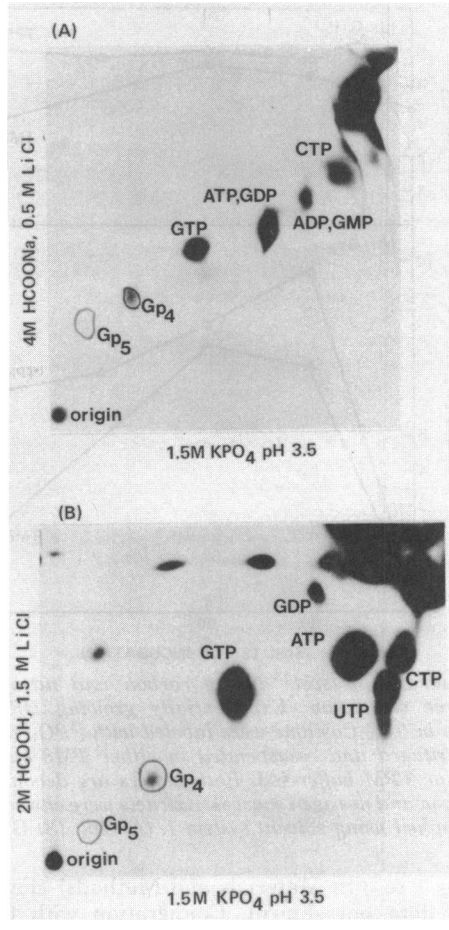

FIG. 1. *Two-dimensional thin-layer chromatography of nucleotides. Two sample chromatograms are shown illustrating the separation of nucleotides and other ^{32}P-labeled substances obtained using different solvent combinations. (A) Solvent system 1. (B) Solvent system 2. Radioactive spots have been identified from the migration of unlabeled standard nucleotides. The positions of authentic Gp_4 and Gp_5 determined by their UV absorption have been drawn in outline on the autoradiograms. (A) Cells starved in TM8 buffer for 30 min; (B) cells starved in TM8 buffer for 10 min.*

Structure of myxobacterial Gp_4. Additional tests were made to investigate whether the material tentatively identified as Gp_4 in Fig. 2 has the chemical properties of authentic guanosine 5'-diphosphate, 3'-diphosphate (represented as pp5'G3'pp or Gp_4). The myxobacterial compound comigrated with standard Gp_4 in thin-layer chromatography using three different two-dimensional solvent systems (solvent sys-

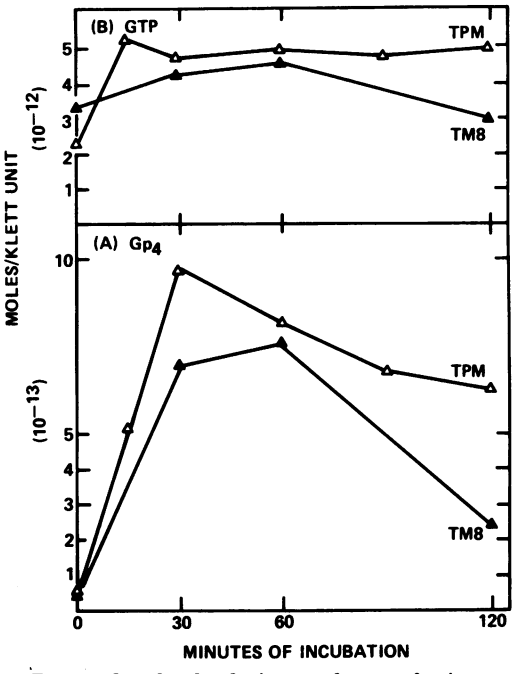

FIG. 2. *Gp$_4$ levels during carbon and nitrogen source starvation. Exponentially growing DK501 cells in 0.5% Casitone were labeled with $^{32}PO_4$, then centrifuged and resuspended in either TM8 buffer (▲) or TPM buffer (△). Both buffers are devoid of carbon and nitrogen sources. Extracts were chromatographed using solvent system 1. (A) Gp$_4$; (B) GTP.*

tems 1 to 3 in Materials and Methods) (Fig. 1 and data not shown). Comigration with Gp$_4$ standard in these solvents distinguished pp5′G3′pp from all of the common (A,G,C,U,T) nucleoside 5′-mono-, di-, and triphosphates, as well as from adenosine tetraphosphate (pp5′A3′pp), adenosine pentaphosphate (ppp5′A3′pp), and guanosine 5′-tetraphosphate (pppp5′G) (4; data not shown).

Further chemical tests were performed on the myxobacterial compound extracted by formic acid and recovered by the method of Pao et al. (19). Figure 3 shows that myxobacterial putative Gp$_4$ was hydrolyzed to compounds comigrating with the breakdown products of authentic Gp$_4$. Lane A shows that the major component of the myxobacterial Gp$_4$ preparation marked by ^{32}P label (constituting about 75% of the total radioactivity) comigrated with added marker Gp$_4$ identified by UV absorption and drawn in outline on the autoradiogram. The additional two faintly radioactive spots, one barely visible, are probably due to breakdown products previously observed (4). When this preparation was treated with alkali (lane B) or with acid at low temperature (lane C), the major radioactive spots comigrated with the marker spot, a mixture of pp5′G2′p and pp5′G3′p (4). Treatment with acid at higher temperature (lane D) released a radioactive compound comigrating with the marker breakdown products p5′G2′p and p5′G3′p. This behavior distinguishes pp5′G2′pp and pp5′G3′pp from other tetraphosphate isomers of guanosine. Taken together, these results argue that the structure of the myxococcal compound is the same as that for *Escherichia coli*, namely, pp5′G3′pp.

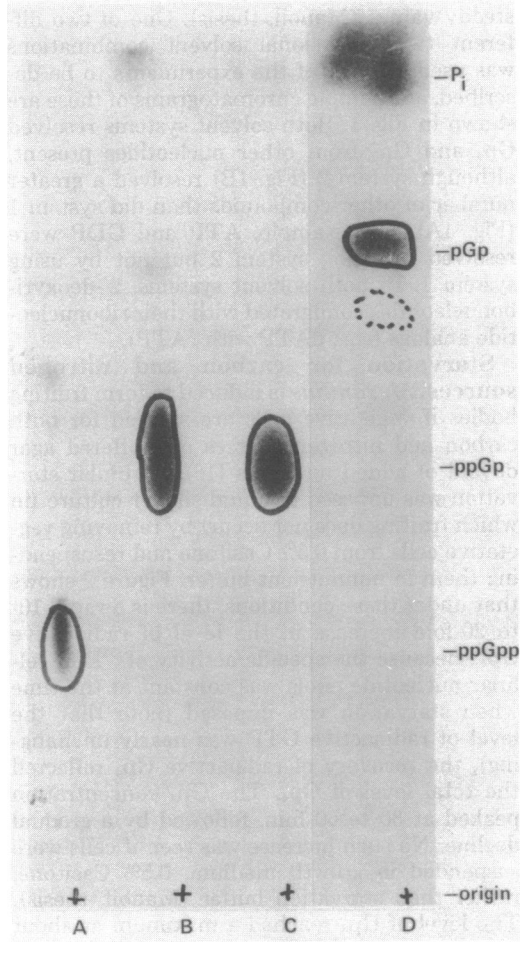

FIG. 3. *Acid and alkaline hydrolysis of myxobacterial Gp$_4$. Myxobacterial Gp$_4$ labeled with ^{32}P was isolated as described in the text and mixed with unlabeled standard Gp$_4$. (A) Untreated mixture; (B) after treatment with 0.3 M KOH for 5 h at 60°C; (C) after treatment with 1 M HCl for 30 min at 37°C; and (D) after treatment with 1 M HCl for 15 min at 100°C. Treated samples were neutralized before one-dimensional chromatography. The outline in each lane shows the position of standard nucleotide visualized by UV adsorbance, and known breakdown products (4) of the standard Gp$_4$ are identified at the right.*

Effects of antibiotics on Gp_4 production. In enteric bacteria, Gp_4 is a product of ribosomal metabolism (3). A variety of antibiotics known to act on ribosomes or on other steps in protein synthesis were examined for their effects on Gp_4 accumulation during starvation to see whether Gp_4 synthesis is likely to be a ribosomal process in *M. xanthus*. In an initial test, the molar ratios of Gp_4 to GTP after 1 h of starvation in buffer were found to be reduced at least threefold from an untreated control by growth-inhibitory levels of tetracycline, rifampin, chloramphenicol, or spectinomycin (Manoil, thesis), antibiotics which inhibit Gp_4 accumulation in *E. coli* (3). Characterization of the inhibition by tetracycline is shown in Fig. 4. Tetracycline markedly inhibited the rate and extent of Gp_4 accumulation during the period of starvation without markedly influencing the GTP level. However, there did appear to be some Gp_4 accumulation that was refractory to inhibition by tetracycline. This residual accumulation was observed even in the presence of 10-fold-higher levels of tetracycline (data not shown).

Gp_4 accumulation during starvation for carbon and nitrogen on a solid surface. Table 1 shows that when fruiting body formation was induced by starvation for carbon and nitrogen sources on a surface, cells accumulated Gp_4 before any morphological change was evident. The absolute recoveries of both Gp_4 and GTP are presented: whereas maximal Gp_4 was recovered after 1 h, GTP recovery steadily declined with increasing length of starvation. This decrease is likely to be nonspecific, perhaps due to cell lysis, since it was also observed for ATP and other nucleotides (data not shown). The ratio of Gp_4 to GTP is also presented in Table 1 to correct for the loss; the ratio was relatively constant from 1 to 13 h of starvation at 0.15 to 0.20. These values are comparable to the maximum values observed during starvation in liquid, which range from 0.12 to 0.28 (e.g., see Fig. 2 and 4). The accumulation of Gp_4 preceded aggregation, clearly visible at 48 h after spotting, and spore formation, observed 60 to 72 h after spotting.

TABLE 1. *Gp_4 levels during starvation on a surface*[a]

Cells	Gp_4[b]	GTP[b]	GP_4/GTP
Exponential growth	1.6×10^{-14}	3.9×10^{-12}	0.004
Concentrated cells	5.1×10^{-14}	4.8×10^{-12}	0.011
Time after spotting:			
15 min	5.0×10^{-14}	1.6×10^{-12}	0.031
1 h	2.6×10^{-13}	1.6×10^{-12}	0.160
3 h	1.8×10^{-13}	9.0×10^{-13}	0.200
6 h	4.4×10^{-14}	2.6×10^{-13}	0.170
13 h	4.2×10^{-14}	2.2×10^{-13}	0.190

[a] As described in the text, cultures were concentrated by centrifugation and spotted onto filters on 0.8% agarose plates containing TM8 buffer. Aggregation activity in the concentrated cell spots occurred by 48 h, and spore-containing fruiting bodies were present at 72 h.

[b] Values are in moles of nucleotide per 5×10^6 cells.

Failure of Gp_4 accumulation in a nonfruiting mutant. Of a number of nonfruiting mutants screened, one (strain DK527) was found to be deficient in Gp_4 accumulation during starvation in buffer. Figure 5 shows that under these conditions, DK527 showed a greatly reduced accumulation of Gp_4 compared to wild type. These measurements also revealed the surprising finding that Casitone cultures of the mutant showed about eight times more GTP than in wild type. ATP levels were also increased. The extra GTP and ATP did not sediment with cells, implying that they were extracellular (Manoil, thesis).

Guanosine polyphosphate accumulation during glycerol-induced sporulation. Addition of glycerol to a growing culture induces sporulation without fruiting body formation (7). Figure 6 presents guanosine nucleotide levels measured during glycerol induction of sporulation. At 90 min after glycerol addition, there were sharp parallel increases in Gp_4 and Gp_5 levels in the wild-type (DK501) culture. These higher levels were transient, decreasing to the earlier low levels by 4 h. As was previously observed (11), the GTP level in wild type showed a gradual twofold increase which preceded the Gp_4 and Gp_5 increases and did not decrease at later times. None of these changes was observed in cultures of DK510, a mutant which fails to form spores in the presence of glycerol. Dimethyl

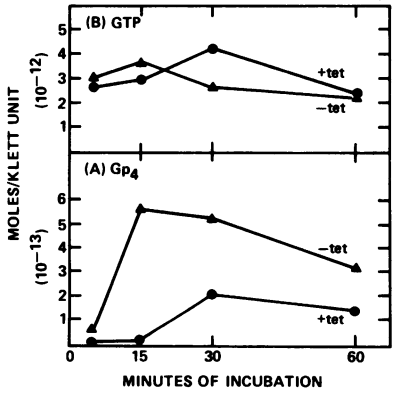

FIG. 4. *Effect of tetracycline treatment on Gp_4 accumulation. DK501 cells grown in 0.5% Casitone containing $^{32}PO_4$ were centrifuged and resuspended in the same volume of TM8 buffer with or without additional 10 μg of tetracycline (tet) per ml. Acid extracts were chromatographed using solvent system 1. (A) Gp_4; (B) GTP.*

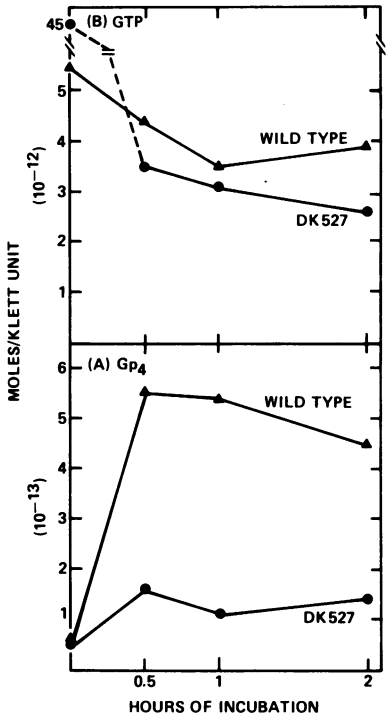

FIG. 5. *Nucleotide levels in cultures of the nonfruiting mutant DK527. Cultures were labeled with 100 μCi of $^{32}PO_4$ per ml in 1.0% Casitone before being centrifuged and resuspended in TM8 buffer. Culture extracts were chromatographed using solvent system 1. (A) Gp_4; (B) GTP. The values at zero hours of incubation are derived from extracts of culture samples taken before centrifugation, when cells were in 1% Casitone medium.*

sulfoxide-induced sporulation led to changes in GTP, Gp_4, and Gp_5 virtually identical to glycerol-induced sporulation (Manoil, thesis).

A number of compounds other than Gp_4 and Gp_5 showing low migration during chromatography were observed to accumulate steadily during glycerol-induced sporulation (Manoil, thesis). Maeba and Shipman (15) have found that many of these compounds are extracellular inorganic polyphosphates.

DISCUSSION

Experiments presented in this paper show that when cultures of *M. xanthus* are starved for carbon and nitrogen, Gp_4 accumulates. This accumulation occurs within 1 h in cultures that are starved on the surface of solid medium, much earlier than aggregation (visible at 48 h) or sporulation (observed from 60 to 72 h). A similar accumulation of Gp_4 occurs in cells starving in liquid shaker culture, where normal aggregation and fruiting are prevented (14) but in which cells are more accessible to quantitative measurements. The identity of the compound accumulating during starvation of *M. xanthus* as guanosine 5'-diphosphate, 3'-diphosphate is implied by (i) cochromatography with authentic guanosine 5'-diphosphate, 3'-diphosphate in three different two-dimensional buffer systems on polyethyleneimine thin layers, and (ii) comigration of the major acid and alkali hydrolysis products of the purified compound with those of authentic guanosine 5'-diphosphate, 3'-diphosphate.

Tetracycline treatment inhibits much of the accumulation of Gp_4 during starvation of *M. xanthus*. Since tetracycline inhibits Gp_4 synthesis in *E. coli* (3, 18), *M. xanthus* may make Gp_4 by mechanisms similar to those of *E. coli*.

If the accumulation of Gp_4 seen during starvation is required for fruiting, then among mutants defective in fruiting there might be some deficient in Gp_4 accumulation. Indeed, a screen of mutants revealed one (DK527) that is defective in Gp_4 accumulation during starvation in buffer. In addition, vegetative cultures of this mutant contain high levels of extracellular nucleotides such as GTP and ATP that are se-

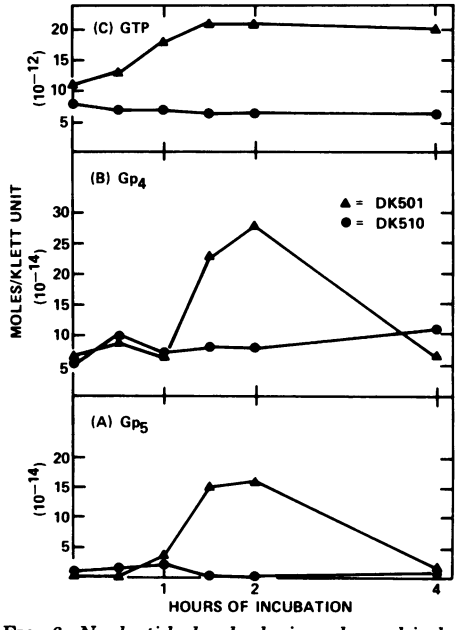

FIG. 6. *Nucleotide levels during glycerol-induced sporulation. Cultures of DK501 and DK510 were grown in 0.5% Casitone and labeled with $^{32}PO_4$ (150 μCi/ml) before glycerol was added to each. Extracts were analyzed using solvent system 1 (DK510) or both solvent systems 1 and 2 (DK501; values plotted are mean determinations from the two separations). (A) Gp_5; (B) Gp_4; (C) GTP.*

creted or leaked out of the cells. However, the detailed relationship between the increased level of extracellular nucleotide and the inability to accumulate Gp_4 has not been investigated, so consequently it is not yet established that the defect in Gp_4 accumulation is responsible for this mutant's inability to fruit.

The pattern of guanosine polyphosphate accumulation during glycerol-induced sporulation differs from that observed during starvation. At 90 min through glycerol sporulation, after cells have shortened but before they have become optically refractile, there are parallel increases in the levels of both Gp_4 and Gp_5. This increase is short-lived and disappears by 4 h. The same pattern of nucleotide synthesis was observed during sporulation induced by dimethyl sulfoxide (Manoil, thesis), showing that metabolism of glycerol does not affect the Gp_4 and Gp_5 levels. Furthermore, a mutant (DK510) unable to form glycerol-induced spores does not show any of the nucleotide level changes, implying that they are associated with the developmental process and not an independent response to high levels of glycerol.

The relatively late appearance of increased levels of guanosine polyphosphates during glycerol sporulation argues against a direct role for them in inducing the change from a vegetative rod to an immature spore. In fact, it is possible that the formation of an immature spore with the attendant surface changes may itself decrease the permeability of the developing cell to exogenous nutrients, thereby starving the cell. This starvation, rather than glycerol addition itself, may cause guanosine polyphosphate accumulation. Guanosine polyphosphate accumulation may nevertheless function in the normal maturation of the immature spore, since the spore begins to acquire refractility and full resistance properties in the interval when the guanosine polyphosphates have achieved their highest levels (14).

The transient elevation of Gp_4 and Gp_5 during glycerol-induced sporulation coincides with a decrease in the rate of stable RNA synthesis (8, 21). A decrease in the rate of synthesis of 16 and 23S (ribosomal) RNA has been observed under fruiting starvation conditions in which the level of guanosine polyphosphate is expected to increase (Smith and Dworkin, submitted for publication). These correlations suggest that in *M. xanthus*, as in other bacteria, guanosine polyphosphate accumulation inhibits rRNA synthesis.

The considerable delay before guanosine polyphosphate levels rise distinguishes glycerol-induced sporulation from fruiting body formation, in which guanosine polyphosphate levels rise immediately (compare Fig. 6 with Fig. 2 and Table 1). This argues that the immediate effect of glycerol on cells is not to starve them, but rather to "short-circuit" the developmental pathway to turn on sporulation directly without the normal preliminaries of starvation and aggregation (14).

The accumulation of guanosine polyphosphate during fruiting body development within 1 h of starvation, before any morphological or biochemical changes are observed, suggests that guanosine polyphosphate may play the role of initiator of fruiting body formation. It is attractive to think that the capacity to fruit evolved from a previously existing physiological response to starvation mediated by guanosine polyphosphate. The hypothesis that fruiting is initiated by guanosine polyphosphate is tested by the experiments presented in the following paper (16).

ACKNOWLEDGMENTS

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