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Myxospore Coat Synthesis in *Myxococcus xanthus*: In Vivo Incorporation of Acetate and Glycine

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Myxospore coat synthesis in *Myxococcus xanthus* was studied by incorporation of [^{14}C]acetate into intermediates in the biosynthesis of coat polysaccharide and into acid-insoluble material during vegetative growth and after glycerol induction of myxospores. During short labeling periods at 27°C, the radioactivity was shown to be located primarily in *N*-acetyl groups rather than sugar moieties. Two hours after glycerol induction, the pools of *N*-acetylglucosamine 6-phosphate and uridine 5'-diphosphate-*N*-acetylgalactosamine (UDPGalNAc) plus uridine 5'-diphosphate-*N*-glucosamine increased about twofold and were labeled at twice the rate measured for vegetative cells. The increased rate of synthesis of UDPGalNAc and its precursors could be correlated with increased enzyme activities measured in vitro. Controlled acid hydrolysis revealed that the galactosamine portion of the myxospore coat was *N*-acetylated. After glycerol induction, the incorporation of acetate into acid-insoluble material increased threefold. This enhanced incorporation was sensitive to neither penicillin nor D-cycloserine. In contrast, bacitracin inhibited the incorporation of [^{14}C]acetate into acid-insoluble material more effectively 2 h after myxospore induction than during vegetative growth. Chloramphenicol added to cells 90 min after induction blocked further increase in the rate of [^{14}C]acetate incorporation. Since the myxospore coat contains glycine, polymer synthesis was also measured by chloramphenicol-insensitive [^{14}C]glycine incorporation into acid-insoluble material. Although protein synthesis decreased after glycerol induction, glycine incorporation increased. Two hours after induction, glycine incorporation was only 75% inhibited by chloramphenicol and rifampin. The chloramphenicol-insensitive rate of incorporation of [^{14}C]glycine increased during the first hour after myxospore induction and reached a peak rate after 2 to 3 h. The chloramphenicol-resistant incorporation of [^{14}C]glycine was resistant to penicillin but sensitive to bacitracin.

Myxospores of *Myxococcus xanthus* produced by glycerol induction (4) contain a thick electron-dense outer coat (2, 12, 25). This coat, which is unique to the myxospore, is synthesized from about 1.3 to 5 h after induction (12). White et al. (12, 27) demonstrated that purified myxospore coats are composed of 75% carbohydrate, 13 to 14% protein, and 7 to 8% glycine. After acid hydrolysis, carbohydrate compounds were identified as galactosamine (two-thirds) and glucose (one-third). The large amount of glycine present relative to protein suggests that not all the glycine was derived from protein.

Coincident with the appearance of the myxospore coat are greatly increased activities of glyoxylate cycle enzymes (16, 17) and the seven enzymes required to synthesize uridine 5'-diphosphate (UDP)-*N*-acetylgalactosamine (UDPGalNAc) from fructose 1,6-diphosphate (5). Activities of these enzymes in partially purified cell-free extracts increased 4.5- to 7.5-fold during the 15- to 120-min interval after induction with 0.5 M glycerol. The present paper provides data on in vivo kinetics of incorporation of [^{14}C]acetate and [^{14}C]glycine into the myxospore coat and its precursors during vegetative growth and myxospore formation. Increased enzyme levels (1, 6, 7, 19) as well as variation in precursor pool concentration (11, 30-32) have both been suggested as critical variables in cellular differentiation.

(This study is part of the thesis to be presented by D. F. to Tel Aviv University in partial fulfillment of the requirements for the Ph.D. degree.)

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MATERIALS AND METHODS

Organism and cultivation. *M. xanthus* ER-1, previously called *M. xanthus* FBmp (29), was used for all experiments. Unless otherwise stated, cells were grown at 32°C on 1% Casitone medium (Difco) (20) with vigorous gyratory shaking.

Myxospore induction. Eight-hundred-milliliter cultures in 4-liter Erlenmeyer flasks were harvested by centrifugation at 12,000 × *g* for 15 min at 4°C when the optical density at 560 nm reached 0.5 to 0.7. The cells were resuspended in 50 ml of induction medium (1% Casitone medium containing 0.5 M glycerol) in a 250-ml graduated cylinder (20) and incubated at 32°C with forced aeration.

Incorporation of sodium [^{14}C]acetate into intermediates in the biosynthesis of the myxospore coat polysaccharide. Cultures (1.0 ml) were incubated with 0.1 ml of sodium [^{14}C]acetate (250 μCi/ml) at 27°C. At timed intervals, 0.1 ml of the labeled culture was transferred to 0.4 ml of cold ethanol to stop the reaction and extract the products. After standing for 1 h at 4°C, the samples were centrifuged at 10,000 × *g* for 10 min. The resulting supernatant fractions were dried in vacuo at room temperature over NaOH. The dried material was dissolved in 0.1 ml of water, and 0.02 ml was applied to Whatman no. 1 paper for descending paper chromatography. After development, radioactive regions of the chromatogram corresponding to the known mobilities of standard compounds were cut out and placed into vials containing 3 ml of scintillation liquid [5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-5-(phenyloxazolyl)benzene per liter of toluene] and counted in a Packard Tri-Carb liquid scintillation spectrophotometer. Parallel samples were rechromatographed in a second solvent system to ensure that the eluted sample did not contain interfering radioactive impurities.

Incorporation of labeled acetate, valine, and glycine into acid-insoluble material. Cultures (1.0 ml) of vegetative cells in growth medium and developing myxospores in induction medium were incubated at 27°C with either 0.05 ml of sodium [^{14}C]acetate (250 μCi/ml), 0.02 ml of [^{14}C]valine (50 μCi/ml), or 0.02 ml of [^{14}C]glycine (50 μCi/ml). At fixed time intervals, 0.05-ml samples were transferred to Whatman 3MM filter pads and immediately placed in 200 ml of cold trichloroacetic acid. After standing for 45 min at 4°C, the pads were washed sequentially three times with 200 ml of 5% cold trichloroacetic acid, ethanol, ethanol-ether (1:1, vol/vol), and ether. After air drying, the pads were transferred to vials containing 3 ml of scintillation fluid and radioactivity was determined.

Paper chromatography. The following solvents were used for descending paper chromatography on Whatman no. 1 filter paper: (i) isobutyric acid–1 M NH_4OH–0.1 M ethylenediaminetetraacetic acid, pH 7.2 (10:6:0.16, vol/vol/vol); (ii) 95% ethanol–1 M ammonium acetate, pH 7.5 (15:6, vol/vol); (iii) *n*-butanol–ethanol–water (13:8:4, vol/vol/vol); (iv) *n*-butanol–pyridine–water–acetic acid (60:40:30:3, vol/vol/vol/vol); and (v) *n*-butanol–acetic acid–water (4:1:5, vol/vol/vol).

Solvents (i), (ii), and (iii) were used to determine phosphate sugars and nucleotides. Solvents (iv) and (v) were used to determine sugars and amino acids. After developing and air drying, nucleotides were detected with an ultraviolet lamp, reducing sugars by the silver nitrate procedure (8), and amino acids with ninhydrin reagent (15).

Analytical procedures. *N*-acetyl amino sugars were estimated by the Morgan-Elson reaction (9, 13). Amino sugars were determined by a modification of the Morgan-Elson method (13). Glucose and galactose derivatives were estimated with the Glucostat and Galactostat reagents, respectively, purchased from Worthington Biochemical Corp.

Chemicals. Amino acids were products of Calbiochem, Los Angeles, Calif. Sugars were products of Sigma Chemical Co., St. Louis, Mo. Chloramphenicol was purchased from Serva Feinbiochemica, Heidelberg, Germany, penicillin G from Teva Ltd., Jerusalem, Israel, and bacitracin and rifampin from Sigma Chemical Co.

Radioisotopes. [*U*-^{14}C]glycine (114 mCi/mmol) and [*U*-^{14}C]valine (280 mCi/mmol) were products of Amersham Nuclear Corp. Sodium [1-^{14}C]acetate (60.4 mCi/mmol) was obtained from Nuclear Research Center, Negev, Israel.

RESULTS

Determination of the extent of *N*-acetylation of the *M. xanthus* myxospore coat. Preparations of *M. xanthus* myxospore coats contain more than 50% of their dry weight as galactosamine (12, 27). At least 85% of the amino sugar was found to be *N*-acetylated (Fig. 1). The maximum amount of free *N*-acetylgalactosamine was obtained after 3 h in 0.05 N HCl at 105°C.

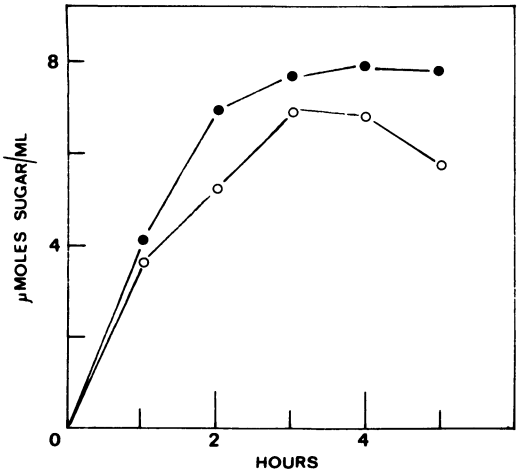

FIG. 1. *Determination of the extent of N-acetylation of the M. xanthus myxospore coat. Myxospore coats (0.4 mg/ml) prepared according to Kottel et al. (12) were hydrolyzed at 105°C in 3 N (●) and 0.05 N (○) HCl and analyzed for amino sugars and N-acetyl amino sugars, respectively.*

Hydrolysis for longer times or in 3 N HCl caused a decrease in the *N*-acetyl amino sugar.

Incorporation of [^{14}C]acetate into intermediates in the biosynthesis of the myxospore coat polysaccharide. Incorporation of radioactive acetate into *N*-acetylglucosamine 6-phosphate (GlcNAc6P) and into UDP-*N*-acetylglucosamine (UDPGlcNAc) plus UDPGalNAc is presented in Fig. 2 and 3. After 2 h of glycerol induction, there was an increase of approximately twofold in the rate of incorporation of labeled acetate into these intermediates relative to vegetative controls. During the first 5 min of labeling, the acetate was incorporated primarily into the *N*-acetyl groups. This was demonstrated by eluting the labeled intermediates from paper chromatograms, hydrolyzing in 3 N HCl at 105°C for 3 h, and determining the distribution of radioactivity in acetate and hexosamines after paper chromatography in solvent (iv). With longer periods of labeling, radioactive acetate entered the sugar moiety. By addition of unlabeled acetate, incorporation studies were performed also with one-half and one-tenth the specific activity of [^{14}C]acetate described in Fig. 2 through 6. The results of these experiments indicate that (i) incorporation is proportional to the specific activity of exogenous [^{14}C]acetate and (ii) the endogenous pool of acetate does not vary significantly upon addition of exogenous acetate. In addition to the enhanced rate of labeling of GlcNAc6P and UDPGlcNAc plus UDPGalNAc after glycerol induction, the size of each of these pools (estimated after 20 to 30 min of labeling) was about twice that of the vegetative cells. It should be pointed out that cell division ceases upon glycerol induction. In these experiments, UDPGlcNAc plus UDPGalNAc was determined because of the technical difficulty in separating these two compounds.

Effect of antibiotics on the incorporation of [^{14}C]acetate into acid-insoluble material during vegetative growth and after 2 h of glycerol induction. After 2 h of glycerol induction, the rate of [^{14}C]acetate incorporation into acid-insoluble material increased about threefold relative to a vegetative control (Fig. 4 and 5). Controlled acid hydrolysis and paper chromatography of the acid-insoluble material revealed that after 10 min of labeling the specific activity of the acetyl groups was seven times greater than that of the sugar moiety. Preincubation for 50 min with penicillin strongly inhibited acetate incorporation into vegetative-cell polymers, but had almost no effect on the incorporation into acid-insoluble material of induced cells (Fig. 4). Similar data were obtained using 50 μg of D-cycloserine per ml in place of penicillin. Preincubation with 500 μg of bacitracin per ml, on the other hand, caused a greater inhibition in incorporation of induced cells than in the vegetative control (Fig. 5). In all of these experiments, preincubation with the antibiotics did

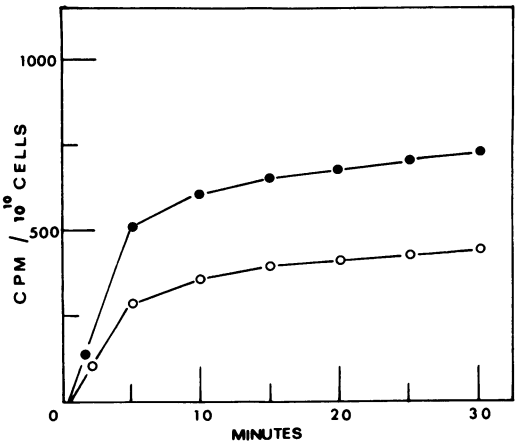

FIG. 2. *Incorporation of [^{14}C]acetate into GlcNAc6P during vegetative growth (○) and after 2 h of glycerol induction of myxospores (●). Cultures (1.0 ml) were incubated with 0.1 ml of [^{14}C]sodium acetate at 27°C. At timed intervals, 0.1 ml of the labeled culture was transferred to 0.4 ml of cold ethanol to stop the reaction and extract the products. The amount of labeled GlcNAc6P was determined after chromatography in solvent (i).*

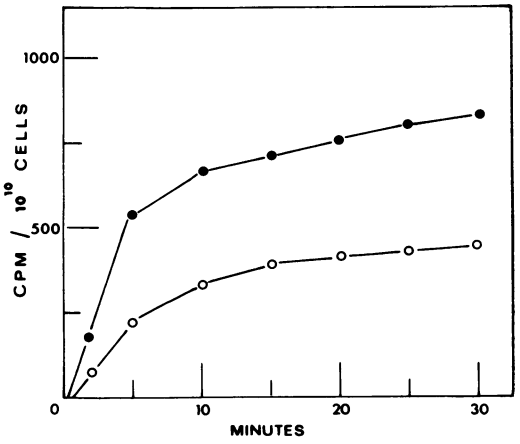

FIG. 3. *Incorporation of [^{14}C]acetate into UDPGalNAc plus UDPGlcNAc during vegetative growth (○) and after 2 h of glycerol induction (●). Cultures (1.0 ml) were incubated with 0.1 ml of [^{14}C]sodium acetate at 27°C. At timed intervals, 0.1 ml of the labeled culture was transferred to 0.4 ml of cold ethanol. The amount of labeled UDPGalNAc plus UDPGlcNAc was determined after chromatography in solvent (i).*

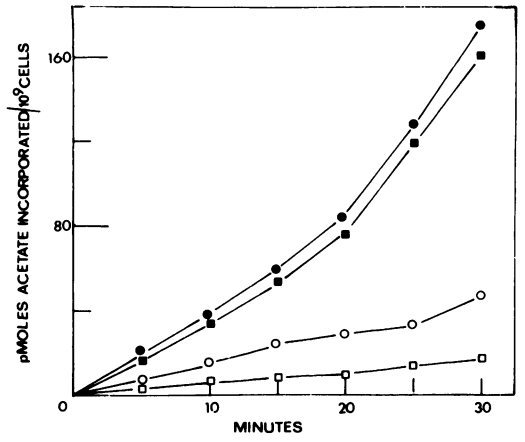

FIG. 4. *Effect of penicillin on the incorporation of [^{14}C]acetate into acid-insoluble material during vegetative growth and after 2 h of myxospore induction with glycerol. To measure the effect of the drug, cultures (1.0 ml) of vegetative and 80-min-induced cells were preincubated for 40 min in the presence of 50 μg of penicillin per ml. The drug-treated and control cultures were labeled with 0.05 ml of [^{14}C]sodium acetate at 27°C. Incorporation of [^{14}C]acetate into acid-insoluble material was determined as described in Materials and Methods. Symbols: (●) 2-h-induced culture; (■) 2-h-induced culture + 50 μg of penicillin per ml; (○) vegetative culture; (□) vegetative culture + 50 μg of penicillin per ml.*

not affect the morphology (as determined by phase microscopy) of the induced culture. Approximately 10% of the vegetative cells formed spheroplasts after 1 h in the presence of the drugs. Relatively high concentrations of antibiotics were needed in these experiments because of high cell density, 3×10^9 cells/ml.

Figure 6 summarizes the rate of [^{14}C]acetate incorporation as a function of the time after glycerol induction. After a lag of about 30 min, the rate of incorporation steadily increased for 2 h. When chloramphenicol was added to a 90-min-induced culture, the increase was blocked, indicating a need for continued protein synthesis. These events closely correlate with the increased activities of the glyoxylate cycle enzymes (16).

Effect of chloramphenicol on the incorporation of [^{14}C]glycine into acid-insoluble material during vegetative growth and after 2 h of glycerol induction. Preparations of *M. xanthus* myxospore coats contain more than 7% of their dry weight as the amino acid glycine (12, 27). Cells after 2 h of glycerol induction incorporated glycine slightly faster than did a vegetative control (Fig. 7). Chloramphenicol completely inhibited the incorporation of glycine into vegetative cells, but only inhibited 2-h-induced myxospores by 75%. On the other hand, incorporation of valine (not present in the myxospore coat) was completely inhibited by chloramphenicol during both vegetative

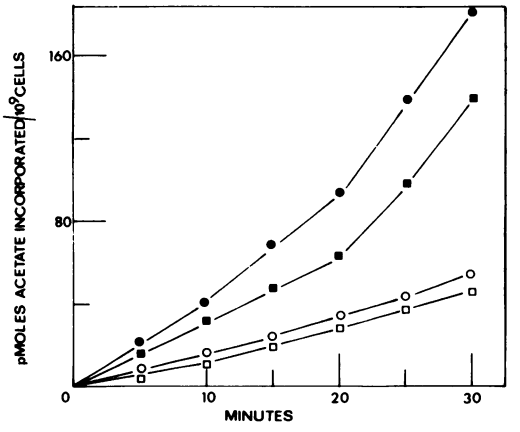

FIG. 5. *Effect of bacitracin on the incorporation of [^{14}C]acetate into acid-insoluble material during vegetative growth and after 2 h of myxospore induction with glycerol. The experiment was performed exactly as described in Fig. 4 except that 50 μg of bacitracin per ml was used instead of penicillin. Symbols: (●) 2-h-induced culture; (■) 2-h-induced culture + 500 μg of bacitracin per ml; (○) vegetative culture; (□) vegetative culture + 500 μg of bacitracin per ml.*

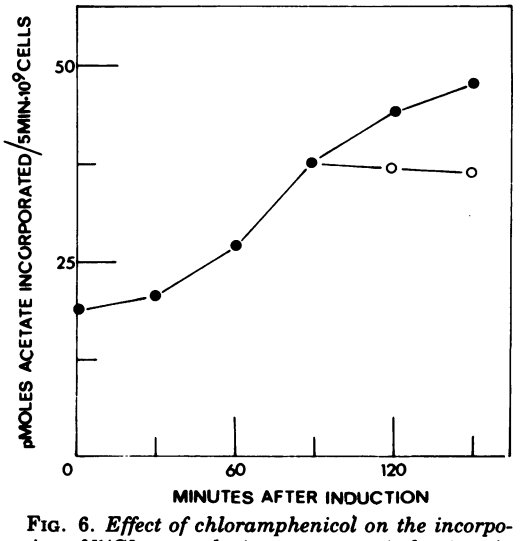

FIG. 6. *Effect of chloramphenicol on the incorporation of [^{14}C]acetate during myxospore induction. At 30-min intervals after glycerol induction, 0.1-ml samples were labeled for 5 min with 0.01 ml of [^{14}C]sodium acetate and acid-insoluble radioactivity was determined (●). Ninety minutes after induction, a portion of the induced culture was transferred to 100 μg of chloramphenicol per ml (○).*

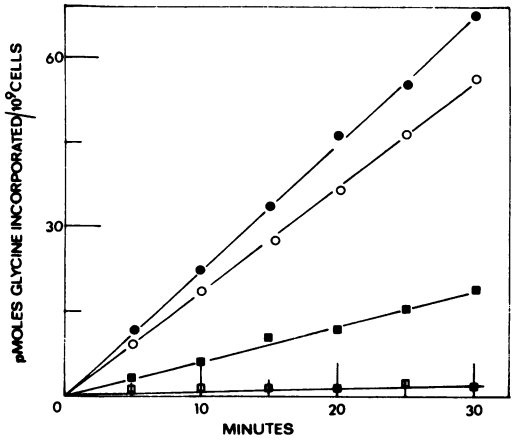

FIG. 7. *Effect of chloramphenicol on the incorporation of [^{14}C]glycine during vegetative growth and after 2 h of myxospore induction with glycerol. Cultures (1.0 ml) were incubated with 0.02 ml of [^{14}C]glycine at 27°C in the presence and absence of 200 μg of chloramphenicol per ml. At timed intervals, radioactivity was determined in acid-insoluble material. Symbols:* (○) *vegetative culture;* (●) *2-h-induced culture;* (□) *vegetative culture + 200 μg of chloramphenicol per ml;* (■) *2-h-induced culture + 200 μg of chloramphenicol per ml.*

growth and myxospore formation (Fig. 8). Similar results were obtained when rifampin was used in place of chloramphenicol.

Effect of bacitracin on the incorporation of [^{14}C]glycine into acid-insoluble material after 2 h of glycerol induction. Figure 9 summarizes the effect of bacitracin on the incorporation of radioactive glycine after myxospore induction with glycerol. Bacitracin inhibited glycine incorporation 75%, whereas valine incorporation was inhibited 10%. Penicillin at 20 μg/ml had essentially no effect on valine or glycine incorporation into 2-h-induced myxospores. Since 75% of glycine incorporation is sensitive to bacitracin whereas only 10% of valine incorporation is sensitive, one might conclude that about 65% of glycine incorporation does not represent protein synthesis and thus should be resistant to chloramphenicol and rifampin treatment. However, only 25% of glycine uptake was chloramphenicol resistant. One possible explanation for this apparent discrepancy is that incubation with chloramphenicol causes a partial inactivation of certain enzymes necessary for glycine processing into coat material.

[^{14}C]valine and chloramphenicol-insensitive [^{14}C]glycine incorporation during myxospore induction. Figure 10 compares the incorporation of [^{14}C]valine (representing general protein synthesis) with chloramphenicol-insensitive [^{14}C]glycine incorporation during myxospore induction with glycerol. Valine incorporation decreased continuously during myxospore formation, reaching 35% of the vegetative rate after 6 h of induction. The chloramphenicol-insensitive glycine incorporation reached a peak after 2 h and then slowly declined.

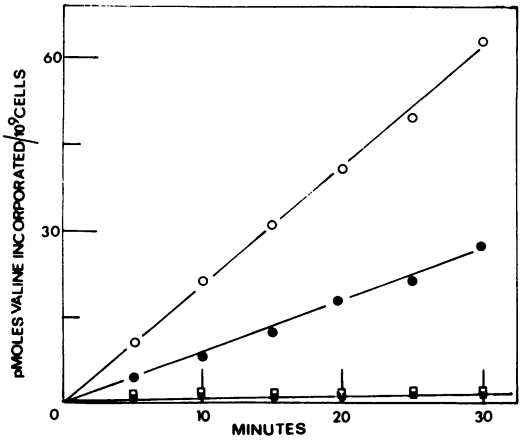

FIG. 8. *Incorporation of [^{14}C]valine into acid-insoluble material during vegetative growth and after 2 h of myxospore induction with glycerol. Cultures (1.0 ml) were incubated with 0.02 ml of [^{14}C]valine at 27°C. At timed intervals, radioactivity was determined in acid-insoluble material. Symbols:* (○) *vegetative culture;* (●) *2-h-induced culture;* (□) *vegetative culture + 200 μg of chloramphenicol per ml;* (■) *2-h-induced culture + 200 μg of chloramphenicol per ml.*

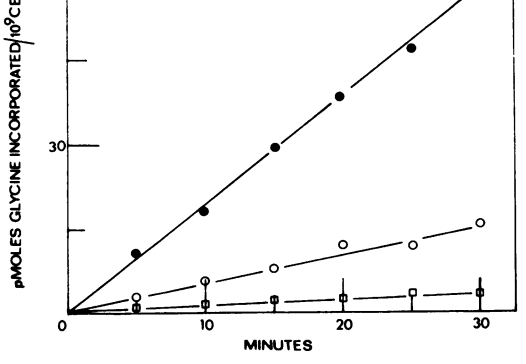

FIG. 9. *Effect of bacitracin on the incorporation of [^{14}C]glycine after 2 h of myxospore induction with glycerol. The experiment was performed exactly as described in Fig. 7 except that 500 μg of bacitracin per ml was used instead of chloramphenicol. Symbols:* (●) *2-h-induced culture;* (○) *2-h-induced culture + 500 μg of bacitracin per ml;* (□) *2-h-induced culture + 500 μg of bacitracin + 200 μg of chloramphenicol per ml.*

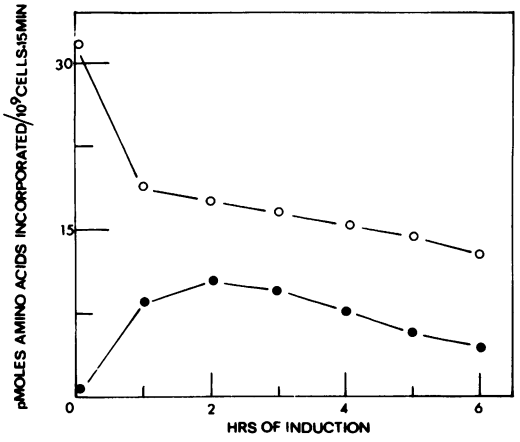

FIG. 10. *Incorporation of [^{14}C]valine and [^{14}C]glycine during myxospore induction. Parallel cultures (0.25 ml) were labeled during myxospore induction, either with 0.005 ml of [^{14}C]glycine in the presence of 200 μg of chloramphenicol per ml* (●) *or with 0.005 ml of [^{14}C]valine* (○). *Incorporation of radioactive amino acids into acid-insoluble material was determined after 15 min of incubation at 27°C.*

DISCUSSION

M. xanthus does not utilize carbohydrates effectively (3, 26) as either carbon or energy sources. To study the biosynthesis of the myxospore coat polysaccharide, we took advantage of the observation that 85% of galactosamine was *N*-acetylated (Fig. 1). Acetate was taken up rapidly by cells and converted into GlcNAc6P and UDPGal(Glc)NAc and subsequently into acid-insoluble material. At short time (5 min) and at 27°C, the radioactivity incorporated was shown to be located primarily in the *N*-acetyl group and not in the sugar moiety. Two hours after glycerol induction, the pools of GlcNAc6P and UDPGlcNAc plus UDPGalNAc were about twofold larger and were labeled at twice the rate measured for vegetative cells (Fig. 2 and 3). The increased rate of synthesis of UDPGalNAc and its precursors can be correlated with both increased pool level (measured in vivo) and increased enzyme activities (measured in vitro) (5).

Myxospore coat polymer synthesis was measured by incorporation of either [^{14}C]acetate or [^{14}C]glycine into acid-insoluble material. After glycerol induction, the incorporation of acetate into acid-insoluble material increased threefold. More interestingly, the enhanced incorporation was not sensitive to penicillin (Fig. 4) or cycloserine. After strong acid hydrolysis of the trichloroacetic acid-insoluble material, three major spots of radioactivity were identified: galactosamine, glucose, and acetate (presumably from the *N*-acetyl group of galactosamine). The acid-insoluble polymer was labeled more rapidly in the *N*-acetyl group than in the sugar moieties. Radioactive acetate enters the sugar skeleton via increased activity of the glyoxylate cycle enzymes (16).

During myxospore induction with glycerol, several changes in cell morphology and cell wall structure occur. After 30 to 40 min of glycerol induction, the cells shorten; after 80 to 90 min, spheres can be detected. Changes in cell morphology have been correlated with alterations in cell wall structure (10, 28). After 60 min of glycerol induction, there is an increase in the extent of cross-linkage in the cell wall (10), as was ascertained from an 11% decrease in free amino groups of the diaminopimelic acid and a 13% decrease in the percentage of the peptidoglycan existing as disaccharide peptide monomers (10, 28). Incorporation of acetate into acid-insoluble material was strongly inhibited by penicillin and cycloserine during vegetative growth and for the first 60 min after induction with glycerol. At the same time the cells became insensitive to penicillin and cycloserine. When these drugs were added 60 min after induction with glycerol, there was no effect on acetate incorporation (Fig. 4). After 60 min of induction there was an accumulation of the myxospore coat components glucose, galactosamine, glycine, and a specific protein (12, 27). In contrast to the other cell wall inhibitors, bacitracin inhibited the incorporation of [^{14}C]acetate into acid-insoluble material more effectively 2 h after myxospore induction than during vegetative growth (Fig. 5). This can be explained by the fact that bacitracin is not specific to cell wall biosynthesis. Processes that require a phospholipid intermediate (bactoprenol-pyrophosphate) are inhibited by bacitracin (21). For example, biosynthesis of the complex polysaccharide capsule of *Aerobacter aerogenes* requires C_{55}-polyisoprenyl pyrophosphate (22, 23). Cell-free extracts of *M. xanthus* prepared 2 h after glycerol induction incorporated glucose from UDPGlc and galactosamine from UDPGalNAc into acid-insoluble material. This incorporation was also affected by the presence of bacitracin (D. Filer, S. H. Kindler, and E. Rosenberg, unpublished data). These experiments provide circumstantial evidence for a phospholipid intermediate in the biosynthesis of *M. xanthus* coat polymer.

Chloramphenicol added to cells 90 min after induction (Fig. 6) blocked further increases in the rate of [^{14}C]acetate incorporation. Thus, there is an apparent requirement for protein synthesis for enhanced acetate incorporation into coat polymer. Part of the increase in en-

zyme activities associated with the formation of UDPGalNAc during myxospore formation (5) may be due to de novo protein synthesis, as was observed in the case of the glyoxylate cycle enzymes (16). Since we have been unable to separate the polysaccharide from the protein, it is possible that the coat contains a glycoprotein. Thus, continued protein synthesis may be necessary for acetate incorporation. Chloramphenicol added during the first 70 min after glycerol induction completely blocked myxospore development.

Myxospore coat polymer synthesis was also measured by incorporation of [^{14}C]glycine into acid-insoluble material. The rate of [^{14}C]glycine incorporation in 2-h-induced cells was higher than in vegetative controls (Fig. 7). This incorporation was only 75% inhibited by chloramphenicol and rifampin, whereas complete inhibition was observed in valine incorporation (Fig. 7 and 8). Thus, chloramphenicol-insensitive incorporation of radioactive glycine can be used to measure the synthesis of myxospore coat polymer. The chloramphenicol-insensitive rate of incorporation of [^{14}C]glycine increased during the first hour after myxospore induction and reached a peak rate after 2 to 3 h. This correlates well with coat synthesis measured directly by Kottel et al. (12). Valine incorporation (representing general protein synthesis) was chloramphenicol sensitive and decreased after induction with glycerol. The chloramphenicol-resistant incorporation of [^{14}C]glycine was sensitive to bacitracin. It should be pointed out that a certain amount of glycine incorporation into trichloroacetic acid-insoluble material could take place indirectly via the glyoxylate shunt. To minimize this nonspecific incorporation, experiments were performed at 27°C. It is interesting that glycine incorporation into the cell wall of *Staphylococcus aureus* is via a phospholipid carrier, polyisoprenyl pyrophosphate (14). In the case of *M. xanthus*, the in vitro incorporation of [^{14}C]glycine is inhibited by bacitracin, but not by chloramphenicol, rifampin, penicillin, or D-cycloserine.

M. xanthus synthesizes a cell wall antibiotic during its developmental cycle (18). The antibiotic has been purified and partially characterized (24). The *M. xanthus* antibiotic blocks cell wall synthesis in both gram-positive and gram-negative bacteria (including *M. xanthus* itself). It is presently not known whether this *M. xanthus* antibiotic is specific to cell wall synthesis (e.g., penicillin) or is general (e.g., bacitracin). Such an antibiotic could play a role in regulating the flow of nucleotide sugars into cell wall and coat polysaccharides during myxospore formation.

ACKNOWLEDGMENTS

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