CONTROL BY PHOSPHATE OF CANDICIDIN PRODUCTION

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SUMMARY: Phosphate concentration was found to control the biosynthesis of the antibiotic candicidin by resting cells of *Streptomyces griseus*. Phosphate concentrations above 1 mM decreased the rate of incorporation of [^{14}C]propionate and [^{14}C]*p*-aminobenzoic acid into candicidin in relation to the concentration of phosphate. The inhibitory effect of phosphate on incorporation of labeled precursors into candicidin was not caused by inhibition of cellular uptake of precursors. Protein synthesis, sensitive to chloramphenicol, was not affected by phosphate levels that inhibit antibiotic synthesis. Similarly, phosphate concentrations inhibitory to antibiotic synthesis did not affect rifampin-sensitive RNA synthesis.

INTRODUCTION

Inhibition of the biosynthesis of secondary metabolites (idiolites) by inorganic phosphate has been observed in many fermentations (1,2). Some of these idiolites are produced only at concentrations of inorganic phosphate suboptimal for growth. Biosynthesis of the polyene macrolide antibiotic candicidin by *Streptomyces griseus* provides a very sensitive system for studying the phosphate effect. Candicidin is produced when net accumulation of DNA stops although cell mass continues to increase due to accumulation of reserve materials (3). Addition of phosphate to such an antibiotic-synthesizing culture results in a reversal from antibiotic production (secondary metabolism) to growth (primary metabolism). Liu *et al.* (4) found increases in respiration, glucose utilization, and dry weight following phosphate addition

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to a candicidin-synthesizing system. Transfer of young growing cells to a phosphate-free, chemically defined medium initiated antibiotic synthesis and halted growth (Martin and McDaniel, submitted for publication). We are involved in the study of the molecular mechanisms by which metabolic signals influence gene expression. This report describes the influence of phosphate on incorporation of labeled precursors into candicidin and on the synthesis of protein and RNA.

MATERIALS AND METHODS

Resting cell system. S. griseus IMRU 3570 was grown in a glucose-soy peptone medium, which supports high antibiotic production, as described previously (3). At 18 hr, when the cells started to produce antibiotic, they were collected, washed, and resuspended in a phosphate-limited defined medium, as described by Martin and McDaniel (submitted for publication). Candicidin synthesis proceeded linearly in this system without increase in dry cell weight.

Incorporation of labeled precursors into candicidin. Candicidin production and incorporation of labeled precursors, [^{14}C]propionate and [^{14}C]*p*-aminobenzoic acid (PABA), were estimated as described by Martin and McDaniel (5). Labeled candicidin was purified by thin-layer chromatography, identified by spectrodensitometry (6), visualized under UV light, scraped and counted. Cellular uptake of [^{14}C]propionate and [^{14}C]PABA was determined as previously described (5).

Protein and RNA biosynthesis. Incorporation of a ^{14}C-amino acid mixture into macromolecules insoluble in trichloroacetic acid was measured as described by Martin and McDaniel (5). Incorporation of [^{14}C]uracil was determined in 100-µl aliquots of the phosphate-limited culture. Cells were incubated during 6-min pulses with 0.5 µCi/ml of [^{14}C]uracil in 0.02 mM unlabeled uracil. Incorporation was stopped by addition to the incubation mixture of 2.5 ml ice-cold 20 mM uracil in 5 percent TCA. Counting and quenching correction were performed as described before (5).

[7-^{14}C]PABA (4.26 Ci/mol) was obtained from Mallinckrodt Nuclear. Sodium [U-^{14}C]propionate (48.7 Ci/mol) and [U-^{14}C]L-amino acid mixture (226 Ci/mol) were products of New England Nuclear. [2-^{14}C]Uracil (62 Ci/mol) was purchased from Amersham/Searle.

RESULTS

Phosphate inhibition of candicidin synthesis. Biosynthesis of candicidin by phosphate-limited cells proceeded in a linear fashion for at least 36 hr without cell growth. Addition of 5 mM phosphate at 12 hr decreased the rate of antibiotic formation (Fig. 1). The decrease in rate of antibiotic formation was

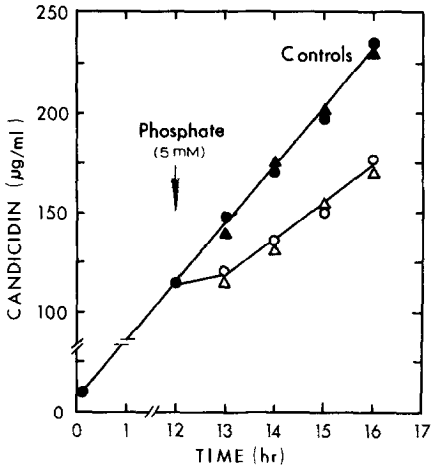

Fig. 1. Phosphate inhibition of candicidin synthesis. At 12 hr, phosphate (5 mM) was added to candicidin-synthesizing cells. Controls (▲) and (●); with 5 mM phosphate (Δ) and (O).

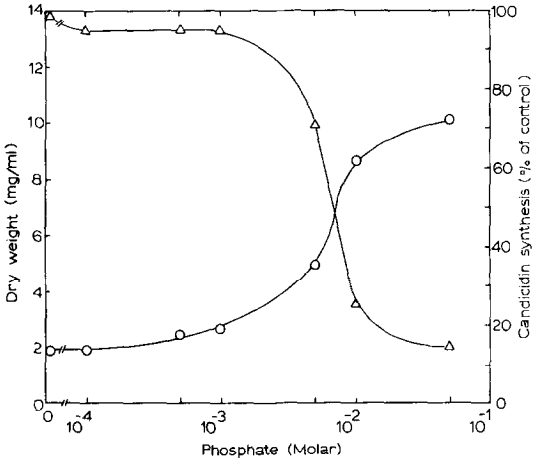

Fig. 2. Effect of different phosphate concentrations on candicidin synthesis and dry cell weight increase. Phosphate was added to the resting cell system at the final concentrations indicated. Dry cell weight (O) and percentage of inhibition of total candicidin synthesis (Δ) were determined after 23 hr of incubation.

proportional to the concentration of phosphate. Fig. 2 shows that phosphate was not inhibitory up to 1 mM whereas considerable inhibition was observed at 5 to 10 mM. Inhibition of antibiotic syn-

thesis by phosphate was accompanied by an increased rate of glucose utilization (not shown) and a large increase in dry cell weight. A high rate of glucose utilization and increasing dry cell weight are characteristic of the growth phase of batch cultures prior to antibiotic formation.

Phosphate inhibition of precursor incorporation into candicidin. To determine whether phosphate addition acts by degrading the preformed antibiotic or by inhibiting the biosynthesis of the antibiotic, we studied the incorporation of labeled precursors into the candicidin molecule. Addition of 5 or 10 mM phosphate drastically and immediately decreased incorporation of [^{14}C]propionate and [^{14}C]PABA into candicidin (Fig. 3).

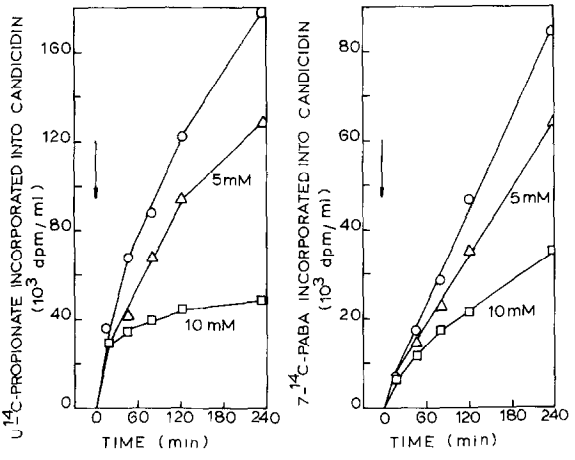

Fig. 3. Effect of phosphate on incorporation of labeled precursors into candicidin. Phosphate was added to the resting cell system at the time indicated by the arrow.

Lack of effect of phosphate on cellular uptake of precursors. Inhibition of the incorporation of labeled precursors could have been the result of decreased uptake of precursors by the cell. Our results (Fig. 4) indicate that a phosphate concentration which

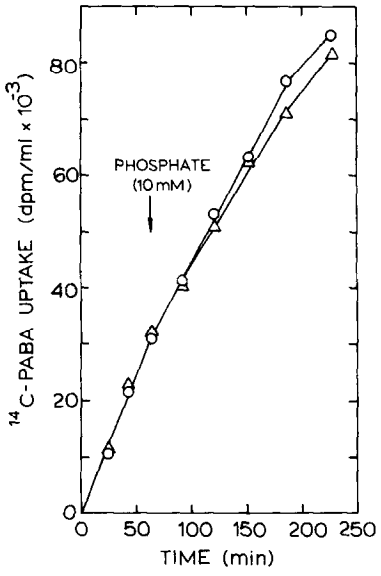

Fig. 4. Lack of phosphate effect on cellular uptake of PABA. [^{14}C]PABA was added at time zero. Phosphate (10 mM) was added at the time indicated by the arrow. Control (O); phosphate added (Δ).

inhibits antibiotic formation does not affect the cellular uptake of PABA.

Phosphate effect on macromolecular biosynthesis during antibiotic production. Phosphate-limited resting cells carry out protein turnover that is sensitive to chloramphenicol (Fig. 5a) and streptomycin (not shown). Antibiotic synthesis decreases in the presence of inhibitors of protein synthesis indicating that antibiotic synthases are continuously being replenished in phosphate-limited cultures (Martin and Demain, unpublished). However, the phosphate effect on antibiotic synthesis is not caused by an inhibition of protein synthesis. In short-term experiments, addition of phosphate concentrations which inhibit antibiotic synthesis had no effect on protein synthesis as determined by incorporation of leucine (Fig. 5a). Pulse labeling studies detected a small turnover of rifampin-sensitive RNA syn-

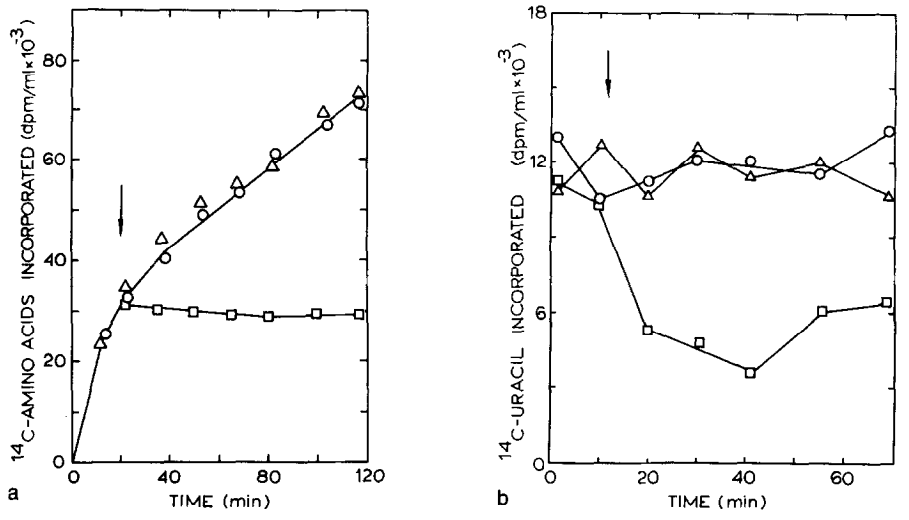

Fig. 5. a. Effect of phosphate on total protein synthesis. The ^{14}C-amino acid mixture was added at time zero and phosphate (10 mM) or chloramphenicol (100 µg/ml) was added to separate flasks at the time indicated by the arrow. Control (O); phosphate added (Δ). With chloramphenicol (□). b. Effect of phosphate on RNA synthesis by candicidin-producing cells. [^{14}C]Uracil was incorporated in 6-min pulses. Phosphate (10 mM) and rifampin (10 µg/ml) were added at the time indicated by the arrow. Control (O); with phosphate (Δ); with rifampin (□).

thesis in phosphate-limited cells (Fig. 5b). Overall, RNA synthesis is not affected by adding phosphate at a concentration which inhibits antibiotic synthesis (Fig. 5b).

DISCUSSION

Our data show that phosphate regulates the biosynthesis of candicidin. Biosynthesis of this polyene macrolide antibiotic occurs only at growth-limiting concentrations of phosphate. The molecular mechanism responsible for this phenomenon, which has been observed in many antibiotic processes, is not yet known; several mechanisms including regulation of activity of antibiotic synthases and control at the transcription level have been proposed (7,8,9).

Our results indicate that the inhibition of antibiotic accu-

mulation is caused by a decreased rate of precursor incorporation into the antibiotic rather than an inactivation of the antibiotic. Since phosphate does not alter the rate of uptake of labeled precursors into the cell, we conclude that the inhibition of precursor incorporation indicates a true inhibition of antibiotic biosynthesis. On the basis of batch fermentation data, Liu *et al.* (4) postulated that phosphate acts by reversing the cell commitment to antibiotic biosynthesis which results in a return to primary metabolism and cell growth. The results of our short-term experiments (Fig. 5a,b) do not support this theory. Protein synthesis by phosphate-limited cells was not stimulated by 10 mM phosphate for at least 100 min; by this time antibiotic synthesis was greatly inhibited (Figs. 1 and 3). RNA synthesis that remained sensitive to rifampin was likewise not stimulated by addition of 10 mM phosphate. We have noted that the intracellular content of ATP increases rapidly following phosphate addition to cells committed to antibiotic synthesis (Martin and Demain, unpublished). We conclude that phosphate control of the biosynthesis of candicidin is not mediated by an effect on growth (protein or RNA synthesis) but by the specific action of phosphate (or a related effector) on formation or action of antibiotic synthases.

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