Correlation of Carbohydrate Catabolism and Synthesis of Macromolecules During Enzyme Synthesis in *Pseudomonas fluorescens*

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ABSTRACT

KIRKLAND, JERRY J. (Oklahoma State University, Stillwater), AND NORMAN N. DURHAM. Correlation of carbohydrate catabolism and synthesis of macromolecules during enzyme synthesis in *Pseudomonas fluorescens*. J. Bacteriol. **90**: 23-28. 1965.—Glucose, ribose, and fructose shorten the lag period required for synthesis of protocatechuate oxygenase. Radioactivity from uracil-2- C^{14} is incorporated into the hot trichloroacetic acid-soluble fraction after a lag period of approximately 20 min after addition of protocatechuic acid. Addition of glucose or ribose simultaneously with the inducer shortens the lag period to approximately 5 min and increases the rate of uracil incorporation. The inducer must be present to initiate incorporation of radioactivity, and the exogenous carbon source accelerates incorporation but is not sufficient to initiate synthesis by itself. The addition of protocatechuic acid increases the rate and total incorporation of radioactivity from uniformly labeled glucose or ribose-1- C^{14} into the hot trichloroacetic acid-soluble fraction. Ribose decreases the incorporation of radioactivity from uniformly labeled glucose into the hot trichloroacetic acid-soluble fraction, and glucose shows a similar effect on incorporation of radioactivity from ribose-1- C^{14} , indicating the two sugars are serving in the same capacity to enhance enzyme synthesis. Radioactivity from glucose-1- C^{14} is not incorporated into the hot trichloroacetic acid-soluble fraction. The results suggest that glucose and ribose shorten the lag period for inducible enzyme formation by serving as a "specific" carbon source for synthesis of macromolecules such as ribonucleic acid.

The dependency of protein synthesis on ribonucleic acid (RNA) is well documented, and, since inducible enzyme formation represents *de novo* protein synthesis (Pardee, 1962), it is dependent on RNA synthesis (Gale, 1962). Pardee (1954) studied β -galactosidase formation in a uracil-requiring mutant of *Escherichia coli* and observed that enzyme formation was dependent on the presence of uracil. Ben-Ishai (1957) investigated β -galactosidase synthesis by a thymine-requiring mutant of *E. coli* and concluded that RNA synthesized simultaneously with protein is involved in protein synthesis, whereas preformed RNA does not participate in inducible enzyme formation. Studies on protein and RNA synthesis during enzyme synthesis by lactic acid bacteria suggest that RNA synthesis precedes protein synthesis (Duerre and Lichstein, 1961), and addition of purines and uracil stimulates enzyme induction.

A number of workers have investigated the effect of exogenous carbon sources, particularly glucose, on inducible enzyme formation. Durham

and McPherson (1960) and Kirkland and Durham (1965) studied the effect of glucose and other carbon sources on inducible enzyme formation by *Pseudomonas fluorescens* and noted that addition of certain carbon sources shortened the lag period required for enzyme synthesis. This communication relates the effect of exogenous carbon sources on inducible enzyme formation to RNA synthesis.

MATERIALS AND METHODS

Test organism. The organism used in this study was *Pseudomonas fluorescens* capable of producing inducible enzymes in response to a number of aromatic compounds (Durham, 1958; Kirkland and Durham, 1965).

Growth of cells. Noninduced cells were obtained by inoculating nutrient agar plates with a 16-hr nutrient agar slant culture. The plates were incubated for 13 hr at 37 C, the cells were harvested with 0.01 M potassium phosphate buffer (pH 7.0), washed twice by centrifugation, and suspended in buffer.

Radioisotope experiments. Radioisotope experi-

ments were conducted as follows: noninduced cells were suspended in 0.01 M potassium phosphate buffer (pH 7.0) and placed in a 250-ml Erlenmeyer flask. The flask was equilibrated to temperature (37 C) on a reciprocating shaker water bath. All substrates were added, and 5.0-ml samples (7.2 mg of protein) were removed at desired time intervals, placed in heavy wall Pyrex test tubes, and immediately frozen in an acetone-dry ice bath. Protein was determined by the method of Lowry et al. (1951). All samples were stored at -20 C until fractionated.

Cell fractionation procedure. The cells were fractionated by a modification of the procedure of Park and Hancock (1960). The frozen cell suspensions were thawed and centrifuged for 10 min at $10,000 \times g$ at 4 C. The supernatant solutions were discarded, and the cells were washed with 5.0 ml of cold distilled water by centrifugation. The cells were suspended in 2.5 ml of 5% cold trichloroacetic acid and allowed to stand at 4 C for 30 min. The cells were centrifuged at 4 C for 10 min at $10,000 \times g$, and the supernatant solution was poured into a liquid scintillation counting vial. The cells were washed with 2.5 ml of 5% cold trichloroacetic acid by centrifugation, and the supernatant wash fluid was added to the vial.

The cell pellet was suspended in 2.5 ml of 5% trichloroacetic acid and incubated at 90 C for 30 min. The precipitate was removed by centrifugation, and the supernatant solution was poured into another counting vial. The precipitate was washed with 5% trichloroacetic acid by centrifugation, and the wash solution was added to the supernatant solution from the hot trichloroacetic acid-soluble fraction. All supernatant solutions were dried under vacuum over anhydrous calcium chloride until approximately 0.1 ml of liquid remained.

Counting procedure. Radioactivity was determined by counting in a Packard Tri-Carb model 314-A liquid scintillation spectrometer. An amount (10 ml) of solvent consisting of 60% sulfur-free toluene, 40% absolute ethyl alcohol, and 0.5% 2,5-diphenyloxazole plus 0.2% 1,4-bis-2'(5'-phenyloxazolyl-) benzene as the phosphor was added to the counting vials. The system had a counting efficiency of approximately 40% for C^{14} .

RESULTS

Effect of glucose on incorporation of radioactivity from uracil-2- C^{14} . *P. fluorescens* synthesizes protocathechuate oxygenase (E. C. 1.99.2.3) when protocathechuate acid is present in the incubation mixture. Glucose, ribose, or fructose shortens the lag period for synthesis of this enzyme (Kirkland and Durham, 1965). The incorporation of uracil-2- C^{14} was measured in the presence and absence of glucose during induction studies to ascertain whether RNA synthesis was involved in this phenomenon. Noninduced nutrient agar-grown cells were washed and suspended (1.4 mg of

protein per ml) in 0.01 M phosphate buffer (pH 7.0) for this study.

Results indicated that very little uracil-2- C^{14} (specific activity 6.15 mc/mole) was incorporated into the cold trichloroacetic acid-soluble fraction of the following systems: protocathechuate acid, protocathechuate acid plus glucose, or the cell control with no exogenous carbon source. The presence of protocathechuate acid stimulated the incorporation of radioactivity into the hot trichloroacetic acid-soluble fraction after a lag of approximately 20 min (Fig. 1). Glucose produced a small increase in the incorporation of radioactivity, but the total incorporation was lower than with protocathechuate acid. A significant increase in incorporation of radioactivity was observed when protocathechuate acid and glucose were added simultaneously.

The cold trichloroacetic acid-soluble fraction, which contains the free, low molecular weight compounds representing the metabolic "pool" (Park and Hancock, 1960), contained very little radioactivity, whereas the hot trichloroacetic acid-soluble fraction, consisting of the high molecular weight components of the cell such as RNA, showed radioactivity, suggesting that RNA

FIG. 1. Effect of glucose on incorporation of uracil-2- C^{14} into the hot trichloroacetic acid-soluble fraction. Uracil-2- C^{14} (0.1 μ Ci/ml) plus 0.5 μ mole of unlabeled uracil per ml was added to all flasks. Symbols: $\square$, protocathechuate acid (14.0 μ moles); $\bullet$, glucose (0.45 μ mole); Δ , protocathechuate acid (14.0 μ moles) plus glucose (0.45 μ mole/ml); $\square$, uracil-2- C^{14} control. Concentrations given as μ moles per ml.

FIG. 2. Effect of ribose on incorporation of uracil-2- C^{14} into the hot trichloroacetic acid-soluble fraction. Uracil-2- C^{14} (0.1 μ c/ml) plus 0.5 μ mole of unlabeled uracil per ml was added to all flasks. Symbols: ○, protocatechuic acid (14.0 μ moles); ●, ribose (0.45 μ mole); △, protocatechuic acid (14.0 μ moles) plus ribose (0.45 μ mole); □, uracil-2- C^{14} control. Concentrations given as μ moles per ml.

synthesis was proceeding more rapidly in the system containing the inducer and glucose.

Effect of ribose on incorporation of radioactivity from uracil-2- C^{14} . Since ribose also shortens the lag period for induction when protocatechuic acid is employed as the inducer (Kirkland and Durham, 1965), the incorporation of radioactivity from uracil-2- C^{14} was studied in the presence and absence of ribose. The cold trichloroacetic acid-soluble fraction, prepared in the presence or absence of ribose, contained little radioactivity. The addition of protocatechuic acid and ribose simultaneously decreased the lag time for uracil-2- C^{14} incorporation into the hot trichloroacetic acid-soluble fraction when compared with protocatechuic acid or ribose alone (Fig. 2). This finding suggests that ribose may shorten the time required for inducible enzyme formation by enhancing RNA synthesis in the cell.

Effect of pyruvic acid on incorporation of radioactivity from uracil-2- C^{14} . Pyruvic acid was tested for its ability to increase the incorporation of radioactivity from uracil-2- C^{14} into macromolecular cell components. This compound was selected, because it does not shorten the lag period for

induction, although it is readily oxidized by washed cell suspension (Durham and McPherson, 1959). Pyruvic acid has little effect on the incorporation of radioactivity from uracil-2- C^{14} into the hot trichloroacetic acid-soluble fraction either in the presence or absence of protocatechuic acid (Fig. 3). This would indicate that the exogenous carbon sources which shorten the lag period required for induction are not serving as a source of energy or as a "nonspecific" carbon source.

Incorporation of radioactivity from uniformly labeled glucose into the hot trichloroacetic acid-soluble fraction. Nutrient agar-grown cells were incubated in the presence of 0.1 μ c/ml of uniformly labeled glucose plus 0.45 μ mole/ml of unlabeled glucose as carrier. Labeled glucose was incorporated into the hot trichloroacetic acid-soluble fraction in the absence of the inducer (Fig. 4). Addition of protocatechuic acid produced a slight stimulation in the rate of incorporation of radioactivity from uniformly labeled glucose and a significant increase in total incorporation.

To ascertain whether ribose and glucose could be serving the same purpose during induction, an

FIG. 3. Effect of pyruvic acid on uracil-2- C^{14} incorporation into the hot trichloroacetic acid-soluble fraction. Uracil-2- C^{14} (0.1 μ c/ml) plus 0.5 μ mole of unlabeled uracil per ml was added to all flasks. Symbols: ○, protocatechuic acid (14.0 μ moles); ●, pyruvic acid (0.45 μ mole); △, protocatechuic acid (14.0 μ moles) plus pyruvic acid (0.45 μ mole); □, uracil-2- C^{14} control. Concentrations given as μ moles per ml.

FIG. 4. Incorporation of uniformly labeled glucose into hot trichloroacetic acid-soluble fraction. Uniformly labeled glucose ($0.1 \mu\text{C}/\text{ml}$) plus $0.45 \mu\text{mole}$ of unlabeled glucose per ml was added to all flasks. Symbols: $\circ$, protocatechuic acid ($14.0 \mu\text{moles}$); $\blacksquare$, ribose ($0.45 \mu\text{mole}$); $\square$, protocatechuic acid ($14.0 \mu\text{moles}$) plus ribose ($0.45 \mu\text{mole}$); $\bullet$, control. Concentrations given as μmoles per ml of incorporation medium.

equimolar concentration of ribose ($0.45 \mu\text{mole}/\text{ml}$) was added to the washed-cell suspension, and incorporation of labeled glucose followed. The presence of ribose decreased incorporation of radioactivity from uniformly labeled glucose by approximately 37% in the absence of the inducer. The addition of ribose to a system containing uniformly labeled glucose and protocatechuic acid decreased incorporation approximately 42% (Fig. 4). A competition was evident between glucose and ribose, and the observation that ribose spares glucose would suggest that these two sugars serve a similar role in decreasing the lag time for enzyme induction.

When the glucose concentration was increased to $45.0 \mu\text{moles}/\text{ml}$, uniformly labeled glucose was incorporated into the hot trichloroacetic acid cell fraction at a linear rate in the absence of protocatechuic acid (Fig. 5). A significant increase in incorporation was observed when protocatechuic acid was added. Addition of an equimolar concentration of ribose ($45.0 \mu\text{moles}/\text{ml}$) to the glucose plus protocatechuic acid system decreased both the rate and total amount of

incorporated glucose (Fig. 5). Thus, protocatechuic acid increases the incorporation of radioactivity from uniformly labeled glucose into the hot trichloroacetic acid-soluble fraction and ribose competes with the incorporation.

Effect of glucose on incorporation of radioactivity from ribose-1- C^{14} . To ascertain whether glucose showed a competitive effect on the incorporation of ribose-1- C^{14} into the hot trichloroacetic acid-soluble fraction, washed-cell suspensions were incubated in the presence of $0.075 \mu\text{C}/\text{ml}$ of ribose-1- C^{14} plus $0.45 \mu\text{mole}/\text{ml}$ of carrier ribose in the presence and absence of protocatechuic acid. The addition of protocatechuic acid stimulated the rate and total incorporation of ribose (Fig. 6).

The addition of an equimolar concentration of glucose ($0.45 \mu\text{mole}/\text{ml}$) to the system containing ribose and the inducer decreased the incorporation of ribose-1- C^{14} into the hot trichloroacetic acid-soluble fraction approximately 32%. These results suggest that glucose and ribose have a similar function in shortening the lag period for inducible enzyme synthesis.

FIG. 5. Influence of ribose on incorporation of radioactivity from uniformly labeled glucose into hot trichloroacetic acid-soluble fraction during induction. Uniformly labeled glucose ($0.1 \mu\text{C}/\text{ml}$) plus $45.0 \mu\text{moles}$ unlabeled glucose per ml added to all flasks. Symbols: $\circ$, protocatechuic acid ($14.0 \mu\text{moles}$); $\blacksquare$, ribose ($45.0 \mu\text{moles}$); $\square$, protocatechuic acid ($14.0 \mu\text{moles}$) plus ribose ($45.0 \mu\text{moles}$); $\bullet$, control. Concentrations given as μmoles per ml.

FIG. 6. Influence of glucose on incorporation of radioactivity from ribose-1- C^{14} into the hot trichloroacetic acid-soluble fraction. Ribose-1- C^{14} (0.075 μ C/ml) plus 0.45 μ mole of unlabeled ribose per ml added to all flasks. Symbols: ○, protocatechuic acid (14.0 μ moles); ▲, glucose (0.45 μ mole); △, protocatechuic acid (14.0 μ moles) plus glucose (0.45 μ mole); ●, control. Concentrations given as μ moles per ml.

Incorporation of glucose-1- C^{14} . One explanation for the stimulation of inducible enzyme formation by glucose and other carbon sources (Durham and McPherson, 1959; Kirkland and Durham, 1965) could be that these sugars are converted to ribose which is then incorporated into the hot trichloroacetic acid-soluble fraction. *P. fluorescens* possesses enzymes for the pentose shunt (Burrous and Wood, 1962); therefore, the C-1 carbon of glucose should not be incorporated into the hot trichloroacetic acid-soluble fraction of the cell to any large amount.

A study was made of the incorporation of radioactivity from uniformly labeled glucose (specific activity, 3.65 mc/mmole) and glucose-1- C^{14} (specific activity, 3.55 mc/mmole). Results (Fig. 7) indicated that little radioactivity from glucose-1- C^{14} was incorporated, whereas a significant amount of radioactivity from uniformly labeled glucose was incorporated into the nucleic acid fraction. The addition of protocatechuic acid did not enhance the incorporation of radioactivity from glucose-1- C^{14} , but did enhance incorporation of radioactivity from uniformly labeled glucose. Thus, very little of the C-1 of glucose is incorporated in the hot trichloroacetic acid-soluble fraction in the presence or absence of the

FIG. 7. Incorporation of uniformly labeled glucose and glucose-1- C^{14} into the hot trichloroacetic acid-soluble fraction of *Pseudomonas fluorescens*. Glucose (0.45 μ mole) was added to all flasks as carrier. Symbols: ○, protocatechuic acid (14.0 μ moles) plus uniformly labeled glucose (0.1 μ C); □, uniformly labeled glucose (0.1 μ C); △, protocatechuic acid (14.0 μ moles) plus glucose-1- C^{14} (0.1 μ C); ▲, glucose-1- C^{14} (0.1 μ C). Concentrations given as μ moles per ml. Values corrected for differences in specific activity.

inducer. This finding supports the contention that glucose is converted to ribose during the induction phenomenon.

DISCUSSION

Glucose, ribose, and fructose, in low or high concentrations, shorten the lag period required for synthesis of protocatechuate oxygenase in *P. fluorescens* (Kirkland and Durham, 1965). It was suggested that these sugars act as "specific" carbon sources during inducible enzyme formation; for example, ribose-5-phosphate for RNA synthesis. Carbon sources such as pyruvate or succinate are oxidized, but did not shorten the lag period for induction. Ribose is not oxidized and does not support growth of this organism, whereas glucose is oxidized and does support growth (Kirkland and Durham, 1965).

Radioactivity from uracil-2- C^{14} is incorporated into the hot trichloroacetic acid-soluble fraction of noninduced cells very slowly in the absence of an exogenous carbon source. When the inducer,

protocatechuic acid, is added, uracil is incorporated after a lag period of approximately 20 min. The addition of glucose or ribose simultaneously with the inducer shortens the lag period to approximately 5 min and also increases the rate of uracil incorporation. Thus, the inducer is necessary for the initiation of RNA synthesis, and the sugar serves to accelerate the process but is not sufficient to initiate synthesis by itself. It is doubtful that the exogenous carbon sources are serving as an energy source for uracil incorporation, since pyruvate, although it is readily oxidized by this organism (Durham and McPherson, 1959), has no effect on the incorporation of radioactivity from uracil-2- C^{14} .

Radioactivity from uniformly labeled glucose or ribose-1- C^{14} is incorporated into the hot trichloroacetic acid-soluble cell fraction in the absence of the inducer. However, the presence of protocatechuic acid significantly increases the rate and total incorporation of radioactivity. This again demonstrates the requirement for the inducer. Ribose decreases the incorporation of radioactivity from uniformly labeled glucose in the presence of protocatechuic acid. Similarly, glucose decreases the incorporation of radioactivity from ribose-1- C^{14} in the presence of the inducer. Thus, glucose and ribose apparently are serving in the same capacity to decrease the lag period for enzyme synthesis.

RNA synthesis is required and precedes enzyme synthesis (Gale, 1962). Creaser (1955) observed that glucose shortened the lag period for the induced biosynthesis of β -galactosidase by *Staphylococcus aureus*, and the addition of purines and pyrimidines further shortened the lag. He suggested that glucose was serving to accelerate the synthesis of RNA for protein synthesis. Beaconsfield and Reading (1964) noted a parallel between the pentose phosphate pathway and synthesis of nucleic acids in mammalian tissues. The results of this investigation suggest that glucose and ribose shorten the lag period for inducible enzyme formation by serving as a "specific" carbon source for RNA synthesis, probably in the form of ribose-5-phosphate. This is further supported by the fact that radioactivity from glucose-1- C^{14} is not incorporated into the hot trichloroacetic acid-soluble fraction when noninduced cells are incubated in the presence of protocatechuic acid. Thus, sugars that shorten the lag period required for protein synthesis are utilized by a nonoxidative pathway, and the formation of a precursor (ribose-5-phosphate) of RNA synthesis permits more rapid protein synthesis.

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