Effects of the Calvin Cycle on Nicotinamide Adenine Dinucleotide Concentrations and Redox Balances of Xanthobacter flavus

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The levels of reduced and oxidized nicotinamide adenine dinucleotides were determined in Xanthobacter flavus during a transition from heterotrophic to autotrophic growth. Excess reducing equivalents are rapidly dissipated following induction of the Calvin cycle, indicating that the Calvin cycle serves as a sink for excess reducing equivalents. The physiological data support the conclusion previously derived from molecular studies in that expression of the Calvin cycle genes is controlled by the intracellular concentration of NADPH.

Xanthobacter flavus assimilates CO₂ via the Calvin cycle during autotrophic growth. The energy required to operate the Calvin cycle is provided by the oxidation of methanol, formate, thiosulfate, or hydrogen. In addition, heterotrophic growth is supported by a wide range of organic substrates, e.g., gluconate, thiosulfate, or hydrogen. In this case, CO₂ fixation is not necessary and supported by a wide range of organic substrates, e.g., gluconate, thiosulfate, or hydrogen. In addition, heterotrophic growth is not induced. To date, three unlinked transcription units encoding Calvin cycle enzymes have been identified in X. flavus: the cbb (14, 21) and gap-pgk (13, 17) operons and the gpi gene (16). The key enzymes of the Calvin cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and phosphoribulokinase, are encoded by the cbb operon.

The LysR-type transcriptional regulator CbbR has been identified in several chemo- and photoautotrophic bacteria (10). This protein controls expression of the cbb operon and, in X. flavus, also the gap-pgk operon (17, 22). We previously showed that purified CbbR protects nucleotides from 75 to 29 relative to the transcriptional start of the cbb operon in a DNase I footprinting assay. In addition, it was shown that purified CbbR responds to NADPH but not NADH in vitro: DNA binding of CbbR increases threefold and CbbR-induced DNA bending is relaxed by 9° in the presence of NADPH. The apparent Kᵦ[NADPH] was determined to be 75 μM; saturation occurs at approximately 200 μM (23).

The results from these in vitro experiments strongly suggest that the in vivo expression of the cbb and gap-pgk operons is mediated by CbbR in response to the intracellular concentrations of NADPH. To examine this in greater detail, the levels of reduced and oxidized nicotinamide adenine dinucleotides were determined during a transition from heterotrophic to autotrophic growth. X. flavus was grown on a mixture of gluconate (5 mM) and formate (20 mM) with pH control by automatic titration with formic acid (25% [vol/vol]) as described previously (15). RuBisCO (5), phosphoglycerate kinase (13), and NAD-dependent formate dehydrogenase (4) enzyme activities were subsequently determined in cell extracts as described previously. Protein was determined by the Bradford method, using bovine serum albumin as a standard (3). Significant decrease in redox balance reached a maximum 2 h after addition of formate to the medium, 15 to 25% of the nicotinamide adenine dinucleotide pools were in the reduced form. This percentage increased rapidly following addition of formate to culture and paralleled the increasing activity of formate dehydrogenase (Fig. 1). The rapid increase in redox balance, defined as the ratio of reduced to total nicotinamide adenine dinucleotide, is therefore most likely due to oxidation of formate and the concomitant production of NADH. Similar observations were made when the chemoautotrophic bacterium Pseudomonas oxalaticus was transferred from oxalate to formate medium (9). The redox balance reached a maximum 2 h after addition of formate to the medium and subsequently decreased rapidly, even though the activity of formate dehydrogenase and the total concentration of nicotinamide adenine dinucleotide continued to increase (Fig. 1 and 2). The sharp decrease in redox balance coincided with the appearance of RuBisCO activity and the increase in phosphoglycerate kinase activity, which is indicative of operation of the Calvin cycle. This pathway consumes 6 mol of NADH and 9 of ATP for every mole of triosephosphate produced. It is therefore likely that the high demand of autotrophic CO₂ fixation for NADH accounts for the observed decrease in redox balance. The most obvious function of the Calvin cycle is to supply the cell with a source of carbon during autotrophic growth. A second, equally important function is to decrease in redox balance. The most obvious function of the Calvin cycle is to supply the cell with a source of carbon during autotrophic growth. A second, equally important function is to act as an electron sink in order to dissipate excess reducing power (11, 20). For example, purple nonsulfur bacteria fail to grow photoheterotrophically in the absence of a functional Calvin cycle unless an alternative electron acceptor such as dimethyl sulfoxide is present (6, 19, 24). Interestingly, secondary mutants of RuBisCO-deficient Rhodobacter sphaeroides were subsequently determined in cell extracts as described previously.

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strains which had regained the ability to grow photoheterotrophically were isolated (24). These mutants induced nitrogenase to reduce protons to H₂, resulting in dissipation of excess reducing equivalents (8). Induction of the Calvin cycle in *X. flavus* resulted in a rapid decrease of the redox balance to below levels seen before the addition of formate. This suggests that CO₂ fixation via the Calvin cycle is very effective in removing excess reducing power.

The Calvin cycle was induced as the concentration of NADPH approached its maximum (16.4 nmol/g [dry weight]), 1 h following addition of formate to the culture (Fig. 1A and 2A). This corresponds to an intracellular NADPH concentration of 189 to 216 μM, assuming a cellular volume of 3.5 to 4 μl/mg of protein (1, 7, 18). NADPH at this concentration saturates CbbR in vitro, resulting in maximum DNA binding affinity and relaxed DNA bending. The NADPH concentration subsequently decreased rapidly (Fig. 1B and 2A). However, although the redox balance was reduced to below levels observed before the addition of formate, the NADPH concentration remained twofold higher, at a concentration of 81 to 93 μM. This concentration is slightly above the Kₘ[NADPH] of 75 μM. The RuBisCO activity increased only 1.3-fold during this period. The expression of the *cbb* operon therefore correspond to the degree of NADPH saturation of CbbR in vitro. This observation supports, but does not prove, our previous conclusion based on molecular studies that the intracellular NADPH concentration determines the activity of CbbR and hence expression of the *cbb* operon (23). Future research will aim to analyze the interaction between NADPH and CbbR in greater detail.

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**REFERENCES**


