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

GEERTJE VAN KEULEN, LUBBERT DIJKHUIZEN, AND WIM G. MEIJER*

Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9750 AA Haren, The Netherlands

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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 is provided by the oxidation of methanol, formate, thiosulfate, or hydrogen.

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 that the Calvin cycle genes are controlled by the intracellular concentration of NADPH.

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 concentration 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). Ribulokinase activity, which is indicative of operation of the Calvin cycle, was determined during a transition from heterotrophic to autotrophic growth, samples withdrawn from the fermenter were immediately frozen in liquid nitrogen and subsequently freeze-dried. Nicotinamide adenine dinucleotides were extracted from freeze-dried samples (12) and then quantified using a sensitive spectrophotometric cycling assay (2). Following addition of formate to the medium, the concentrations of NAD(H) and NADP(H) increased four- and twofold, respectively, over a period of 5 h (Fig. 2). Prior to 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 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.
strains which had regained the ability to grow phototrophically 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 Kp[NADPH] of 75 μM. The RuBisCO activity increased only 1.3-fold during this period. The expression levels 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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