REDOX-DEPENDENT CONTROL PHENOMENA IN PHOTOSYNTHETIC BACTERIA

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Introduction

The purple non-sulfur bacterium *Rhodospirillum rubrum* displays a remarkable metabolic versatility, which allows the cells to live under a variety of environmental conditions. When growing aerobically, ATP is synthesized by oxidative respiration, while a drop in the availability of oxygen as terminal electron acceptor leads to a dramatic change in the overall physiology and morphology. With light and under anaerobic conditions these organisms switch to a photosynthetic lifestyle where photoheterotrophic growth with various organic compounds as carbon and electron sources or photoautotrophic growth with molecular hydrogen and CO_2 takes place. However anaerobic respiration in the dark with alternative electron acceptors like DMSO or trimethylamine as well as fermentative growth is also possible. The change of respiration to photosynthesis requires the formation of an intracellular membrane system where the components of photosynthetic energy transduction are localized. The vesicular structures of these membranes are formed by invaginations of the cytoplasmic membrane.

In *R. rubrum*, the photosynthetic apparatus consists of the pigment-protein complexes, light-harvesting antenna (LH) and photochemical reaction centres (RC). Photosynthetic pigments are bacteriochlorophyll a and carotenoids such as spirilloxanthin which cause the characteristic deep red colour of anaerobic liquid cultures.

The polypeptides of LH-complexes reaction centres are encoded by the *puf-* and *puhA-* operons respectively. The nucleotide sequence of these operons have been determined for *R. rubrum* and the related species *Rhodobacter sphaeroides*, *Rhodobacter capsulata* and *Rhodopseudomonasps viridis* (Beatty, 1995).

The transduction of light energy into chemical energy comprises a cyclic transfer of electrons from the RC, via ubiquinone, the cytochrom bc_1-complex and a soluble cytochrome c_2 back to the photoxidized RC. This process results in a membrane gradient (ΔpH) and allows the synthesis of ATP by the action of the ATP-synthase complex (cyclic photophosphorylation, fig. 1).

Due to the existence of easily distinguishable different metabolic states, facultative phototrophic bacteria are suitable objects for studying differentiation processes in procaryotes. The regulatory mechanisms involved, not only have to promote the

expression of *puf* and *puhA*-genes, but also to coordinate the expression of genes for the synthesis of membranes, bacteriochlorophyll, carotenoids as well as for key metabolic pathways e.g. for CO_2-fixation. The major determinant for the synthesis of the photosynthetic apparatus is oxygen. When the oxygen tension drops below a certain threshold value, semiaerobic growth occurs, which is characterized by aerobic respiration and simultaneous formation of photosynthetic membranes even in the absence of light. Additional influences are caused by light or the composition of the growth medium (Ghosh et al., 1994). Since all these signals probably influence the cellular redox potential, it can be assumed that differentiation of photosynthetic bacteria is in fact controlled by a redox-dependent regulatory network.

In *Rb. sphaeroides* at least four major regulatory systems could be identified (Oh et al., 2000). The PrrBA two-component system, the AppA-PpsR antirepressor-repressor system, FnrL and TspO. All these components are considered to be responsible for redox-sensing. However, the nature of the actual cellular redox signal is still unclear.

Investigating redox dependent metabolism in phototrophic bacteria

In order to improve our understanding of redox-mediated effects on signal transduction and differential gene expression, we have used a combination of fermentation experiments and mathematical modelling methods. One major advantage of the selected model organism *R. rubrum* is that the redox poise of different components of the photosynthetic apparatus can be monitored *in vivo* using optical spectroscopy.

An absorption spectrum of photosynthetic membranes of *R. rubrum* is shown in fig. 1. From this spectrum various optical parameters can be derived. The absorption maxima at 880 nm, 810 nm, 750 nm, 580 nm and 378 nm represent the LH and RC-complexes respectively. Carotenoids exhibit absorption maxima at around 450 nm and the maximum at 550 nm is caused by cytochrome c_2.
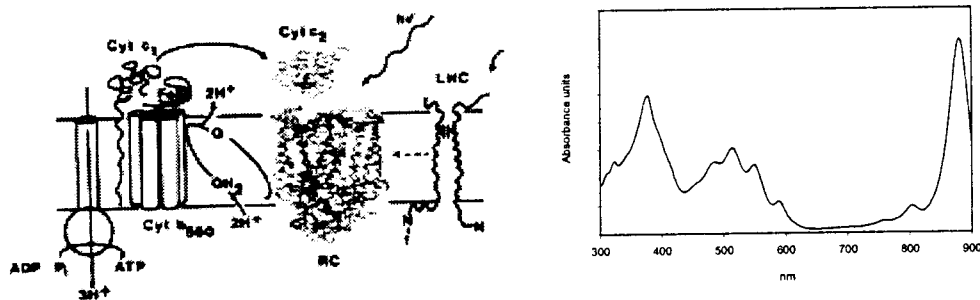

Fig. 1: Right: Structure of photosynthetic membranes. Protein components involved in cyclic photophosphorylation are shown. Left: Absorption spectrum of photosynthetic membranes of *R. rubrum*

Fermentation of *R. rubrum*

Fermentations with *R. rubrum* were carried out in the dark in stainless steel reactors. A synthetic growth medium with two carbon sources, succinate and fructose was used as this combination leads to high cell densities with a high yield of photosynthetic membranes (Ghosh et al., 1994). No diauxic growth behaviour occurs with this medium (fig. 2). During the first phase of the fermentation shown in fig. 2, the cells grew aerobically at a fixed pO_2-value of 5 %. Process control was achieved by a digital control system. Under these conditions a depigmentation of the cells took place as can be seen from the ratio of optical density at 880 nm to the optical density at 660 nm. After inoculation of the fermenter with a light grown preculture this ratio decreased from 1.1 to 0.75. After approximately 17 hours the dissolved oxygen concentration was shifted to 0.1 %, whereupon a switch to semiaerobic growth occurred. This switch was accompanied by the synthesis of photosynthetic membranes as well as by the excretion of acetate and formate into the fermentation broth.

With other nutrient combinations tested, such as succinate/acetate or succinate or fructose as sole carbon sources, the pigmentation of semiaerobic cells was significantly lower than observed above, while cell densities were comparable (data not shown).

These observations are in good agreement with the hypothesis of redox control. As fructose represents a relatively reduced carbon source, the limiting oxygen concentration under semiaerobic conditions leads to the accumulation of NAD(P)H inside the cells. The synthesis of membrane as well as the formation of extracellular products may help the cells to get rid of excessive reducing power and to achieve redox balance. After exhaustion of the growth substrates in the medium (data not shown), acetate is metabolized again and the A 880/OD 660 value slightly decreases.

Determination of intracellular flux distributions

As a prerequisite for the detailed analysis of signal transduction and regulation using mathematical modelling and simulation, the metabolic fluxes of catabolism and energy metabolism have to be identified. It might be expected that aerobic substrate degradation proceeds via different pathways compared to anaerobic or semiaerobic conditions. In particular the physiology of semiaerobic growth is still largely obscure.

Clearly redox balancing is of critical importance for the transition from chemoheterotrophic growth to phototrophic growth. As well as the formation of membranes and extracellular products, there are numerous additional pathways which can remove reducing equivalents. The reduction of CO_2 could play a major role in this context. Under photosynthetic conditions the Calvin-Benson-Bassham cycle is induced with ribulose 1,5-bisphosphate carboxylase/oxygenase as key enzyme for CO_2-fixation although there are also several alternative mechanisms for the assimilation of CO_2 in microorganisms. For example, two ferredoxin-linked enzymes of the reductive tricarboxylic acid, α-ketoglutarate synthase and pyruvate synthase as well as most of the other known CO_2-fixation pathways are present in *R. rubrum* (Joshi and Tabita, 2000). Furthermore the synthesis of intracellular storage compounds like poly-β-hydroxyalkanoates may serve as a redox sink.

We address here the question of the distribution of intracellular metabolic fluxes by the use of the mathematical method „metabolic flux analysis" (Vallino and Stephanopoulos, 1992). This procedure requires the experimental determination of substrate uptake rates, product formation rates and the composition of the biomass of steady state cultures. After the formulation of all catabolic and anabolic reactions in the cell in form of a stoichiometric network, the estimation of all unknown intracellular reaction rates is possible.

For this purpose a programming package, the „metabolic flux analyzer" was developed by the use of the MATLAB environment. The graphical representation of the catabolic network is shown in fig. 3. Possible degradation routes for fructose, the Embden-Meyerhoff-Parnass-pathway, the Entner-Doudoroff pathway and the pentosephosphate pathway are depicted. Succinate as an additional carbon source is metabolized in the tricarboxylic acid cycle by the action of succinate dehydrogenase.

The graphical interface allows the interactive input of measured or assumed reaction rates and subsequent calculation of fluxes, so that metabolic scenarios, e.g. the existence or absence of whole pathways can be simulated. As an additional application, the metabolic flux analyzer has implemented an optimization algorithm and a procedure to detect measurement errors for handling under- and overdetermined networks.

Outlook

Future work will concentrate on modelling the dynamics of the physiology of *R. rubrum* under different growth conditions. Therefore the new system- and signal-oriented modelling approach described by Kremling et al., (2000) will be applied. This approach allows the dynamical modelling and simulation of complex cellular systems on the basis of a modular structuring of metabolic networks and regulatory networks.

The characterization of intracellular redox signals during fermentation experiments can be achieved using spectroscopic techniques *in-situ*. As well as monitoring the LH- and RC-formation and the biosynthesis of carotenoids and cytochromes using absorption spectroscopy, further redox signals such as the ratios of NAD(P)/NAD(P)H or UQ/UQH2 will be determined. These data will provide a quantitative basis for the modelling process. After analysis of dark metabolism of *R. rubrum* (aerobic-, semiaerobic growth), the same techniques will be applied for investigating fermentations under photosynthetic conditions in a photobioreactor (phototrophic growth).

Furthermore, modified strains of *R. rubrum* can be constructed using recombinant DNA-technology. Comparative fermentation and quantitative survey of mutant strains deficient in redox-signalling and redox-regulation will yield valuable information for modelling global regulation in phototrophic bacteria.
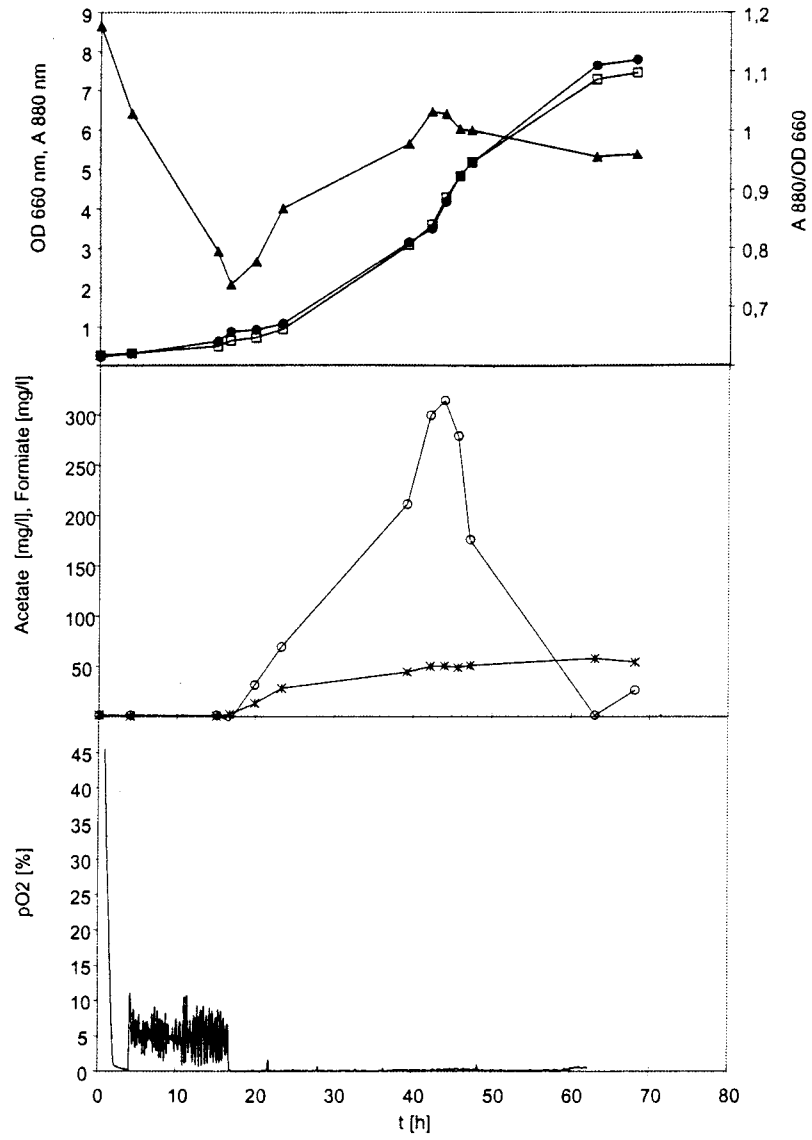
Fig. 2: Batch fermentation of *R. rubrum*. -●- OD 660 nm, -▣- A 880 nm, -▲- A 880/OD 660, -⊖- acetate, -✳- formate, - pO₂
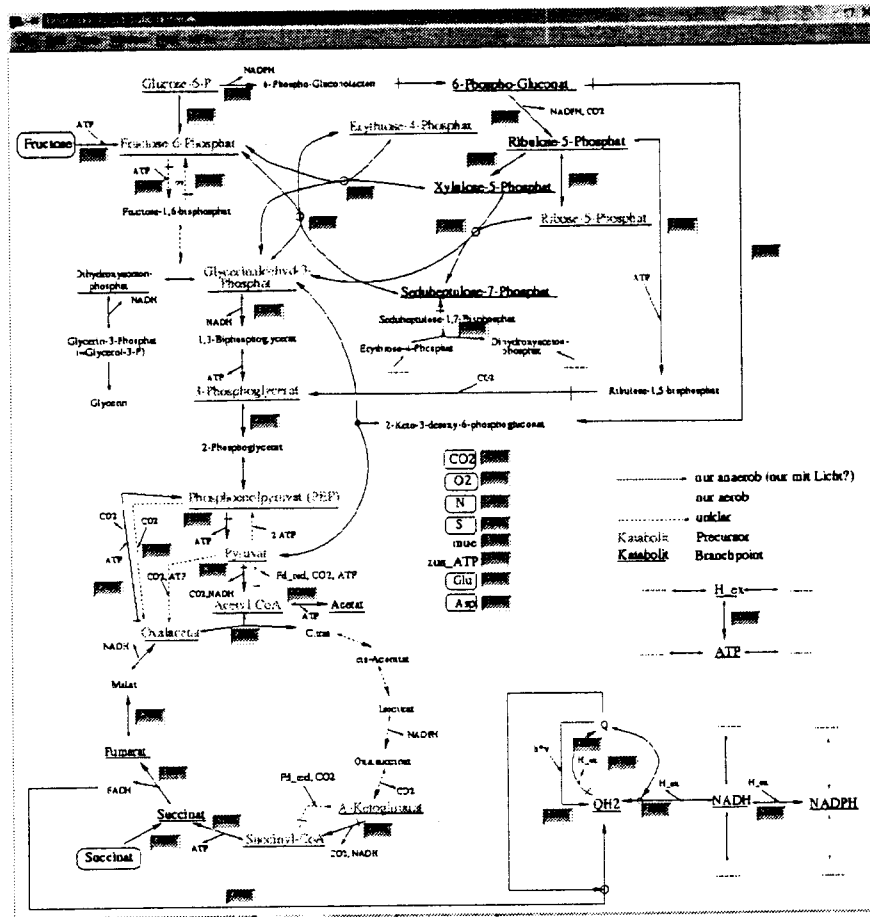
Fig. 3: Metabolic Flux Analyzer. Catabolic network of *R. rubrum*

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