Regulation of pyruvate catabolism in Escherichia coli: the role of redox environment

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Summary

Bacteria have a tremendous capacity to adapt their metabolism to an ever changing environment. To guarantee continuation of energy conservation, it is necessary to adjust catabolism rapidly to a changing environment. *Escherichia coli,* a well studied Gram negative bacterium, can invoke different catabolic modes: respiration, anaerobic respiration and fermentation. Pyruvate is the key intermediate in energy conversion, as the carbon fluxes are branched at this intermediate. In this thesis we focus on the pyruvate dehydrogenase complex (PDHc). This multi-enzyme complex catalyses under aerobic conditions the conversion of pyruvate into acetyl-CoA and NADH , and it is activated by pyruvate and is sensitive towards NADH. Its anaerobic counterpart, pyruvate formate lyase (PFL) catalyses the cleavage of pyruvate into acetyl-CoA again but without NADH generation, and is very sensitive to oxygen.

This thesis tries to elucidate the role of the redox environment at the different levels of regulation of pyruvate catabolism. Steady states have been studied, with different electron acceptors added to the medium to manipulate the external redox potential (chapter 2) or by varying the oxygen availability in the culture thus varying the external redox potential (chapter 3). These experiments were done to study the regulation at the level of enzyme synthesis as well as at the level of enzyme activity.

Studies on steady state cultures of a wild type *E. coli* as well as a PFL mutant and a PDHc mutant revealed that the PDHc can be active under anaerobic conditions when an external electron acceptor (nitrate or fumarate) is added to the medium. Moreover, the flux through the PDHc was found to be correlated to the NADH/NAD ratio of the cultures and is regulated by both gene expression and enzyme activity (chapter 2).

When *E. coli* was cultured at different dissolved oxygen tensions (DOT) again a correlation could be seen with the NADH/NAD ratio, although only at DOT <1%: the NADH/NAD ratio increases with decreasing DOT. At higher DOT values the NADH/NAD ratio is as low as in fully aerobic cultures.

Suprisingly, at low DOT values a flux was measured through the pyruvate formate lyase, although this enzyme is highly oxygen sensitive. We argue that at these DOT values the high NADH/NAD ratio blocks the pyruvate dehydrogenase complex and the intracellular oxygen concentration is sufficiently low to allow for a functional PFL (chapter 3).

To study direct effects on the kinetics of the PDHc, transient state cultures were studied during a switch from aerobic to anaerobic conditions and *vice versa*. Upon switching from aerobic to anaerobic conditions, the cells were able to activate anaerobic pathways immediately (including the oxygen sensitive PFL). PDHc activity was blocked immediately, and again the decrease in PDHc was correlated with the NADH/NAD ratio, which increased sharply during this switch. The ATP/ADP ratio dropped immediately and this caused the linking number of DNA to rise. During the reverse switch the cells were not able to immediately carry out aerobic catabolism, as was seen by excretion of pyruvate. The NADH/NAD ratio decreased more slowly than the increase during the switch from aerobic to anaerobic conditions. In addition, the ATP/ADP ratio responded in a complex manner, first increasing rapidly, and after a period of a few minutes decreasing back to the steady state level. The linking number of the DNA showed the opposite trend compared to the ATP/ADP ratio. (chapter 4).

The PDHc of *E. coli* is not active under fully anaerobic conditions, due to the enzyme being highly sensitive towards NADH. Yet, the enzyme is present under anaerobic conditions. The differences in sensitivity towards NADH of the PDHc from various bacteria explain the differences in catabolic end-products under various conditions. *Enterococcus faecalis* has, for example, a PDHc that is far less sensitive towards NADH than the PDHc of *E. coli*, and the E3 subunit (lipoamide dehydrogenase) of the enzyme complex is responsible for this sensitivity. In chapter 5 the cloning and sequencing is reported of the E3 subunit of the PDHc of *E. faecalis*. By cloning this relatively insensitive enzyme a better understanding of the mechanism of inhibition by NADH can be obtained.

The link between catabolism and the NADH/NAD ratio is an obvious one, yet not fully understood. The correlation between *in vivo* activity of the PDHc of *E. coli* and the NADH/NAD ratio is shown in chapter 2, 3 and 4. Moreover we show that the NADH/NAD ratio reflects the external redox conditions (*e.g.* anaerobic, aerobic). We have not yet elucidated the molecular mechanism of the regulation by the NADH/NAD ratio. However, in chapter 6 we provide a model as to the strategies that have evolved in *E. coli* in order to adapt effectively to various redox-related conditions and to respond adequately to changes thereof.