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

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Chapter 6

General discussion and conclusions

Reading the huge encyclopedia of *E. coli* (Neidhardt *et al.*, 1996) one is tempted to think that this bacterium has no secrets to us anymore. Yet any attempt to understand its fine details will lead to the discovery of numerous gaps in our knowledge on the one hand and the complexity of its regulatory machinery on the other. In this thesis an effort has been made to fill one of these gaps: the effect of the redox environment on the pyruvate catabolism of *E. coli*, by studying the *in vivo* flux through the pyruvate dehydrogenase complex and pyruvate formate lyase and the effect of the NADH/NAD ratio on the physiological behaviour.

In the 60's some research has been done to evaluate the values for NAD(H) in bacteria (London and Knight, 1966; Takaebe and Kitahara, 1963; Wimpenny and Firth, 1972). From these studies, it was concluded that NAD(H) plays no major role in regulation of metabolism, although Wimpenny (1972) recognised a possibly significant role, especially during transient states from anaerobic to aerobic conditions. Being such an important metabolite it is surprising so little attention has been paid to NAD(H) for many years. Only recently it has come into the picture again (Snoep, 1992).

As becomes clear throughout this thesis the NADH/NAD ratio can serve as a monitor of the redox state, in the sense of being a reflection of the external redox state. The exact mechanism remains obscure, and it is impossible to make out whether the NADH/NAD ratio is the effect or the effector, as the total makeup of the cell (enzyme concentrations and substrate/products) determines this ratio, but is also influenced by the same ratio. The link with the (redox) regulators FNR and Arc is obvious, and awaits further research, but we do know that FNR is most likely influenced by the redox potential (Unden *et al.*, 1990). Although the internal redox potential *per se*, has been proven to be an almost hypothetical parameter (Walz, 1979), it remains intriguing how the external redox potential influences the internal redox state.

The present studies prove that the NADH/NAD ratio is an important parameter under different redox conditions. And although the mechanism may be unknown, this implies that the NADH/NAD ratio can be manipulated in many different ways, as one can read in this thesis. First by changing the redox potential of the electron acceptor (chapter 2), secondly by the concentration of the electron acceptor (chapter 3). Moreover in

transient states, the NADH/NAD ratio can change rapidly (chapter 4). The question that remains, however, is whether the NADH/NAD ratio is just a reflection of the redox state of the cell, or whether it has a more physiological importance.

For alcohol dehydrogenase a direct link between expression of the gene and the NADH/NAD ratio (Leonardo *et al.*, 1996) has been found, but it can not be excluded that NADH/NAD is only a mediator and the actual gene expression is controlled by another regulation system. This effect of the NADH/NAD ratio can easily be understood physiologically, as the function of alcohol dehydrogenase in the catabolism of *E. coli* is to remove any excess of NADH.

A role for the NADH/NAD ratio in the regulation of fluxes has been pointed out before in *Clostridium acetobutylicum*; Girbal and Soucaille (1994) have shown that whether the cell follows the acetogenic or the solventogenic pathway depends on the NADH/NAD ratio: a low ratio corresponds to the acetogenic pathway and a high NADH/NAD ratio to the solventogenic pathway.

Another enzyme that is known to be dependent on the NADH/NAD ratio in the cell is the pyruvate dehydrogenase complex; for that reason special attention had been paid in this thesis to the PDHc/PFL couple, catalyzing the conversion of pyruvate into acetyl-CoA under different conditions. Although it is not easy to measure the exact flux distribution over the PDHc and the PFL, at least in wild type *E. coli*, from the studies in this thesis it is very likely that this distribution takes place in response to changes in the (redox) environment, giving *E. coli* the possibility not only to switch between these enzymes but use them both at the same time to allow pyruvate catabolism to be optimal under all conditions. This phenomenon has been seen before in *E. faecalis* (Snoep, 1992). One can only speculate about the possible reason for this phenomenon, but it implies that *E. coli* tries to maintain the flux from pyruvate to acetylCoA under all conditions and is able to have the optimal stoichiometry for ATP synthesis.

Experiments with transitions from aerobic to anaerobic conditions have proven that the pyruvate formate lyase is ready to take over the role of the pyruvate dehydrogenase complex immediately and revealed the presence of PFL in (micro)aerobic cells, again safeguarding the flux from pyruvate to acetylCoA. In short, this gives *E. coli* the potential to cope with many

natural habitats, where the environmental conditions are varying all the time. Being very oxygen sensitive PFL will be inhibited whenever oxygen is present, and now PDHc will come into action, which produces more NADH to feed into the respiratory chain with subsequent nergyceonservation. The PDHc being sensitive towards NADH is inhibited under anaerobic conditions and the pyruvate flux is redirected via PFL. High NADH production is undesirable under these conditions, because redox neutrality has to be maintained.

Studies with the different pyruvate dehydrogenase complexes from *Enterococcus faecalis, Escherichia coli, Lactococcus lactis* and *Azotobacter vinelandii* have shown that the sensitivity towards NADH of the complex varies from species to species: *Enterococcus faecalis* showed the lowest sensitivity and *E. coli* the highest of the ones studied. (Snoep *et al.*, 1993). This explains the high activities of *E. faecalis* under anaerobic conditions and the low activities in anaerobic cultures of *E. coli*. Since the lipoamide dehydrogenase component is responsible for this and now that this component of *E. faecalis* has been cloned (chapter 5), structural studies can give a clue why the different lipoamide dehydrogenases are so different with respect to this NADH sensitivity and the possibility is open to make mutants with an altered sensitivity.

From these studies it becomes once again clear that *E. coli* is capable to adapt its catabolism in such a way, that it will take energetically as much advantage of the environmental conditions as possible. In order to do so, *E. coli* possesses a wide range of regulation systems and modes of regulation, operating on different timescales and different levels.

A flux through an enzyme is built up of two components: the amount of enzyme in the cell and the activity of this enzyme in the cell, which is dependent on concentrations of substrate, products, effectors and inhibitors. In enzyme synthesis different regulation systems are involved, which react to (external) redox conditions. The most important systems are FNR and Arc, which have been described in the general introduction. These systems sense in some way the redox environment: FNR most likely senses the redox potential of the cell (Unden *et al.*, 1990), although recently oxygen has been put forward as being directly involved in the signal transduction (Unden and Schirawski, 1997). Arc is probably influenced by a change in intracellular catabolism (Iuchi, 1993; Iuchi *et al.*, 1994). Several

metabolites like NADH and pyruvate can enhance the phosphorylation of Arc (see general introduction) and this offers the possibility for the system to sense changes in the catabolism. The exact primary stimulus of Arc remains however unknown.
In figure 6.1 a model of the timescales of the events during adaptation to different redox conditions is given.

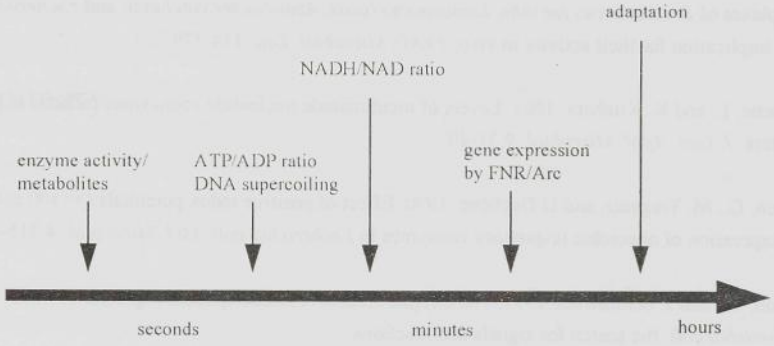

Fig 6.1. Model of the subsequent events in adaptation of *E. coli* to different redox conditions

As a result of the alteration of external conditions, internal concentrations of metabolites and cofactors are changed and this will modulate enzyme activities (seconds). Among these metabolites the ATP/ADP ratio will change, and subsequently DNA supercoiling, which enables the cell to response rapidly by inducing a wide variety of genes (chapter 4). Meanwhile the redox state of the cell (NADH/NAD ratio) can change rapidly, which will have its effect on the activity of many enzymes. This change in redox state is likely sensed by regulatory systems such as FNR and Arc. These systems react to a distinct stimulus (redox state, oxygen) and induce (repress) only those genes that are (un)necessary for the new situation (minutes). They are superimposed on the gene regulation by DNA supercoiling.

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