On the Efficiency of Catabolism at the Cellular Level

P. Sharma

Summary

The major focus of this work is on the understanding and manipulation of the energetic efficiency of growth of *E.coli* in glucose limited continuous culture, and in glucose excess batch cultures. The presence and absence of oxygen as the signaling molecule for the availability of an external electron acceptor strongly affects the energetic efficiency of cellular metabolism, thus making the study and manipulation of respiratory electron transport in *E. coli,* important from the research point of view, in order to optimize the biotechnological processes for the production of reduced biochemical products and the efficient growth of large-scale cultures. This thesis project deals with the functional characterization of various components of the respiratory chain of *Escherichia coli* which consists of various dehydrogenases, three quinone pools, several reductases and three cytochrome oxidases. The role of these components in metabolic regulation under conditions of variable oxygen supply has been investigated in detail. The project has been divided into five, partially parallel and partially successive, topics as detailed below.

Chapter 1: General Introduction, consists of an overview of various types of metabolism and the significance of free energy and the generation of reducing power in the regulation of metabolism. It introduces various components of the electron transport chain (of *E. coli*) and their role as described in literature. This is followed by a description of the various cellular responses to the presence and absence of oxygen, and the role of the regulatory two component system ArcBA. It ends with a description of current models for the role of quinones in ArcB kinase regulation.

The fifth chapter of this thesis deals with metabolomics experiments carried out with CHO cells. Therefore this chapter also briefly introduces CHO cells as a model cell line for basic research in the life sciences and for pharmaceutical applications. An overview of methods is then presented that can be used for quenching of intermediary metabolism and separation of mammalian cells from their suspending medium, as (common) methods that may be used for the quantification of intracellular metabolites in mammalian cells.

Chapter 2: A recent publication on the biochemical analysis of the H^+/e^- stoichiometry of the bd-II type quinol oxidase of *E. coli* led us to re-evaluate the contribution of “uncoupling pathways” to the catabolism of glucose in *E. coli* under glucose-limited growth conditions. In contrast to the assumptions generally made in microbial physiology, we found that two such pathways, i.e. the methylglyoxal bypass and the pyruvate oxidase pathway, do significantly

contribute to glucose catabolism under these conditions with limited glucose availability. Moreover, this study also revealed that respiratory electron transfer in *E.coli* cannot be fully uncoupled from trans-membrane proton translocation, as proposed by Bekker et al, 2009, because all three cytochrome oxidases translocate at least 1 (scalar) proton per electron transferred to oxygen.

Chapter 3: The role of dimethyl-menaquinone (DMK) in aerobic respiration of *E. coli*. DMK was heretofore known to have a major role in anaerobic respiration but not in aerobic respiration. This notion is consistent with its redox midpoint potential, which is considerably lower (i.e. more negative) than the one of ubiquinone. We have shown, with the help of various deletion mutants, analyzed in batch- and in continuous culture, that DMK has a significant role also in aerobic respiration, by accepting electrons from succinate dehydrogenase, and passing them on to all three oxidases.

Chapter 4: The role of the redox state of the ubiquinones and the menaquinones in ArcA-mediated regulation of the aerobic/anaerobic transition in *E.coli*. This chapter describes the direct measurement of ArcA phosphorylation levels in intact cells of *E. coli*, as a function of the type of quinone present, its concentration and its redox state. The results obtained have shown that the oxidized form of both napthaquinones and ubiquinones each have a role in inhibiting the kinase activity of ArcB, thus inhibiting ArcA phosphorylation and thus, suspending the activation of fermentative genes, under aerobic conditions, which for both is reversed in reducing or anaerobic conditions. A small set of aerobiosis experiments performed have shown differential regulation of the ArcBA two component system by the various quinones, under the whole range of oxygen supply rates (i.e. the aerobiosis scale). Representative transition experiments have shown that all three quinones can switch on/off the activity of ArcBA, be it that for the napthaquinones, with their more negative redox midpoint potential, a more stringent oxygen limitation is required.

Chapter 5: It deals with the metabolomics experiments with CHO cells, a model mammalian cell line. We have developed a procedure for the quantitation of a large series of intracellular metabolites, using the silicone oil centrifugation method, in combination with LC/MS analysis. This method turned out to be superior over the “methanol quenching method” that is often used for such studies reported in the recent literature. Fluorescence enzyme assays have been used for independent calibration of the method developed. These metabolomics analysis

will be used to carry out metabolic flux analysis at different growth rates of CHO cells growing in a chemostat, to make a start with dynamic metabolic modeling of CHO cells, to identify bottlenecks that limit their growth rate.

Chapter 6: General Discussion, after discussing some general aspects of the respiratory chain in *E. coli*, primarily focuses on the application of each aspect of this research in the biotechnology industry. Use of uncoupled alternate pathways, manipulation of the ArcBA two component system, as well as of the quinone composition of the cells, can surely lead the way to the generation of optimal industrially relevant biochemical production processes. Similarly, the metabolomics method for the CHO cells can give way to improved efficiency of the growth of mammalian cells, for applications such as in vitro meat production, therapeutic protein expression and pharmaceutical research.