Energy transducing processes in growing and starving lactic acid streptococcci

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SUMMARY

Lactic acid streptococci are widely used in the manufacture of dairy products. The most important applications of these organisms involve the rapid fermentation of lactose ("milk-sugar") to lactic acid and the degradation of casein ("milk-protein") to peptides and amino acids for the flavour development during cheese ripening. Lactic acid streptococci have not only been studied for economic reasons but also for the resolution of fundamental questions about bacterial metabolism. The diversity in metabolic pathways in lactic acid streptococci is limited and various facets of the major pathways have already been described in the early days of bacterial physiology. Owing to the relative simplicity of streptococcal metabolism, these organisms proved to be very useful in studying the (complex) interrelations between metabolic pathways and the regulation of these processes at a molecular level. The investigations described in this thesis deal with these aspects with emphasis on the regulation of energy transducing processes. In addition, extensive attention has been paid to the (eco)physiological significance of the observed regulatory mechanisms. The literature data concerning these studies have been reviewed in a general context in **Chapter I**, whereas specific topics are dealt with in the introduction of the chapters concerned. The contents of the thesis has been summarized in the following sections.

(i). Transient states between growth and starvation. Chapter II describes the initial events in the metabolite pools and the response of the energy metabolism upon renewed addition of a fermentable sugar to lactose-starved cells of *Streptococcus cremoris*. It is shown that *S.cremoris* cells remain fully viable in the absence of an exogenous and/or endogenous reservoir of metabolic energy for at least 20 to 30 hours. After this period, the loss of viability during (lactose) starvation correlates quantitatively with the ability to re-generate a proton motive force (or electrochemical gradient of protons) across the cytoplasmic membrane of the cell. This proton motive force provides the driving force for the accumulation of various solutes which are essential for growth of lactic acid streptococci. The decreased capacity to generate a proton motive force has been attributed to a decreased rate of carbohydrate fermentation (glycolysis) which is (primarily) caused by the inactivation of glyceraldehyde 3-phosphate dehydrogenase during starvation (**Chapter III**). By titrating the activity of the glycolytic pathway with a specific irreversible inhibitor of glyceraldehyde 3-phosphate dehydrogenase evidence has been obtained that, already in freshly harvested cells of *S.lactis* and *S.cremoris*, glycolysis (i.e. acid production) is significantly limited by the activity of glyceraldehyde 3-phosphate dehydrogenase.

(ii). The glutamate-glutamine transport system. Despite the lack of metabolic energy, starved cells of *S.cremoris* and *S.lactis* are able to maintain large outwardly directed concentration gradients for a number of solutes, i.e. glutamate, aspartate, alanine, glycine and phosphate, indicating that the corresponding transport systems are inactive in energy-depleted cells and/or do not facilitate exit at all. The magnitude of the glutamate and phosphate pool (> 100 mM each) suggested that these solutes

could be quantitatively important in neutralizing cationic solutes (like K^+) in the cytoplasm and serve a major role as cellular osmolytes. These considerations provided the basis of studying glutamate and phosphate transport in *S.lactis* (and *S.cremoris*). **Chapter IV** and **V** describe the mechanism of energy coupling, the kinetic properties and the regulation of glutamate (and glutamine) transport activity in *S.lactis* (and *S.cremoris*). Although the data for aspartate and phosphate uptake have not been incorporated in this thesis, the corresponding transport systems are very similar to that of glutamate. The features of these transport systems are: (i) The driving force for uptake is not supplied by the proton motive force (electrochemical energy) but rather by (chemical) phosphate-bond energy directly. Consequently, these solutes can be accumulated to concentration gradients exceeding 10^5, which violate the thermodynamic limits set by the proton motive force (assuming a H^+/solute stoichiometry of 1). (ii) Solute translocation is essentially unidirectional, i.e. the rates of carrier-mediated exit are at least two orders of magnitude slower than carrier-mediated influx. (iii) The activity of the transport systems increases with the intracellular pH with pK_a values of about 7. The significance of these findings with respect to the mechanism of energy coupling and regulation of proton motive force-dependendent processes in bacteria is discussed in **Chapter VIII.** In addition to the internal pH dependency, uptake of glutamate also exhibits a striking external pH dependence resulting from the specificity of the transport system for glutamic acid instead of glutamate anion (**Chapter IV**).

Chapter VI demonstrates that the absolute preference of the transport system for glutamic acid imposes severe limitations on the growth of *S.lactis* and *S.cremoris* at alkaline pH values. At pH 7 or above the maximum specific growth rates of these organisms can be predicted (calculated) from the kinetic parameters of glutamate transport, the requirement for glutamate and the concentrations of amino acids (which competitively inhibit glutamate uptake) in the medium. Growth inhibition at alkaline pH values can be overcome by replacing in the growth medium glutamate for glutamine as source of glutamate and glutamine for biosynthesis. Glutamine is taken up by the glutamate transport system with an affinity constant (K_t) and a maximal rate (V_{max}) comparable to that of glutamic acid and independent of the external pH.

Also in **Chapter VI**, the capacity of *S.lactis* to accumulate leucine, isoleucine, valine and phenylalanine is related to the requirements for these essential amino acids. At high growth rates the demands for these amino acids can (to varying extents) be satisfied by the V_{max} of the corresponding transport systems which in case of the branched chain amino acid carrier is determined by the composition (source of amino acids?) of the growth medium. Under some conditions passive diffusion of (hydrophobic) amino acids may also contribute to net uptake of these solutes.

(iii). Arginine/ornithine exchange and the arginine deiminase pathway. Chapter VII describes the interrelation between arginine uptake and subsequent metabolism to ornithine, ammonia and carbon dioxide by the arginine deiminase (ADI) pathway. The ADI pathway provides organisms with an additional substrate level phosphorylation site and the production of ammonia which can oppose a

decrease in medium pH by glycolysis (**Chapter VIII**). Streptococci possessing the ADI pathway have been shown to couple the uptake of arginine to the excretion of ornithine by a very efficient antiport mechanism. Since the driving force for the translocation of arginine and ornithine is supplied by the corresponding concentration gradients, no additional metabolic energy is required for the transport step. Evidence is presented indicating that ADI pathway activity is regulated by the internal concentrations of (adenine) nucleotides to which arginine/ornithine antiport activity is matched by adjusting the internal concentrations of ornithine and arginine. Arginine/ornithine antiport and ADI pathway activity is regulated at the level of enzyme synthesis by glucose (respressor, antagonized by cAMP) and arginine (inducer).