DYNAMICS OF INTRACELLULAR METABOLITE POOLS IN MDCK SUSPENSION CELLS DURING GROWTH AND INFLUENZA VIRUS INFECTION

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Influenza virus infections are responsible for millions of flu cases with hundred thousands of deaths worldwide [1]. Additionally, pandemic outbreaks of aggressive influenza virus strains are very dangerous both for livestock and human population. Seasonal vaccination campaigns are in place to reduce infections, especially among young, old or immunodeficient individuals, generating a huge demand of 500 million (2015) vaccine doses every year [2]. Besides egg-based vaccine manufacturing, production platforms based on animal cell culture increasingly contribute to an overall growing market. Thus, the use of suspension MDCK cells ($MDCK_{sus}$) cultivated in chemically defined medium emerges as a modern vaccine manufacturing platform. In order to improve overall productivity and reduce costs, process analysis, process optimization, and process intensification strategies are necessary. In particular, a better understanding of the effect of virus replication on cell growth, cell morphology and cell metabolism is crucial for developing production processes.

In this study, the effect of a synchronous influenza A virus infection on cell growth and central carbon metabolism was investigated. Additionally, intracellular virus replication dynamics of influenza were analyzed and correlated to metabolic pool dynamics. For analysis of intracellular metabolites, an established HPLC-MS method was used to identify and quantify extracted metabolites [3]. A mathematical model, established for adherent MDCK cells, was modified to describe cell growth, consumption and production of main extracellular metabolites [4] as well as dynamics of intracellular metabolite pools of glycolysis and TCA.

Our results showed fast infection (< 2 h) of the whole $MDCK_{sus}$ population under the used infection conditions. Intracellular infection was very similar to the already reported dynamics in adherent MDCK cells [5]. Virus particles were released six hours post infection (hpi) for 30 h, with an overall yield of 10,000 virus particles per cell. Despite massively impaired cell growth at 6 hpi, the concentrations of extracellular metabolites did not differ significantly from mock-infected cells used as a control. The majority of intracellular TCA metabolites also followed a similar dynamics. For glycolysis, however, metabolite pools of lower glycolysis decreased rapidly after infection, whereas glucose-6-P and fructose-6-P pools where maintained at a similar level as controls. Overall it seems that influenza infected MDCK cells show primarily an alteration in the glycolysis pathway, channeling metabolites not to the lower part of glycolysis but to the pentose phosphate pathway. Energy metabolism (ATP pools and energy charge) and TCA pools seemed not be affected by virus infection. Quantitative data for mock-infected cells are described by the mathematical model. Work is in progress to explain the dynamics observed in infected cells.

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