S4-10 UNCOUPLING OF NAPHTHALENE DIOXYGENASE ACTIVITY AND PRODUCTION OF HYDROGEN PEROXIDE

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In this study the reactions catalyzed by naphthalene dioxygenase (NDO) with benzene were investigated. In the presence of benzene, NADH oxidation and O_2 utilization were partially uncoupled from substrate oxidation. Approximately 40 - 50% of the consumed O_2 was detected as hydrogen peroxide. The rate of benzene-dependent O_2 consumption decreased with time, but it partially increased by the addition of catalase in the course of the O_2 consumption by NDO. The total amount of O_2 consumed and the rate of benzene-induced O_2 consumption increased in the presence of hydrogen peroxide-scavenging agents, and further addition of the terminal oxygenase component (ISP_{NAP}) of NDO. Kinetic studies showed that ISP_{NAP} was irreversibly inactivated in the reaction that contained benzene. In addition, hydrogen peroxide added at a range of 0.1 - 0.6 mM in the reaction mixtures inactivated the reduced ISP_{NAP} containing mononuclear iron. These results show that hydrogen peroxide released during the uncoupling reaction acts as both an inhibitor of benzene-dependent O_2 consumption and an inactivator of ISP_{NAP}. Furthermore, NDO catalyzed the formation of a trace level of *cis*-benzene 1,2-dihyrodiol. This result shows that NDO also couples a trace amount of benzene to both O_2 consumption and NADH oxidation.

S5-1 ACETATE METABOLISM IN *ESCHERICHIA COLI*: ITS METABOLIC LINK TO C3-C4 ANAPLEROTIC REACTIONS

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The analysis of response of overall metabolic network to the modification of the target pathway is prerequisite to achieve the goal of metabolic engineering. A metabolic flux analysis showed that *Escherichia coli* accumulates acetate due to an oversupply of pyruvate exceeding the amount of precursor metabolites required to synthesize biomass. As expected from metabolic flux analysis, the blocked acetate metabolism resulted in a severe perturbation of pyruvate/acetyl-CoA flux that was manifested by accumulation of pyruvate and D-lactate. Moreover, the *pta* mutant excreted glutamate, showing that the accumulated pyruvate/acetyl-CoA flux was redirected to C3-C4 anaplerotic pathways. Introducing of PHB synthesis pathway which would balance perturbed acetyl-CoA flux suppressed the excretion of by-products. For further increase of flux through C3-C4 anaplerotic links, additional *ldhA* mutation was introduced in the *pta* mutant: The resulting *pta ldhA* double mutant excreted pyruvate as the major by-product in aerobic conditions, and fermented glucose mainly to pyruvate and succinate anaerobically. Taken together, we propose that acetate metabolism is closely linked to C3-C4 anaplerotic pathways and is a potential target site for redirection of central metabolic flux to C3-C4 linkage.