

Malate Dehydrogenase-Citrate Synthase Multienzyme Complex Dynamics Is Affected By TCA Cycle Flux In Living Yeast Cells

ABSTRACT

A multienzyme complex is formed by enzymes that catalyze sequential reactions of a pathway, such as malate dehydrogenase (MDH) and citrate synthase (CS) of the Krebs tricarboxylic acid (TCA) cycle. The MDH-CS multienzyme complex is assumed to be dynamic, but the relationship between its assembly and cellular metabolic flux is not yet established. We hypothesize that the dynamic MDH-CS interaction is related to the TCA cycle flux. A time-resolved *in vivo* protein-protein interaction study was performed by split-luciferase assay using nanoBIT system (Promega) in *Saccharomyces cerevisiae* (BY4741) cells. Cells were grown on different carbon sources, and the interaction of MDH-CS multienzyme complex was measured as a function of luminescence. To induce change in metabolic flux, various carbon sources, electron transport chain inhibitors, and fermentation inhibitors were applied. We found that cells grown on glucose showed a significantly lower association of the complex compared to raffinose or acetate grown cells. Glucose, which represses the TCA cycle and oxidative phosphorylation via the Crabtree effect, triggered dissociation of the complex when it was added to raffinose grown cells, and the addition of a fermentation inhibitor restored this interaction. On the other hand, acetate which stimulates TCA cycle flux, enhanced the association of the complex. Glycerol/lactate also caused an abrupt increase in the MDH-CS complex association. Interestingly, the electron transport chain inhibitors altered the MDH-CS complex dynamics in varying ways. Taken together, these results show that the dynamics of the MDH-CS complex is related to TCA cycle flux, and the MDH-CS complex may play a role in regulating metabolic flux through the TCA cycle.

Keywords: Krebs tricarboxylic acid cycle, multi-enzyme complex, metabolite channeling, metabolon, malate dehydrogenase, citrate synthase, Crabtree effect.