Metabolites Modulate Malate Dehydrogenase-Citrate Synthase Multienzyme Complex Formation

ABSTRACT

Two tricarboxylic acid (TCA) cycle enzymes, citrate synthase (CS) and malate dehydrogenase (MDH), form a multi-enzyme complex (MDH-CS) that catalyzes two sequential reactions of the cycle. It is not known if this complex is static or dynamic and if it is dynamic, what are the factors that regulate its association/dissociation? Various metabolic factors, including the concentrations of substrates and products of the MDH-CS reaction, TCA cycle intermediates, energy and redox status, and pH, fluctuate with change in metabolic status in the mitochondrial matrix and function as allosteric modulators of the TCA cycle enzymes. Therefore, we hypothesize that these factors regulate the association/dissociation of the MDH-CS complex. We performed an *in vitro* study on porcine heart MDH and CS to evaluate the effects of the afore mentioned factors on the affinity of the multi-enzyme complex by determining equilibrium dissociation constant (K_d) of the multienzyme complex using MicroScale Thermophoresis (MST). We found that substrates of the MDH-CS reaction, NAD^+, acetyl CoA, and malate, enhanced complex association while products of the reaction, NADH and citrate, weakened the binding affinity of the enzymes. Oxaloacetate, which is channeled by this complex, showed no significant effect on the affinity. However, it enhanced the positive effect of acetyl CoA when both compounds were present while oxaloacetate showed no effect on binding affinity when added together with NADH. Succinyl CoA, and α-ketoglutarate, which proceed from connecting pathways, also enhanced association of the complex. pH did not alter binding affinity. These results support the hypothesis that formation of the MDH-CS complex is dynamic and modulated by metabolic factors in response to respiratory metabolism.