

Tricarboxylic Acid Cycle Enzymes of the Ectomycorrhizal Basidiomycete,

Suillus bovinus

Norbert Grotjohann^{a,*}, Yi Huang^b and Wolfgang Kowallik^{a,*}

^a Lehrstuhl für Stoffwechselfysiologie, Fakultät für Biologie, Universität Bielefeld, Postfach 100131, D-33501 Bielefeld, Germany. Fax: 0521-106-6039.

E-mail: W.Kowallik@Biologie.Uni-Bielefeld.DE

Norbert.Grotjohann@Biologie.Uni-Bielefeld.DE

^b Department of Urban and Environmental Sciences, Peking University, Beijing, 100871 PR. China

^{a*} Authors for correspondence and reprint requests

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In crude cell extracts of the ectomycorrhizal fungus, *Suillus bovinus*, activities of citrate synthase, aconitase, isocitrate dehydrogenase, succinate dehydrogenase, fumarase, and malate dehydrogenase have been proved and analyzed. Citrate synthase exhibited high affinities for both its substrates: oxaloacetate ($K_m = 0.018$ mM) and acetyl-CoA ($K_m = 0.014$ mM). Aconitase showed better affinity for isocitrate ($K_m = 0.62$ mM) than for citrate ($K_m = 3.20$ mM). Analysis of isocitrate dehydrogenase revealed only small maximum activity ($60 \text{ nmol} \times \text{mg protein}^{-1} \times \text{min}^{-1}$), the enzyme being exclusively NADP⁺-dependent. Using the artificial electron acceptor dichlorophenol indophenol, activity and substrate affinity of succinate dehydrogenase were rather poor. Fumarase proved Fe²⁺-independent. Its affinity for malate was found higher ($K_m = 1.19$ mM) than that for fumarate ($K_m = 2.09$ mM). High total activity of malate dehydrogenase could be separated by native PAGE into a slowly running species of (mainly) cytosolic (about 80%) and a faster running species of (mainly) mitochondrial origin. Affinities for oxaloacetate of the two enzyme species were found identical within limits of significance ($K_m = 0.24$ mM and 0.22 mM). The assumed cytosolic enzyme exhibited affinity for malate ($K_m = 5.77$ mM) more than one order of magnitude lower than that for oxaloacetate. FPLC on superose 12 revealed only one activity band at a molecular mass of 100 ± 15 kDa. Activities of 2-oxoglutarate dehydrogenase and of succinyl-CoA synthetase could not be found. Technical problems in their detection, but also existence of an incomplete tricarboxylic acid cycle are considered. Metabolite affinities, maximum activities and pH-dependences of fumarase and of malate dehydrogenase allow the assumption of a reductive instead of oxidative function of these enzymes *in vivo*.