Abstract

The enzyme lactate dehydrogenase (LDH) is responsible for the reversible interconversion of pyruvate and NADH to lactate and NAD^+. LDH exists as five different isozymes, and is made up of four subunits, defined as either H or M. LDH-5 (4M) is present in liver and muscle cells, and plays a key role in the Cori cycle, which is responsible for the continued production of small amounts of ATP in the muscle cell under anaerobic conditions. During anaerobic glycolysis in peripheral tissues, LDH converts pyruvate to lactate, replenishing NAD^+ levels so that glycolysis may continue. Lactate travels through the bloodstream to the liver, where LDH converts it back to pyruvate, uses the pyruvate to synthesize glucose, and returns the glucose off to peripheral tissues to complete the cycle. In our experiments, standard biochemical purification techniques (precipitation, ion exchange chromatography, affinity chromatography) were used to purify LDH from chicken breast. The presence of LDH was monitored by enzymatic assays and protein concentration was determined with Bradford assays. This purification was compared to the purifications of bovine heart LDH and recombinant *Plasmodium falciparum* LDH done at the same time, revealing a relatively high specific activity in chicken breast LDH, but greater fold purification in beef heart LDH. Bands from SDS-PAGE were submitted for mass spectrometric analyses to confirm the presence of expected products and identify some of the impurities in the different preparations, and comparative Michaelis-Menten and inhibition kinetic analyses are currently underway. These analyses will enable the inclusion of the purification and comparison of chicken muscle LDH in future iterations of the BIOC/CHEM/MMG 207 laboratory course.