PURIFICATION AND CHARACTERIZATION OF ACIDIC ADENYLATE KINASE (ATP: AMP PHOSPHOTRANSFERASE, EC 2. 7. 4. 3) IN PORCINE HEART

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Porcine heart adenylate kinase can be separated into acidic and basic components on isoelectrofocusing, and the lattar is the same with porcine skeletal muscle adenylate kinase.

The acidic adenylate kinase was purified approximately 3,200 fold from porcine heart extract in an overall yield of 58% by DEAE-cellulose chromatography, affinity elution from a column of phospho-cellulose, affinity chromatography on a column of blue dextran-Sepharose 4 B and gel filtration.

The purified acidic enzyme had a specific activity of 1,100 units per mg of enzyme and migrated as a single component on disc and sodium dodecylsulfate gel electrophoresis and blue dextran-Sepharose 4 B affinity chromatography. However, electrophoretic variants were observed on gel isoelectrofocusing, and this variance depended on the pH environment in gels.

The purified acidic enzyme had a molecular weight of 26,900 and an optimum pH of 5.8 in the reverse reaction (ATP + AMP → 2 ADP).

The amino acid composition of the acidic enzyme was Asx_{22}, Thr_{13}, Ser_{14}, Glx_{29}, Pro_{17}, Gly_{17}, Ala_{22}, $\frac{1}{2}Cys_4$, Val_{13}, Met_8, Ile_{13}, Leu_{24}, Tyr_4, Phe_7, Lys_{19}, His_5, Arg_{14}, and no Trp, totaling 245 residues. The acidic enzyme had two -SS- bonds no free SH, and it was not inhibited by 5, 5′-(2-nitrobenzoic acid) at all. From these results, it may be concluded that the acidic adenylate kinase in porcine heart is similar to liver mitochondrial adenylate kinase.