COMPARISON OF SOME KINETIC PROPERTIES OF CYTOPLASMIC AND MITOCHONDRIAL ASPARTATE AMINOTRANSFERASES IN HOLSTEIN STEER LIVER

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Introduction

Aspartate aminotransferase (EC 2.6.1.1) is known to be present as two distinct isozymes in animal cells, one located in the cytoplasm(cAsp-AT) and the other in the mitochondria(mAspAT). Biochemical properties of the enzyme in various mammalian tissues have been reported so far (Wada and Morino, 1964; Braunstein, 1973; Jenkins and Fonda, 1985). In spite of important physiological roles of the enzyme in the liver, however, relatively small number of works have been published for the kinetic properties of the respective isozyme from the ruminant liver in comparison with many reports on the heart enzyme in various domestic animals. In special, information on the isozymes in Holstein steer liver is limitted. In this paper, we report the comparison of some kinetic properties of aspartate amino-transferase isozymes in Holstein steer liver.

Materials and Methods

Materials

Substrates, cofactors, DTT, Trizma base and BSA were purchased from Sigma. Malate dehydrogenase and lactate dehydrogenase were obtained from Boehringer. DEAE-cellulose and CM-cellulose were obtained from Whatman. Hydroxyapatite was the product of Seikagaku Kogyo Co. DEAE-Sephacel, Sephadex G-100 and Sephadex G-150 were purchased from Pharmacia, Sweden. As the enzyme source, Holstein steer liver was used.

Enzyme assay

Initial velocity of aspartate aminotransferase activity was measured spectrophotometrically, following the change of absorbance at 340nm.

Isozyme purification

After extraction, ammonium sulfate fractionation and Sephadex G-100 column chromatography, Holstein steer liver cAspAT and mAspAT were separated by ion exchange chromatography, as described by Barra et al. (1976) and Porter et al. (1981) and were purified separately with two successive combinations of hydroxyapatite and Sephadex G-100 column chromatography, respectively.

Results and Discussion

In order to know catalytic function of Holstein steer liver cAspAT and mAspAT, which was purified approximately 400-500 folds to have the specific activity of 200-220 units/mg at pH 7.5 and 30°C, respectively, various kinetic investigations were performed. In the standard assay condition at pH 7.5 and 30°C, Km (Michaelis constant) for α-ketoglutarate of cAspAT was 0.04 mM, while that of mAspAT was 0.62 mM. On the other hand, Km for L-aspartate of cAspAT and mAspAT was 2.8 mM and 0.9 mM, respectively. Although Km for L-aspartate of Holstein steer liver cAspAT was in good agreement with that of pig heart cAspAT, Km for α-ketoglutarate of Holstein steer liver cAspAT was significantly smaller than that of pig heart cAspAT. Concerning Km for α-ketoglutarate or L-asparatate of mAspAT, a slight variation was observed between Holstein steer liver and pig heart isozymes, but significant difference was not obtained respectively (Braunstein, 1973; Jenkins and Fonda, 1985).

Since the enzyme activity is influenced by the changes in pH, ionic composition and the affinity to substrate, and has a significant implication in metabolic regulation, Km for α-ketoglutarate and L-aspartate of the both isozymes under wide pH range was examined. Consequently, as shown in figure 1 and figure 2, a marked dependence on pH of Km for the respective substrate was observed. In addition, these results show a great difference in

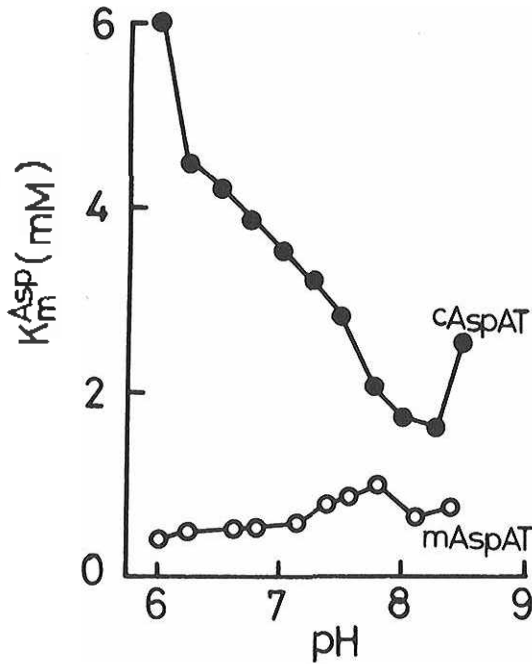

Figure 1. Effect of pH on Km for L-aspartate of cytoplasmic and mitochondrial aspartate aminotransferases in Holstein steer liver. cAspAT (●) and mAspAT (○) stand for the cytoplasmic and mitochondrial aspartate aminotransferases, respectively.

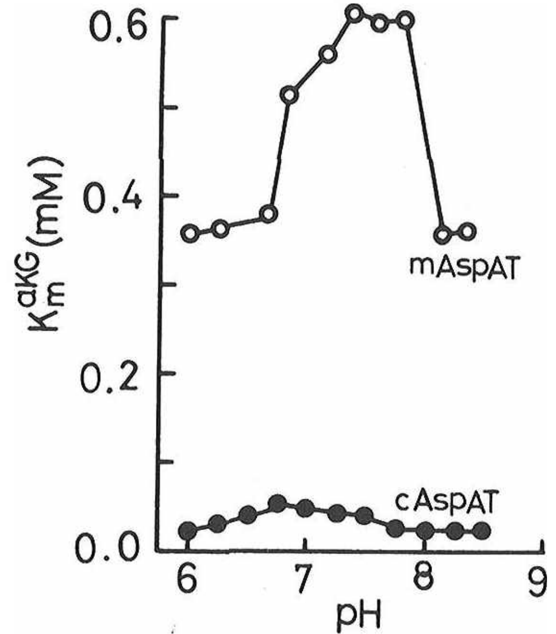

Figure 2. Effect of pH on Km for α-ketoglutarate of cytoplasmic and mitochondrial aspartate aminotransferases in Holstein steer liver. Signals are the same as described in figure 1.

Km for the same substrate between cAspAT and mAspAT. As the ionization and deionization states of the respective substrate and protein surface have been reported to have important effect on the reaction of the enzyme, Dixon plots of the respective isozyme were carried out. In special, the AspAT isozyme molecule and substrate species specific pH vs log V/Km profiles were observed. These molecular behaviors in concert with the changes in pH and ionic environment was evaluated to suggest the physiological possibility for the respective isozyme to share preferencial metabolic contribution in cell environment of Holstein steer liver (Cooper and Meister, 1985).

(**Key Words**: Aspartate Aminotransferase, Isozymes, Kinetic Property, Holstein Steer Liver)

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