PURIFICATION AND CHARACTERIZATION OF A HIGH Km ALCOHOL DEHYDROGENASE FROM MOUSE LIVER

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Two kinds of alcohol dehydrogenase(ADH) isozyme in mouse liver have been reported by the electrophoretic studies using cellulose acetate plates at pH 8.5. By ADH activity staining at pH 8.0, one of them has been observed near the cathode, using ethanol as substrate, and the other near the anode, using hexenol.

In this study, NAD-containing disc gel electrophoresis at pH 9.7 was used and ADH activity was stained at pH 10.7, using ethier hexenol or ethanol of high concentration, and three ADH isozymes were found in mouse(ddY strain) liver extracts. Then these three isozymes were purified and characterized.

Result: The anodic(PI) and the cathodic(PII) bands were found as main broad ones, and the middle bands(PIII) as a weak one. At low concentration of ethanol as substrate, it was difficult to detect PI band. Three ADH isozymes were separated one another by CM-chromatography and purified by Blue sepharose affinity chromatography. By electrophoresis after purification, both PI and PII were found to be composed of several bands, which suggested the existence of variant forms in each of these two isozymes.

The physical and kinetic properties of PII and PIII were similar each other and also to the other mammalian liver ADH, as reported earlier in detail, whereas PI was different from them with respect to following properties: acidic isoelectric points (pI 5.3-6.4), very high Km for ethanol(6.7M) and resistant to pyrazole inhibition. PI was further purified by preparative electrophoresis on polyacrylamide gel(pH 8.9) until it was immunologically homogeneous. By the double diffusion test using ant-PI, it was revealed that PI is different immunologically from PII and PIII. Moreover, by the antibody inhibition tests, it was suggested that PI contributes to the cytosol ethanol oxidation in about 10% and to its hexenol oxidation in about 70%.

Memo:

ISOELECTROPHORETIC ACIDIC ISOENZYME OF HUMAN RIBONUCLEASE

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In our previous study, isoelectrophoretic heterogeneities were observed in serum and tissue Ribonuclease (RNase), and acidic RNase was detected in serum of the patients with pancreatic carcinoma and hepatoma. The aim of this study was to investigate the heterogeneities of the enzyme and to characterize acidic RNase as carcinofetal protein.

MATERIALS AND METHODS The activity of RNase was measured with Poly(C) as substrate. Examined samples were serum, tissue and pancreatic juice. Serum was obtained from normal persons and patients with various diseases. The RNase activity of tissue was estimated with crude extracts of homogenized samples obtained from surgical specimens and fetal organs. The separation of this enzyme was performed by isoelectric focusing method using LKB 8100 Ampholine electrofocusing column.

RESULT Tissue RNase activities in pancreatic cancer (mean±SD; n=4,49.9±36.9) and fetal pancreas (n=4,17.0±24.0) were significantly low as compared with adult pancreatic tissue (n=5, 742.6 ±432.1). Three main fractions of RNase, basic(pI 9.4),neutral (pI 6.3) and acidic(pI 4.6), were separated by isoelectric focusing. The basic and neutral isoenzymes were observed in serum and normal pancreatic tissue. In contrast, this acidic isoenzyme appeared in the sera of patients with pancreatic cancer and hepatoma. Moreover, this acidic fractions were exclusively detected in the tissues of pancreatic carcinoma, hepatoma and fetal organs.

CONCLUSION Acidic RNase was a unique isoenzyme found in the tissues of pancreatic carcinoma, hepatoma and fetal organs, and was proved as a carcinofetal protein. The detection of this isoenzyme is usefull for the diagnosis of pancreatic cancer and hepatoma.

Memo:

INCIDENCE OF ENZYME-LINKED IMMUNOGLOBULIN IN HUMAN SERUM

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Although incidences of various kinds of enzyme-linked immunoglobulin(Ig) in human serum has been reported, they could not be compared each other, because methods for the detection and analyzed samples were different. For comparing the incidence of enzyme-linked Ig, lactate dehydrogenase(LDH)-, alkaline phosphatase(ALP)- and amylase(AMY)-linked Ig were examined in the sera from the same patients' group, simultaneously.

Samples were obtained from patients' sera submitted for HBs-antigen test in our laboratory. ALP activity on the precipitate was examined for the detection of ALP-linked Ig after immunoelectrosyneresis(IES) with antisera to both light-chains. Both identifications of class and type of ALP-linked Ig were performed by immunoelectrophoresis(IEP) and IES. Abnormal patterns of LDH- and AMY-isoenzymes were found by isoenzyme analysis on cellulose acetate membrane. Ig linked to LDH or AMY was detected by IEP or immunoprecipitin reaction in free liquid media(IPR).

In series of 10,000 patients, there demonstrated ALP-linked Ig in 33(0.33%) and LDH-linked Ig in 29(0.29%). AMY-linked Ig was found in 14 out of 6,000 patients(0.23%). These patients were arranged in relation to their age. The age where the most number of patients was found was seventies in ALP-linked Ig (10 patients) and sixties in LDH-linked Ig(9 patients). AMY-linked Ig was found most(3 patiwnts) in fifties and sixties, respectively. Male patients with enzyme-linked Ig were more than female ones in every enzyme. The isotype of enzyme-linked Ig which was found in the highest incidence was IgG-lambda in ALP(45%), IgG-kappa and lambda in LDH(31%) and IgA-kappa in AMY(43%).

Memo:

MITOCHONDRIAL CREATINE KINASE IN HUMAN TISSUE

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Creatine kinase (EC 2.7.3.2) has three isoenzymes, namely CK-BB(CK_1), CK-MB(CK_2) and CK-MM(CK_3). Recently, many investigators have reported another type of CK from mitochondria. We purified CK in mitochondria (CKmi) from various human tissues and studied their enzymatic physicochemical and immunological properties.

(MATERIALS AND METHOD)

CKmi was extracted from mitochondrial fraction obtained by the ultracentrifugal fractionation method as previously reported.[1] Electrophoresis with agarose gel and inhibition studies with anti-M antibodies were carried out as previously described.[1]

(RESULTS)

CKmi in skeletal muscle, in heart and in brain were migrated CK-MM position on agarose gel electrophoresis, and they were not inhibited with anti-M antibodies. The apparent molecular weight of CKmi in three tissues estimated by Sephadex-G-200 column chromatography were indicated about 370.000 dalton. Apparent Km value of CKmi for creatine phosphate were 2.66×10^{-3}(skeletal muscle), 1.67×10^{-3}(heart) and 9.8×10^{-4} (brain) mol/l, respectively. Activation energies of CKmi according to Stein et al.[2] was over 100 kJ/mol.

(REFERENCE)

1. Yasui, T., et al.:Jan. J. Clin. Pathol., 30(suppl):91, 1982.
2. Stein, W., et al.:Clin. Chem., 28:19, 1982.

Memo: