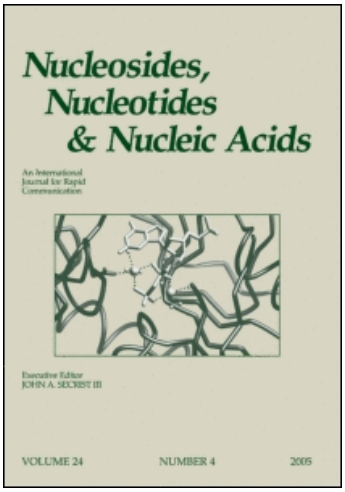

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Comparative Immunologic and Kinetic Evaluation of AMP-Deaminase Isolated from Normal Human Liver and Hepatocellular Carcinoma (HCC)

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Comparative Immunologic and Kinetic Evaluation of AMP-Deaminase Isolated from Normal Human Liver and Hepatocellular Carcinoma (HCC)

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ABSTRACT

In the present paper physico-chemical properties of AMP-deaminase purified from human liver neoplasm–hepatocellular carcinoma (HCC) were investigated and compared with these obtained for the enzyme from normal, unaffected tissue.

Key Words: AMP-deaminase; Hepatocellular carcinoma (HCC).

INTRODUCTION

AMP-deaminase (AMP-aminohydrolase, EC 3.5.4.6) is an enzyme playing a role in stabilization of adenylate energy charge (AEC) in cells of animal tissues. AMP-deaminase from human liver has been shown to be an oligomeric, allosterically regulated protein, composed of four identical subunits of molecular mass of 68 kDa, each,[1] forming differently aggregated, active structures.[2]

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Table 1. Effect of some important regulatory ligands on kinetic parameters of reaction catalyzed by AMP-deaminase isolated from normal and neoplasmatic (HCC) (values in the brackets) human liver.

Effectors added	$S_{0.5}$ (mM)	V_{max} (% of the control)	n_H
None	8 (14)	100 (100)	1.9 (1.9)
1 mM ATP	2 (2)	102 (102)	1.1 (0.9)
1 mM ADP	4 (11)	105 (125)	1.3 (1.0)
2.5 mM Pi	16 (13)	101 (107)	2.0 (1.9)

Reaction was started by adding of 5 μg of enzyme protein into the incubation medium. Kinetic data presented are means from three experiments. S.D. values were within ± 10% of the value of parameters calculated.

MATERIALS AND METHODS

Liver samples (frozen in −20°C) were weighed and subsequently homogenized in 3vol (v\w) of the extraction buffer (0.089 M phosphate buffer, pH 6.5, containing 0.18 M KCl, 1 mM thioethanol, 1 mM PMSF and 1 μg/ml of trypsin inhibitor), using a Warring blender-type homogenizer. The homogenate was twice centrifuged (first 30 min at 3000 g, and then 30 min at 18000 g), and supernatant obtained was applied onto a phosphocellulose column (2.6 × 20 cm), essentially according to the procedure of

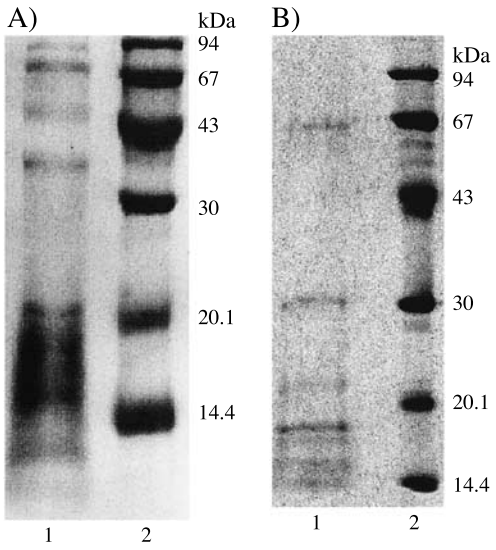

Figure 1. SDS-polyacrylamide gel electrophoresis of AMP-deaminase isolated from A) normal and B) neoplasmatic (Hepatocellular carcinoma) human liver. In both cases 5 μg of enzyme protein was used for electrophoretic separations. Phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsine inhibitor (20.1 kDa) and lactoalbumin (14.4 kDa) were used as standards (lane 2).

Smiley et al. Activity of AMP-deaminase was determined colorimetrically according to a phenol-hypochlorite method of Chaney and Marbach. Electrophoresis in the presence of 0.4% SDS was carried out on 12% (w/v) polyacrylamide slab gel.

RESULTS AND DISCUSSION

AMP-deaminase from the neoplasmatically changed human liver is less specific towards substrate analogues and, in contrast to the enzyme from the healthy organ, is hardly affected by pH and adenylate energy charge changes tested. Results presented in Table 1 illustrate kinetic and regulatory properties of AMP-deaminase isolated from two sources compared. In enzyme from healthy liver beside immunologically reactive 92 kDa protein fragment, corresponding to the full size of the enzyme subunit, also a smaller, immunologically reactive protein fragment 68 kDa was identified in the gel (Fig. 1A). When SDS-PAG electrophoresis of AMP-deaminase isolated from neoplasmatic liver was performed only presence of protein fragment 68 kDa was detected (Fig. 1B). One consequence of intensive hepatocellular necrosis may be increased intracellular proteolysis. This, in turn, appears to alter the regulatory behaviour of human liver AMP-deaminase.

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