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Remarkably enhanced expression of the type II hexokinase in rat hepatoma cell line AH130

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Expression of mRNAs encoding hexokinase isozymes was studied in various cells such as rat brain, liver, skeletal muscle, kidney and heart, and the rat hepatoma cell line AH130 by Northern blotting. High specific expression of type II hexokinase was observed only with AH130 cells. In contrast, specific expression of type I hexokinase was detected in energy-requiring normal tissue cells such as brain and heart. These results suggest that the expression of hexokinase isozyme in the tumor cells is different from that in normal cells.

Ascites hepatoma (AH130); Northern blotting; Hexokinase isozyme; Tissue specific expression

1. INTRODUCTION

Hexokinase (EC 2.7.1.1), a key enzyme in glycolysis, catalyzes the conversion of glucose to glucose-6-phosphate. Therefore, the activity of hexokinase is high in tissues in which efficient energy conversion is required: the activity in brain is about 10-fold that of other tissues [1,2]. As the activity in tumor cells such as AS-30D is extremely high being more than 100-fold that in normal cells [2,3], much attention has been paid to its induction in tumor cells.

There are three types of hexokinase isozymes, types I, II and III, and cDNAs encoding these isozymes have been cloned from cDNA libraries of rat brain [4,5] and human kidney [6], rat skeletal muscle [7], and rat liver [8], respectively. The homologies of these isozymes are very high and they all consist of two identical chains with a molecular mass of about 100 kDa. Glucokinase (EC 2.7.1.2), which has half the molecular mass of hexokinase and which consists of a single peptide chain of half the duplicated sequence of hexokinase, is sometimes called type IV hexokinase. To understand the detailed mechanism of energy conversion in relation to the activity of hexokinase, especially in tumor cells, it is important to know whether the expression of hexokinase isozymes is tissue specific and if so, which isozyme is responsible for its high activity for efficient energy conversion.

As a first step in the study of this problem, we determined the types of hexokinase isozymes expressed in various tissue cells of rats such as liver, kidney, skeletal muscle, heart, and brain, and in the rat hepatoma cell line AH130 by Northern analysis of their mRNAs. We found that type I hexokinase is significantly expressed in normal energy requiring cells such as brain and heart cells, and that the type II enzyme is highly expressed in hepatoma AH130 cells. In contrast, no significant expression of type II hexokinase was observed in any of these cells.

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2. MATERIALS AND METHODS

[α-^{32}P]dCTP (111 TBq/mmol) was obtained from New England Nuclear. Nitrocellulose membrane (BA85) was purchased from Schleicher & Schuell. Other materials and reagents were of the highest grade commercially available.

Total RNA was purified from rat hepatoma AH130 and liver, kidney, heart, skeletal and brain of male Wistar rat by the guanidium thiocyanate method [9].

Specific molecular probes for each of the three isozymes were designed carefully to avoid cross-hybridization with other isozymes as shown in Table I. These probes were radiolabelled with [α-^{32}P]dCTP by the specific priming method [9]. The specific radioactivities of these probes were more than 10^8 cpm/μg.

Total RNAs were obtained from various tissue cells and the AH130 cell line, and samples of 8.0 μg were separated by electrophoresis in formaldehyde containing gel and transferred to a nitrocellulose membrane. Hybridization was carried at 42°C in the presence of 50% of formamide and then the membrane was washed with a solution of 300 mM NaCl and 30 mM sodium citrate, pH 7.0 (2 × SSC) containing 0.1% SDS for 10 min at room temperature. This treatment was repeated 3 times, and then the membrane was washed once with the same solution at 60°C for 40 min. Hybridization bands were visualized autoradiographically by exposing the filter membrane to X-ray film at −80°C for 3 days with an intensifying screen. After autoradiography, the amount of the RNA loaded was confirmed by staining with methylene blue [9].

3. RESULTS AND DISCUSSION

We isolated total RNAs from various tissue cells of rats such as the liver, kidney, skeletal muscle, heart and

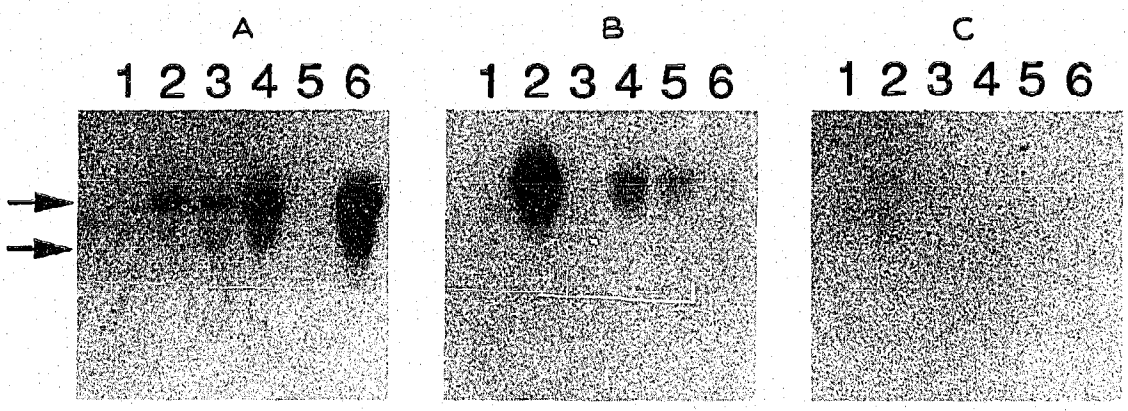

Fig. 1. Northern hybridization of total RNAs isolated from various rat tissues and the rat hepatoma AH130 cell line. After transfer to nitrocellulose membranes, RNAs were hybridized with probe I (A), II (B) and III (C). (Lane 1) liver; (lane 2) hepatoma AH130; (lane 3) kidney; (lane 4) heart; (lane 5) skeletal muscle; (lane 6) brain cells. The positions of 28 S and 18 S ribosomal RNAs are shown by arrows.

brain, and the rat hepatoma cell line AH130, and examined which hexokinase isoform is expressed in these cells. Based on the reported sequences of cDNAs for the three hexokinase isozymes [4–8], we synthesized three sets of oligonucleotides, referred to as probe I, II and III, as molecular probes for type I, II and III hexokinase, respectively (cf. Materials and Methods and Table I). As shown in Fig. 1, the molecular probes for each isozyme did not cross-hybridize with the other two isozymes under the present hybridization and washing conditions (cf. Materials and Methods), indicating that they were suitable as specific probes for the three hexokinase isozymes.

On nitrocellulose membranes RNA samples from brain and heart gave strong hybridization bands with probe I (fig. 1A). Expression of the type I isozyme was low in the kidney and hepatoma AH130, and was not detectable in the liver or skeletal muscle. In contrast, with probe II, AH130 cells gave two extremely strong bands of very similar molecular masses, while heart and skeletal muscle cells gave two faint bands. RNAs from other tissues such as liver, kidney and brain did not react with probe II (Fig. 1B). None of the significant bands of RNAs hybridized with probe III were observed (cf. Fig. 1C).

There have been many reports of structural characterization of the three isozymes [4–8,10–14]. But to the best of our knowledge the only paper on expression of hexokinase isozymes is the recent report by Thelen and Wilson [7] of the expression of type II hexokinase isozyme. They showed by Northern blotting, that type II hexokinase was expressed in both the Novikoff ascites tumor cell line and rat skeletal muscle cells, and they concluded that, contrary to expectation [3], there is no distinction between the isozymes in tumor and normal cells. However, their conclusion was based on the similar intensities of the hybridization bands of the two types of cells, although they used about 7 times more mRNA from skeletal muscle cells than from Novikoff hepatoma cells [7]. Thus, contrary to their conclusion,

Table I

Molecular probes for hexokinase isozymes

Probe	Synthesized oligonucleotides[a]	Corresponding position of cDNA[b]	Reference
I	5' AGAGGAGACCCTTCGATCGCCTAAAAGCCAGGA 3' ATTTTCGGTCCT	1336–1368	5
II	5' ATCCGGGAGGCTGGGCAGAGATAGAAGCTTGGG 3' ATCTTCGAACCC	2928–2960	7
III	5' GGTCTGCACCCGGGAGAAAGAGACTCAAGTGC 3' CTGAGTTCGACG	102–134	8

[a]All probes were designed as 33-mers and shown with their specific primers (12-mers each).
[b]Positions of the nucleotide sequences are indicated according to those in the references cited.

their results indicate that the expression of type II hexokinase is about 7 times greater in the Novikoff hepatoma cells than in skeletal muscle.

As we used the same amounts of total RNAs in this study, the relative amounts of mRNA of each isozyme expressed could be compared directly. Accordingly, our results show that the type I isozyme is expressed mainly in energy requiring normal tissues such as brain and heart, and type II hexokinase predominantly in tumor cells such as hepatoma AH130 cells, although slight expression of the type I isozyme was also observed in these tumor cells. Furthermore, no significant expression of type III isozyme was observed under the present experimental conditions using total RNAs, suggesting that the expression of this type of isozyme was very low, although it should exist at least in the liver [8].

In the present study, we demonstrated for the first time the tissue specific expression of type I and II hexokinase isozymes in various tissue cells of rats and a rat derived hepatoma cell-line, and found that expression of hexokinase isozymes in tumor cells is distinct from that in normal cells. These findings are consistent with the results of Thelen and Wilson [7] on Novikoff ascites tumor cells. Information on the mechanism of regulation of type II hexokinase expression in tumor cells and that of type I hexokinase in brain and heart cells is important for understanding the mechanism of efficient energy conversion in tumor cells and how it differs from that in brain and heart cells.

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