γ-GLUTAMYLTRANSPEPTIDASE ACTIVITY IN NORMAL, REGENERATING AND MALIGNANT HEPATOCYTES

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1. Introduction

γ-Glutamyltranspeptidase (EC 2.3.2.2) which catalyzes the transfer of the γ-glutamyl moiety of peptides to a variety of amino acid and peptide acceptors is widely distributed in mammalian tissues [1]. Adult rat liver has been shown to have low levels of γ-glutamyltranspeptidase activity, however, during the course of hepatocarcinogenesis, the enzyme is strikingly activated [2,3]. A high enzyme activity was also observed in fetal rat liver [4]. The enzyme has been purified from azo dye-induced rat hepatomas and its properties described [5]. γ-Glutamyltranspeptidase exists in two different forms in rat liver and small intestine, depending on the stage of development [6]. The enzyme is exclusively localized on the cell surface of a variety of lymphoid cells [7], and is stimulated by treatment of the cells with various mitogens. In an effort to define the factors effecting the activity of this enzyme, we report here the levels of γ-glutamyltranspeptidase activity in quiescent hepatocytes, regenerating liver, Morris hepatoma 7777, hepatocytes in monolayer culture, and hepatoma cells in monolayer and spinner culture.

2. Materials and methods

L-γ-Glutamyl-*p*-nitroanilide and glycylglycine were purchased from Sigma Chem. Co. Collagenase (type II) was obtained from Worthington Biochemical Corp. All other reagents were of analytical grade.

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Suspensions of normal isolated hepatocytes were prepared from the liver of male Sprague-Dawley rats (200–250 g) fed ad libitum, using a collagenase perfusion technique as in [8]. Hepatoma tissue culture (HTC) cells [9] were grown in suspension culture in Swim's 77 medium, supplemented with 5% fetal calf serum and 5% calf serum [10]. The following rat liver epithelial cell lines were grown in monolayer culture as in [11,12] and were kindly provided to us by Dr Z. Tökés for these studies. 'Normal' hepatocytes in culture are designated RL-34. Cells (RL-34) treated with the carcinogen, 4-nitroquinoline-1-oxide, designated RL-34-HII, showed very low levels of tumorigenicity [11]. Spontaneously-transformed hepatocytes (RL-34) designated RLC C-1 exhibited a high degree of tumorigenicity. Monolayer cultures were harvested by gentle scraping with a rubber spatula, washed three times with phosphate-buffered saline and resuspended in this buffer. Regenerating liver was obtained following partial (70%) hepatectomy under ether anesthesia as in [13]. Morris hepatoma 7777 which is a rapidly growing tumor was transplanted intramuscularly every three weeks in male Buffalo rats. Tumors were harvested and the neoplastic tissue carefully excised. Cells and minced tissue derived from regenerating liver and Morris hepatoma 7777 were homogenized (40 strokes) in a Dounce homogenizer in 1 mM $NaHCO_3$, pH 7.4, containing 0.5 mM $CaCl_2$ or in phosphate-buffered saline. Protein was determined by the method in [14] as modified in [15].

The γ-glutamyltranspeptidase activity was determined as in [16]. Briefly, 0.1 ml cell suspension or homogenate was added to the assay solution (pH 8.0)

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containing 2.5 mM of L-γ-glutamyl-*p*-nitroanilide, 30 mM glycylglycine, 50 mM Tris·HCl, pH 8.0 and 75 mM NaCl, to give a final ionic strength of 0.15. The assay mixture was incubated at 37°C in a shaker bath for 1 h, chilled to 0°C and the cells removed by centrifugation. In assays with cell homogenates, the reaction was terminated by the addition of 0.1 ml glacial acetic acid and the denatured protein removed by centrifugation. The resultant supernatant $A_{410\,nm}$ was measured at on a Zeiss PMQ II spectrophotometer.

3. Results and discussion

In an effort to define the effect of rapid cellular proliferation on the expression of γ-glutamyltranspeptidase activity as compared to hepatocyte transformation, γ-glutamyl-transpeptidase activity has been measured using the chromogenic substrate γ-glutamyl-*p*-nitroanilide and the high activity acceptor glycylglycine in a variety of hepatocytes in different states of growth and differentiation as shown in table 1.

Isolated normal hepatocytes possess very low enzymatic activity. A comparison of the activity observed in whole cells with the corresponding cell homogenate indicates that 42% enzyme is associated with the cell surface. HTC cells exhibited a 150-fold increase in enzymatic activity, with 52% activity associated with the cell surface. The rapidly-growing Morris hepatoma 7777 also showed a large increase in enzymatic activity. Comparable increases have been reported for azo dye-induced hepatomas [2]. Normal and transformed hepatocytes growing in monolayer culture have also been investigated. Normal (RL-34) and carcinogen treated (RL-34-HII) cells are not, or only slightly, tumorigenic, respectively [11,12]. These cells exhibited a 6–7-fold increase in enzyme activity and a cellular distribution, indicating a 70% and 50% surface association, respectively. This moderate increase may be due to alterations resulting from their adaption to cell culture conditions. The spontaneously-transformed cell line, RLC C-1, which was shown to be highly tumorigenic and fast-growing [11,12] expressed highly-elevated activity levels, comparable to the HTC cell system, with 70% activity associated with the cell surface. Thus, the increased level of γ-glutamyltranspeptidase activity appears to correlate with the degree of tumorigenicity expressed by the cell line or tissue. The cellular distribution of the enzyme differs from that reported for a variety of lymphoid cells where essentially all of the enzymatic activity is associated with the cell surface [7].

Table 1
Comparison of the γ-glutamyltranspeptidase activity of intact and homogenized rat hepatocytes

	Activity[a]	% Surface activity
Normal hepatocytes		
Intact cells	26	42
Homogenate	62	
HTC cells		
Intact cells	4836	52
Homogenate	9346	
Morris hepatoma		
Homogenate	5180	–
RL-34		
Intact cells	306	71
Homogenate	430	
RL-34-HII		
Intact cells	165	49
Homogenate	337	
RLC C-1		
Intact cells	7517	70
Homogenate	10 749	
Regenerating liver		
Homogenate	80–150	–

[a] The enzymatic activity is expressed as nmol *p*-nitroaniline released/h/mg protein

In contrast to the rapidly proliferating hepatoma systems, regenerating liver (table 1) at different time intervals following partial hepatectomy, expressed low activity levels as shown in fig.1. A 2.5-fold increase in activity was observed after 12 h, which rapidly returned to a slightly elevated plateau. Time-dependent alterations in the activity of several membrane-associated enzymes such as 5′-nucleotidase and leucyl-β-naphthylamidase in regenerating liver have been reported [17]. The increase in γ-glutamyl-

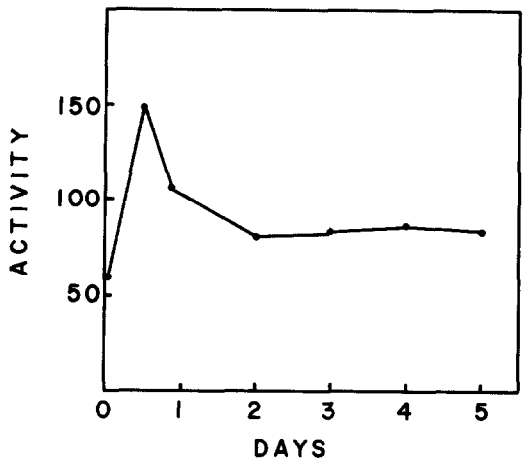

Fig.1. Time-dependent alteration in the activity of γ-glutamyltranspeptidase in regenerating liver following partial hepatectomy.

transpeptidase activity was, however, quite small compared to the values observed in several malignant hepatocyte systems, suggesting that the marked increases in the activity of this enzyme is characteristic of hepatocyte transformation and not of rapid cell proliferation.

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