PROTEIN KINASE ACTIVITY IN MORRIS HEPATOMAS*

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Summary - Rat liver protein kinase has been fractionated into five peaks of activity on isoelectrofocusing columns. The major liver peak, which was activated by cAMP, was decreased in two fast growing Morris hepatomas. The second major liver peak, independent of cAMP, was increased in the tumors.

One of the earliest measureable molecular lesions in cancer is the modification of hormonal and/or dietary controls of genetic expression in highly differentiated Morris "minimal deviation" hepatomas (Criss and Morris, 1973; Criss, 1973a, 1973b, 1973c). These molecular lesions have been observed in both protein and steroid hormone action. Therefore, the concept of "depressive interception" has been used to allow focusing of research efforts in neoplastic tissues upon the events between the interaction of hormone at the cell membrane to the resultant functioning genetic product.

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The cAMP system has been shown to be one of the major intracellar systems for relaying a membrane-hormone message to the nuclear-genetic unit in hepatic tissue (Sutherland and Rall, 1960; Robison et al, 1968; Holzer and Duntze, 1971). A major component of the cAMP system is protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37). Protein kinase has been observed to exist in multiple forms in rabbit skeletal muscle (Corbin et al, 1972; Yamamura et al, 1973), lobster tail muscle (Kuo and Greengard, 1970; Miyamoto et al, 1973), bovine heart (Rubin et al, 1972), bovine brain (Miyamoto et al, 1973), human erythrocytes (Guthrow et al, 1972), mouse mammary glands (Majumder and Turkington, 1971, 1972), rat adipose tissue (Corbin et al, 1973), and rat liver (Chen and Walsh, 1971; Kumon et al, 1972). The present manuscript describes multiple forms of protein kinase in rat hepatomas.

Methods

Normal Buffalo rats were innoculated with tumors in Washington, immediately shipped to Florida, and maintained in the Florida laboratories on commercial rat chow until time of sacrifice. The animals were sacrificed when the tumors were approximately 1.5 cm in diameter. Liver and hepatomas were removed, cooled and homogenized in 0.25 M sucrose containing 5 mM Tris-HCl (pH 7.5), 1mM EDTA, and 10 mM β-mercaptoethanol using a Potter-Elvehjem homogenizer with Teflon pestle. The supernatant fraction, from the resulting centrifugation at 100,000 Xg for 1 hr, was mounted upon a 110 ml LKB isoelectrofocusing column. Iso-

electrofocusing of the liver and tumor extracts was performed according to Criss et al, 1970. Protein kinase activity was measured in a reaction mixture (200 µl) containing 2.5 nmoles of [γ-^{32}p] ATP (10^5cpm), 10µg calf thymus histone, 10 µmoles Mg-acetate, 10 umoles potassium phosphate at pH 7.5 and an appropriate amount of enzyme preparation. Cyclic AMP (5µM) was added where indicated. The incubation was stopped after 15 min at 30° by addition of 5 ml of 10% trichloroacetic acid. The mixture was filtered through Whatman GF83 filter paper, washed 6 times with 5ml of 10% trichloroacetic acid, then counted in a toulene-Permablend I (Packard) cocktail in a Nuclear Chicago Three Channel Liquid Scintillation Spectrometer.

Results and Discussion

Normal rat liver gave 5 peaks of protein kinase activity upon isoelectrofocusing columns (Figure 1). The isoelectric points were determined to be pHs 4.3, 5.0, 7.8, 8.5, and 9.1 for peaks I, II, III, IV, and V, respectively. Peak II was a broad peak and present in the largest quantity. Present experience with three other isozymic systems and isoelectrofocusing techniques leads us to believe that this peak is not homogeneous. Peaks I and II were stimulated by cAMP. Peaks III, IV and V were not stimulated by cAMP.

Morris hepatoma 8999 (Morris, 1963, 1965; Morris and Wagner, 1968) is a slow growing and highly differentiated tumor. It showed some increase in peak II protein kinase activity but the activity in this peak was only stimulated about two fold by cAMP (normal liver peak II was stimulated

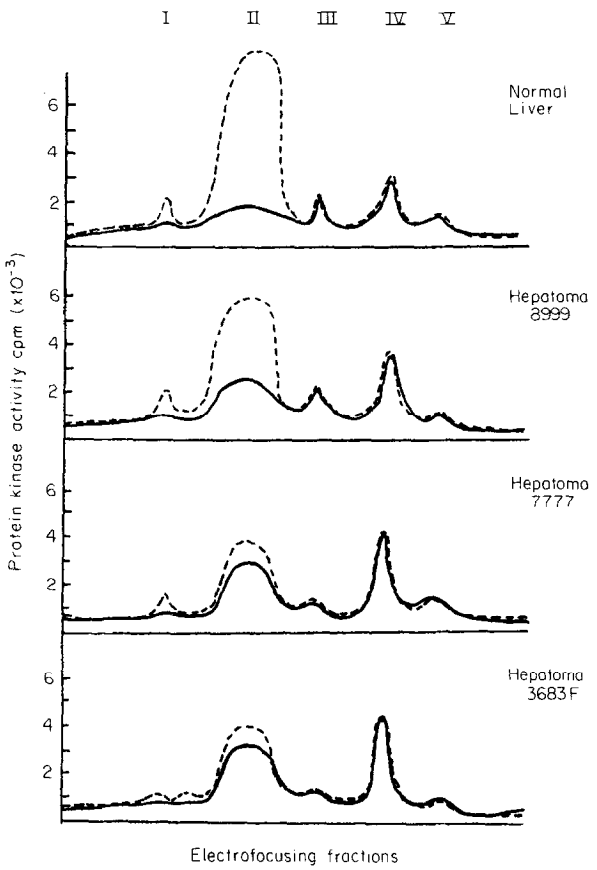

Figure 1: Fractions collected from the isoelectrofocusing of liver and hepatoma cytosols. Measurement of protein kinase activity is described under Methods. Dotted line is in presence of cAMP; solid line is in absence of cAMP.

over four fold). There appeared to be little change in the other peaks.

Morris hepatomas 7777 and 3683F are rapid growing and poorly differentiated tumors. There was increased activity in peak II from these tumors when compared to peak II of normal liver. Also peak II in the fast growing tumors was even less responsive to cAMP activation. Peak IV was slightly, but consistently increased over the normal

liver peak IV. In hepatoma 3683F, we observed an extra peak with protein kinase activity which appeared to have an isoelectric point near 4.0.

Strict interpretation of data in these protein kinase systems is very difficult. The native state of the enzyme is thought to be both a haloenzyme (containing regulatory and catalytic subunits) which is cAMP dependent and a catalytic subunit which is independent of cAMP. The various purified protein kinases appear to have similar catalytic subunits and varying regulatory subunits, each protein component having a specific isoelectric point. One must argue from the data in the current manuscript that there are three cAMP independent forms and two cAMP dependent forms of protein kinase in normal rat liver. The poorly differentiated tumors also contain five (perhaps six) forms of protein kinase, but the dependency upon cAMP may have changed.

Therefore, these results indicate a shift in the multiple forms of protein kinase during tumor progression. However, one must keep in mind that such parameters as the endogeneous levels (pre-bound moieties) of cGMP, cAMP, protein kinase heat stable modulator, and divalent metals can and do directly effect the activity of protein kinase (Ashby and Walsh 1973; Donnelly et al, 1973a, 1973b). Therefore, one will have to examine the many components of the cAMP system (including adenyl cyclases, phosphodiesterases, the multiple activators and inhibitors of the enzymatic components) as well as the cGMP system analogues in neoplastic tissues and in the tissues of

neoplastic origin before one can begin to access the role of hormonal involvement in the neoplastic process.

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