PROTEOLYTIC DEGRADATION OF NUCLEAR MATRIX PROTEINS OF RAT LIVERS, ZAJDELA'S HEPATOMA, AND HEPATOMA 22A IN THE PRESENCE OF ATP

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Considerable protease activity against an exogenous substrate, namely casein [3], has been found in the nuclear matrix (NM) of rat liver. According to the results of inhibitor analysis, the NM preparation contains proteases of all the principal systematic groups. On the addition of 10 mM dithiothreitol (DTT) to the incubation medium, according to the data obtained by the workers cited, proteolysis of casein was reduced by 20%.

Traces of considerable proteolytic degradation of both proteins and phosphoproteins have been found [4] in preparations of NM of rat liver and, in particular, of Zajdela's hepatoma, incubated in medium with DTT (5 mM) and ATP (5 mM). On the other hand, it has been stated that incubation with DTT in a concentration of 10-20 mM does not induce proteolytic breakdown of the proteins of NM [9, 11]. NM proteins of rat liver are quite resistant to endogenous proteolysis, and incubation at room temperature in medium with 2 M NaCl for 24 h gives rise to only a small decrease in the protein content of NM without any change in the electrophoretic pattern [1].

NM proteins in Zajdela's hepatoma are less resistant in tnedium with 2 M NaCl. Proteolysis of the laminae (triplets of NM proteins with mol. wt. of 68-75 kD) and, evidently, of high-molecular-weight proteins, takes place in them [1]. Moreover, the same proteolytic process is observed in NM of this hepatoma in vivo at the late stages of its development [7].

The aim of this investigation was to attempt to find ATP-dependent proteolytic activity in NM from nuclei of the normal liver and two ascites hepatomas.

EXPERIMENTAL METHOD

The liver of noninbred albino rats weighing 100-120 g was used. Zajdela's hepatoma was transplanted to the salne rats, and hepatoma 22A to C3HA mice; ascites cells were harvested on the 5th and 7th days respectively after transplantation. The nuclei were isolated from liver cells of normal rats and from ascites hepatolna cells, and NM was obtained from them [2]. The incubation lnedium contained 30 mM Tris-HCl, pH 7.4, 0.1% of the nonpolar detergent NP = 40, 5 mM $MgCl_2$, 5 mM DTT, and 1 kBq of γ-[^{32}P] ATP. The samples were incubated in this medium for 1 h at 30°C, then diluted 1:40 with medium containing 0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 20 mM Tris-HCl, pH 7.4. The samples were then centrifuged at 5000*g* for 15 min. The supernatant was treated in the cold with 20% TCA in the ratio of 1:1 and the mixture centrifuged at 2000*g* for 15 min. The residue was washed with acetone. The residues obtained by the first and second centrifugations were dissolved in lytic buffer for electrophoresis [4] and used for electrophoresis in a polyacrylamide gel gradient (7.5-15%), and their radioactivity was counted [4].

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TABLE 1. Distribution of Protein Concentration and ^{32}P Label between Residue and Supernatant Fraction after Incubation of NM with γ-[^{32}P] ATP (in percent of total value)

Source of NM	Residue		Supernatant	
	protein	label	protein	label
Rat liver	85	82	12	18
Zajdela's hepatoma	92	93	5	7
Hepatoma 22A	65	29	30	70

EXPERIMENTAL RESULTS

As Table 1 shows, during incubation of the NM preparations from rat liver, hepatoma 22A, or Zajdela's hepatoma in the presence of DTT and ATP, solubilization of some of the proteins took place; the specific radioactivity of the solubilized product was higher than that left in the residue.

During electrophoretic fractionation of proteins of the residue, attention was drawn to the similarity of the protein composition of preparations from rat liver, Zajdela's hepatoma, and hepatoma 22A. Proteins 105, 54, and 50 kD were best represented in preparations from the three test sources (Fig. 1). Since, according to data obtained by other workers, proteins with this molecular weight appeared in NM preparations of Zajdela's hepatoma under the influence of proteolytic degradation [1, 3], in the present case they may have been the result of proteolysis of proteins in the preparations chosen for study. The differences which we observed among low-molecular-weight proteins may perhaps indicate, not different pathways of proteolytic degradation, but only differences in its intensity. In native preparations of NM (not subjected to proteolysis) from rat liver and Zajdela's hepatoma, no significant differences were observed between proteins in the region of low molecular weight.

In preparations precipitated by TCA, no protein bands were found by Coomassic staining from parts of NM passing into solution during incubation.

Examination of autoradiographs of the gels after electrophoresis of NM proteins passing into the supernatant after incubation with DTT and ATP showed that radioactive material could be found only in preparations of hepatoma 22A, and moreover, it had low molecular weight (Fig. 2). The autoradiographs also show that the profile of phosphorylated NM proteins of hepatoma 22A remaining in the residue differed significantly from the pattern observed in NM from rat liver and Zajdela's hepatoma (Fig. 2). Phosphoproteins remaining in the residue of NM from the liver and Zajdela's hepatoma have mol. wt. of 110-130, 65-75, and about 40 kD, whereas in hepatoma 22A these were low-molecular-weight phosphoproteins which were probably proteolysis products of proteins with higher molecular weight (Fig. 2).

Thus although proteolysis of proteins takes place more actively in the preparation from hepatoma 22A than in NM preparations from rat liver and Zajdela's hepatoma, no significant or basic differences could be observed in the products of this process. Proteolysis of phosphoproteins in the NM preparation from hepatoma 22A, however, leads to their virtually complete lysis (no phosphorylated products could be found with mol. wt. of over 15 kD), and it thus differs in principle from the process taking place in preparations of rat liver and of Zajdela's hepatoma.

Proteins with mol. wt. of 105, 54, and 50 kD were not found in NM preparations from rat liver subjected to additional treatment, but were most marked in NM preparations of Zajdela's hepatoma in the late stages of development of this tumor (Fig. 3). Thus proteolysis of proteins in NM preparations of the liver and both hepatomas led to the formation of similar products, resembling those formed in NM of Zajdela's hepatoma in vivo or during prolonged incubation of nuclei of this hepatoma with 1 M NaCl [1]. Proteins with 50 and 54 kD, most widely represented in the preparations studied, and which were first found by Berezney in NM of Zajdela's hepatoma, in his opinion are proteolysis products of lamins [7]. The most probable cause of their degradation is hyperphosphorylation in dividing cells [10], a description which ayplies to the cells of Zajdela's hepatoma also. In the medium which we used, one stage of the process leading to proteolysis of proteins in NM of Zajdela's hepatoma in vivo is evidently simulated, not only in the isolated preparation of this hepatoma, but also in analogous preparations of rat liver and hepatoma 22A. It has been shown for well-studied preparations of rat liver that neither DTT [9] nor ATP [13, 14] separately can induce proteolytic degradation of NM proteins.

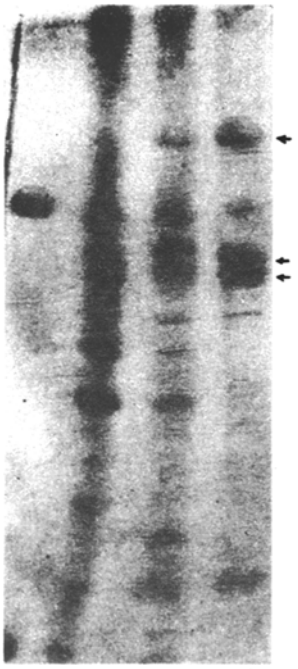

Fig. 1

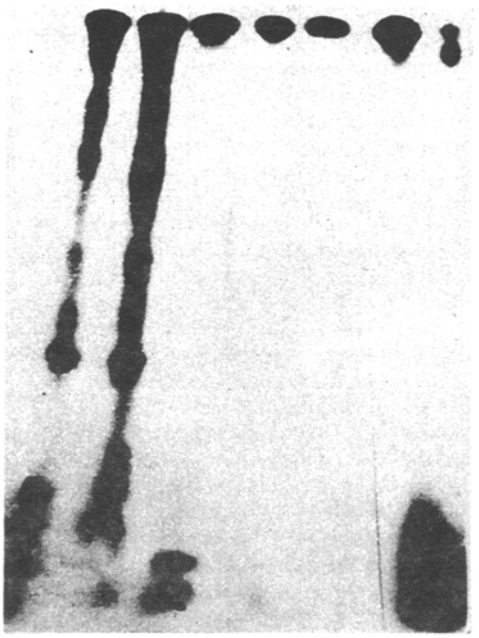

Fig. 2

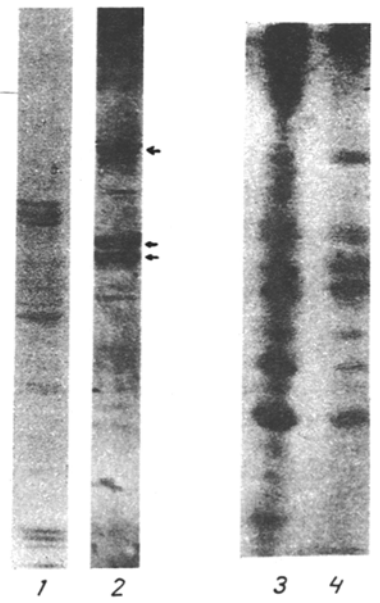

Fig. 3

Fig. 1. Electrophoretic profile of proteins sedimented at 5000*g* from NM preparations of rat liver, Zajdela's hepatoma, and hepatoma 22A after incubation in medium containina DTT, NP-40, and ATP. Stained with Coomassie. 1) Reference substances: bovine serum albumin above, cytochrome *c* below; 2-4) NM preparations from: 2) rat liver, 3) Zajdela's hepatoma, 4) Hepatoma 22A.

Fig. 2. Incorporation of ^{32}P into proteins of NM preparations from rat liver, Zajdela's hepatoma, and hepatoma 22A in the course of 1 h at 30°C, and subsequent fractionation of resulting phosphorylation products by centrifugation at 5000*g*. Autoradiographs. 1-3) Preparations of residues, 4-6) Preparations of supernatant. 1, 4) Rat liver; 2, 5) Zajdela's hepatoma; 3,6) Hepatoma 22A.

Fig. 3. Comparison of protein composition of NM preparations from rat liver and Zajdela's hepatoma in late stages of tumor development (5 days) and of preprations incubated with DTT and ATP for 1 h at 30°C. 1, 2) Preparations not incubated after isolation, 3, 4) incubation for 1 h, 1, 3) rat liver, 2, 4) Zajdela's hepatoma.

In the NM preparation of Zajdela's hepatoma, incubated with ATP and DTT, incorporation of phosphorous into proteins resistant to proteolysis and proteolysis products was quantitatively more intensive than in preparations from rat liver; mainly proteins with the same molecular weight were phosphorylated in both preparations in this case. This suggests similarities of the pathways both of protein phosphorylation and of phosphoprotein lysis in NM from rat liver and Zajdela's hepatoma.

However, proteolysis of virtually all the phosphoproteins of NM was observed in the preparation from hepatoma 22A (Fig. 2, Table 1). This evidently can be explained by the presence of protease or of a eroup of proteases characteristic of the NM of this hepatoma or of tumors related to it. Proteases localized in the nuclei, and not found in the nuclei of normal cells, have been demonstrated in certain tumor cells [5]. Since active thiol proteases, for which phosphoproteins are the optimal substrate, have been found hoth in cell nuclei and in cytoplasm [8, 12], it can be tentatively suggested that these enzymes hydrolyze both proteins and phosphoproteins under the conditions now studied.

Proteolysis is evidently intensified by the transition of a high proportion of the proteins of the NM preparations into the soluble state under the influence of DTT. Phosphorylation of proteins also modifies the reactivity of their sulfhydryl groups [6], and this also may probably promote more rapid proteolytic degradation of proteins which have lost their native conformation. Thus the presence of DTT and ATP in the incubation medium creates conditions for degradation of NM proteins in all the preparations which we studied. The most intensive proteolysis in NM preparations from hepatoma 22A evidently indicate either

very high proteolytic activity or the presence of one or more proteases, specific for phosphoproteins, in this preparation, which are not present in NM of rat liver and of Zajdela's hepatoma.

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EFFECT OF OVARIECTOMY ON FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN JAVA MACAQUES WITH CHOLESTASIS

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KEY WORDS: ovariectomy; cholestasis; phospholipids.

Steroid hormones of the estrogen group are involved in regulation of the metabolism of biliary lipids, including cholesterol and bile acids [14], through regulation of activity of the corresponding enzyme systems, in the formation of protein components of lipoproteins [12], and also regulation of the fatty acid composition of different classes of lipids [9]. There is now much biochemical evidence on some aspects of regulation of lipid metabolism by estrogens in cholestasis — an important factor in the development of hepatocholecystitis. In particular, estrogens stimulate esterification of cholesterol by the hepatocytes, which causes a decrease in bile acid synthesis such as is observed during pregnancy and following administration of estrogenic hormones, often giving rise to cholestasis [11]. Meanwhile, the role of estrogens in the process of incorporation of their most important components (bile acids) of the biliary lipids, participating in the formation of biliary micelles and vesicles in cholestasis, has received little study.

The aim of this investigation was to study the effect of ovariectomy on formation of the composition of lipids in the bile and on the phospholipid level, with analysis of the spectra of their component fatty acids in the organs and tissues of the enterohepatic system in Java macaques with experimental cholestasis.

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