EFFECT OF DITHIOTHREITOL DURING ISOLATION OF NUCLEAR MATRIX PREPARATIONS FROM RAT LIVER AND ZAJDELA'S HEPATOMA ON THEIR PROTEIN COMPOSITION AND PHOSPHORYLATION

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One of the principal forces stabilizing the internal nuclear matrix (NM) consists of S—S-bonds of oligomers of a 38 kD protein [5]. Dithiothreitol (DTT), in a concentration of 5-20 mM, ruptures thiol bonds, disturbs the stability of RNP-particles [7], and promotes solubilization of the intranuclear matrix. As a result, the fibrillar granular network and the residue of the nucleolus are eliminated from NM [6], whereas nonmembranous structures of the nuclear membrane and the pore complexes remain visibly unchanged. Sodium tetrathionate (an agent cross-linking S—S-bonds) acts in the opposite manner on the structure of NM. An NM preparation obtained from cell nuclei treated by this agent was distinguished by its high content of intranuclear matrix [8]. No appreciable changes were found in the protein composition of NM under these conditions [6, 8].

Comparison of NM preparations from normal leukocytes and leukemia cells showed that a much greater quantity of NM protein in normal cells compared with tumor cells is linked by S—S-bonds [13]. However, only a single communication does not allow conclusions to be drawn regarding the less important role of disulfide bonds in the association of proteins of the intranuclear matrix of tumor cells than of normal cells.

By studying phosphorylation, one of the principal post-translation modifications regulating activity of proteins, the change in functional state of NM caused by treating nuclei of both normal and tumor cells with DTT can be assessed.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female albino rats weighing 100-120 g and on a transplantable Zajdela's hepatoma of the same rats, taken on the 5th day. The nuclei were obtained from rat liver by a modified [2] method of Blobel and Potter. The isolated nuclei were incubated for 20 min at 4°C in medium containing 10 mM DTT, 170 Triton X-100, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 20 mM Tris-HCl, pH 7.4; no DTT was added to the control preparations. NM was then isolated as described previously [2]. Phosphorylation was carried out in medium containing γ-[^{32}P] ATP in a dose of 2 kBq/100 μg, 5 mM $MgCl_2$, 30 mM Tris-HCl, pH 7.4, and 100 mM NaCl, at 0°C. The reaction was stopped by the addition of 2 volumes of electrophoretic application buffer, containing 5 mM EDTA. Electrophoresis was carried out in the presence of sodium dodecylsulfate (SDS) [10] in an acrylamide gradient gel (5-20%). The gels were dried and autoradiographs taken on RT-1 x-ray film.

Phosphatase and protein-phosphatase activity was determined in the same incubation medium at 37°C. The substrate was either 1 mM phenolphthalein phosphate or 1 mg casein to 20 μg of NM protein in a volume of 50 μl. During incubation with phenolphthalein phosphate the reaction was stopped by addition of an equal volume of 0.25 NaOH, and the intensity of the color was determined visually. In experiments with casein the reaction was stopped by 15% TCA [11], one-third by volume compared with the incubation medium. Phosphate was determined by the method in [11].

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TABLE 1

Source of NM	Protein of NM in percent of total nuclear protein		Reduction of NM protein during isolation with DTT, %
	control	with DTT	
Rat liver	10±0,5	6,0±0,5	40
Zajdela's hepatoma	8±0,6	6,2±0,2	25

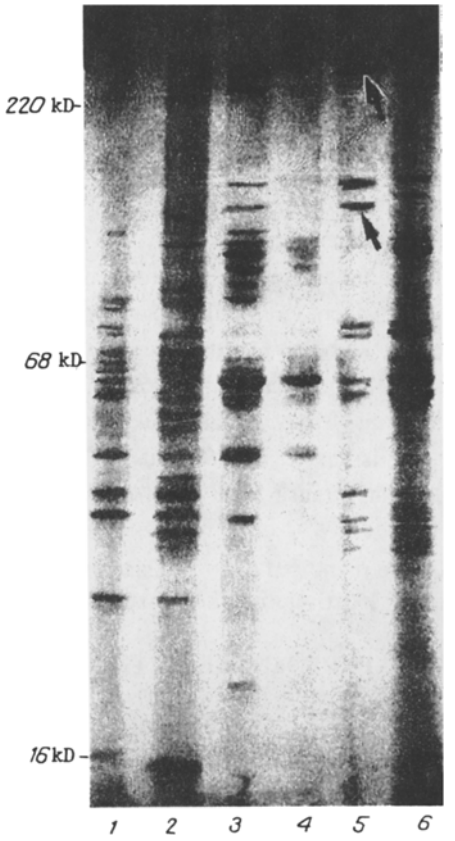

Fig. 1. Proteins solubilized from isolated nuclei of rat liver and Zajdela's hepatoma by 1% Triton X-100 in the presence and absence of 10 mM DTT. 1) Proteins from rat liver nuclei, solubilization without DTT; 2) proteins from rat liver nuclei, solubilization by 10 mM DTT; 3) nuclear proteins of Zajdela's hepatoma, solubilization by 10 mM DTT; 4) nuclear proteins from Zajdela's hepatoma, solubilization without DTT; 5) control preparation of NM from Zajdela's hepatoma; 6) preparation of NM from Zajdela's hepatoma, isolated in the presence of 10 mM DTT Coomassie stain.

EXPERIMENTAL RESULTS

When NM was isolated from the liver cell nuclei with the aid of DTT the yield of protein in NM of the liver was reduced by 40%, whereas in Zajdela's hepatoma it was reduced by 25% compared with the control preparation (Table 1).

Meanwhile the quantity of protein extracted with Triton X-100 was considerably increased, both from rat liver nuclei and from nuclei of Zajdela's hepatoma (Fig. 1).

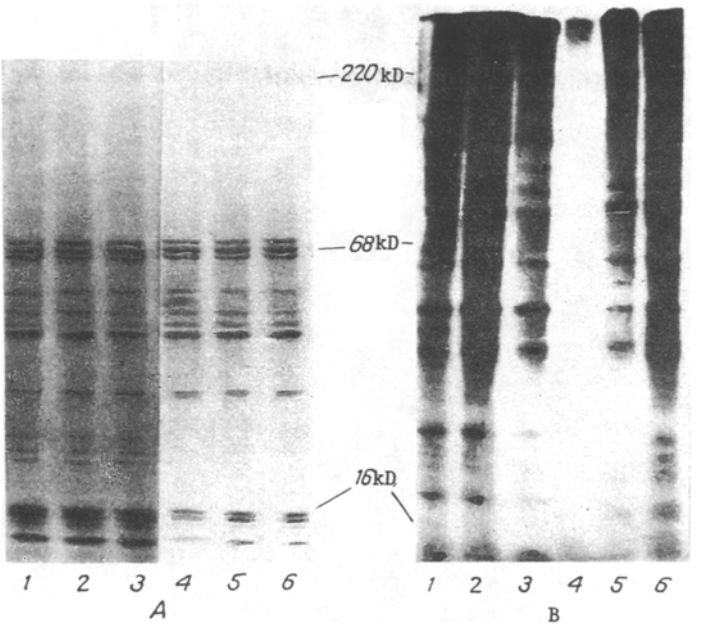

Fig. 2. Protein composition and phosphorylation of NM preparations isolated from rat liver in the presence of 10 mM DTT and in control. A) Coomassie stain, B) autoradiograph. 1-3) Control preparations, 2-4) isolated in presence of DTT 1, 4) Incubation for 1 min with γ-[^{32}P] ATP; 2, 5) for 5 min, 3, 6) for 30 min; 110 kD) ferritin, 68 kD) bovine serum albumin, 16 kD) RNase.

Electrophoresis of proteins of NM of rat liver (Fig. 2) showed that despite a considerable difference in the quantity of protein in the preparations, isolated in the presence of DTT and in the control, there was no difference in the qualitative picture the same proteins were discovered. These results are in good agreement with data obtained by other workers [6].

Three proteins with more than 220, 150, and 140 kD were absent in the NM preparation from Zajdela's hepatoma, isolated with the aid of medium containing DTT (Fig. 1). It is interesting to note that these three proteins are characteristic of tumor cells [1, 3, 14]. However, this does not describe the whole of the action of DTT solutions on the protein composition of NM of Zajdela's hepatoma. For instance, in preparations treated with DTT there was a sharp increase in the quantity of 105 kD protein. Since the appearance of this protein correlates closely with disappearance of proteins with higher molecular weights, it can be postulated that the 220, 140, and 150 kD proteins are not completely extracted by medium containing DTT, and that they undergo partial proteolysis either under the influence of DTT-activated proteases, or due to rupture of disulfide bonds in proteins on which this SH-reagent acts.

Incorporation of phosphate into proteins of the NM preparations isolated from rat liver in the presence of DTT, differed in its kinetic characteristics from phosphorylation in the control preparations. Whereas in the control incorporation of ^{32}P reached a maximum at the 5th minute, and thereafter slow dephosphorylation took place, in preparations isolated with DTT an increase was observed in the incorporation of ^{32}P into NM protein until 30 min, although it was slower (Fig. 2). Furthermore, the protein with mol. wt. of 35 kD was not phosphorylated in preparations isolated in the presence of DTT.

Considerable quantitative differences were observed during phosphorylation of NM proteins from Zajdela's hepatoma from preparations isolated in the presence of DTT and the controls. In the NM preparation isolated with DTT the proteins were phosphorylated much more strongly than in the control. Moreover, the 115 kD protein, differing greatly in its kinetics of incorporation of ^{32}P, and the remaining proteins, characterized by roughly identical kinetic characteristics of phosphorus incorporation, differed only a little at the 1st, but considerably at the 5th and 30th minutes of incubation (Fig. 3).

Thus dephosphorylation was evidently weakened in preparations from both normal and tumor cells after treatment with DTT. There are other facts to confirm this view. For instance, staining indicating cleavage of phosphophenolphthalein in the presence of the control NM preparation from rat liver appeared after 2 min, but with the NM preparation from Zajdela's hepatoma at the 5th minute, whereas in preparations isolated in the presence of DTT, it could not be found even after 30 min (Table 2). If casein was used as the substrate, protein phosphatase activity after 30 min, in preparations of both liver and

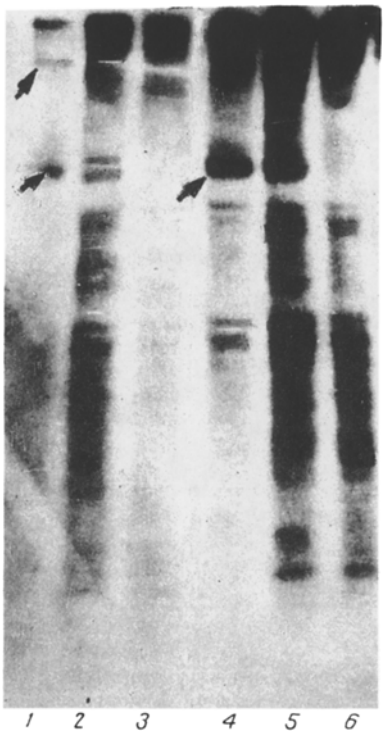

Fig. 3. Phosphorylation of proteins of Zajdela's hepatoma from NM preparations isolated in the presence of DTT and controls. Autoradiograph, 1-3) Control preparation, 4-6) isolated in presence of DTT. 1, 4) Incubation for 1 min with γ-[^{32}P]ATP; 2, 5) 5 min, 3, 6) 30 min. Arrows indicate proteins with 115 and 220 kD.

TABLE 2. Determination of Phosphatase and Protein-Phosphatase Activity in NM Preparations Isolated in the Presence of DTT and Controls (200 μg)

Source of NM	Time of removal of phosphate from substrates			
	phosphophenol-phthalein		casein	
	DTT	control	DTT	control
Rat liver	Absent 30 min	Present 2 min	Absent 30 min	Present 5 min
Zajdela's hepatoma	Absent 30 min	Present 5 min	Absent 30 min	Present 10 min

Zajdela's hepatoma, treated with 10 mM DDT, likewise could not be found, whereas the control preparations processed 30-40 μmoles phosphate after only 5-10 min. Thus in these NM preparations we found neither phosphatase nor protein phosphatase activity when exogenous substrates were used. Since the kinetics of incorporation of ^{32}P into NM proteins of preparations treated with DTT also indicates weakening of dephosphorylation of these proteins compared with the control preparations we can legitimately conclude that protein phosphatase activity is absent in NM preparations isolated in the presence of DTT, or at least, it is very greatly weakened. Although the 115 kD phosphoprotein did not contain ^{32}P in NM preparations incubated for 30 min, it evidently was not dephosphorylated, but underwent proteolysis.

If we examine the possibility of inhibition of protein phosphatases by DTT, it seems unlikely because the negligible concentration of this substance which could remain in an NM preparation after incubation of the nuclei with 10 mM DTT could hardly exert any significant influence on enzyme activity. Meanwhile, it has been shown that after treatment of the nuclear membrane with nonpolar detergent£, no protein phosphatase activity can be found in the unsolubilized residue [12]. But since it is mainly nonmembranous elements of the nuclear membrane that remain in an NM preparation treated with DTT [9], it can be

concluded that protein phosphatases are solubilized from NM by medium containing DTT and are absent in preparations of these structures.

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