ACTIVITY OF CHROMATIN-BOUND PROTEASE FROM RAT LIVER AND MORRIS HEPATOMAS

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1. The activity of serine protease was studied in chromatin and in histone preparations from rat liver and Morris hepatomas, lines 5123 D and 7777.

2. Electrophoretic patterns of histones and the amounts of acid-soluble peptides released during incubation of chromatin and histones showed that there was no significant correlation between protease activity and tumor differentiation and its growth rate.

The function of neutral chromatin-bound protease, found in animal and plant tissues, has not so far been elucidated. Bartley & Chalkley (1970) suggested that this enzyme is involved in autolytic cell destruction. However, other authors (Garrels et al., 1972; Kurecki & Točzko, 1974; Chong et al., 1974; Brandt et al., 1975) suggest that it plays a significant role in gene regulation. According to Chong et al. (1974) and Chae et al. (1975), neutral protease takes part in the turnover of non-histone chromosomal proteins.

In view of controversial data on the activity of protease in chromatin derived from rapidly dividing neoplastic tissues, we have studied the activity of this enzyme from rat liver and two Morris hepatomas, namely: highly differentiated and slowly growing line 5123 D, and low differentiated, fast growing line 7777.

MATERIALS AND METHODS

Material. The experiments were performed on rat liver (Buffalo strain) and Morris hepatomas 5123 D and 7777, which were provided by the Institute of Oncology in Gliwice (Poland). Tumours were excised 3 weeks (line 7777) or 6 weeks (line 5123 D) after inoculation of neoplastic cells into thigh muscles of rats.
Isolation of chromatin and total histones. Nuclei and chromatin were isolated as described by Lipińska et al. (1976): the nuclei by the modified sucrose method (Majkowska et al., 1971), and chromatin according to Spelsberg & Hnilica (1971). Total histones were obtained from chromatin by threefold extraction with 0.25 M-HCl and precipitated with 10 volumes of cold acetone.

Assay of histone proteolysis. Histone (1 mg/ml) or chromatin samples (2.5 mg DNA/ml, containing about 4.2 mg protein) in 10 mM-Tris-HCl buffer of pH 7.6, were incubated under toluene at 37°C for the appropriate length of time. Then 1 vol. of 10% trichloroacetic acid was added and in the supernatant the acid-soluble peptides were determined. For disc electrophoresis, after incubation histones were acidified with acetic acid (final concentration 0.9 M) and from chromatin histones were extracted with 0.25 M-HCl and isolated as described above.

Gel electrophoresis was performed in 15% polyacrylamide gel (0.6 x 10 cm) at pH 2.7 with 2.5 M-urea according to Panyim & Chalkley (1969) at 160 V, 1.5 mA per tube, for 3.5 h and 20 μg of protein per gel. The gels were stained with 1% Naphthalene Black 10B in 7% acetic acid and 20% ethanol, and scanned at 560 nm using an ERJ 65 densitograph (Carl Zeiss, Jena).

The protein and acid-soluble peptides were estimated by the method of Lowry et al. (1951) with serum albumin as a standard.

Reagents. Tris-hydroxymethyl-aminomethane was from Loba-Chemic (Wien, Austria); bovine serum albumin from Serva Feinbiochemica (Heidelberg, G.F.R.); diisopropyl fluorophosphate (DFP) and acrylamide from Koch-Light Lab. (Colnbrook, Bucks., England). N,N'-bis-acrylamide was a product of Fluka A.G. (Buchs S.G., Switzerland); 1,4-tetramethylenediamine (TEMED) of K & K Lab.Inc. (Hollywood, Calif., USA); Naphthalene Black 10B of George T. Gurr (High Wycombe, Bucks., England). Other reagents were analytical grade products supplied by POCh (Gliwice, Poland).

RESULTS AND DISCUSSION

Incubation for 4 h of chromatin obtained from rat liver and Morris hepatomas resulted in the release of some acid-soluble peptides; on further incubation, the amount of these peptides increased (Fig. 1A). In chromatin complex the enzyme degraded mainly histone H1, which after 18 h practically disappeared (Fig. 2). The protease activity was significantly higher in rat liver and Morris hepatoma 5123 D than in Morris hepatoma 7777.

Under similar conditions (pH 8, incubation for 4-6 h), Bartley & Chalkley (1970) and Chae et al. (1975) found practically no protease activity in chromatin from Ehrlich ascites tumour cells and Morris hepatomas, respectively. On the other hand, Carter & Chae (1976) and Ballal et al. (1975) observed degradation of chromosomal proteins from Ehrlich ascites tumour cells and Novikoff hepatoma, but in the presence of high concentration of salt and urea.

Chromatin protease from rat liver and Morris hepatomas, as well as from other tissues (Furlan & Jericijo, 1967; Bartley & Chalkley, 1970; Kurecki et al., 1971;
Fig. 1. Release of acid-soluble peptides from A, chromatin and B, histones of rat liver and Morris hepatomas, after incubation for the indicated length of time in 0.01 M-Tris-HCl buffer, pH 7.6 at 37°C.

Fig. 2. Densitometric tracings of electrophoretic patterns of histones extracted from chromatin of rat liver and Morris hepatomas, incubated in 0.01 M-Tris-HCl buffer, pH 7.6, at 37°C, for the indicated length of time. Note disappearance of histone f1 in the course of incubation.
Krajewska et al., 1976), is coextracted with histones with 0.25 M-HCl. As compared with the histones bound in chromatin complex, the free histones were more readily degraded (Fig. 3). Lysine-rich histone f1 of both neoplastic tissues and rat liver was more resistant to proteolysis than other histone fractions. All histone fractions from Morris hepatomas 5123 D and 7777 were more susceptible to proteolysis than the corresponding fractions from rat liver, and after 18 h incubation no protein fractions were visible on the electrophoretograms. On the other hand, in the preparation from rat liver, protein bands, although faint, appeared even after 24 h incubation. Higher proteolysis of histones from Morris hepatomas was also demonstrated by the determinations of acid-soluble peptides (Fig. 1B). The amount of acid-soluble peptides corresponded to about 30% of histones from Morris hepatomas, whereas for rat liver this value was only about 12%. Although our knowledge of the chemistry of histones from neoplastic tissues is insufficient to explain the above-presented results, it may be supposed that modification of amino acids in histones (phosphorylation, acetylation, methylation) in neoplastic tissues makes them more susceptible to proteolytic degradation after their isolation from chromatin complex, as compared with histones from normal tissues. Laine et al. (1976) found that in rat chloro-
leukaemia histone f2a2 threonine at position 16 is substituted by serine, and this substitution induces a potential phosphorylation site. Histone f2a1 from human leukaemic lymphoblasts has a higher ratio of dimethyllysine to monomethyllysine than the corresponding histone from foetal calf thymus (Desai & Foley, 1970).

The neutral protease from chromatin of rat liver and Morris hepatomas was inhibited by 1 mM-DFP (Fig. 4), the serine-specific reagent. The same was found for neutral proteases from other tissues (Chong et al., 1974; Kurecki et al., 1975; Carter & Chae, 1976).

Fig. 4. Densitometric tracings of electrophoretic patterns of histone preparations from rat liver and Morris hepatomas incubated for 8 h with or without dimethylphosphoryl fluoride in 0.01 M-Tris-HCl buffer, pH 7.6, at 37°C.

The presented results indicate that the activity of the neutral protease coextracted with histones from chromatin of the slowly growing Morris hepatoma line 5123 D was not significantly different from the activity of the enzyme from the fast growing hepatoma line 7777. However, in the chromatin complex the activity of this enzyme was higher in the slowly growing hepatoma than in the fast growing line 7777.

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REFERENCES


AKTYWNOŚĆ PROTEAZY CHROMATYNOWEJ WĄTROBY SZCZURA I WĄTROBIAKÓW MORRISA

Streszczenie

1. Badano aktywność serynowej proteazy w preparatach chromatyny i histonów otrzymywanych z wątrob szczaura oraz wątrobików Morrissa 5123 D i 7777.
2. Na podstawie analizy elektroforetycznej histonów i ilości kwasoopuszczalnych produktów degradacji ułatwiających się podczas inkubacji preparatów chromatyny oraz histonów, stwierdzono brak wyraźnej zależności między aktywnością enzymu a stopniem różnicaowania i szybkością wzrostu wątrobików.

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