Lack of Relationship Between Activity of Chromatin-Bound Proteinase and Cell Growth Rates

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Proteinase associated with chromatin isolated from liver and hepatoma of rat is stimulated by salt, and, of the histone fractions, lysine-rich (F1) histone is preferentially degraded by this enzyme at an ionic strength comparable with that found in the nucleus. However, there appears to be no strict relationship between the activity of the proteinase and growth rates of hepatomas. Chromatin isolated from a fast-growing tumour, Ehrlich ascites carcinoma, shows no apparent proteinase activity in the presence of salt.

Proteinase is closely associated with chromatin that is isolated from various tissues (Bartley & Chalkley, 1970; D. B. Carter & C.-B. Chae, unpublished) work, but the function of this chromatin-bound proteinase is obscure. Bartley & Chalkley (1970) have proposed that it may have an autolytic function rather than any gene regulatory role. However, chromosomal proteins, especially non-histone chromosomal proteins, turn over throughout the cell cycle (Borun & Stein, 1972) and it is possible that the chromatin-bound proteinase may be responsible for this turnover. If this is true rapidly growing cells will show a higher activity of chromatin-bound proteinase than slower-growing cells. In the present report we have compared the activity of chromatin-bound proteinases of Morris hepatomas growing at different rates.

Methods

Preparation of chromatin. Chromatin was prepared from livers of normal and tumour-bearing rats and from hepatomas as described previously (Chae, 1974). Briefly, nuclei obtained by centrifugation through 2.3M-sucrose containing 3mM-CaCl₂ and 10mM-Tris-HCl, pH 6.5, were washed twice each in the following solutions: 1% Triton X-100 containing 0.25M-sucrose, 3mM-MgCl₂ and 10mM-potassium phosphate, pH 5.8; 0.075M-NaCl-0.024M-EDTA, pH 7; 0.3M-NaCl, pH 6; 5mM-potassium phosphate, pH 6.5. The final chromatin pellet was suspended in cold water and sheared in a VirTis homogenizer at 60V for 90s.

Extraction of histones. Chromatin containing 250–500µg of DNA/ml was extracted with cold 0.2M-H₂SO₄ for 30min at 4°C and centrifuged at

20000g for 20min. The clear supernatant containing histones was used in this study.

Acid-urea-gel electrophoresis of histones. The acid-extracted histones were dialysed against 7M-urea-0.9M-acetic acid-1% 2-mercaptoethanol overnight at 4°C. About 20µg of histones was applied to the acid-urea gel (15%) in 2.5M-urea prepared by the method of Panyim & Chalkley (1969). Electrophoresis was carried out at 150V for 3.5h for gels of size 0.5cm×10cm and 10–12h for gels of size 0.5cm×15cm. The gels were stained with Amido Black and destained electrophoretically in 10% acetic acid-10% ethanol. The gels were scanned at 600nm in a Gilford recording spectrophotometer.

Results and discussion

Bartley & Chalkley (1970) have reported that histones of calf thymus chromatin are extensively degraded during incubation of chromatin in 10mM-Tris-HCl, pH 8, at 37°C. However, histones of rat liver and hepatoma chromatin remain relatively intact during incubation of chromatin in 10mM-Tris-HCl, pH 8, for 4–6h, and it was difficult to measure activity of chromatin-bound proteinase in terms of the amount of histones degraded. However, the proteinase associated with liver chromatin is activated by salt and mixtures of salt and urea at wide pH ranges (Chae & Carter, 1974). As shown in Plate 1, the degradation of F1 histone in rat liver chromatin is stimulated by increasing concentrations of NaCl up to a concentration of 0.5M, but other histone fractions remain relatively intact under the same conditions. Histones from hepatomas show similar patterns of degradation (not shown here). The degradation of non-histone chromosomal

Fig. 1. Degradation of F1 histones by chromatin-bound proteinase in 0.3 M-NaCl-10 mM-Tris-HCl (pH 8)

Chromatin containing 250 μ g of DNA (1 ml) was incubated in 0.3 M-NaCl-10 mM-Tris-HCl (pH 8) at 37°C for different periods of time, and 50 μ l of cold 3 M-H₂SO₄ was added. After extraction for 30 min at 4°C, the histones in the supernatant were dialysed against cold 7 M-urea-0.9 M-acetic acid-0.1% 2-mercaptoethanol, and the same volume of the histone solution was applied to the acid-urea gel. The areas under the F1 histone peaks in densitometric tracings of triplicate gels were averaged and compared with the areas of F1 histone peaks obtained from unincubated chromatin (control). (a) Normal liver (○) and hepatoma 7777 (●). (b) Host liver of hepatoma 7794A (●), hepatoma 7794A and 9121 (○), and hepatoma 7800 (■).

proteins is also stimulated in the presence of NaCl, the proteins of high molecular weight (>80000) being preferentially degraded (D. B. Carter & C.-B. Chae, unpublished work). Since it has been reported that the ionic strength in the nucleus could be 0.2-0.4 (Langendorf *et al.*, 1961; Siebert, 1966), in the present study we have determined the activity of chromatin-bound proteinase by the rate of degradation of F1 histone in the presence of 0.3 M-NaCl-10 mM-Tris-HCl, pH 8.

The hepatoma lines compared in this study are Morris hepatomas 7777, 7800, 9121 and 7794A, whose growth rates (average weeks between transfers) are 3, 6, 6 and 14 respectively. Fig. 1 shows the results obtained with hepatoma and liver chromatin. Chromatins isolated at the same time from normal or host liver and hepatomas were incubated in 0.3 M-NaCl-10 mM-Tris-HCl, pH 8, at 37°C for different periods of time, and the amount of F1 histone remaining was determined from the densitometric tracings of histone gels. As shown in Fig. 1(a), the chromatin-bound proteinases from normal liver and the fast-growing hepatoma, 7777, degrade F1 histone at the same rate, about 40% being degraded during the 2 h incubation. On the other hand, the F1 histone from chromatin of the slower-growing hepatomas, 7794A and 9121, is degraded at a considerably higher rate than the F1 histone from host liver chromatin (Fig. 1b), about 65% being degraded during the 2 h incubation. The F1 histone in hepatoma 7800 chromatin was, however, completely degraded in 90 min, as shown in Fig. 1(b). The order of growth

rates of the four hepatomas is 7777 > 9121 = 7800 > 7794A. However, the order of the degradation of F1 histone is 7800 > 7794A = 9121 > 7777 = normal liver. Therefore it appears that there is no strict relationship between the rate of degradation of F1 histone by chromatin-bound proteinase and the growth rates of the four hepatomas studied here. There are also no apparent differences between the activities of chromatin-bound proteinases from regenerating liver at S phase (22 h after partial hepatectomy) and normal liver (not shown here). In view of the fact that chromosomal non-histone proteins are turned over rapidly at S phase (Borun & Stein, 1972), it would appear that the rate is not regulated by changes in the activity of chromatin-bound proteinase. Bartley & Chalkley (1970) have reported that histones of the chromatin isolated from a rapidly dividing Ehrlich ascites carcinoma are not degraded during incubation in 10 mM-Tris-HCl, pH 8. We have also found that histones of Ehrlich ascites-tumour chromatin show no sign of degradation during incubation of the chromatin in 0.3-0.5 M-NaCl at pH 8 for 2-6 h at 37°C, suggesting that Ehrlich ascites-tumour chromatin may not contain any proteinase (not shown here).

In summary, the results obtained from Morris hepatomas growing at different rates, from regenerating liver at S phase, and from a rapidly growing Ehrlich ascites carcinoma suggest that there is no relationship between the activity of chromatin-bound proteinase and rate of cell growth and the turnover of chromosomal proteins. It is uncertain at the present

EXPLANATION OF PLATE I

Histones isolated from normal liver chromatin after incubation in NaCl of various concentrations for 2 h at 37°C

(a) Control (chromatin in water); (b) 10 mM-Tris-HCl (pH 8); (c) 0.15 M-NaCl-10 mM-Tris-HCl (pH 8); (d) 0.3 M-NaCl-10 mM-Tris-HCl (pH 8); (e) 0.5 M-NaCl-10 mM-Tris-HCl (pH 8).

time whether the proteinase associated with chromatin preparations is involved in the turnover of chromosomal proteins in cells in view of the apparent absence of proteinase activity from chromatin isolated from a fast-growing Ehrlich ascites carcinoma.

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- Bartley, J. & Chalkley, R. (1970) *J. Biol. Chem.* **245**, 4286-4292
Borun, T. W. & Stein, G. S. (1972) *J. Cell Biol.* **52**, 308-315
Chae, C.-B. (1974) *Biochemistry* **13**, 1110-1115
Chae, C.-B. & Carter, D. B. (1974) *Biochem. Biophys. Res. Commun.* **57**, 740-746
Langendorf, H., Siebert, G., Lorenz, I., Hannover, R. & Beyer, R. (1961) *Biochem. Z.* **335**, 273-284
Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130**, 337-346
Siebert, V. G. (1966) *Z. Klin. Chem.* **4**, 93-105