THE DIGESTION OF BASIC PROTEINS BY EXTRACT OF GUERIN TUMOR LYSOSOMES+

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SUMMARY: Lysosomes of Guerin tumor at its initial stage of growth are much more stable than lysosomes from the advanced stage. Lysosomal extracts from the tumor decompose easily basic proteins, especially arginine-rich proteins from the tumor cell cytoplasmic fraction. Basic protein degradation products probably affect the permeability of the tumor cell membranes and penetrate into the blood stream.

INTRODUCTION

Basic proteins are good substrates for acid lysosomal proteases and other proteolytic enzymes (1). We have found that basic cytoplasmic proteins of Guerin tumor are digested by intracellular proteases of the tumor (2). These findings induced the authors to study in vitro the digestion of some basic and acid proteins which differ in the composition of the aminoacids. The results have indicated that basic proteins isolated from the tumor cell cytoplasm can be a source of basic peptides which penetrate into the blood circulation.

MATERIALS AND METHODS

The Guerin epitheliomas were grown in Wistar rats. The transplantation was performed with a subcutaneous injection of tumor cells suspended in Krebs solution. Tumors grown for

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10, 20 and 30 days after transplantation were used for investigation.

The stability of lysosomes in 10% tumor homogenates (0,25 M sucrose, 1 mM EDTA, pH 7,4), to which various amounts of Triton X-100 were added, was estimated by assessing the activity of cathepsin D (3).

Purified lysosomal extract (PLE) from tumors grown for 10 days was prepared according to Sawant et al. (4). The purification of lysosomes was 20-fold on the basis of cathepsin D activity.

Degradation process of the following proteins was investigated:

a) Arginine-rich basic proteins, containing 17% arginine, prepared from the cytoplasmic fraction of Guerin tumor as described previously (5).

b) Whole histone from calf thymus, produced by Polish Chemical Reagents, Gliwice.

c) Cytoplasmic fraction of Guerin tumor cells, obtained by centrifugation of post-mitochondrial supernatant at 105.000 x g, 40 min.

d) Bovine serum albumin, Koch Light Laboratories, Ltd., England.

e) Casein produced by BDH, England.

PLE-induced degradation of proteins was investigated in 1,5 ml of medium (15 mg of protein, 0.25 mg of PLE, 250 µMoles NaCl, 1.5 µMoles EDTA at sodium acetate buffer, pH 5.0), to which 5 ml of 10% TCA was added after 30 min of incubation at 37°. The levels of α-amino nitrogen (6), arginine (7) and tyrosine with phenol reagent were determined in the supernatant.

The effect of pH on the rate of PLE-induced protein degradation was investigated in Britton-Robinson buffer.

The method of Lowry et al. (8) was used for the protein determination.

RESULTS AND DISCUSSION

Results presented in Fig. 1 indicate that six-fold increase of the cathepsin D activity in homogenates is observed with the tumor growth. Addition of Triton X-100 to the incubation medium increases the cathepsin D activity. The total activity is nearly the same in each stage of tumor growth. The addition of the Triton X-100 to the homogenates of tumor 10 days after transplantation increases cathepsin D activity by about 80%, and addition 30 days after transplantation by only 20%. It indicates that lysosomes in the initial stage of tumor growth are more stable than in the final stage of its growth. For these reasons PLE was prepared from the tumors 10 days after the transplantation of the neoplastic cells.

The degradation of various proteins by PLE is shown in Table 1. The greatest amounts of α-amino nitrogen and arginine are released from arginine-rich basic proteins isolated from cytoplasm of Guerin tumor. Considerable amounts are also liberated from calf thymus histones. Proteins of tumor cytoplasmic fraction are digested more slowly than arginine-rich

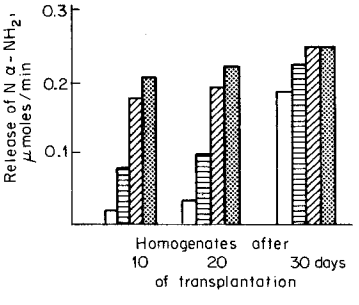

Fig. 1. The activity of cathepsin D in homogenates of Guerin tumors at various stages of the tumor growth. without Triton X-100, with Triton X-100 0.025% with Triton X-100 0.05%, with Triton X-100 0.1%.

TABLE 1

The degradation of proteins by extract of Guerin tumor lysosomes.

Protein	μMoles/mg, min		
	α-amino nitrogen	arginine	tyrosine
Preparation of arginine-rich basic protein from Guerin tumor	0.071	0.030	0.005
Whole histone from calf thymus	0.053	0.012	0.005
Proteins of cytoplasmic fraction from Guerin tumor	0.042	0.023	0.003
Bovine serum albumin	0.023	0.003	0.002
Casein	0.026	0.001	0.005

Mean values of three experiments.

basic proteins isolated from this fraction. Probably other proteins of this fraction (more acid) are less specific for lysosomal action. More acid proteins like albumin and casein are less susceptible to lysosomal action than basic proteins.

Lysosomal activity with respect to protein hydrolysis increases by about 10% after the addition of 5 mMoles dithiothreitol, indicating the involvement of sulfhydryl groups in those processes. Many proteolytic enzymes are activated by thiol reagents.

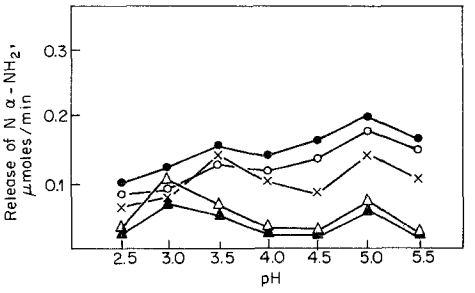

Fig. 2. The effect of pH on the hydrolysis of various proteins by lysosomal extracts from Guerin tumor, measured by the release of α-amino nitrogen. The incubation was performed using Britton-Robinson buffer at pH 2.5 to 5.5.
●—● preparation of arginine-rich basic protein from cytoplasmic fraction of Guerin tumor, ○—○ whole histone from calf thymus, ×—× cytoplasmic fraction of Guerin tumor, ▲—▲ casein, △—△ bovine serum albumin.

The degradation of basic proteins by lysosomal proteases is the highest at pH 5.0. Proteins of cytoplasmic fraction of Guerin tumor (the mixture of basic and other proteins) are digested to the same extent at pH 3.5 and 5.0. Albumin and casein (acid proteins) are decomposed best at pH 3.0 (Fig. 2). Autodegradation of whole Guerin tumor proteins is most pronounced at pH 3.5 (2).

The present results indicate that basic proteins and mainly arginine-rich proteins from Guerin tumor are degraded very easily by tumor lysosomal proteases. It is considered that this degradation may be biologically significant. On the basis of studies of Cochrame and Aikin (9), and Wood (10), it may be suggested that protein degradation products especially rich in arginine peptides change the permeability of cell tumor membranes. As a result of disturbances in the permeability, these peptides can penetrate into the blood stream. We have found that low molecular peptides rich in arginine appear in the blood stream of rats bearing Guerin tumor at the final stage of its growth (11).

REFERENCES

1. Davies, P., Krakauer, K. and Weissmann, G. (1972) Anal. Biochem. 45, 428-440.

2. Farbiszewski, R., Worowski, K. and Rzeczycki, W. (1975) Bull. Acad. Polon. Sci., ser. sci. biol. 22, 829-836

3. Anson, M.L. (1937). J. Gen. Physiol. 20, 565-574.

4. Sawant, P.L., Shibko, S., Kumta, U.S. and Tappel, A.L. (1964) Biochim. Biophys. Acta. 85, 82-92

5. Farbiszewski, R. and Rzeczycki, W. (1974) Neoplasma 21, 363-366.

6. Moore, S. and Stein, W.H. (1948) J. Biol. Chem. 176, 367.

7. Farbiszewski, R., Worowski, K. and Rzeczycki, W. (1972) Chem. Anal. 17, 133-137.

8. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R. (1951) J. Biol. Chem. 193, 265-275.

9. Cochrame, C.G. and Aikin B.S. (1966) J. Exp. Med. 124, 733-752

10. Wood, S., (1973) In: The microcirculation in clinical medicine, pp. 275-287, Academic Press, New York, London.

11. Farbiszewski, R. Worowski, K. and Rzeczycki, W. (1971) Neoplasma 18, 265-269.