Mitochondrial and Nuclear Glucocorticoid-Sensitive Alkaline Proteases in Thymocytes

S. I. Ogurtsov, A. S. Dukhanin, and A. A. Temnov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 132, No. 7, pp. 23-25, July, 2001
Original article submitted March 6, 2001

Glucocorticoid-sensitive alkaline proteases are localized in the nuclear and mitochondrial fractions of rat thymocytes. Dexamethasone load increased alkaline protease activity in the nuclear fraction and decreased or even eliminated it in the mitochondrial fraction. The inhibitory analysis showed that SH groups and disulfide bonds play a crucial role in the functioning of glucocorticoid-activated nuclear alkaline protease. Hence, this enzyme can be assigned to as cysteine proteases. Mitochondrial alkaline protease is a serine hydrolase, although it does not belong to the class of trypsin- or chymotrypsin-like enzymes. The role of alkaline proteases in apoptotic death of thymocytes is discussed.

Key Words: *alkaline proteases; glucocorticoids; apoptosis; thymocytes*

As differentiated from necrosis, apoptosis, or programmed cell death, is a component of physiological regulation of homeostasis. Apoptosis mediates the death of neutrophils, short-living cells, elimination of autoreactive T lymphocytes, involution of cells deprived of growth factors, *etc.* [4].

Apoptosis is also involved in the thymolytic effects of glucocorticoid preparations. Intracellular mechanisms of apoptosis include activation of proteases inducing irreversible changes in the nuclear and mitochondrial membranes [8].

Our previous experiments showed that glucocorticoids increase alkaline protease (AP) activity in the thymus [2,3]. SH groups and serine hydroxyl groups play an important role in the functioning of thymic AP [1]. However, localization of these glucocorticoid-sensitive enzymes remains unclear.

Here we studied intracellular localization and properties of glucocorticoid-sensitive thymic AP.

MATERIALS AND METHODS

Experiments were performed on male albino rats. The animals were intraperitoneally injected with dexamethasone-21-sodium phosphate (0.5 ml, 2 mg/kg) 24 h before euthanasia. The rats were decapitated under light ether anesthesia. AP activity in the homogenate and subcellular fractions was estimated by azocasein hydrolysis [2].

5,5-Dithio-bis(2-nitrobenzoic acid), L-5-amino-1-(p-toluenesulfonylamide)-2-phenylchloromethyl ketone (TACK), L-1-(p-toluenesulfonylamide)-2-phenylethyl chloromethyl ketone (TPCK), and phenylmethylsulfonyl fluoride were dissolved in 0.05 M Tris-HCl buffer (pH 8.5) containing dimethylsulfoxide (final concentration 8%).

p-Chloromercuribenzoate was prepared in 0.05 M Tris-HCl buffer (pH 8.5).

RESULTS

Disintegration of the thymus into thymocytes and stroma and measurements of AP activity showed that 94% total AP activity was localized in the thymocyte fraction.

Calculation of specific AP activity per weight of isolated thymocytes and homogenate showed that 85% AP activity of the homogenate was localized in thymocytes. Therefore, subcellular fractions for further experiments were isolated from thymocyte suspension.

Russian State Medical University, Moscow

0007-4888/01/1321-0627$25.00

For elucidation of the intracellular localization of AP, enzyme activity was measured in the nuclear, mitochondrial, microsomal, and cytosolic fractions of thymocytes from adrenalectomized, intact, and dexamethasone-treated (2 mg/kg) rats. In all animals AP activity was detected only in the nuclear and mitochondrial fractions (Fig. 1).

In animals treated with dexamethasone (2 mg/kg) specific AP activity in the nuclear fraction gradually increased up to 12 h postinjection and sharply increased 24 h after treatment (Fig. 2). Specific enzyme activity in the mitochondrial fraction progressively decreased and disappeared 24 h after treatment.

Dexamethasone in doses of 1.0 and 2.0 mg/kg increased specific AP activity in the nuclear fraction of thymocytes. Enzyme activity of the mitochondrial fraction underwent opposite changes. No AP activity was detected in the mitochondrial fraction from animals receiving dexamethasone in a dose of 1 mg/kg or higher.

Thus, in animals receiving various doses of glucocorticoids AP was localized only in the nuclear and mitochondrial fractions. However, the distribution of AP depended on the degree of dexamethasone load. Increasing the dose of dexamethasone led to transition of AP activity into the nuclear fraction and its disappearance in mitochondria.

TABLE 1. Effects of Reagents on AP Activity (% of Control) in Nuclear and Mitochondrial Fractions of Thymocytes

Reagents, final concentration	Activity	
	nuclei	mitochondria
Triton X-100, %		
1	53*	42*
0.1	91	91
0.01	95	103
5,5-Dithio-bis(2-nitrobenzoic acid), mM		
10	36*	101
5	33*	91
1	21*	94
p-Chloromercuribenzoate, mM		
10	18*	89
5	16*	97
1	19*	105
TACK, 10 mM	101	94
TCPK, 10 mM	95	97
Phenylmethylsulfonyl fluoride, 10 mM	89	19*

Note. *$p<0.05$ compared to the control.

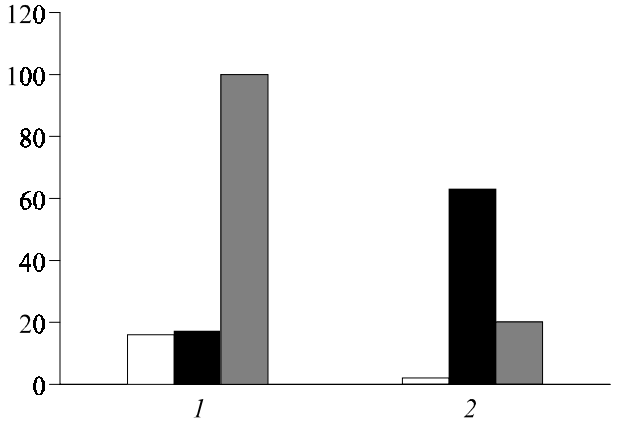

Fig. 1. Alkaline protease activity in thymocyte nuclei (*1*) and mitochondria (2) from adrenalectomized (light bars), control (dark bars), and dexamethasone-treated animals (2 mg/kg, shaded bars).

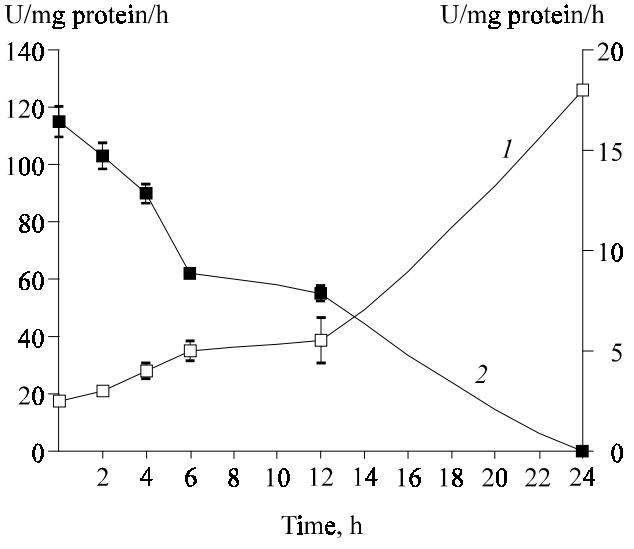

Fig. 2. Changes in alkaline proteases activity in nuclear (*1*, left ordinate) and mitochondrial fractions (*2*, right ordinate) caused by 2 mg/kg dexamethasone.

It is unlikely that the observed phenomenon is related to "true" transition of enzyme activity. First, AP activity in the mitochondrial fraction is insufficient to provide such an increase in enzyme activity in the nuclear fraction. Second, in animals receiving 1 and 2 mg/kg dexamethasone, enzyme activity was undetected in the mitochondrial fraction, but sharply increased in the nuclear fraction.

The inhibitory assay showed that Triton X-100 in concentrations of 0.1% and lower had no effect on AP activity in the studied fractions (Table 1). Thus, these enzymes are not bound to membranes. The role of cysteine residues in AP activity was evaluated by azocasein hydrolysis in the presence of sulfhydryl p-chloromercuribenzoate and disulfide 5,5-dithio-bis (2-nitrobenzoic acid). Both agents markedly decreased AP activity in the nuclear fraction, but had no effect on

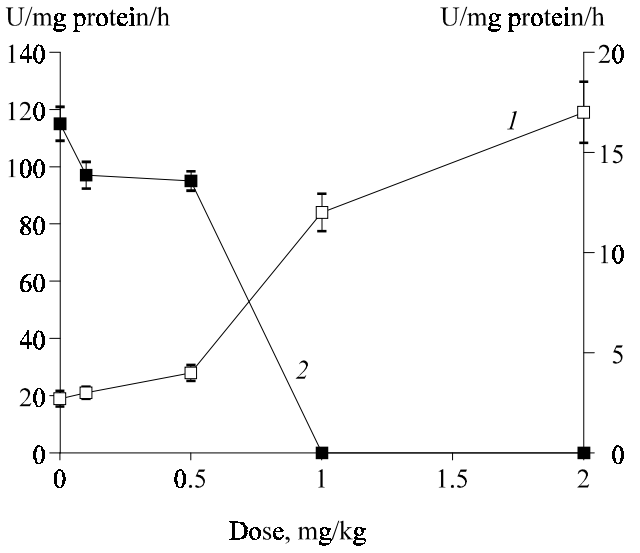

Fig. 3. Alkaline protease activities in the nuclear (*1*, left ordinate) and mitochondrial fractions (*2*, right ordinate) 24 h after administration of dexamethasone in various doses.

this parameter in mitochondria (Table 1). Trypsin and chymotrypsin inhibitors (TACK and TCPK, respectively) did not change the rate of azocasein hydrolysis, while serine protease inhibitor phenylmethylsulfonyl fluoride inhibited this reaction in the mitochondrial fraction (Table 1).

Thus, thiol groups play an important role in the functioning of glucocorticoid-activated nuclear AP. AP activity in the nuclear fraction is determined by nativity of SH groups and disulfide bonds, hence this enzyme can be assigned to as the cysteine protease family. Mitochondrial AP is a serine hydrolase not belonging to the class of trypsin- or chymotrypsin-like enzymes.

Our results are consistent with the current concept of apoptosis. According to this concept activation of nuclear cysteine proteases induced by mitochondrial cytochrome *c* is a key biochemical event of apoptosis [6]. It is hypothesized that this process leads to irreversible changes resulting in cell shrinkage, chromatin condensation, and formation of apoptotic bodies. Dead cells are rapidly phagocytized by macrophages or adjacent cells without inducing inflammatory response [7]. Processing of the cytochrome *c* precursor is performed by mitochondrial protease [5].

Our findings indicate that AP activity in rats with different glucocorticoid status is localized in the nuclear and mitochondrial fractions of thymocytes. After treatment with synthetic glucocorticoid dexamethasone the specific AP activity increases in nuclei, but decreases in mitochondria. Nuclear and mitochondrial AP differ in their properties. Nuclear AP is a cysteine protease, while mitochondrial AP belongs to the serine hydrolase family.

REFERENCES

1. S. I. Ogurtsov, A. S. Dukhanin, and S. Yu. Mitroshina, *Byull. Eksp. Biol. Med*., **115**, No. 2, 162-163 (1993).
2. S. I. Ogurtsov, A. S. Dukhanin, and P. V. Sergeev, *Farmakol. Toksikol*., **54**, No. 2, 52-54 (1991).
3. P. V. Sergeev, S. I. Ogurtsov, and A. S. Dukhanin, *Byull. Eksp. Biol. Med*., **114**, No. 11, 489-490 (1992).
4. A. A. Yarilin, *Immunologiya*, No. 6, 10-23 (1996).
5. I. D. Bowen and S. M. Bowen, *Programmed Cell Death in Tumors and Tissues*, London (1990), pp. 4-45.
6. B. Fadeel, S. Orrenius, and B. Zhivotovsky, *Biochem. Biophys. Res. Commun*., **266**, 699-717 (1999).
7. V. A. Fadok, D. L. Bratton, S. C. Frasch, *et al*., *Cell Death Differ*., **5**, No. 7, 551-562 (1998).
8. C. Stefanelli, *Biochem. J*., **322**, Pt. 3, 909-917 (1997).