PROPERTIES OF GLUCOCORTICOID-SENSITIVE ALKALINE PROTEINASES IN RAT TARGET ORGANS

S. I. Ogurtsov, A. S. Dukhanin, and S. Yu. Mitroshina

UDC 615.357:577.175.53].015.4.065.076.9

KEY WORDS: dexamethasone; alkaline proteinases; thymus; liver

Drugs with glucocorticoid activity, when used in medical practice, may give rise to complications such as inhibition of the immune system, atrophy of skeletal muscles, and degeneration of connective tissue [3, 4, 7]. These side effects are caused by increased protein breakdown. However, the mechanisms of the catabolic effects of glucocorticoids and their synthetic analogs are unknown, nor have the causes of the anabolic action of this group of steroids on liver cells been studied [1].

It has recently been shown that glucocorticoid-sensitive cells contain alkaline proteinases (AlP), which are proteolytic enzymes with pH optimum in the alkaline region [8, 10-12]. Administration of glucocorticoids in vivo increases the AlP activity in the thymus [2] and skeletal muscles [5, 6].

However, properties of glucocorticoid-sensitive AlP have received little study. Particular features of AlP, activated by glucocorticoids, in skeletal muscles have been described to some extent [9, 13].

This paper gives preliminary characteristics of AlP of the thymus and liver induced in rats by dexamethasone-21-sodium phosphate.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats, which were given an intraperitoneal injection of 0.5 ml of a solution of dexamethasone-21-sodium phosphate (DMSP) in a dose of 2 mg/kg 24 h before sacrifice. Decapitation was carried out under superficial ether anesthesia. The thymus and liver were removed and kept in the cold. AlP activity in tissue homogenates was determined as the degree of hydrolysis of azocasein [2].

5,5′-dithio-*bis*-(2-nitrobenzoic) (DTNB), L-5-amino-1-(*p*-toluenesulfonyl) amidopentylchloromethyl-ketone (TACK), L-1-(*p*-toluenesulfonyl)amido-2-phenylethylchloromethylketone (TPCK), and phenylmethylsulfonyl fluoride (PMSF) were dissolved in 0.05 M Tris-HCl, pH 8.5, with the addition of dimethylsulfoxide (final concentration 8%). Solutions of *p*-chloromercuribenzoate (PCMB), ATP, EDTA, and EGTA were prepared in 0.05 M Tris-HCl buffer, pH 8.5, and the salts were dissolved in distilled water.

EXPERIMENTAL RESULTS

The study of the effect of temperature on the degree of hydrolysis of azocasein showed that in thymus homogenates AlP activity between temperatures of 37 and 60°C rose steadily, to reach 238% of the control value (Fig. 1). In liver homogenates the change in enzyme activity was not so great and a maximum was reached at 50°C. At 70°C AlP activity in the thymus and liver did not differ significantly from the initial values. Consequently, AlP of the thymus and liver are thermostable enzymes.

Department of Molecular Pharmacology and Radiobiology, Russian National Medical University, Moscow.
(Presented by Academician of the Russian Academy of Medical Sciences P. V. Sergeev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 12, pp. 615-617, December, 1992. Original article submitted May 27, 1992.

0007-4888/92/0012-1820$12.50

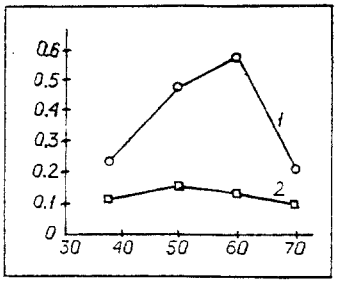

Fig. 1. Effect of temperature on proteolytic activity of homogenates of rat thymus (1) and liver (2), induced by dexamethasone (2 mg/kg). Abscissa, temperature (in °C); ordinate, optical density at wavelength of 366 nm (in relative units). Relative error of determination of enzyme activity does not exceed 3%.

TABLE 1. Effect of Reagents on AlP Activity in Rat Thymus and Liver

Reagent	Final concentration	Activity, in percent of control	
		thymus	liver
Triton X-100	1 %	41	33
	0,1 %	93	91
	0,01 %	96	115
DTNB	10 mM	34	5
	5 mM	30	4
	1 mM	22	4
PCMB	10 mM	17	14
	5 mM	15	14
	1 mM	20	3
TACk	10 mM	94	74
TPCK	10 mM	92	116
PMSF	10 mM	32	90
ε-ACA	40 mM	94	06
Contrykal	500 ED	98	98
ATP	10 mM	114	82
	5 mM	104	98
	1 mM	105	85
$CaCl_2$	10 mM	128	08
$MgCl_2$	10 mM	120	94
$FeSO_4$	10 mM	197	374
	1 mM	102	179
	0,1 mM	104	86
$FeCl_3$	10 mM	60	33
$CuSO_4$	10 mM	9	5
EDTA	10 mM	78	52
	5 mM	82	60
	1 mM	76	68
EGTA	10 mM	6	14
	5 mM	42	20
	1 mM	92	44

Triton X-100, in concentrations below 1%, had virtually no effect on AlP activity in the thymus, but in a concentration of 0.01% it caused a small (not significant) increase in its value in the liver (Table 1). High concentrations of the detergent significantly lowered AlP activity in homogenates of both thymus and liver.

To study the role of active cysteine residues in the functioning of AlP, hydrolysis of azocasein was carried out in the presence of sulfhydryl and disulfide reagents. In thymus homogenates DTNB inhibited AlP activity within the range of concentrations tested, and this effect was a function of concentration. In the liver, DTNB in all concentrations suppressed AlP activity virtually completely. PCMB significantly inhibited proteolytic activity in thymus and liver homogenates (Table 1).

Since PCMB is an organomercury compound and can react with one SH-group with the formation of mercaptides, whereas DTNB (Ellman's reagent) is a disulfide and takes part in a thiol-disulfide exchange reaction, the experimental results indicate that thiol groups play an essential role in the function of AlP in the liver and thymus; in the liver, moreover, the native nature of the SH-groups and disulfide bonds largely determines the active state of the enzyme.

To evaluate the participation of serine residues in AlP function in the thymus and liver we studied the effect of TACK (a trypsin inhibitor), TPCK (a chymotrypsin inhibitor), and PMSF (a serine protease inhibitor) on the activity of the systems studied.

In the thymus TACK and TPCK had virtually no effect on proteinase activity whereas PMSF inhibited AlP by 68%. Consequently, AlP of the thymus may belong to the class of serine proteases, although it does not possess trypsinlike or chymotrypsinlike activity. In our experiments, serine reagents did not significantly change AlP activity in liver homogenates.

Inhibitors of proteolytic enzymes, used in clinical practice, namely Contrykal and ε-aminocaproic acid (ε-ACA), in concentrations of 500 U and 40 mM respectively, had no action on AlP activity in the test system.

Addition of ATP within the concentration range indicated in Table 1, did not significantly change AlP activity.

AlP activity depends only a little on the presence of Ca^{2+} and Mg^{2+} ions in the medium. In liver homogenates the addition of these ions did not cause significant changes in enzyme activity, but in thymus homogenates the increase observed did not exceed 30% of the control value.

Significant activation of AlP in the thymus and liver was observed on the addition of Fe^{2+} ions to the reaction mixture; the effect, moreover, was dependent on concentration. Meanwhile Fe^{3+} ions significantly depress AlP activity in the thymus. The shift of equilibrium between the oxidized and reduced forms of ions of metals can be regarded as a regulatory mechanism for the change in AlP activity.

Cu^{2+} ions completely inhibited activity of the enzymes, confirming the data given above on the role of free SH-groups in AlP function.

The chelating compounds EDTA and EGTA lower AlP activity in the thymus and liver; the inhibitory action of EGTA, moreover, was stronger and depended on the concentration of the complex. This action of the selective Ca^{2+}-binding compound is difficult to explain. Perhaps EGTA binds other bivalent ions whose action on the activity of proteinase systems was not studied more strongly. It can be concluded that the glucocorticoid-sensitive AlP of the thymus and liver are not ATP-dependent, or at least, they mainly are not bound with thermostable cell membrane enzymes, in whose function an important role is played by thiol groups. However, AlP of the thymus and liver are not completely identical. They differ in the degree of involvement of serine residues in hydrolysis of peptide bonds and in their sensitivity to the action of bivalent ions.

REFERENCES

1. P. P. Golikov, Receptor Mechanisms of the Glucocorticoid Effect [in Russian], Moscow (1983), pp. 107-142.
2. S. I. Ogurtsov, A. S. Dukhanin, P. V. Sergeev, and A. A. Eiyubova, Farmakol. Toksikol., **54**, No. 2, 52 (1991).
3. P. V. Sergeev and M. V. Nezhentsev, Farmakol. Toksikol., **43**, No. 6, 737 (1981).
4. P. V. Sergeev, T. G. Pukhal'skaya, and V. P. Bol'shev, Farmakol. Toksikol., **45**, No. 2, 120 (1983).
5. T. P. Sööne, K. P. Alev, and A. J. Pöhme, Uch. Zapiski (Tartu), **702**, No. 7, 81 (1985).
6. T. P. Sööne and K. P. Alev, Byull. Éksp. Biol. Med., **102**, No. 11, 557 (1986).
7. N. S. Chermnykh, Farmakol. Toksikol., **42**, No. 1, 109 (1980).
8. A. Asghar. Adv. Food Res., **31**, 343 (1987).
9. T. Edmunds and R. J. T. Pennington, Biochim. Biophys. Acta, **661**, No. 1, 28 (1981).
10. D. Holmes, M. E. Parsons, D. C. Park, and R. Pennington, Biochem. J., **125**, No. 4, 981 (1971).
11. R. Hough, G. Pratt, and M. Reshsteiner, J. Biol. Chem., **262**, 8303 (1987).
12. I. Ito, K. Kitamura, et al., Biochim. Biophys. Acta, **661**, No. 2, 267 (1981).
13. M. Mayer, R. Amin, and E. Shafrir, Arch. Biochem., **161**, No. 1, 20 (1974).