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The Properties of Glucocorticoid-Sensitive Alkaline Proteinases of Rat Target Organs

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When used in clinical treatment, pharmacological agents with glucocorticoid-like activity cause such complications as immunodepression, myatrophy, and connective tissue degeneration [3,4,7]. These side effects result from aggravated protein degradation. However, the mechanisms whereby glucocorticoids and their synthetic analogs exert their catabolic or, in the liver cells, anabolic effects are still unknown [1].

The glucocorticoid-sensitive cells have been recently shown to possess alkaline proteinases (AP), proteolytic enzymes with the activity optimum within the alkaline range of pH values [8,10-12]. Glucocorticoid administration *in vivo* enhances AP activity in the thymus [2] and skeletal muscles [5,6].

However, the properties of glucocorticoid-sensitive AP are poorly understood. The peculiarities of glucocorticoid-activated AP in the skeletal muscles have been partially described [9,13].

In the present study the preliminary characteristics of rat thymus and liver AP induced by dexamethasone-21-Na-phosphate were obtained.

MATERIALS AND METHODS

Unbred albino male rats were used for the experiments. The rats received 2 mg/kg dexamethasone in 0.5 ml intraperitoneal injection 24 h before decapitation. Decapitation was carried out under light ether narcosis. The thymus and liver were removed and placed on ice. AP activity in the tissue homogenates was determined by measuring the rate of azocasein hydrolysis [2].

5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), L-5-amino-1-p-toluenesulfonyl amidopentylchloromethaneketone (TACK), L-1-(p-toluenesulfonyl)amido-2-phenylethanechloromethaneketone (TPCK), and phenylmethanesulfonyl fluoride (PMSF) were dis-

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TABLE 1. Effect of Reagents on Thymus and Liver AP Activity

Reagent	Final concentration	Activity, % of control	
		thymus	liver
Triton X−100	1%	41	33
	0.1%	93	91
	0.01%	90	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	100
Contrykal	500 IU	98	98
ATP	10 mM	114	82
	5 mM	104	98
	1 mM	105	85
$CaCl_2$	10 mM	128	108
$MgCl_2$	10 mM	120	94
$FeSO_4$	10 mM	197	374
	1 mM	102	179
	0.1 mM	104	86
$FeCl_3$	10 mM	60	83
$CuSO_4$	10 mM	9	5
EDTA	10 mM	78	62
	5 mM	82	60
	1 mM	76	68
EGTA	10 mM	6	14
	5 mM	42	20
	1 mM	92	44

solved in 0.05 M Tris-HCl buffer (pH 8.5) supplemented with dimethyl sulfoxide (DMSO) (final concentration 8%). The solutions of p-4-(chloromercuri)benzoate (PCMB), ATP, EDTA, and EGTA were prepared in 0.05 M Tris-HCl buffer (pH 8.5); the salts were dissolved in distilled water.

RESULTS

The investigation of the temperature dependence of the azocasein hydrolysis rate reveals that AP activity in the thymus homogenates gradually increases over a temperature range from 37°C to 60°C up to 238% compared to the control. In the liver homogenates enzyme activity changes are less pronounced,attaining the maximum at 50°C. At 70°C AP activity in the thymus and liver homogenates differs insignificantly from the initial level. Thus, the thymus and liver AP are thermostable enzymes.

Triton X-100 in a concentration of less than 1% practically does not affect AP activity in the thymus but, in a concentration of 0.01%, slightly increases it in the liver (Table 1). High detergent concentrations significantly reduce AP activity in both the thymus and liver homogenates.

To investigate the role of AP active cysteine residues, the hydrolysis of azocasein was carried out in the presence of sulfhydryl and disulfide reagents. In the thymus homogenates DTNB within the range of concentrations studied inhibits AP activity in a dose-dependent manner. In the liver AP activity is almost completely blocked by any of the DTNB concentrations studied. PCMB markedly inhibits proteolytic activity in both the thymus and liver homogenates (Table 1).

Since PCMB is an organomercuric compound that can react with one SH-group to form mercaptides; and DTNB (Ellman's reagent) is a disulfide involved in thiol-desulfide exchange, the results of the experiments described above indicate that SH-groups play an important role in AP function in the organs studied, in the liver the nativeness of SH-groups being more essential for enzyme activity.

To assess the involvement of serine residues in the thymus and liver AP function the effects of TACK (trypsin inhibitor), TPCK (chymotrypsin inhibitor) and PMSF (serine protease inhibitor) on the activity of the studied systems were investigated.

In the thymus proteinase activity is practically unaffected by TACK and TPCK, whereas PMSF causes 68% inhibition of AP activity. Consequently, the thymus AP may be considered as a serine

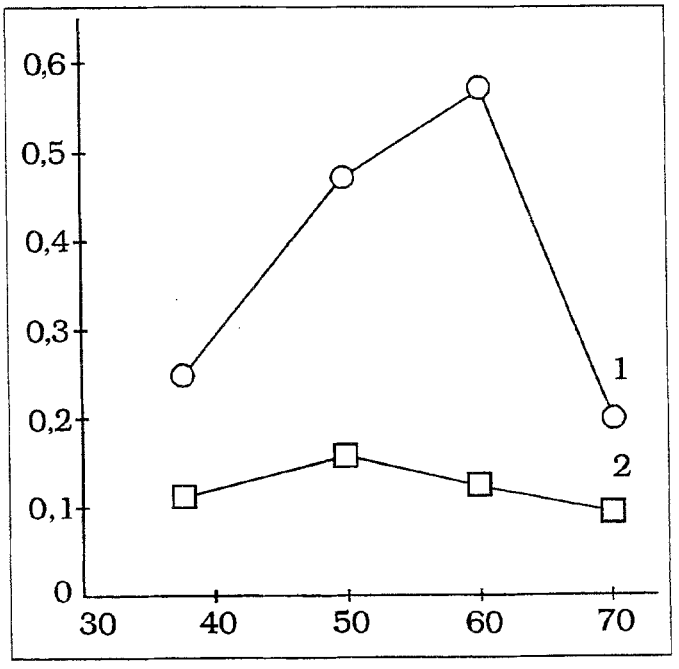

Fig.1. Temperature dependence of proteolytic activity of dexamethasone−induced rat thymus (*1*) and liver (*2*) homo−genates. On the abscissa: temperature (°C), on the ordinate: optical density at wavelength 366 nm (rel.units). The relative error of enzyme activity estimation is not more than 3%.

proteinase, although it does not possess trypsin- or chymotrypsin-like activity. In our experiments AP activity in the liver was not significantly affected by serine reagents.

The inhibitors of proteolytic enzymes used in clinical treatment, contrykal and e-aminocaproic acid (ε-ACA), in a concentration of 500 IU and 40 mM, respectively, have no effect on AP activity in the studied system.

The addition of ATP does not change AP activity reliably in the concentrations shown in Table 1.

AP activity depends little on the presence of Ca^{2+} and Mg^{2+} in the medium. When added, these ions have no significant effect on enzyme activity in the liver homogenates and cause less than 30% AP activation in the thymus homogenates as compared to the control.

A considerable dose-dependent AP activation is detected in the thymus and liver for the addition of Fe^{2+} to the reaction mixture. On the other hand, Fe^{3+} significantly decreases AP activity in the thymus. The shift of equilibrium between the oxidized and reduced forms of the metal ions can be considered to be one of the mechanisms of AP activity regulation.

Cu^{2+} ions completely inhibit the enzymes, which confirms the data mentioned above concerning the role of free SH-groups in AP function.

The chelating agents EDTA and EGTA decreased the thymus and liver AP activity, with the effect of EGTA being more pronounced and dependent on the complexone concentrations. This effect of the selective Ca^{2+}-binding compound is difficult to explain. EGTA seems to bind more strongly other bivalent ions, the effect of which on proteinase activity was not investigated in the present study. It may be concluded that the glucocorticoid-sensitive thymus and liver AP are ATP-independent thermostable enzymes, which, at least for the most part, are not connected with the cell membranes; SH-groups play an important role in their function. Howerever, the thymus and liver AP are not completly identical. They differ from each other in the extent of involvement of the serine residues in the hydrolysis of peptide bonds and in their sensitivity to bivalent ions.

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