COMPARISON OF LYSOSOMAL PROTEINASE ACTIVITY IN CONTINUOUS CELL CULTURES SUSCEPTIBLE AND RESISTANT TO A CERTAIN GROUP OF VIRUSES

V. A. Tutel'yan, A. V. Vasil'ev, and G. P. Sovetova

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The protective function of lysosomes, predicted at the end of last century by Mechnikov, is reflected in their participation in phagocytosis and subsequent degradation of microorganisms and viruses. The lysosomes of practically any cell of the body can participate both in the destruction of foreign agents and in their biological inactivation, but cells which are functionally specialized in this respect are distinguished by a specific set of lysosomal enzymes [5]. Meanwhile investigation of the principles governing the manifestation of acquired resistance to viral infection at the cellular level shows that each cell, irrespective of the functions it performs in the body, is capable of resisting virus infection under the influence of definite factors [2, 4].

On the basis of previous investigations it was suggested that an important role in the mechanism of resistance may be played by the lysosomal system of the cell; the aim of the present investigation was accordingly to study activity of the group of lysosomal proteolytic enzymes, differing in the character of their action, in cell cultures of "normal" and virus-resistant lines [3].

EXPERIMENTAL METHOD

Four models of cell cultures were used in the experiments: "normal" lines – a continuous cell line of mouse origin (L_{929}), susceptible to vesicular stomatitis virus, and a culture of HeLa cells of human origin, with high susceptibility to Coxsackie group B viruses, and the corresponding virus-resistant clones L_R and HeLa-R. Virus-resistant clones L_R and HeLa-R were obtained by injecting subtoxic doses of diphtheria toxoid (strain Park-Williams, 28 Lf/ml) into growing monolayers of L_{929} cells (2-day culture) and HeLa cells (3-day culture) in dilutions of 1:4 and 1:512, respectively. The L_{929} cell culture was treated 3 times, and the HeLa culture once with the above doses of diphtheria toxoid. The L_R cell culture is currently at the 85th passage, and the HeLa-R cell culture at the 115th passage with no decrease in their degree of resistance to the corresponding viruses.

Homogenates of the cell cultures were prepared in a suspension medium consisting of 0.25 M sucrose (pH 7.4) with 1 mM EDTA (90 sec, 1200 rpm, 0-2°C). The protein content of the homogenates was determined by Lowry's method [8] and activity of the four lysosomal proteinases, cathepsins A, B_1, D, and C, also was assayed. Activity of cathepsins A and D was determined spectrophotometrically [6], in the writers' modification, using carboxybenzoyl-Glu-Tyr and hemoglobin respectively as the substrates. Activity of cathepsins B_1 and C was determined spectrofluorometrically [9], using benzoyl-DL-arginine-β-naphthylamide and glycyl-L-phenylalanine-β-naphthylamide respectively as the substrates.

EXPERIMENTAL RESULTS

Changes in enzyme activity in the cell cultures at different pH values were studied in one series of experiments. As Fig. 1 shows, the pH optima for cathepsins A, D, and B_1 had similar values in different cell cultures for each enzyme. The pH optimum for cathepsin A was identical in value (pH 5.0) in all cases. Cathepsin D in the "normal" culture of HeLa cells exhibited maximal activity at pH 2.5, and in the other cultures tested at pH 3.0. Equally slight differences in the pH-optimum were discovered for cathepsin B_1 also. The pH-optimum for this enzyme in the HeLa cell culture was 4.0, in the HeLa-R culture 4.2, the L_{929} cell culture 3.6, and the L_R cell culture at pH 4.2.

Attention is drawn to the sharply different pH optimum of cathepsin C in HeLa and HeLa-R cell cultures and the pH-optimum of this same enzyme in L_{929} and L_R mouse fibroblast cultures. Whereas in HeLa and HeLa-R cultures the pH optimum for cathepsin C lay in the 4.0 region, in L_{929} and L_R cultures it lay in the neutral zone (7.0). A characteristic property of cathepsin C, which distinguishes it from the group of known lysosomal proteinases, is its ability to act as an

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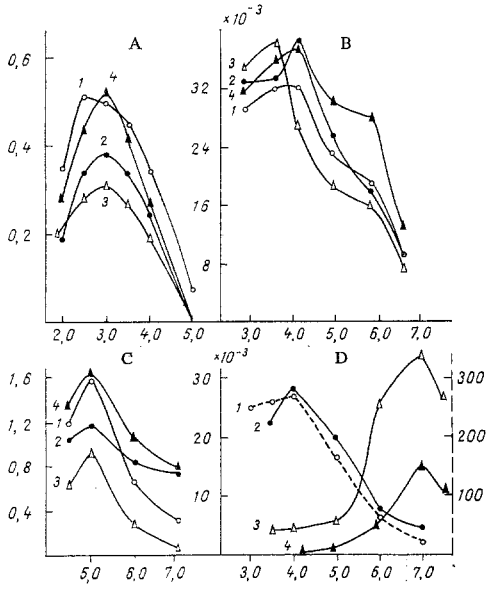

Fig. 1

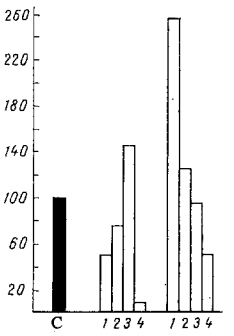

Fig. 2

Fig. 1. Effect of pH on lysosomal proteinase activity in cell cultures of different origin. Abscissa, pH: ordinate, activity of enzymes (in μmoles/min/g protein): A) cathepsin D, B) cathepsin B_1, C) cathepsin A, D) cathepsin C. Cell cultures: 1) HeLa, 2) HeLa-R, 3) L_{929}, 4) L_R.

Fig. 2. Activity of cathepsins in cultures of virus-resistant HeLa-R and L_R cells. Ordinate, enzyme activity (in % of control). C) Control (enzyme activity in normal lines), 1) cathepsin A, 2) cathepsin D, 3) cathepsin B_1, 4) cathepsin C.

active transferase, catalyzing the transpeptidation reaction by transferring dipeptidyl residues to an acceptor containing an amino group. In the acid pH zone the predominant reaction is hydrolysis of the substrate, whereas at pH 7.0-8.0, the transpeptidation reaction becomes predominant [7]. The possibility cannot be ruled out that activity of the enzyme in a fibroblast culture is mainly transferase in character, and its hydrolytic activity exists only in traces.

Meanwhile differences also were found in cathepsin activity in cell cultures which differed in susceptibility to virus infection. The HeLa-R cell culture was characterized by a decrease in activity of cathepsins A and D by 50 and 26%, and of cathepsin C by 92%, whereas cathepsin B_1 activity was increased by 45% compared with the level of activity of these enzymes in the normal cell culture.

In turn, cells of the L_R culture differed from the normal L_{929} line in their increased activity of cathepsins A and D (by 155 and 25%, respectively). Activity of cathepsin C in the L_R cell culture was reduced by 50%, whereas activity of cathepsin B_1 was the same in cultures of L_{929} and L_R cells. The groups of cells studied were characterized by changes in activity of other metabolic systems. It was shown previously that the HeLa-R culture is distinguished by a lower intensity of protein and RNA synthesis, whereas in the L_R culture protein and RNA synthesis are more active than in intact cultures of HeLa and L_{929} cells [1]. These results correlate with changes in activity of cathepsins A and D, reflecting to a certain degree the intensity of protein catabolism in the cell.

Genetic changes in the cells, leading to their resistance to individual groups of viruses, thus give rise to changes in lysosomal proteinase activity. It can be tentatively suggested that in the case of HeLa cells the increase in endopeptidase activity of cathepsin B_1 is one cause of the loss of biological activity of the infecting virus, whereas in resistant cells of the fibroblast culture death of the virus may be partly attributable to the synergic action of cathepsins A and D. It should be noted that a common feature of the virus-resistant cell cultures was a sharp decrease in their cathepsin C activity. The results point to different degrees of participation of the cathepsins in the intracellular digestion of foreign material during realization of the protective function of the lysosomes.

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EFFECT OF COLD STRESS ON CONTENT AND ACTIVITY OF MICROSOMAL CYTOCHROME P-450 IN RAT LIVER

L. I. Deev, M. Ya. Akhalaya,
and Yu. B. Kudryashov

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Under the influence of various stressors long-term changes in enzyme activity in the blood and digestive system take place in living organisms [2, 11]. The effect of stressor agents on enzymes located in the endoplasmic reticulum of the animal liver has received less study. It has been shown that in acute (pain) stress the content of microsomal cytochrome P-450 – an enzyme playing an important role in detoxication and metabolism of a wide range of endogenous compounds and xenobiotics [8] – in the rat liver falls sharply. Data on the formation of toxic metabolites in animal tissues during stress have been reported in the literature [1, 4, 5]. The study of the effect of various stressor agents on the level and activity of enzymes of the endoplasmic reticulum of the animal liver is accordingly very interesting.

This paper describes a study of possible changes in the content of microsomal cytochromes P-450 and b_5 and in the aminopyrine-demethylase activity of the liver microsomes of rats after short-term cooling of the animals. To elucidate one of the possible mechanisms of the changes discovered, the effect of cold stress was studied on the ratio between the forms of cytochrome P-450 which differ in their resistance to the destructive action of linoleic acid hydroperoxides.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-180 g were used. Ice baths (0°C) lasting 5 min were used as the stressor agent [7]. The microsomal fraction of the liver was isolated by gel filtration on Sepharose 2B [15]. Protein was determined by Lowry's method. The content of cytochromes P-450 and b_5 in the microsomes was determined spectrophotometrically [13, 14], using extinction coefficients of $E_{450-500} = 91$ $mM^{-1}\,cm^{-1}$ for cytochrome P-450 and $E_{424-409} = 185$ $mM^{-1}\,cm^{-1}$ for cytochrome b_5. The ratio between the two forms of cytochrome P-450, differing in their resistance to linoleic acid hydroperoxides, was determined by the method described previously [10].

The demethylase activity of rat liver microsomes was estimated from the rate of formaldehyde formation during demethylation of aminopyrine. The incubation mixture contained, in a volume of 1.3 ml, 2 mg/ml of microsomal protein, 0.1 M Na,K-phosphate buffer (pH 7.5), 5 mM $MgCl_2$, 3 mM NADPH, and 10 mM aminopyrene. After incubation for 10 min at 37°C the quantity of formaldehyde formed was determined [12].

The experimental results were subjected to statistical analysis. The significance of differences was estimated by Student's t-test.

EXPERIMENTAL RESULTS

The results showed that cold stress causes phasic changes in the content of cytochromes P-450 and b_5 in rat liver microsomes (Fig. 1). The levels of cytochromes P-450 and b_5, which normally are 0.768 ± 0.033 and 0.304 ± 0.016 nmole/mg protein, respectively, remained virtually unchanged during the first 4 h after stress, although a tendency was observed for the content of both cytochromes to increase.

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