GP, GR, and GDAR activity is considerably depressed in the bone marrow, specific activity of GP was found to be reduced in the liver only on the 3rd day of CRD, and GR and GDAR activity was increased on the 7th day after CRD. Exposure to irradiation or mechanical trauma separately does not lead to any significant change in activity of the enzymes studied in the liver, unlike in the bone marrow (data not given).

The comparatively low power of the enzyme system protecting the cell against free-radical damage in the bone marrow and its lability (Figs. 1-3) form the pathochemical basis of selective radiosensitivity of the bone marrow and the radioprotective action of preparations of SOD [13, 14] and exogenous glutathione [9].

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INACTIVATION OF PLASMA α_1-PROTEINASE INHIBITOR BY TWO SPLENIC THIOL PROTEINASES ACTIVE IN A NEUTRAL MEDIUM

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For several years the writers have studied splenic thiol proteinases active in a neutral medium. Two enzymes, identified as cathepsin L and cathepsin H, have been isolated from bovine spleen in a virtually homogeneous form [3]. The study of the properties and specificity of these proteinases has shown that they can take part in the formation and inactivation of several physiologically active proteins and peptides [3-5] and, in experiments in vitro, they can inhibit the transformation of phytohemagglutinin-stimulated lymphocytes [1]. To investigate the biological functions of these proteinases and their role in the development of immunologic and inflammatory reactions further, their action was studied on α_1-proteinase inhibitor

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TABLE 1. Interaction of Cathepsin L and Cathepsin H with α_1-Proteinase Inhibitor

Proteinase	MR 1/2	Activity, % of inhibitor, reaction time, min				Activity, % of proteinase, reaction time, min	
		1/2	30	60	120	1/2	60
Cathepsin L (without EDTA + DTT)	1/2	0	—	0	—	100	100
	1/2	100	—	—	0	10	—
	1/10	80	70	0	—	—	—
Cathepsin H	1/1,5	60	—	—	—	100	100

Legend. MR) Molar ratio enzyme/α_1-PI, calculated per weighed sample of proteins, using the following values of molecular weight: α_1-PI 53 kilodaltons (kD), cathepsin L 23 kD, cathepsin H 30 kD.

(α_1-PI), one of the main plasma inhibitors lowering the activity of serine proteinases. α_1-PI, previously called α_1-antitrypsin, is known to be a polyvalent inhibitor and, under physiological conditions, its function is linked mainly with inactivation of the elastase of granulocytes, the affinity of α_1-PI for which is an order of magnitude higher than for other proteinases [15].

α_1-PI is an "acute phase" protein, whose synthesis is stimulated by a marked degree in acute inflammation, and is aimed at neutralizing the released intracellular proteinases [15]. The function of α_1-PI may also be connected with its role in immunologic reactions, for it has been shown that it is synthesized and expressed on the surface of monocytes and of stimulated lymphocytes [8], and in experiments both in vivo and in vitro it modulates activity of responses of cellular immunity [2, 9].

The aim of this investigation was to study interaction of cathepsins L and H with α_1-PI from human blood plasma, and the experiments showed that the proteinases induced irreversible inactivation of the inhibitor.

EXPERIMENTAL METHOD

Proteinases were isolated from bovine spleen by the method in [3]. Cathepsin L activity was determined by hydrolysis of histone at pH 7.2, and cathepsin H activity by hydrolysis of L-leucine-2-naphthylamide at pH 7.2, by methods described previoulsy [3]. Preparations of α_1-PI (with activity of 15, 10, and 5 IU/mg) were isolated by a modified method [14]. Preparations of α_1-PI (5 IU, from Sigma, USA) also were used before and after further purification on Sephadex G-100. The reaction of α_1-PI with proteinases was carried out at pH 7.2 at room temperature and at 37°C in the presence of EDTA and 0.1 mM dithiothreitol (DTT), with various ratios of proteinase to inhibitor. The concentration of α_1-PI was 100-500 μg/ml. Aliquots were taken at definite time intervals and activity of the proteinases and inhibitors was determined. The degree of inactivation of the inhibitor was calculated on the basis of lowering of its ability to inhibit the esterase activity of trypsin (from Spofa, Czechoslovakia), which was determined by measuring hydrolysis of N-benzoyl-L-arginine ethyl ester (BAEE, from Reanal, Hungary). Hydrolysis was measured as the change in $A_{253\ nm}$ at pH 8.0 after preincubation of α_1-PI for 5 min with trypsin [14]. Electrophoresis in the presence of sodium dodecylsulfate was carried out in a polyacrylamide gel gradient (5-15%), with and without reduction of the samples by the method in [13]. N-terminal amino acids were determined by the dansyl method [11] and the DNS-amino acids were separated on polyamide plates (Schleicher und Schüll, West Germany). The immunochemical determinations were carried out with the aid of LC- and M-partigens (Boehringer, West Germany).

EXPERIMENTAL RESULTS

It will be clear from Table 1 that during interaction of the proteinases with α_1-PI rapid and irreversible inactivation of the inhibitor took place whereas activity of the enzymes was unchanged. With a stoichiometric ratio of proteinase to inhibitor, total inactivation of α_1-PI under the influence of cathepsin L takes place instantaneously, whereas under the influence of cathepsin H, it takes place after 30-60 min. In the absence of EDTA and DTT, essen-

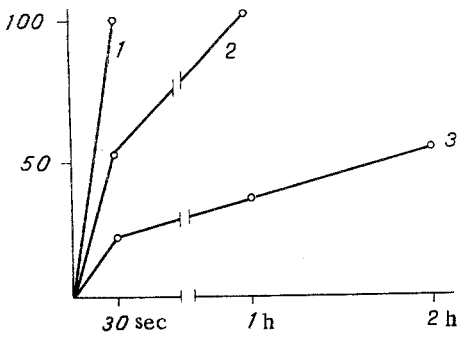

Fig. 1

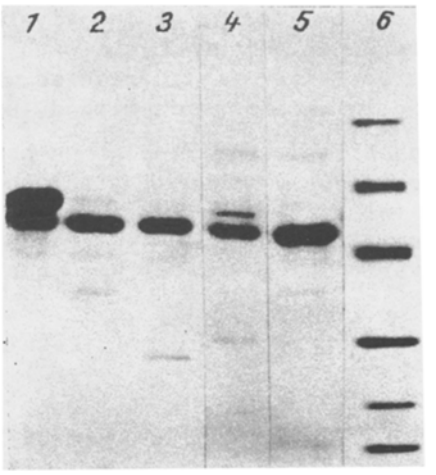

Fig. 2

Fig. 1. Inactivation of α_1-PI by cathepsin L after successive addition of new portions of inhibitor to the sample. Abscissa, reaction time; ordinate, inactivation of α_1-PI (in %). 1, 2, 3) After addition of 1st, 2nd, 3rd portions of inhibitor, respectively. Sample (volume 0.5 ml) contained 5 μg of cathepsin L, 50 μg of α_1-PI, EDTA + DTT (10^{-4} M), pH 7.2. The molar ratio proteinase/inhibitor was 1/4. A new portion of inhibitor was added (2) 15 min after beginning of reaction (100% inactivation of α_1-PI). Third portion of α_1-PI was added 75 min after beginning of reaction, after complete inactivation of 2nd portion (3).

Fig. 2. Electrophoretic analysis of inactivation products of α_1-PI. Electrophoresis without reduction of samples. 1) α_1-PI; 2, 3) α_1-PI + cathepsin L, 30 sec and 2 h (100% inactivation); 4, 5) α_1-PI + cathepsin H, 30 min and 2 h (50% and 100% inactivation, respectively); 6) marker proteins: phosphorylase B 94 kd, albumin 67 kD, ovalbumin 43 kD, carbonic anhydrase 34 kD, soy trypsin inhibitor 20.1 kD, α-lactalbumin 14.4 kD. The 23 and 30 kD components correspond to proteinases L and H.

tial for complete manifestation of proteinase activity, inactivation of the inhibitor took place slowly, and with cathepsin L it was observed only after 2 h (Table 1). Inactivation of α_1-PI was observed when inhibitor preparations with different values of inhibitory activity were used (15, 10, and 5 IU), and in all cases it occurred more effectively under the influence of cathepsin L. Complete inactivation of the inhibitor was observed in samples with a high proteinase content: a molar ratio (M/M) of enzyme to inhibitor of 1/1 to 1/20. With a ratio of enzyme to substrate of 1/100, no appreciable reduction of inhibitory activity occurred in the course of 3 h. The dynamics of inactivation of the inhibitor by cathepsin L was the same at pH 7.2 and 5.8.

Inactivation products of α_1-PI were found to have an inhibitory action on the velocity of its reaction with cathepsin L. It will be clear from Fig. 1 that during consecutive addition of new portions of inhibitor to the reaction medium, the velocity of its inactivation fell.

Electrophoretic study of products of interaction of α_1-PI with cathepsins L and H showed that inactivation of the inhibitor was accompanied by a decrease of its molecular weight from about 53 to about 46 kilodaltons (Fig. 2). The component with mol. wt. of 46 kilodaltons which was formed is resistant to proteolysis and does not undergo further degradation by the proteinases tested. This fragment was found in small quantities in all the original preparations of inhibitor available. It is evidently a product of partial degradation of α_1-PI under the influence of any of the blood plasma proteinases. No low-molecular-weight product (or products) of the reaction could be found on electrophoresis. No components with a higher molecular weight than that of the original inhibitor, which could be regarded as a proteinase–inhibitor complex, likewise could be found (Fig. 2).

The observed proteolytic inactivation of α_1-PI was evidently due to limited proteolysis of the inhibitor. This is shown also by the results of determination of N-terminal amino acids in the inactivated inhibitor. Besides N-terminal glutamic acid, present in the original α_1-PI after the action of the proteinases an N-terminal threonine appeared.

The study of binding of inactivated α_1-PI with immobilized trypsin showed that at pH 8.0 it is not retained on the column with trypsin-sepharose. The inactive inhibitor preserved its antigenic properties, for it reacted with antiserum against the native inhibitor. This was shown by the immunodiffusion method, using LC- and M-partigens.

The inactivation of α_1-PI thus observed is similar to the inactivation of this inhibitor, described previously, under the influence of papain, cathepsin B [12], and macrophagal metalloproteinase (elastase) [6, 7]: in these cases limited proteolysis of the inhibitor and reduction of its molecular weight by 5-6 kD also were observed. In the present experiments, however, inactivation of α_1-PI was not observed under the influence of cathepsin B from bovine spleen.

The results showing that intracellular thiol proteinases inactivate α_1-PI by limited proteolysis are interesting from several points of view. They indicate that on release from the cell these enzymes may be involved in monitoring an effective concentration of α_1-PI and, by disturbing its balance with the corresponding serine proteinases, can take part in the regulation of their activity. These reactions can evidently proceed in foci of chronic inflammation and, together with oxidative inactivation of α_1-PI, they must be regarded as a factor enhancing the destructive function of the serine proteinases and, in particular, of the granulocytic elastase, in a number of pathological states [7, 12, 14]. These reactions may probably also play a definite role in immune processes, by causing modification of α_1-PI on the surface of activated mononuclear cells. In this connection, it should be pointed out that the immunochemical and immunofluorescence methods of α_1-PI assay, used in many cases, are not always adequate, for they demonstrate not only the active, but also the inactivated inhibitor.

The results are interesting also in connection with our understanding of the general pathways of regulation of proteinase activity in the body. Together with data on proteolytic inactivation of various other plasma inhibitors — α-antiplasmin, C_1 inactivator [10], antithrombin III, etc. [14] — they are evidence that limited proteolysis is one of the main mechanisms of inactivation of proteinase inhibitors and of the control of their activity.

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