by 40%. Lysolecithin is known to have a membranolytic action, but the exact mechanism of this action is unknown. Differences in the degree of change of sensitivity of adipose tissue to ACTH and adrenalin after treatment with lysolecithin are thus evidence merely that the organization of the membrane part of the mechanism responsible for the action of adrenalin is less sensitive to disturbance of its structure by lysolecithin than in the case of ACTH.

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CHARACTERISTICS OF HUMAN SPLENIC PROTEINASES ACTIVE IN A NEUTRAL MEDIUM*

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Fractionation of proteins of aqueous and salt (1 M KCl) extracts of human spleen on Sephadex G-100 revealed several proteinases hydrolyzing histone and casein and active in a neutral medium. The enzymes were found in the extracts in a relatively inactive form, due to the presence of an inhibitor, chiefly in the aqueous extract. Proteinases active in a neutral medium were found in two protein fractions. "High-molecular-weight" proteinases are inhibited by di-isopropylfluorophosphate (DFP), so that they can be classed in the group of serine proteinases. The fraction of "low-molecular-weight" proteinases contains neutral SH-dependent proteinase (proteinases) and enzymes inhibited by DFP. Kininogenase activity and activity hydrolyzing N-Boc-L-alanine nitrophenyl ester, N-benzoyl-L-tyrosine ethyl ester, and N-benzoyl-DL-arginine-p-nitroanilide also were found in this fraction.

KEY WORDS: neutral proteinases; human spleen.

Studies of proteolytic enzymes of bovine spleen in the protein fraction with a molecular weight of about 25,000 have revealed a neutral SH-dependent proteinase hydrolyzing histones [1] and a proteinase with kininogenase activity [2].

Considering the possible role of these and certain other enzymes in the development of various pathological states, it was decided to study whether these proteinases are present in the human spleen and also to attempt to determine the general characteristics of proteinases active in a neutral medium.

*The following abbreviations are used: NBA) N-Boc-L-alanine nitrophenyl ester; BTEE) N-benzoyl-L-tyrosine ethyl ester; BAPA) N-benzoyl-DL-arginine-p-nitroanilide; DFP) di-isopropylfluorophosphate; p-CHMB) p-chloromercuribenzoate; DTT) dithiothreitol.

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TABLE 1. Histone—Hydrolase Activity of Proteinases at Different Stages of Isolation (mean results of analysis of three spleens)

Stage of isolation	Extract I					Extract II				
	total protein, mg	activity, nmoles arginine/mg/h		yield, %		total protein, mg	activity, nmoles arginine/mg/h		yield, %	
		relative	total	activity	protein		relative	total	activity	protein
1. Extract	6000	10	60 000	100	100	4320	30	130 000	100	100
2. Fraction precipitated by $(NH_4)_2SO_4$ at 0.75 saturation after desalting	2000	100	200 000	330	33	1020	130	133 000	102	24
3. Gel-filtration through Sephadex G-100:										
"high-molecular-weight" fraction	150	300	45 000	75 (25)*	7,5	530	200	106 000	80	52
"low-molecular-weight" fraction	120	500	60 000	100 (30)*	6	80	375	30 000	23	8

*Activity after second stage of isolation taken as 100%.

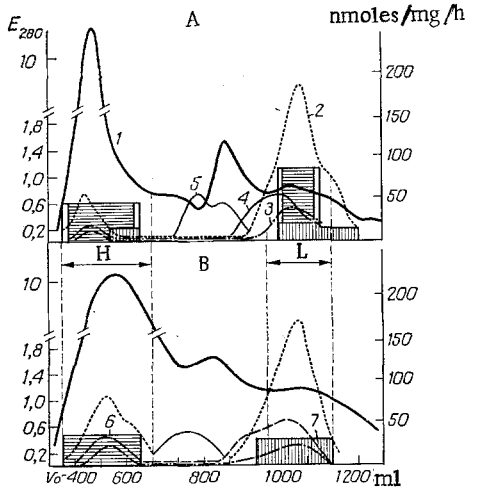

Fig. 1. Gel-filtration of proteins precipitated at 0.75 saturation with $(NH_4)_2SO_4$ through Sephadex G-100: A) extract I, B) extract II. 1-1.5 g protein applied. Column measuring 5 × 100 cm equilibrated with 0.005M phosphate buffer, pH 7.8, containing 0.1M NaCl and 10^{-5}M EDTA; rate of elution 12 ml/h. 1) Optical density at 280 nm; 2) hydrolysis of histone (pH 7.2); 3) of casein (pH 7.5); 4) of BAPA (pH 6.5); 5) of hemoglobin (pH 3.8); 6) of NBA (pH 7.5); 7) of BTEE (pH 7.5). Pooled samples of "high-molecular-weight" (H) and "low-molecular-weight" (L) proteinases. Abscissa, volume of eluate (in ml); ordinate: left) optical density at 280 nm (E_{280}), right) histone-hydrolyzing activity (in nmoles arginine/mg protein/h).

TABLE 2. Effect of Inhibitors and Activators on Activity of Histone-Hydrolyzing Proteinases

Reagent	Final concentration, M	Activity, %			
		extract I		extract II	
		"high-molecular-weight" proteinases	"low-molecular-weight" proteinases	"high-molecular-weight" proteinases	"low-molecular-weight" proteinases
None added	—	100	100	100	100
EDTA + DTT	5.10^{-4}	100	250	100	150
DFP	1.10^{-3}	20	60	10	40—50
p-CHMB	5.10^{-4}	100	20	100	70

Legend. Incubation at pH 7.2 in 0.1M borate buffer at 20°C for 10-15 min, after which activity was determined.

METHODS

Spleens of persons dying from acute trauma were used for isolation of the proteinases. The spleen was kept at between −70 and −20°C. Before use, the tissue was minced, and frozen and thawed 4 or 5 times. The tissue was extracted with water and then with 1 M KCl; pH of the extracts was 6.3. The conditions of fractionation of the extract and the methods used to determine proteinase activity were described previously [1].

RESULTS

To determine the properties of human splenic proteinases, enzymes contained in aqueous (I) and salt (II) extracts were studied. Most (about two-thirds) of the histone-hydrolase activity at pH 7.2 was found initially in extract II (Table 1). During fractionation there was a sharp increase in activity, which was greater for the neutral proteinases of extract I (Table 1). An increase in total activity was observed at the first stage of purification, and indicated that the extract contained an inhibitor or an inactive precursor of the proteinases. Since, on addition of extract I to extract II, activity in the latter was considerably reduced it can be concluded that extract I contained an inhibitor.

The results of fractionation of proteins in extracts I and II through Sephadex G-100 are given in Fig. 1. Besides neutral proteinase activity, the distribution of activity of cathepsins D and B_1 — the principal tissue endopeptidases — also is shown. Histone-hydrolase and caseinolytic activity were found in a neutral medium at two peaks, one in the region of proteins with a molecular weight of about 80,000 ("high-molecular-weight" proteinases), the other in the region of proteins with a molecular weight of about 30,000 ("low-molecular-weight" proteinases). The pattern of distribution of neutral proteinase activity was similar in the two extracts (Fig. 1, A and B). However, the ratio between activity of the "high-molecular-weight" and "low-molecular-weight" proteinases differed: In extract I the activities were distributed approximately equally, but in extract II, activity of the "high-molecular-weight" proteinases was greater (Table 1).

Activity with respect to NBA and BTEE and kininogenase activity* were determined in pooled samples of "high-" and "low-molecular-weight" proteinases. Kininogenase activity was found in the low-molecular-weight fraction of extract I, whereas hydrolysis of NBA and BTEE was found in practically all fractions (Fig. 1).

The study of the action of activators and inhibitors showed that activity of the "high-molecular-weight" proteinases is inhibited by DFP, whereas EDTA and DTT had no appreciable effect on it (Table 2). Activity of "low-molecular-weight" proteinases increased 1.5-2.5 times in the presence of EDTA and DTT, and when preserved fractions were used the increase was as much as by 8-10 times. The increase in activity in the presence of EDTA and DTT and its inhibition by p-CHMB point to the presence of an SH-dependent proteinase. It was pres-

*The authors are grateful to T. P. Egorova for determining kininogenase activity.

ent mainly in the "low-molecular-weight" fraction of extract I. Proteinases inhibited by DFP also were found in the "low-molecular-weight" fractions of both extracts; their relative contents was greater in extract II (Table 2). These results show that, first, the human spleen contains two types of neutral proteinases, identifiable as serine and SH-dependent enzymes respectively, and second, the assortment of proteinases differs in extracts I and II.

In 1976 two serine proteinases — elastase and cathepsin G — were isolated from human spleen [3-5]. They hydrolyzed the corresponding synthetic substrates and attacked several proteins including casein and histone. In the present experiments the highest activity for hydrolysis of NBA — the substrate for elastase — was observed in the "low-molecular-weight" fraction of extract I. It can tentatively be suggested that some of the histone-hydrolase activity of this fraction was due to the action of this enzyme. Although activity for hydrolysis of BTEE — the substrate for cathepsin G — was observed in practically all fractions tested, the data in the literature [6] suggest that this enzyme is present mainly in the "low-molecular-weight" fraction of extract II.

Besides the above-mentioned "low-molecular-weight" serine proteinases, we also found a group of "high-molecular-weight" proteinases, which also belonged to this type of enzymes. These proteinases have not yet been identified. It may be that some of them (especially enzymes of extract I) may belong to the group of blood plasma proteinases.

The SH-dependent proteinase responsible for the greater part of the histone—hydrolase activity of the "low-molecular-weight" fraction of extract I was not described in papers on the isolation of elastase and cathepsin G [3-5], although information on the existence of a similar enzyme was obtained previously [7].

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