DETECTION OF ELASTASE ACTIVITY OF THERMOSTABLE LEUKOCYTIC α-GLYCOPROTEIN FROM HUMAN BLOOD PLASMA

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Human granulocytes secrete large quantities of proteinases. In several diseases of an inflammatory nature increased concentrations of some of them are observed in the blood stream such as granulocytic elastase [14]. Granulocytic elastase also is found in the blood plasma from healthy blood donors, but mainly in the form of complexes with proteinase inhibitors [13], which makes the study of the biological role of this enzyme very difficult [12].

A specific granulocytic protein is that known as thermostable leukocytic α-glycoprotein (TLD) [5, 10], which was identified immunochemically in 1982 [4]. TLG is not present in plasma obtained from healthy blood donors, but increased concentrations of it have been found in the blood plasma of patients with immunomediated diseases (systemic lupus erythematosus, rheumatoid arthritis, myasthenia) [1, 2, 4, 10], and also after operations involving the use of an assisted circulation [9]. However, no reports could be found of the isolation of biologically active TLG from blood plasma. The discovery of protein components found under normal conditions may play a particularly important role in the study of the pathogenesis of diseases and also in the development of diagnostic methods.

It was suggested previously that TLG may be granulocytic elastase [11]. The aim of this investigation was to study the elastase activity of TLG preparations obtained from patients' blood plasma.

EXPERIMENTAL METHOD

Samples of blood serum from patients with immunomediated diseases and patients undergoing operations involving the use of an assisted circulation served as the material for isolation of TLG.

The absorbent for isolation of TLG was synthesized by immobilizing the soluble protein fraction of pus on CNBr-activated Sepharose 4B (Pharmacia, Sweden) [7].

The protein fraction used as ligand was prepared in two stages. By freezing and thawing once, followed by centrifugation, an aqueous extract was prepared from pus obtained during opening of an abscess, and this was then fractionated by ion-exchange chromatography on Sephadexes DEAE A-50 and SP C-50 [6]. The fraction obtained after elution of proteins adsorbed on a cation-exchange resin with 0.5 M NaH_2PO_4 was used for immobilization. The adsorbent was washed with 0.1 M phosphate buffer (pH 7.4). Elution was carried out with 0.1 M glycine-HCl buffer (pH 2.4) containing 0.1 M NaCl.

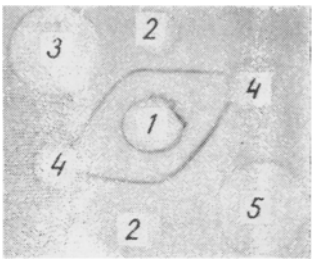

Fig. 1. Immunochemical analysis of eluates obtained after passage of patients' blood serum through adsorbent. 1) Monospecific antiserum against TLG; 2) extract of pus in dilution of 1:10; 3) eluate from blood plasma of patient with rheumatoid arthritis; 4) 0.9% NaCl; 5) eluate from blood plasma of a patient undergoing heart surgery.

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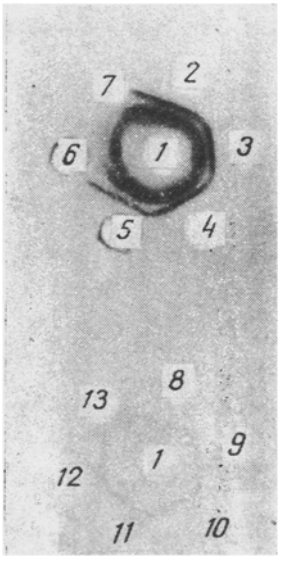

Fig. 2

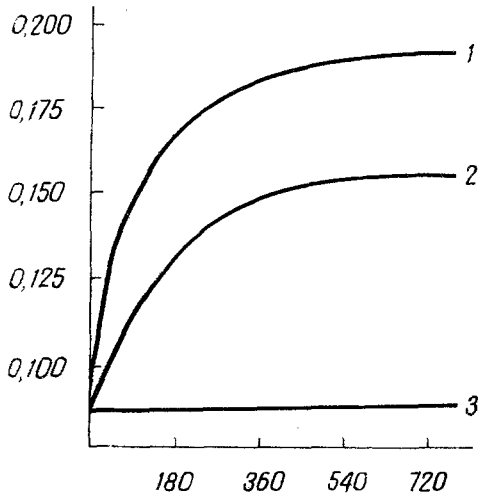

Fig. 3

Fig. 2. Immunochemical analysis of eluate from blood plasma from a patient compared with original plasma. 1) Polyspecific antiserum against blood plasma proteins; 2) blood plasma from patient with rheumatoid arthritis; 3-7) blood plasma in dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32, respectively; 8) eluate from blood plasma of patient with rheumatoid arthritis; 9-13) eluate from blood plasma of patient with rheumatoid arthritis, in dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32, respectively.

Fig. 3. Kinetic curves of interaction between substrate for elastase and eluates from different samples of blood serum. Abscissa, time (in sec); ordinate, optical density (in relative units). 1) Eluate from blood plasma from patients with rheumatoid arthritis; 2) eluate from blood plasma of patients undergoing cardiac surgery; 3) eluate from blood plasma of a healthy blood donor, not containing TLG.

Immunochemical analysis was carried out by double immunodiffusion in agar gel with standard test systems [8]. A monospecific antiserum against TLG, obtained as described previously [10], antisera against lysozyme and lactoferrin (Behringwerke, West Germany), and polyspecific antisera, obtained by immunizing rabbits with pooled blood plasma or lyzed leukocytes from healthy blood donors by three subcutaneous injections with intervals of 8 days, and in a dose of 200 mg protein per injection, mixed with Freund's complete adjuvant, were used. Blood was taken from the marginal vein of the ear on the 9th-11th days after the last injection. Reimmunization was carried out by a single injection 1 month later. For immunodiffusion and immunoelectrophoretic analysis, polyspecific antisera revealing at least 20 arcs of proteins in blood plasma and at least 10 arcs in samples of lyzed donors' leukocytes on electrophoresis, were used.

Elastase activity was determined with the aid of a chromogenic substrate Suc[Ala]$_3$pNA (Serva, West Germany), diluted in 10 mM dimethyl sulfoxide [14]. For the reaction 50 µl of substrate and 100 µl of the protein preparation were used.

The optical density of the reaction mixture was measured by determining accumulation of isolated p-nitroaniline on an FP-901 biochemical analyzer (Labsystems, Finland), at 405 nm. The results were recorded automatically every 10 sec by means of an Olivetti computer (Italy).

EXPERIMENTAL RESULTS

Samples of blood sera from patients with immunomediated diseases and patients undergoing operations with the aid of an assisted circulation, in which the TLG titer was not less than 1:2, were passed through the synthesized adsorbent. On immunochemical analysis with specific antisera against individual leukocytic proteins, the eluates contained neither lactoferrin nor

lysozyme,but TLG was found (Fig. 1). With the aid of polyspecific antiserum against plasma proteins from normal blood donors it was shown that the eluates were virtually free from contamination by plasma proteins (Fig. 2). When the experiments were repeated with different samples of blood plasma (10 experiments) the results of immunochemical analysis in all cases were similar to those given above.

With the aid of an immobilized protein fraction of pus it was thus possible to isolate TLG from patients' blood plasma. The fact that the TLG content in the original material, namely patients' blood plasma, was below 0.1% relative to the total protein content in plasma [4, 10] is evidence of the adequate selectivity of the synthesized adsorbent. This was also shown by the absence of lysozyme and lactoferrin, leukocytic proteins constantly present in small amounts in blood plasma [3], in the eluates. The extract of pus evidently contains a component which can bind TLG. Identification of the TLG-binding substance may provide a basis for the development of ways of isolating pure TLG from biological material containing this protein in high concentration (pus, lysed leukocytes).

During analysis of enzyme activity of TLG preparations obtained from patients' blood plasma it was found that the test preparations exhibit amidolytic activity relative to the substrate for elastase. Meanwhile control samples from blood plasma not containing TLG did not cause hydrolysis of the substrate (Fig. 3). The enzyme activity discovered points to the presence of elastase, isolated from plasma, in the eluates. Considering that the TLG preparations were virtually uncontaminated with plasma proteins, and also the leukocytic nature of this protein, it can be tentatively suggested that TLG is granulocytic and not pancreatic elastase. However, for a final solution to the problem of the identity of TLG and granulocytic elastase, direct immunochemical comparison of monospecific antisera against these proteins is necessary, for the presence of immunochemically undetectable granulocytic elastase in TLG preparations cannot be ruled out.

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