INHIBITION OF HUMAN GRANULOCYTIC ELASTASE BY ANTIBODIES AGAINST LEUKOCYTIC THERMOSTABLE α-GLYCOPROTEIN

O. P. Shevchenko, O. V. Eremenko,
O. G. Ogloblina, and E. I. Sorochinskaya

UDC 612.112.91.015.1:577.152.34]-064:
612.112.015.348:577.112.853

KEY WORDS: elastase; human granulocytes; antibodies to leukocytic thermostable α-glycoprotein.

Human granulocytic elastase takes part in the degradation of very important biological substrates, and in that way it influences biochemical processes in an inflammatory focus, blood clotting and fibrinolysis, humoral immunity, and the elasticity and permeability of the vascular wall [9, 10, 12]. An important role in the regulation of granulocytic elastase (GE) activity is played by natural protease inhibitors [11]. The secretion of GE into the blood plasma from leukocytes is increased in inflammation [13]. We know that activity and the damaging action of important pathophysiological processes depend on GE and its inhibitors, but the biological role of GE is insufficiently clear.

In 1982 a leukocytic thermostable α-glycoprotein (LTF) with mol. wt. of 90 ± 7 kD [4], a specific protein of granulocytes [6], was identified immunochemically. The discovery of LTG in blood plasma in chronic inflammatory and autoimmune diseases [1, 4, 6-8] suggests that this protein plays an important role in pathology, and also that it may be possible to use it for diagnostic purposes.

To study the biological role of LTG, in the investigation described below, antibodies against this protein were isolated and their effect on GE activity was studied.

EXPERIMENTAL METHOD

The method of preparing a monospecific antiserum against LTG was described by the writers previously [6, 7]. Immunodiffusion analysis of LTG was carried out in the modification by Khramkova and Abelev [5], using a standard test system to this antigen [6].

LTG was isolated from an extract of pus obtained on opening an abscess. The fraction of pus proteins not binding with DEAE-Sephadex A-50 (Pharmacia, Sweden) was subjected to ion-exchange column chromatography on SP-Sephadex C-50. The column was washed with 0.15 M NaCl and elution carried out with 1 M Na_2HPO_4. Eluates were estimated for LTG content and contaminating antigens by immunodiffusion and immunoelectrophoretic analysis with monospecific antiserum to LTG and with polyspecific antisera to blood plasma proteins and to proteins of lysed leukocytes from healthy blood donors.

To isolate antibodies against LTG, the preparation of this protein obtained as described above was immobilized on CNBr-activated Sepharose 4B (Pharmacia). The immunosorbent thus obtained was washed with 0.1 M phosphate buffer, pH 7.4, then with glycine-HCl buffer, pH 2.4, after which monospecific antiserum to LTG was passed through it. The column was washed with 0.1 M phosphate buffer and elution carried out with glycine-HCl buffer, pH 2.4.

To determine GE activity, we used methoxy-succinyl-dialanyl-prolyl-valyl-p-nitroanilide, synthesized as described by the writers previously [3]. The reaction mixture contained 100 µl of the test solution of the LTG preparation and 30 µl of substrate. To the control sample 400 µl of buffer was added, and to the experimental samples 200 µl of buffer, 200 µl of antibodies and 400 µl of antibodies respectively. The substrate for elastase was dissolved in 10 mM dimethyl sulfoxide. Changes in optical density of the reaction mixture were recorded automatically every 20 sec by means of an FP-201 biochemical analyzer (Lab Systems, Finland) at 405 nm.

Research Institute of Transplantology and Artificial Organs, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 1, pp. 80-82, January, 1988. Original article submitted Aprul 17, 1987.

0007-4888/88/0001-0099$12.50

The initial reaction velocity was calculated by the equation $V = \frac{dP}{dt}$ [2], where P is the p-nitroaniline concentration, determined by the equation $P = \frac{D}{\varepsilon l}$. In this case D denotes optical density (optical density units), ε the coefficient of molar extinction of p-nitroaniline, namely $10.4 \cdot 10^{-3}$ $cm^{-1}mole^{-1}$, l the width of the cuvette = 1 cm, and t denotes time (sec). The degree of inhibition of GE by antibodies to LTG was estimated by measuring the reduction of the initial reaction velocity in percent of the control.

EXPERIMENTAL RESULTS

Immunodiffusion analysis of antibodies against LTG obtained from monospecific antiserum by immunosorption and subsequent elution was carried out. However, preliminary washing of the newly prepared sorbent with an acid buffer is essential for isolation of the antibodies. If the antiserum is passed through the newly synthesized sorbent, but the sorbent has not been washed with acid buffer, binding of the anti-LTG antibodies will take place, but it will be impossible to detect the presence of antibodies in the eluate. Moreover, after washing the newly synthesized sorbent with acid buffer, the presence of LTG can be detected in the washings. Some of the LTG in pus is evidently in a bound state with a component also present in pus. This binding is sufficiently strong, and in order to remove the bound LTG, careful washing with phosphate buffer alone is not sufficient, but the pH of the buffer must also be lowered to 2.4. If this preliminary removal of the bound LTG is not done, in the process of elution of the antibodies, elution of the bound LTG also will take place, and this will lead to neutralization of the antibodies.

The results indicate that pus contains a component which binds LTG. Besides its immediate practical importance in relation to the correct preparation of the newly synthesized immunosorbent for isolation of the antibodies, this fact also is important on its own account: evidently for the further study of the function and biology of LTG, the identification and study of the LTG-binding substance will be useful.

The LTG preparation obtained by ion-exchange chromatography from extract of pus causes hydrolysis of the GE substrate used, as is shown by an increase in the optical density of the reaction mixture as a result of isolation of p-nitroaniline. Under these circumstances the initial reaction velocity was $12.43 \cdot 10^{-8}$ moles/sec. This property of the LTG preparations, of hydrolyzing the specific substrate, indicates the presence of an elastase. The substrate used in the present investigation is highly specific for leukocytic elastase, by contrast with pancreatic [15]. Thus the test preparation contained active GE.

Addition of anti-LTG antibodies led to a decrease in the rate of p-nitroaniline accumulation in the reaction mixture, evidence of inhibition of GE by anti-LTG antibodies. The inhibitory action of the antibodies increased with an increase in their concentration: the initial reaction velocity was $7.89 \cdot 10^{-8}$ and $5.81 \cdot 10^{-8}$ mole/sec. Inhibition of GE by anti-LTG antibodies was expressed as a percentage. On the addition of 400 ml of antibodies to the reaction mixture the velocity of hydrolysis of the specific substrate was reduced by half. Since in all cases investigated the concentration of the remaining low-molecular-weight components was the same, and samples of reaction mixture differed only in the presence and concentration of the anti-LTG antibodies, this suggested that it was the antibodies that were responsible for inhibiting GE. The presence of another, immunochemically undeterminable protein component in the antibody preparation, acting as an inhibitor of GE, evidently cannot be completely ruled out, although it seems unlikely. The monospecific nature of the antiserum which was the source of the antibodies was confired by immunodiffusion analysis.

Besides LTG present in the test preparations in titers of 1:4-1:32, from one to four contaminating antigens also were detected in it immunochemically. The fact that GE is inhibited by anti-LTG antibodies shows that the elastase activity was associated with this particular leukocytic protein. However, for a final answer to the question of the identity of LTG and GE, a direct immunochemical comparison of anti-LTG antiserum and of the standard antiserum to GE must be carried out. Considering certain differences in the physicochemical properties of LTG and GE [14], and also the heterogeneity of LTG as regards its physicochemical properties, it seems probable that GE in some cases may be part of this antigen: for example, a component of a GE—inhibitor or GE—protein carrier complex.

LITERATURE CITED

1. A. A. Dmitriev, O. P. Shevchenko, and D. D. Petrunin, Ter. Arkh., No. 5, 81 (1984).

2. E. N. Eremin, A Course in Chemical Kinetics [in Russian], Moscow (1977), pp. 22-23.
3. O. G. Ogloblina, L. V. Platonova, V. F. Martynov, et al., Ukr. Biokhim. Zh., No. 3, 285 (1983).
4. D. D. Petrunin, Yu. M. Lopukhin, M. N. Molodenkov, and G. A. Olefirenko, Byull. Éksp. Biol. Med., No. 2, 66 (1982).
5. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
6. O. P. Shevchenko, L. V. Beletskaya, V. F. Kirsanov, et al., Byull. Éksp. Biol. Med., No. 7, 53 (1986).
7. O. P. Shevchenko, A. B. Kosyrev, O. V. Eremenko, and A. A. Dmitriev, Byull. Éksp. Biol. med., No. 5, 552 (1987).
8. V. I. Shumakov, O. P. Shevchenko, and R. Sh. Saitgareev, Transplantation of Organs and Tissues [in Russian], Moscow (1986), pp. 156-159.
9. D. L. Bader, G. E. Kempson, A. J. Barrett, and W. Webb, Biochim. Biophys. Acta, 677, 103 (1981).
10. P. B. Dobrin and W. C. Gley, Experientia, 41, No. 8, 1041 (1985).
11. U. Fryksmark, K. Ohlsson, and M. Rosengren, Hoppe-Seylers Z. Physiol. Chelm., 364, No. 7, 793 (1983).
12. M. Gramse, R. Egbring, and K. Havemann, Hoppe-Syelers Z. Physiol. Chem., 365, No. 1, 19 (1984).
13. M. S. Hibbs, K. A. Hasty, A. H. Kang, and C. Mainardi, Collagen Rel. Res., 4, No. 6, 467 (1984).
14. J. Travis, P. J. Giles, L. Porcelli, et al., Ciba Found. Symp., 75, 51 (1979).
15. H. R. Wenzel, S. Engelbrecht, H. Reich, et al., Hoppe-Seylers Z. Physiol. Chem., 361, No. 9, 1413 (1980).