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Increased Proteinase Inhibitory Activity on Thermolysin in Idiopathic Nephrotic Syndrome

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Summary. **Inhibitory activity of plasma on bacterial thermolysin was significantly higher in patients with idiopathic nephrotic syndrome (INS) at relapse than in those in remission, chance proteinuria and/or hematuria and control subjects. Simultaneously measured plasma concentrations of α2-macroglobulin (α2-M), a large molecular proteinase inhibitor of human plasma, were also significantly elevated in INS. Sephadex G-200 gel-choromatography of the plasma revealed the thermolysin inhibitory activity was confined to the large molecular fractions. Sephacryl S-300 chromatography demonstrated the coincidence of the thermolysin inhibitory actitivity with α2-M. The relation between the plasma thermolysin inhibitory actitivity and α2-M concentration showed a weak, but not significant, correlation ($r=0.34$, $p<0.10$), suggesting a possible alteration of the binding capacity (mol/mol) of increased plasma α2-M to proteinase in patients with INS.**

From these results it is suggested that the increased thermolysin inhibitory activity serves as a defense against invading pathogens by inactivating the proteinases elaborated by these organisms. This may be a compensatory reaction of the body for depressed cell immunity in INS.

INTRODUCTION

α2-macroglobulin (α2-M) is a large molecular plasma protein with a wide variety of specificity for various proteinases such as elastase, cathepsin G, trypsin, chymotrypsin, kallikrein, thrombin, plasmin, etc.[1] For years, plasma α2-M concentrations have been reported to be elevated in various diseases including idiopathic nephrotic syndrome (INS).[2-6] These studies have focused on the quantitative changes of plasma α2-M and on coagulation abnormality. However, since α2-M has a wide variety of bindings with many proteinases, its biological role should be considered more widely. Because of similarities of the molecular structure to certain complement proteins, the proteinase inhibitor has been suggested to have a possible significance of the immune system.[7] This study was conducted to clarify the biological significance of elevated plasma α2-M in INS by assaying the inhibitory activity on exogenous proteinase isolated from other organisms.

SUBJECTS AND METHODS

Twenty-two pediatric patients with INS (12 patients at relapses), 23 with chace proteinuria and/or hematuria (CPH), and 18 normal children as controls were included in this study.

Plasma concentration of α2-M was measured by a single radial immunodiffusion method. The proteinase inhibitory activity of plasma α2-M was determined by using thermolysin (Sigma) as a proteinase and dyed hide-powder (Sigma) as the substrate for thermolysin based on the method by Barrett[8] with slight modifications. Thermolysin is a bacillary metalloproteinase isolated from Bacillus rhokko and is considered to be selectively inhibited by α2-M.[8] The inhibitory activity of plasma on thermolysin was expressed as the values representing ng of inhibited thermolysin activity by 1.0 μl of plasma (ng/μl).

In order to examine whether themolysin is selectively inhibited by α2-M, we conducted two studies as follows: a) first, 2.0 ml of plasma sample from a normal subject was applied to a Sephadex G-200 column eluted with 0.01 M phosphate buffer, pH 7.4, and the fractions were measured for α2-M (mg/dl) and the thermolysin inhibitory activity; b) α2-M was separated and purified from a pool of 300 ml exchan-

ged plasma obtained on a plasmapheresis, based on the method described by Sottrup-Jensen[9] using Zn-chelating affinity gel and Sephacryl S-300 gel chromatography, respectively. The fractions of the final chromatography on a Sephacryl S-300 column were measured for α2-M and the thermolysin inhibitory activity. The purification procedures followed a regimen reported previously.[9]

RESULTS

1) Inhibitory activity of plasma α2-M on thermolysin

As shown in Fig. 1, the mean ($\pm$SD) value of the inhibitory activity in patients with INS at relapse (252.9 ± 54.0 ng/μl) was significantly higher than that in remission (190.0 ± 58.0 ng/μl, $p<0.01$), chance proteinuria and/or hematuria (197.3 ± 51.5 ng/μl, $p<0.001$), and controls (218.0 ± 40.7 ng/μl, $p<0.05$).

2) Plasma α2-M concentration

The mean ($\pm$SD) plasma α2-M concentration was significantly higher ($p<0.001$) in patients with INS at relapse (456.5 ± 148.3 mg/dl) than that in remission (333.6 ± 93.1 mg/dl).

3) Relation between plasma α2-M concentration and inhibitory activity on thermolysin

Although higher plasma α2-M concentrations (mg/dl) paralleled higher inhibitory activities on thermolysin, the relation between them was not statistically significant ($r=0.34$, $p<0.10$), as shown in Fig.2.

4) α2-M and thermolysin inhibitory activity in Sephadex G-200 chromatogram of plasma

Thermolysin inhibitory activities were demonstrated only in the void volume fractions coinciding with α2-M. As shown in Fig. 3, other plasma proteinase inhibitors, eluted in the fractions with lower molecular weights than α2-M, did not show the inhibitory activity on thermolysin.

5) Thermolysin inhibitory activity of purified α2-M

As shown in Fig. 4, the final step of the purification procedure was performed on a Sephacryl S-300 column to separate α2-M from IgG. The chromatogram showed a complete coincidence of the thermolysin inhibitor activity with α2-M.

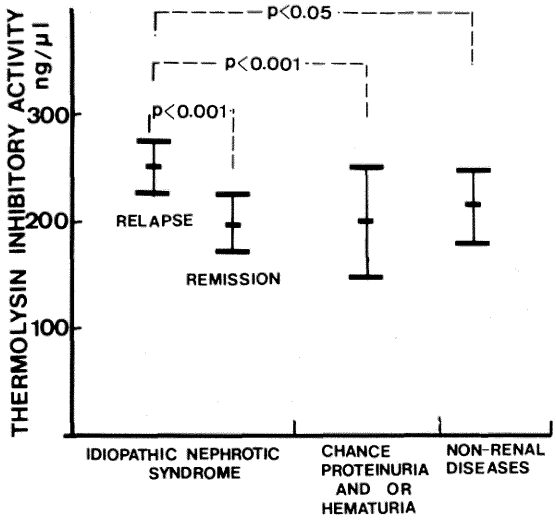

Fig. 1. Thermolysin inhibitory activity of plasma α2-macroglobulin in patients with renal diseases. Patients with idiopathic nephrotic syndrome (INS) at relapse show a significantly incresed mean themolysin inhibitory activity of plasma compared with chance proteinuria (CPH) and controls.

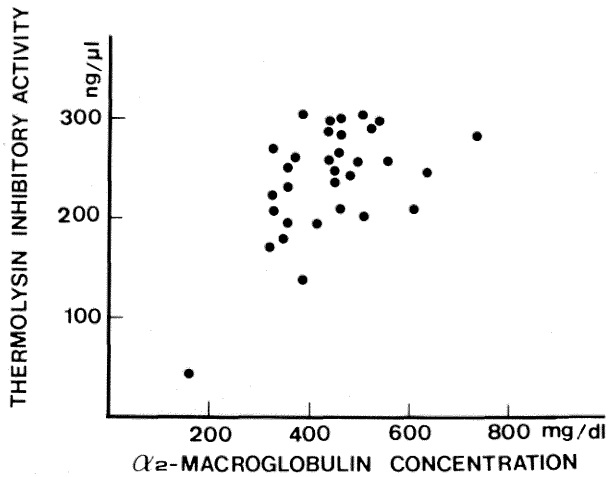

Fig. 2. Relation between plasma α2-macroglobulin and thermolysin inhibitory activity. Though a positive correlation ($r=0.34$) is revealed between plasma α2-macroglobulin and thermolysin inhibitory activity, the difference did not reach statistical significance ($p<0.10$).

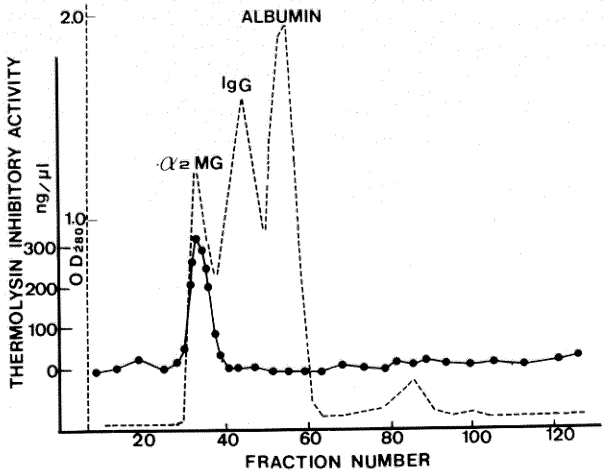

Fig. 3. Sephadex G-200 gel-chromatogram of plasma α2-macroglobulin and thermolysin inhibitory activity. A sample of 1.0 ml plasma applied to a Sephadex G-200 column. Closed circles represent the thermolysin inhibitory activity expressed by ng/μl, and broken line show protein concentrations (OD280)of the fractions in which three peaks are seen coinciding with α2-macrolgobulin (MW: 725,000), IgG (MW: 160,000), and albumin (MW: 66,000), respectively. The thermolysin inhibitory activity was demonstrated only in the α2-macroglobulin fractions.

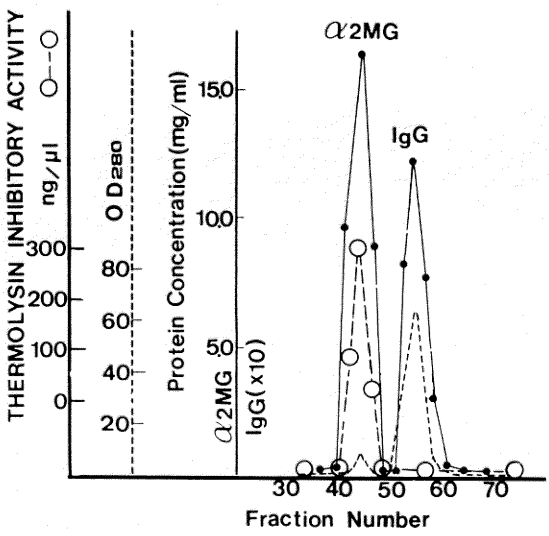

Fig. 4. Coincidence of the thermolysin inhibitory activity with α2-macroglobulin on a Sephacryl S-300 column. Separation of the concentrated eluate from Zn^{2+} chelate affinity step on a Sephacryl S-300 column (2×60 cm) equilibrated and eluted with 0.1 M Na phosphate, pH 8.0, at a flow rate of 10 ml/hr. The eluate in the larg peak was identified as pure α2-M coinciding with thermolysin activity, while the eluate in the adjacent small contained IgG.

DISCUSSION

The major proteinase inhibitor in plasma, α2-M, has repeatedly been reported to be elevated in nephrotic syndrome.[2-6] However, since these studies were conducted based on the quantitative determination of the plasma protein concentration of α2-M (mg/dl), the inhibitory activity of plasma α2-M on proteinase has not been well clarified. In this study, we have not only confirmed the elevation of plasma α2-M concentration, but also found the increased inhibitory activity of plasma α2-M on bacillary thermolysin in patients with INS at relapse. Of particular importance is that the determination of proteinase inhibitory activity was performed by using a bacterial thermolysin and that the inhibitory activity of plasma on thermolysin was shown to be attributable only to α2-M. The results thus obtained indicate that the inhibitory activity of nephrotic plasma on bacterial proteinase is increased, despite the low levels of plasma proteins due to excessive urinary loss in nephrotic syndrome.

Because of anti-thrombin or anti-plasmin activity of α2-M, the elevation of plasma α2-M has been discussed mainly in association with coagulation or fibrinolysis abnormality in INS. However, of the 7 major proteinase inhibitors in plasma, α2-M has the broadest specificity for a wide variety of proteinases from all classes of endopeptidases,[1] suggesting wider biological roles in the body. Native α2-M is a "Ж" shaped tetramer of four identical 185,000 molecular weight peptide chains linked in pairs by disulfide bonds.[10] The mode of proteinase inhibition is characteristic and termed "trapping".[11] The primary structure of α2-M was determined and reported to have a common evolutionary origin to complement protein C3 and C4.[7] Much evidence has been demonstrated the presence of depressed immunity due to impaired lymphocyte function, and recognized as an important cause of various infections frequently seen in INS patients. On the basis of these observations. α2-M may play an important role in the primary defensive system of the body.[4]

Each half of the α2-M molecule has a proteinase binding site, so the maximum binding capacity for a proteinase (thermolysin) in 2 mol/mol α2-M.[10,11] In the present study, however, the relation between the inhibitory activity of plasma α2-M on thermolysin and the plasma α2-M concentration did not show a statistically significant correlation ($p<0.10$). These findings may implicate that the binding capacity of the α2-M molecule may be decreased in the nephrotic plasma as seen in diabetes mellitus.[12] Although fur-

ther studies are needed to clarify the biochemical nature of increased α2-M, it seems possible that elevated α2-M serves as a defense against invading pathogens by inactivating proteinases elaborated by these organisms. This phenomenon may be caused by a compensatory reaction of the body for depressed cell immunity in INS.

Since the major site of synthesis of α2-M is the liver from where it is secreted into the plasma,[13,14] the raised plasma levels may be due to increased synthesis. However, α2-M has been reported to be synthesized and secreted by various cells such as lymphocytes,[15] human macrophages,[16] cultured fibroblasts[17,18] or melanoma cells.[19,20] It is difficult with the available data to explain the mechanism by which α2-M increases in nephrotic plasma.

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