

mediates. However, inhibition of DNA synthesis begins at a time when the mass of the parasite is so small as compared to the host that effective competition seems unlikely, and another mechanism must be sought.

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## Macroglobulin from Human Plasma Which Forms an Enzymatically Active Compound with Trypsin

**Abstract.** *The protein from human plasma which forms an enzymatically active complex with trypsin and is able to protect trypsin from inhibition by soybean trypsin inhibitor has been shown to be an  $\alpha_2$ -macroglobulin. It was obtained from the lipid-poor euglobulin of Cohn Fraction III-0, and appears to be closely related to the 19S-glycoprotein or  $\alpha_2$ -macroglobulin.*

Haverback *et al.* (1) have reported a protein in human plasma which combines with trypsin or chymotrypsin in such a way that the enzyme is active but can no longer be inhibited by certain inhibiting proteins, such as that from soybean. The trypsin-binding activity and a major component in preparations obtained by chromatography of plasma on diethylaminoethylcellulose (2) had a sedimentation coefficient between 15S and 20S. Since Haverback and associates had already shown that this component is an  $\alpha_2$ -globulin, it appeared that the activity might reside in the  $\alpha_2$ -macroglobulin, also known as 19S- $\alpha_2$ -glycoprotein (3-6). Consequently, we have prepared  $\alpha_2$ -macroglobulin from Cohn Fraction III-0 by a modification of the procedure of Brown *et al.* (4) and tested this for trypsin-binding activity.

Fraction III-0 was prepared in the Hyland Laboratories (7) from a pool of fresh human plasma by Cohn methods 6 and 9 (8). The III-0 paste was kept in dry ice until dialyzed against 30 volumes of a mixture of 1.65M NaCl and 0.004M phosphate at pH 7. Dialysis was started with the salt solution partly frozen to keep the tempera-

ture below 0°C until most of the alcohol had dialyzed out, and was completed at 2°C. The dialysis was repeated, and the solution (solvent density of 1.06) was then centrifuged at 97,000g for 18 hours. The pellet, which contains the  $\alpha_2$ -macroglobulin, had a very high content of trypsin-binding protein, as measured by the determination of trypsin activity in the presence of an excess of soybean trypsin inhibitor. Hydrolysis of benzoylarginine-*p*-nitroanilide was followed at pH 7.7 in tris buffer by measuring the increase in absorbancy at 383 m $\mu$  (1, 9). The substrate was prepared in a supersaturated aqueous solution, to avoid the necessity of including an organic solvent in the reaction mixture. Each assay tube contained 15  $\mu$ g of trypsin (10), and this was allowed to react with the sample to be tested for 15 minutes before the addition of 20  $\mu$ g of soybean trypsin inhibitor (10). Substrate was then added, and the extent of hydrolysis was determined after a fixed period of incubation, during which the rate of hydrolysis was constant. Values for the binding protein are given as the amount of trypsin which appears to be active in the presence of the inhibitor, and are calculated from the

rate of hydrolysis relative to that obtained with 15  $\mu$ g of trypsin, buffer, and substrate. Since the amounts of trypsin have not been corrected for the presence of any inactive trypsin in the Worthington trypsin (11), and since the combined trypsin appears to have only about 75 percent of the activity of free trypsin, the values for bound trypsin are apparent.

The lipid-poor material of the pellet from the fraction III-0 was further purified by gel filtration through Sephadex G-200 in 0.06M NaCl + 0.02M tris at pH 7.7. The elution patterns for material absorbing at 280 m $\mu$  and for trypsin-binding activity are shown in Fig. 1. The trypsin-binding activity is expressed as micrograms of combined trypsin per milliliter, and is obviously associated with the material of higher molecular weight. Fractions 60 to 80 were pooled for comparison with material prepared from plasma by a series of chromatographic steps which were designed to purify the trypsin-binding activity. Various preparations of the latter, which will be referred to as binding protein, had given ratios of apparent trypsin bound per milliliter to absorbancy at 280 m $\mu$  of 30 to 50. The material from Sephadex G-200, which we will refer to as III-0 macroglobulin, gave a value of 42 for this ratio. Approximately 1 percent solutions of

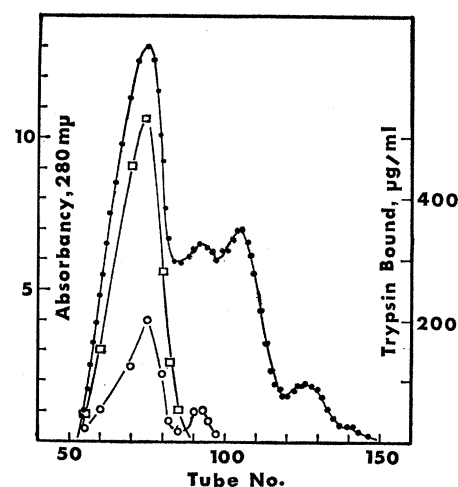


Fig. 1. Gel filtration of lipid-poor euglobulin from Cohn Fraction III-0 through Sephadex G-200 in a mixture of 0.06M NaCl and 0.02M tris, pH 7.7. Solid circles, absorbancy at 280 m $\mu$ ; the scale is on the left. Open squares, trypsin-binding protein, and open circles, total trypsin inhibitor; results are expressed as apparent trypsin bound per milliliter of fraction; this scale is on the right.

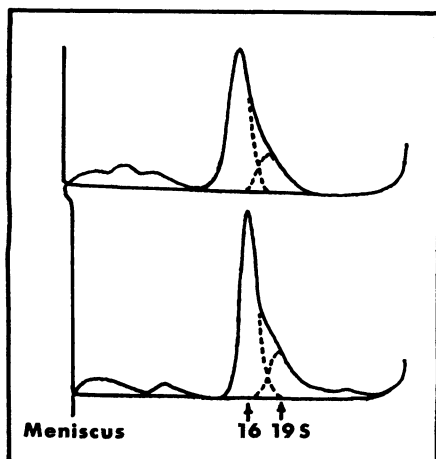


Fig. 2. Ultracentrifuge patterns of III-0 macroglobulin (upper) and binding protein (lower), at 30 minutes after reaching full speed at 56,100 rev/min. Both patterns were made at the same time with a standard and a wedge window. The upper pattern is slightly displaced because the filling of the two cells was not identical.

III-0 macroglobulin and of binding protein were dialyzed against a mixture of 0.105M NaCl and 0.02M phosphate buffer, pH 7.2, and then run together in analytical cells, one with a wedge window, in a Spinco ultracentrifuge at 56,100 rev/min. The two patterns at 30 minutes are shown in Fig. 2. Both preparations contain about 64 percent of a major component which has a sedimentation coefficient of 16.3S in one case and 16.4S in the other (without correction for protein). Additional material is present in both preparations which has an average value of about 19.2S. The other minor components are somewhat different in the two cases.

The results of starch gel electropho-

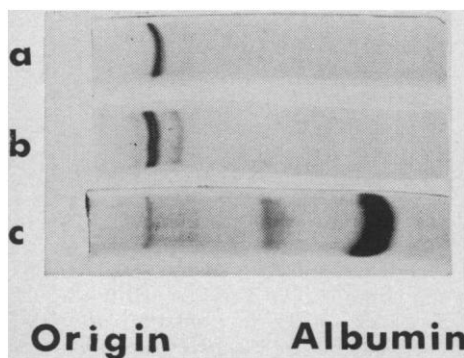


Fig. 3. Starch gel electrophoresis of binding protein (a), III-0 macroglobulin (b), and plasma (c). The sample was applied at the extreme left end of each of the gel sections.

resis (12) in tris-citrate buffer at pH 9.65 (Fig. 3) are also similar, though the major band appears single in the case of the binding protein and double for the III-0 macroglobulin. Some preparations of the binding protein have also shown a doubled  $\alpha_2$ -band. Poulik and Smithies (12) note that the  $\alpha_2$ , or "0," band may be followed by a more diffuse band which is due to adsorption on filter paper. Although the two bands here appear to be of comparable sharpness, their irregular occurrence may result from the use of filter paper for sample application.

From the results of double diffusion (13) against commercial antibody to human plasma and antibody to human  $\alpha_2$ -macroglobulin, both preparations contain a common major antigen in addition to other antigens which are not shared, and both react with antiserum against human  $\alpha_2$ -macroglobulin.

To demonstrate that the binding protein is actually macroglobulin, sufficient trypsin to saturate between one-half and two-thirds of the binding capacity was added to a sample of III-0 macroglobulin, and the mixture was examined in a partition cell in the ultracentrifuge. The sedimentation coefficient was calculated from the tryptic activity of the original mixture and the activity remaining above the partition at the end of the run. A value of 19.8S was obtained. Since this is based on a single run, it would be premature to conclude that the binding activity is associated with the 19S to 20S material rather than the 16S to 17S material, but it is clear that the trypsin is associated with a macroglobulin in such mixtures.

Our preparations are much less pure than the best preparation reported from Schultze's laboratory for the  $\alpha_2$ -macroglobulin, and the sedimentation coefficient of the major component in our preparations is low in comparison with the values reported by Oncley *et al.* (3) and by Schönerberger and co-workers (6). The obvious expedient of examining highly purified material from Schultze's procedure would be fruitless, however, since ammonium sulfate was employed in their method of preparation, and since the trypsin-binding activity has the rather unusual property of being destroyed by ammonium ion at 0.2M or higher concentrations.

Our results provide further confirmation for the existence of a protein in plasma which binds trypsin (or chymotrypsin) in such a way that the enzy-

matic activity is largely preserved, but inhibition by protein inhibitors of these enzymes is prevented. Since combination of trypsin with binding protein appears to prevent the combination of trypsin with protein inhibitors, but does not prevent trypsin from acting on either small substrates or proteins (1), the tentative suggestion can be made that neither the binding protein nor protein inhibitors react directly with the active site. The trypsin-binding protein is an  $\alpha_2$ -macroglobulin with a sedimentation coefficient between 16S and 20S.

The fact that amounts of the best preparations which are required to combine with a given weight of trypsin are relatively large is consistent with the large molecular weight of the binding protein and the binding of a small number of molecules of trypsin by each molecule of the macroglobulin. This would also provide an explanation for the earlier report (1) that both the binding protein and the complex of binding protein with trypsin behave as an  $\alpha_2$ -globulin.

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