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Short Communications

The Combining Ratio between Trypsin and Serum α_2-Macroglobulin

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Human α_2-macroglobulin (α_2-M) forms an enzymatically active complex with trypsin.[1] Haverback *et al.* have called this complex "trypsin-protein esterase" (TPE).[2] The binding of trypsin to α_2-M reduces the specific activity of trypsin on casein by about 95 %, but only by 20 % on a low molecular weight substrate, such as benzoyl-DL-arginine-*p*-nitroanilide (BAPNA).[3] The TPE-activity is determined as the increase in trypsin-like activity of the serum on saturation with trypsin and subsequent addition of soy bean trypsin inhibitor in excess.[4] The serum TPE-activity determined with BAPNA varies so closely with the concentration of α_2-M determined immunochemically that the latter can be calculated from the TPE-activity.[4] The total trypsin binding capacity of α_2-M in the serum expressed in weight of trypsin per volume of serum can be calculated by determining the TPE-activity of the serum with BAPNA and correcting the value found for the reduction of specific activity compared with the specific activity of free trypsin. "The weight combining ratio" and "the molar combining ratio" can then be calculated provided that the absolute concentration of α_2-M in a sample is known.

In a previous investigation the absolute concentration of α_2-M was determined in a serum pool from healthy adult males [5] by comparing the concentration of the pool, as measured by both the TPE-method and an immunochemical method, with the concentration in a pure preparation of α_2-M. It was found to be 180 mg/100 ml. Determination of the TPE-activity in this serum with the previously described method [4] gave an average extinction value of 0.138 for 50 μl serum. After correction for the above mentioned reduction of specific activity of trypsin in the TPE-complex the extinction value was 0.173 which thus corresponds to the activity of the amount of free trypsin that could be bound by α_2-M in 50 μl serum. On simultaneous determination an extinction value of 0.323 was obtained for 10 μg free trypsin. From this value we calculated the trypsin binding capacity of α_2-M in the serum pool as $0.173 \times 10/0.323$ μg/50 μl or 10.7 mg/100 ml. The weight combining ratio (trypsin/α_2-M) was thus 0.059 (10.7/180). A molar combining ratio of 2.02 moles trypsin per mole α_2-M was obtained when the molecular weight of trypsin was taken as 24 000 [6] and the molecular weight of α_2-M as 820 000.[7]

The trypsin binding capacity of α_2-M was also determined with crystalline trypsin (Novo) labelled with ^{125}I [8] in an average amount of less than 1 atom iodine per molecule of trypsin and then purified as described before.[9] By gel filtration (Sephadex G 200) of a mixture of human serum and the ^{125}I-labelled trypsin the trypsin binding proteins of serum were separated as complexes with the labelled trypsin, and the amount of bound trypsin in the fraction was calculated.

Gel filtration was done on a mixture of 0.5 ml serum and 0.40 mg ^{125}I-labelled trypsin (about 80 % saturation of the total trypsin inhibiting capacity of the serum). To avoid the disturbing effect of radio-activity of small peptide impurities in the trypsin preparation, which were also bound to α_2-M, soy bean trypsin inhibitor in an amount equal to twice that of the ^{125}I-labelled trypsin was added to the

mixture after the labelled trypsin had been bound to the serum proteins. The distribution of the radioactivity of the fractions was similar to that of the trypsin inhibiting activity found in a previous study.[10] Trypsin complexes with α_2-M, a trypsin inhibitor presumed to be identical with the inter-α-trypsin inhibitor,[11] α_1-antitrypsin and soy bean trypsin inhibitor were identified. In a control experiment where ^{125}I-labelled trypsin and soy bean trypsin inhibitor were mixed before addition of the serum, the radioactivity found in the macroglobulin fractions was about 5 % of that found in the above experiment. All the trypsin was found as a complex of trypsin and soy bean inhibitor. Soy bean trypsin inhibitor is known not to affect trypsin bound to α_2-M.[1,2] The amount of trypsin bound to α_2-M was therefore calculated from the counts per minute for the fractions with the highest radioactivity of the macroglobulin peak (Fig. 1. No. 20—23) minus the count per minute for the corresponding fractions in the control experiment. In another experiment, where twice the amount of trypsin was added, the amount of trypsin bound to the macroglobulin fraction increased only insignificantly. The α_2-M concentration of the same macroglobulin-fraction was determined immunochemically with the above normal serum pool as a standard.[4] From these values the weight combining ratios (trypsin/α_2-M) were calculated as 0.055, 0.056, 0.057, and 0.061 for fractions Nos. 20, 21, 22, and 23. This corresponds to a molar combining ratio of 1.89, 1.90, 1.95, and 2.07.

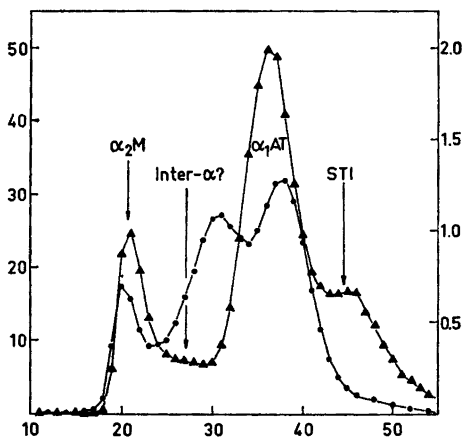

Fig. 1. Distribution of radioactivity found on gel filtration of a mixture of human serum and ^{125}I-labelled trypsin with subsequent addition of soy bean trypsin inhibitor, expressed as counts per minute × 10^{-3} (scale to the left) and distribution of protein expressed as optical density at 280 mμ (scale to the right). Fraction numbers given along the abscissa, ▲ radioactivity, ● protein content.

The close correlation between the concentration of α_2-M and the TPE-activity found in more than 200 individual sera[4] suggests that trypsin is bound to α_2-M in a regular way in certain molar proportions. The molar combining ratios found in the above experiments indicates that the trypsin-α_2-M-complex (TPE) formed on saturation of serum with trypsin consists of one molecule of α_2-M and 2 molecules of trypsin.

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