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STRUCTURAL COMPARISON OF RAT α_1- and α_2-MACROGLOBULINS+

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SUMMARY. Although similar in many properties, homogeneous rat α_1-macroglobulin and acute-phase α_2-macroglobulin are very different in some important structural properties: (a) Rat α_2-macroglobulin is composed of 178,000-dalton subunits, whereas α_1-macroglobulin, like the mouse homologue, contains two types of subunits (Mr 156,000 and 42,000); (b) after reacting with trypsin, native rat α_1- and α_2-macroglobulins generate subunit fragments closely resembling those of mouse α-macroglobulin and human α_2-macroglobulin respectively; (c) after complete trypsin digestion, rat α_1- and α_2-macroglobulins generate strikingly different peptide "fingerprints"; and (d) rat α_1-macroglobulin is serologically more closely related to the mouse homologue as rat α_2-macroglobulin is to the human homologue. This study indicates that these rat proteins are the functional homologues encoded by separate structural genes. Only one of these two genes, albeit considerably modified, appears to be normally represented in the human or the mouse.

INTRODUCTION. Two homologous forms of human α_2-Macroglobulin (α_2M) have been described in rats. The α_1-macroglobulin homologue (α_1M) occurs in the serum of adult normal rats at much higher concentrations (3-5 mg/ml) than rat α_2M (15-36 µg/ml). During acute inflammation, however, the α_1M level remains relatively constant, whereas the α_2M level is greatly elevated to between 1-4 mg/ml in serum (1,2). The level of α_2M is also elevated in pregnant, fetal and neonatal rats (3) as well as those bearing tumors (4). The acute-phase α_2M has previously been purified to homogeneity and studied rather extensively (1,2, 5-7). It is serologically more related to human α_2M than rat

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α_1M. To date, relatively little is known concerning the structure and function of rat α_1M, especially how it might differ from rat α_2M. In this study the structures of α_1M and α_2M were compared.

MATERIALS AND METHODS.

Preparation of α-macroglobulins. Rat α_1M and α_2M were respectively prepared from normal and acute-phase plasma. To obtain acute-phase plasma, adult male rats (Sprague Dawley, 250-400 g) were injected in the thigh muscle with 1 ml of commercial grade turpentine, before the animals were bled 2 days later. Blood was collected by cardiac puncture into plastic syringes containing 1/10 volume of 3.8% sodium citrate in 1 mg/ml soybean trypsin inhibitor. Plasma was harvested by centrifugation (1,200 x g for 30 min).

The macroglobulins were initially extracted from plasma by 4-12% polyethylene glycol precipitation and then chromatographed by a Blue-Sepharose column as described by Koo (8). The fractions containing α-macroglobulins were pooled and then diluted 4-fold with the initial buffer (0.05 M Tris-HCl, pH 8.0, buffer containing 0.05 M NaCl), before the pooled sample was applied to a DE-52 (Whatman) column (17 x 2 cm) previously equilibrated with the initial buffer. The column was eluted with 2 to 3 column volumes of the initial buffer, and then with a linear buffer gradient which was composed of 250-ml each of the initial buffer and the final buffer (0.05 M Tris-HCl, pH 8.0, buffer containing 0.30 M NaCl). The absorbance of all column fractions was determined spectrophotometrically at 280 nm. Rat α_2M was eluted by the initial buffer, whereas α_1M was eluted only by the linear gradient. Other details of the purification procedures are described elsewhere (9). The yields of α_1M and α_2M were about 1.3 and 0.4 mg per ml of plasma.

Electrophoresis. Disc 5% polyacrylamide gel electrophoresis was performed in Tris/glycine buffer at pH 8.9 (10). β-mercaptoethanol-reduced samples were electrophoresed in 7.5% polyacrylamide slab gels (1.5 mm) with the Tris/glycine/sodium dodecylsulfate (SDS), pH 8.9, electrode buffer at 30 mA/gel (11). Gels were stained with Coomassie brilliant blue and destained in a solution of acetic acid:isopropanol:water (1:1:8). The molecular weight standards employed were myosin, β-galactosidase, phosphorylase B, bovine serum albumin and ovalbumin (Bio-Rad Laboratories, Richmond, CA).

Limited trypsin hydrolysis of α-macroglobulins. The susceptibility of the native α-macroglobulins to trypsin hydrolysis was analyzed and compared by SDS-Polyacrylamide gel electrophoresis according to Harpel (12). Briefly, murine and human macroglobulins (50-100 μg each) were incubated in parallel with TPCK-treated trypsin (Worthington Biochemical Corporation) for 1 h at 37°C at various molar ratios as indicated in Fig. 3. The samples were analyzed by SDS gel as above.

Two-dimensional thin-layer peptide mapping of radioiodinated proteins. The macroglobulins were first electrophoresed in 7.5% SDS-polyacrylamide gels and stained as described above. The subunit components were sliced from the remainder of the gel, radio-iodinated, digested by trypsin, and two-dimensional thin-layer peptide maps of the components were obtained according to the procedure of Elder et al. (13).

Other Methods. Protein was determined according to Lowry (14). Homogeneous human α_2M and the mouse α-macroglobulin homo-

logue (αM) were prepared as previously described (15), as was densitometric analysis of the stained gel. Antisera against rat α_1M and α_2M were prepared in New Zealand white rabbits, and immunoelectrophoresis was carried out at pH 8.8 under the same conditions as previously described (8). The trypsin-binding assays were carried out according to Mosher and Wing (16).

RESULTS AND DISCUSSION: Rat α_1M and α_2M have been purified to homogeneity as judged by the criteria of polyacrylamide gel electrophoresis (Fig. 1A) and immunoelectrophoresis (not shown). The isolated proteins are believed to be the true homologues of human α_2M for the following reasons: (a) a rabbit anti-rat α_1M serum crossreacts with rat α_2M, and a rabbit anti-rat α_2M serum in turn crossreacts with human α_2M (data not shown); (b) both rat α_1M and α_2M have molecular weights approximately equal to that of human α_2M as determined by gel filtration in a Sepharose CL6B column (data not shown); and (c) both rat α_1M and α_2M have similar trypsin-binding specific activities, respectively 0.95 and 0.94 ΔA_{247} per min per mg (averages of 3 independent determinations).

Although similar in many properties rat α_1M and α_2M differ in the following properties: (a) Native α_1M exhibits a slightly faster electrophoretic mobility (Figs. 1A); (b) they exhibit different chromatographic properties on a DE-52 column, with α_2M being more basic; (c) the totally reduced α_1M can be dissociated into Mr 156,000 and 42,000 components occurring at approximately equal molar ratio as determined by densitometric analysis, whereas only one subunit size (Mr 178,000) can be obtained from rat α_2M (Fig. 1B); and (d) results in Fig. 2 demonstrate that, at lower trypsin/macroglobulin ratios, the Mr 156,000 subunit of rat α_1M was cleaved into 82,500- and 61,000-dalton subunits, whereas rat α_2M is first split into 88,000- and 77,000-dalton subunits. The cleavage patterns of rat α_1M and α_2M, however, somewhat resemble those of mouse αM and human α_2M respectively. Therefore, rat α_1M is like mouse αM which also possesses two types of

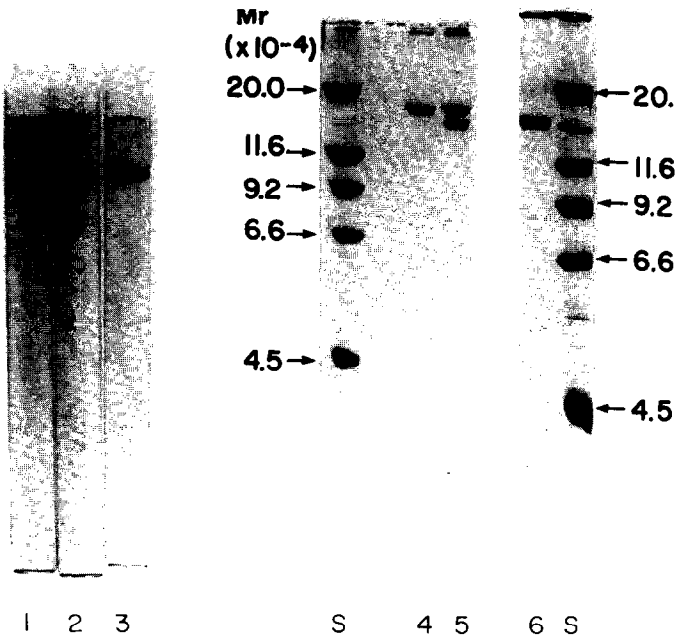

Fig. 1. Electrophoretic analysis of rat α_1M and α_2M. A) Fifty µg of α_1M (column 1), α_2M (column 3) and a mixture of α_1M and α_2M (column 2) were subjected to electrophoresis on 5% discontinuous native polyacrylamide gel as described in "Materials and Methods". B) Five µg each of α_1M (column 4), a mixture of α_1M and α_2M (column 5), and α_2M (column 6) were reduced and subjected to SDS 7.5% polyacrylamide slab gel electrophoresis. S denotes molecular weight standards.

subunits (Mr=163,000 and 35,000) (15), and rat α_2M is similar to human α_2M which also contains subunits of about Mr 180,000 (12). Moreover, neither mouse αM nor rat α_1M produced any precipitin reaction with rabbit antisera against rat or human α_2M. Thus we conclude that rat α_1M is structurally more related to mouse αM than rat or human α_2M, as rat α_2M is more closely related to human α_2M.

The primary structures of the rat alpha-macroglobulins were examined by two dimensional peptide maps. The thin-layered tryptic maps of the ^{125}I-labeled 156,000-dalton subunit of α_1M, the 178,000-dalton subunit of α_2M, and the mixture were obtained after electrophoresis in the first dimension and ascending chromatography in the second dimension. As shown in Fig. 3A and B, the autoradiograms of the α_2M and α_1M maps are very different.

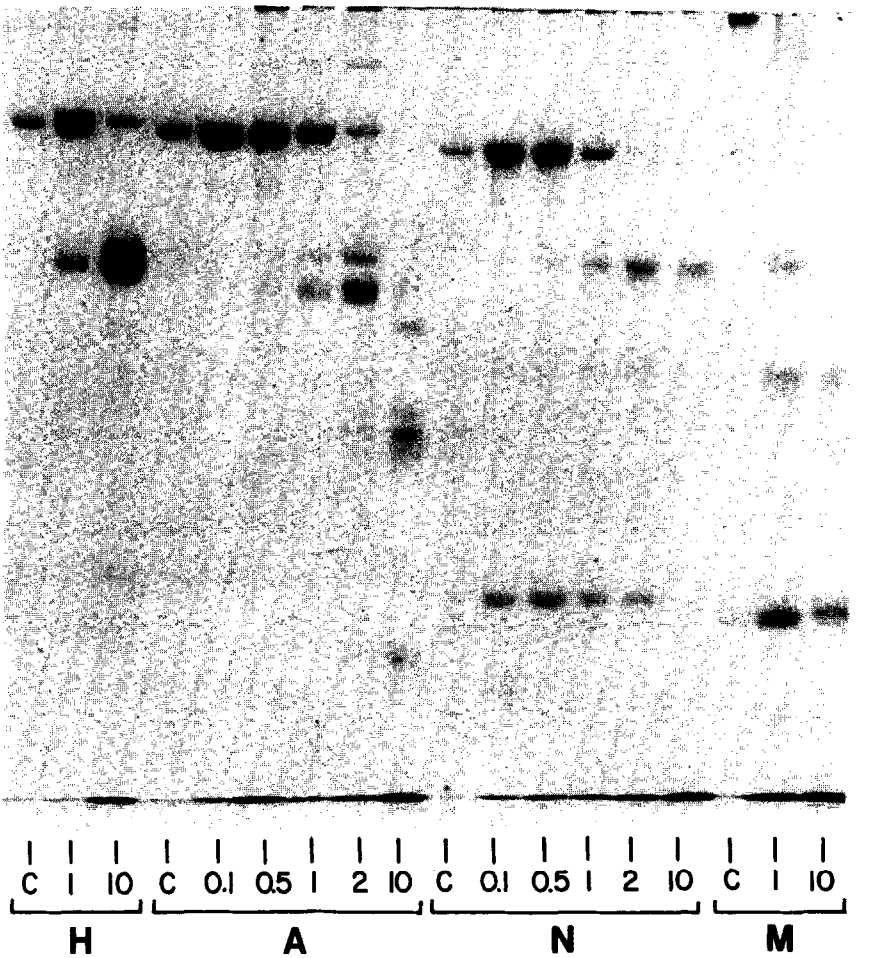

Fig. 2. SDS polyacrylamide gel electrophoresis of α-macroglobulin-trypsin complexes. Various native α-macroglobulin samples were incubated with trypsin at the molar ratios as indicated. The samples were then reduced and subjected to electrophoresis on 7.5% gels. The gel "C" is the nontrypsinized control. The numbers under the gels are the molar ratios of trypsin/macroglobulin. M, N, A and H respectively stand for mouse αM, rat α_1M, rat α_2M and human α_2M.

Although the 156,000-dalton subunit of α_1M is smaller, it contains many peptides not found in α_2M. The peptide map of the mixing experiment (Fig. 3C) shows that there are many extra peptides in map C than either of the individual maps A and B. The schematic diagram (Fig. 3D) assigns many of the peptides in the mixture to either α_1M or α_2M, and shows that α_1M and α_2M may share very few, if any, tyrosine-containing tryptic peptides. In a separate experiment, the 42,000-dalton subunit was found not to

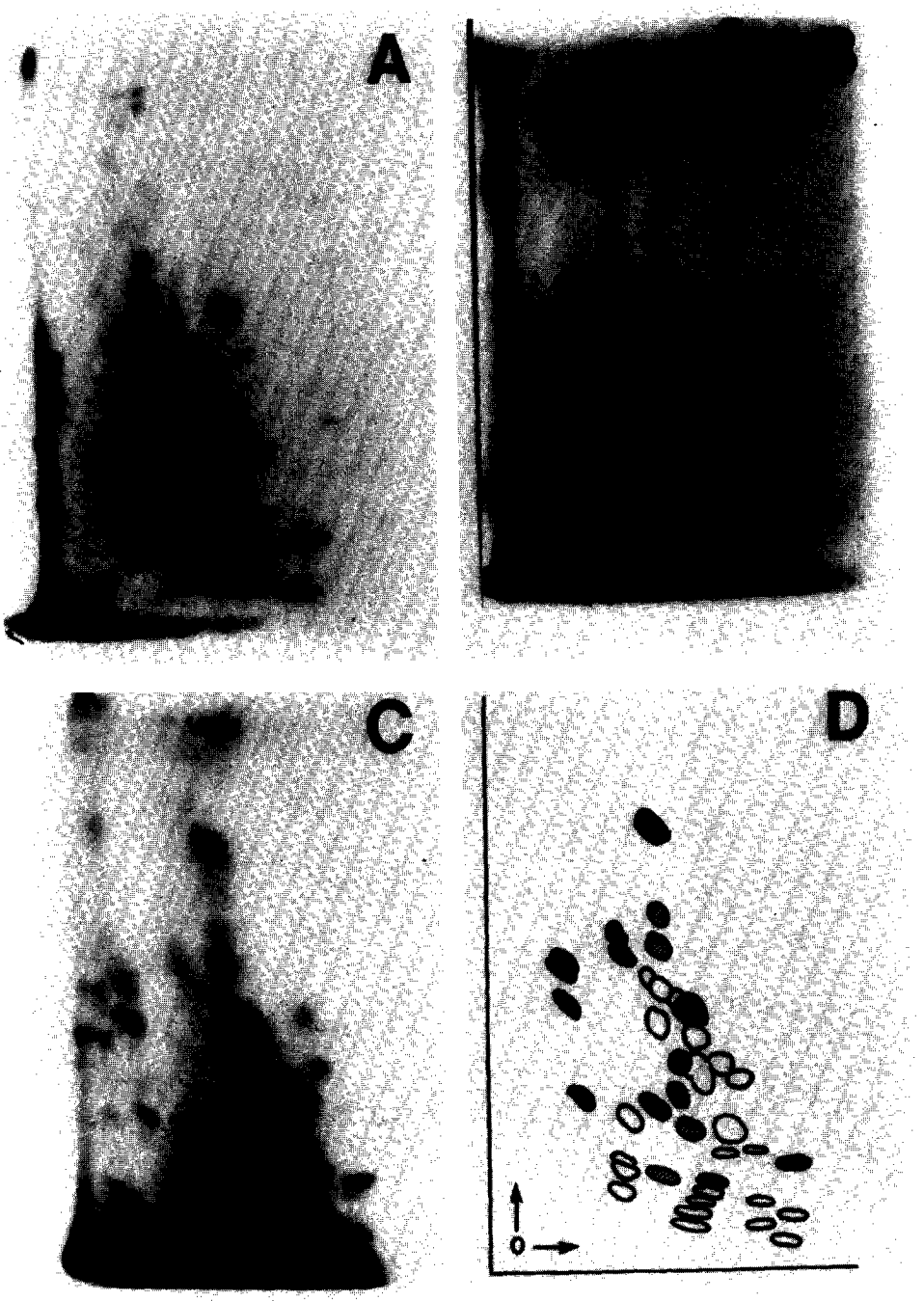

Fig. 3. Two-dimensional peptide maps of the rat alpha-macroglobulins. Electrophoresis was performed in the horizontal direction (left to right) and ascending chromatography in the vertical direction. The origin is at the bottom left of each map. (A) An autoradiograph of the peptide map of the 178,000-dalton subunit of acute-phase α_2M. (B) An autoradiograph of the 156,000-dalton subunit of normal rat α_1M. (C) An autoradiograph of the co-chromatographed mixture of (A) and (B). (D) A schematic diagram of the map (C) indicating the location of the α_2M peptides (open circles), the α_1M peptides (cross-hatched circles), and the presumptive common peptides (hatched circles). The identity of other peptides in map (C) are less obvious, so they are not indicated in (D).

share any discernable tryptic peptides with 156,000- and 178,000-dalton subunit (not shown). Thus the small novel component is not a breakdown product of α_1M or α_2M.

In conclusion, this study demonstrates that rat α_1M and α_2M are the functional homologues which bear very different subunit and primary structures, so they must be coded for by two separate genes. In addition to the protease-binding function, a number of biological activities have been reportedly associated with human α_2M (1) and murine αM (8,17). In the wake of a recent report (18) which suggests that only one form of rabbit α-macroglobulins (α_2M, not α_1M) carries a polyclonal B cell activator, thus each isoprotein may possess certain unique biological properties not shared by the other. Since rat α_1M also can now be readily obtained homogeneous in large quantities, detailed comparisons of structure and function of these macroglobulins will be possible.

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