Immunological Analysis of $\alpha_1$-Microglobulin in Different Mammalian and Chicken Serum

$\alpha_1$-MICROGLOBULIN IS 5-8 KILODALTONS LARGER IN PRIMATES*

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Heterologous radioimmunoassays for a semiquantitative analysis of $\alpha_1$-microglobulin were developed, exploiting the binding between polyclonal rabbit or goat antisera against human, guinea pig, or rat $\alpha_1$-microglobulin and $^{125}$I-labeled human, guinea pig, or rat $\alpha_1$-microglobulin. Homologues of this protein were detected in human, guinea pig, Rhesus monkey, rat, mouse, rabbit, goat, horse, and cow serum by inhibition of a set of heterologous radioimmunoassays. Serum proteins were separated by gel chromatography, and fractions were pooled, concentrated, and radiolabeled with $^{125}$I. By immunoprecipitation of the radiolabeled serum pools with heterologous anti-$\alpha_1$-microglobulin-sera, and separating the precipitates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, analogues of $\alpha_1$-microglobulin were isolated from serum of man, guinea pig, Rhesus monkey, rat, mouse, horse, and chicken. The apparent molecular weight of $\alpha_1$-microglobulin was 31,000–32,000 in human and monkey serum and 24,000–26,000 in guinea pig, rat, mouse, horse, and chicken serum. The possibility of an addition of a 5,000–8,000-Da peptide in primate $\alpha_1$-microglobulin is discussed.

$\alpha_1$-Microglobulin is a plasma protein, isolated from the urine of humans (1), guinea pigs (2), and rats (3, 4) with disturbed renal filtration and/or reabsorption functions. Human $\alpha_1$-microglobulin has been reported to have immunoregulatory functions suppressing the blastogenic effect of some antigens on lymphocytes in vitro and inhibiting the migration of leukocytes in vitro (5). $\alpha_1$-microglobulin from all three species is a glycoprotein, and glycopeptides from human and guinea pig $\alpha_1$-microglobulin were shown to possess the inhibitory activity of antigen stimulation of lymphocytes (6). The protein carries an unidentified brown substance and is electrophoretically heterogeneous (2–4, 7). The plasma concentration of $\alpha_1$-microglobulin is normally between 10 and 50 mg/liter and, in addition to monomeric $\alpha_1$-microglobulin, it also appears in a high molecular weight form (1, 2, 4, 7), which in humans has been described as a complex between IgA and $\alpha_1$-microglobulin (8). Synthesis of $\alpha_1$-microglobulin has been definitely demonstrated in human fetal liver (9), guinea pig liver (10), and rat hepatocytes (4). Contradictory results concerning the production of the protein by lymphocytes have been published (11–13).

The apparent molecular weight of human $\alpha_1$-microglobulin determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis is 31,000 (7), which is larger than guinea pig $\alpha_1$-microglobulin from urine (2) and rat $\alpha_1$-microglobulin from urine or hepatocyte medium (25,000) (4). In this work, as a first step to study this size difference from an evolutionary point of view, we have demonstrated the presence and analyzed the apparent molecular weight of $\alpha_1$-microglobulin in serum from several different species.

**MATERIALS AND METHODS**

Detection of $\alpha_1$-Microglobulin in Mammalian Sera—Heterologous binding between radiolabeled human, guinea pig, or rat $\alpha_1$-microglobulin and antibodies against either of these antigens could be inhibited by serum from other species (heterologous radioimmunoassays). Ten different combinations of $^{125}$I-$\alpha_1$-microglobulin and anti-$\alpha_1$-microglobulin were used. Fig. 2 illustrates the ability of nine different mammalian sera and chicken serum to inhibit these 10 heterologous radioimmunoassays. The sera were diluted twice with radioimmunoassay buffer. Normal rabbit serum was used as a control for 0% inhibition of the heterologous radioimmunoassays where a rabbit antiserum was used (1, 2, 4, 5, 7, and 8), and normal goat serum served as a control for the radioimmunoassays with goat antiserum (3, 6, 9, and 10). Fig. 2 shows that the sera yielded different patterns of inhibition of the 10 radioimmunoassays. All nine mammalian sera were able to inhibit at least some of the radioimmunoassays. No inhibition could be seen by chicken serum. The weak interactions displayed by this serum were not significant.

Immunoprecipitation of $\alpha_1$-Microglobulin from Serum Pools—Monomeric $\alpha_1$-microglobulin in serum from man, guinea pig, rat, mouse, Rhesus monkey, horse, and chicken was partly purified by gel chromatography through Sephadex G-200. The serum proteins thus prepared were radiolabeled with $^{125}$I, immunoprecipitated with anti-$\alpha_1$-microglobulin sera or, as a control, antiserum against guinea pig albumin, and

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Fig. 2. Inhibition of heterologous radioimmunoassays by mammalian and chicken sera, diluted twice. The radioimmunoassays were designated numbers according to Table I and were composed of $^{125}$I-labeled human (1-3), guinea pig (4-6), and rat (7-10) $\alpha_1$-microglobulin, and rabbit anti-rat $\alpha_1$-mI (1, 4), rabbit anti-guinea pig $\alpha_1$-mI (2, 7), rabbit anti-human $\alpha_1$-m (5, 8), goat anti-guinea pig $\alpha_1$-m (1, 4), goat anti-rabbit $\alpha_1$-mI (3, 5, 7, 8), and goat anti-rabbit $\alpha_1$-m for human serum pool, to a final concentration of 0.5 mg/ml. The chicken samples contained approximately 200 cpm and the other samples between 500 and 2000 cpm. The total concentration of acrylamide and bisacrylamide was 13.7% and the degree of cross-linking 3.7%. The gel was dried and subjected to autoradiography. The start of the separation gel is denoted with an O, and molecular weights of markers are given as $M_r \times 10^{-3}$.

then separated by SDS-polyacrylamide gel electrophoresis (Fig. 5). Heterologous anti-$\alpha_1$-microglobulin-sera were used throughout.

In all cases, anti-albumin precipitated a single component with a molecular weight of 65,000-70,000, and anti-$\alpha_1$-microglobulin bound a molecule with a molecular weight between 24,000 and 32,000 in the different serum pools. Anti-$\alpha_1$-microglobulin (rabbit anti-rat $\alpha_1$-mI) also precipitated traces of a molecule of 70,000 Da from the chicken serum pool, and both the anti-albumin and anti-$\alpha_1$-microglobulin precipitates from chicken serum produced stainings that remained at the origin of the electrophoresis. The serum pools were subjected to a preimmunoprecipitation with anti-guinea pig albumin prior to the anti-$\alpha_1$-microglobulin immunoprecipitation or with rabbit anti-rat $\alpha_1$-mI prior to anti-albumin. The preimmunoprecipitates when analyzed by SDS-polyacrylamide gel electrophoresis contained a number of components in addition to the protein bands shown in Fig. 5.

The radiolabeled $\alpha_1$-microglobulins from human, guinea pig, Rhesus monkey, rat, mouse, horse, and chicken serum are compared in Fig. 6. Human and monkey serum $\alpha_1$-microglobulin, with an apparent molecular weight of 31,000 and 32,000, respectively, are larger than the analogues from the other five species. Guinea pig, rat, mouse, horse, and chicken serum $\alpha_1$-microglobulin are smaller and have an apparent molecular weight of 24,000-26,000.

**DISCUSSION**

$\alpha_1$-Microglobulin has previously been detected and isolated from the urine of three different species: man, guinea pig, and

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It has been detected in the blood of these three species by the use of immunochromatographic methods (1, 2, 4) and isolated from the plasma of humans (22). Here, we have described the presence of \( \alpha_1 \)-microglobulin in serum from six other mammals (see Fig. 2) and chicken. When analyzing the apparent molecular weights of the immunoprecipitates from various serum analogues of \( \alpha_1 \)-microglobulin upon SDS-polyacrylamide gel electrophoresis (Fig. 6), they seem to fall into two categories: 31,000-32,000 (human and monkey \( \alpha_1 \)-microglobulin) and 24,000-26,000 (guinea pig, rat, mouse, horse, and chicken \( \alpha_1 \)-microglobulin). Of these, only the human homologue has previously been subjected to an analysis of the molecular weight (22), which yielded the same value. Reports on the apparent molecular weight of guinea pig and rat \( \alpha_1 \)-microglobulin from urine and rat \( \alpha_1 \)-microglobulin from hepatocyte cultures (2, 4) agree well with the data for the serum proteins. Thus, \( \alpha_1 \)-microglobulin from the two primates has proved to be considerably larger than \( \alpha_1 \)-microglobulin from the five nonprimates investigated so far.

The inhibition of cross-idiotypic binding between antisera and antigen was employed in an agglutination assay by Kunkel et al. (23) to describe structures related to the antigen and was further developed as heterologous radioimmunoassays by Gordon and Kindt (24). Here, such heterologous radioimmunoassays were used for the detection of \( \alpha_1 \)-microglobulin in various sera. Fig. 2 illustrates the inhibition of the 10 different heterologous radioimmunoassays by various sera. The patterns were strikingly similar between human and monkey serum and between goat and cow serum, indicating structural similarities between \( \alpha_1 \)-microglobulin from these species. Only small differences could be seen between rat and mouse serum: heterologous radioimmunoassays 2 and 7 (both rabbit anti-guinea pig \( \alpha_1 \)-m) could be inhibited by rat serum but not by mouse serum, which suggests a closer structural relatedness between rat and guinea pig than between mouse and guinea pig \( \alpha_1 \)-microglobulin. Moreover, monkey was the only “third” species able to interact clearly with radioimmunoassay 3 (goat anti-guinea pig \( \alpha_1 \)-m binding of \( ^{125}I \)-human \( \alpha_1 \)-microglobulin), and the inhibitory activity of guinea pig serum implied a pattern very different from the others. This implies exceptional structural properties of guinea pig \( \alpha_1 \)-microglobulin among the 10 species investigated here. The inhibitory activity of serum \( \alpha_1 \)-microglobulin on the radioimmunoassays is determined in part by its association with other molecules. It has been demonstrated by Grubb et al. (22) that human serum \( \alpha_1 \)-microglobulin is complex-bound to IgA, which is also reflected on the elution profile of gel chromatography of human serum (1). A similar behavior has been shown for rat serum \( \alpha_1 \)-microglobulin (4). However, guinea pig \( \alpha_1 \)-microglobulin in serum, upon gel chromatography, shows a much less pronounced IgA-\( \alpha_1 \)-microglobulin peak (2) (see also Fig. 3 in this work). This may account for at least part of the difference between guinea pig and other sera in these systems.

Chicken serum did not significantly inhibit any of the heterologous radioimmunoassays 1-10, although a molecule of 25,000 Da was bound by anti-\( \alpha_1 \)-microglobulin sera in the immunoprecipitation analysis. This suggests that the chicken indeed synthesizes a homologue to human, guinea pig, and rat \( \alpha_1 \)-microglobulin, but that the evolutionary distance between chicken and these mammals has yielded structural differences that allow only low affinity heterologous interactions. These results together with the notable difference in size between primate and nonprimate \( \alpha_1 \)-microglobulin may indicate a rapid evolution of the protein. However, before any conclusions of this kind can be drawn, it is necessary to evaluate the usefulness of the heterologous radioimmunoassays to evolutionary studies on proteins, i.e. the compatibility of the differences obtained by the heterologous radioimmunoassays with, for example, the evolutionary clock hypothesis (for a review, see Ref. 25).

Attempts to isolate \( \alpha_1 \)-microglobulin by immunoprecipitation from radiolabeled whole serum were not successful (results not shown). Instead, a fraction of serum, containing partly purified monomeric \( \alpha_1 \)-microglobulin, was prepared by gel chromatography. \( \alpha_1 \)-Microglobulin was isolated from the radiolabeled serum pools by immunoprecipitation with heterologous anti-\( \alpha_1 \)-microglobulin sera. Immunoprecipitations with homologous reagents in most cases yielded less pure preparations (results not shown). The reasons for this are not known. Perhaps antibodies against impurities in the original \( \alpha_1 \)-microglobulin preparations (cf. Ref. 13) are less liable than the anti-\( \alpha_1 \)-microglobulin antibodies to cross-react with proteins from another species. Immunoprecipitated human \( \alpha_1 \)-microglobulin showed a faint additional band, corresponding to an apparent molecular weight of approximately 27,000 on SDS-polyacrylamide gel electrophoresis (Fig. 6, lane A). By adding light chains from human IgG, this band was eliminated (Fig. 5, Man, lane II), suggesting that the band represents radiolabeled human light chain, presumably precipitated by cross-reaction of the second antibody, goat anti-rabbit IgG.

The difference in apparent molecular weight between \( \alpha_1 \)-microglobulin from serum of the two primates and the five nonprimates could perhaps be explained by the addition of a peptide with an apparent molecular weight of 5000-8000 in the human and monkey protein. This hypothetical peptide may have been added to either the amino terminus or carboxyl terminus of the protein, or inserted somewhere along the \( \alpha_1 \)-microglobulin peptide. A difference at the amino-terminal end would indeed be interesting since amino acid residues 5 and 17 have been proposed as attachment sites of an O-glycosidically and N-glycosidically linked carbohydrate chain, respectively (26, 27). Addition of a 5000-8000 Da peptide at the amino-terminal end would certainly include at least amino acid residue 5 and thus a possible carbohydrate side-chain. However, this structural difference between primate and nonprimate \( \alpha_1 \)-microglobulin may not reflect a difference in the polypeptide backbone, caused by a rearrangement of the \( \alpha_1 \)-microglobulin gene. Another possible explanation could be a difference in the post-translational handling of the protein, such as a difference in size and structure of the enigmatic brown-colored chromatophore.

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REFERENCES


\(^{3}\) B. Åkerström, work in progress.
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**Supplementary Material**

IMMUNOLOGICAL ANALYSIS OF alpha-MICROGLOBULIN IN DIFFERENT MAMMALIAN AND CHICKEN SERUM anne-MICROGLOBULIN IN 8-3% LARGER IN PROMIXES

As Antibody

**MATERIALS AND METHODS**

Reagents and antisera. alpha-microglobulin was purified in this study from the serum of guinea pigs, rabbits, goats, and chickens. alpha-microglobulin from guinea pig, rabbit, and chicken sera was obtained from the company Bio-Rad Laboratories (Richmond, CA). guinea pig alpha-microglobulin was used in all experiments, except for those involving affinity-purification of chicken alpha-microglobulin. The purification procedure has been described (27). Bovine polyclonal antisera were used in all experiments. The identities of the antisera were confirmed by Western blotting and ELISA. alpha-Microglobulin from guinea pig serum was used as a standard for the assay.

Binding of radiolabeled alpha-microglobulin. Binding was determined by incubation in the absence of antiserum. The immune precipitate was washed, and the pellet was then washed again with 200 volumes of saline until no further precipitation occurred. The pellet was then dissolved in 0.2 ml of 0.2 M Tris-HCl, pH 7.5, and the supernatant was analyzed for alpha-microglobulin by Western blotting.

Inhibition of binding of radiolabeled alpha-microglobulin. The binding of radiolabeled alpha-microglobulin was determined in the absence of antiserum, and the immune precipitate was washed. The pellet was then washed again with 200 volumes of saline until no further precipitation occurred. The pellet was then dissolved in 0.2 ml of 0.2 M Tris-HCl, pH 7.5, and the supernatant was analyzed for alpha-microglobulin by Western blotting.

**RESULTS AND DISCUSSION**

I. Immunological Properties of alpha-Microglobulin

II. Inhibition of binding of radiolabeled alpha-microglobulin

III. Comparison of binding of radiolabeled alpha-microglobulin in different species

Figure 1. Binding of 125I-labeled guinea pig (A), rabbit (B), and chicken (C) alpha-microglobulin to anti-alpha-microglobulin serum. The binding was determined in the absence of antiserum. The immune precipitate was washed, and the pellet was then washed again with 200 volumes of saline until no further precipitation occurred. The pellet was then dissolved in 0.2 ml of 0.2 M Tris-HCl, pH 7.5, and the supernatant was analyzed for alpha-microglobulin by Western blotting.

Figure 2. Comparison of binding of radiolabeled alpha-microglobulin in different species. The binding was determined in the absence of antiserum. The immune precipitate was washed, and the pellet was then washed again with 200 volumes of saline until no further precipitation occurred. The pellet was then dissolved in 0.2 ml of 0.2 M Tris-HCl, pH 7.5, and the supernatant was analyzed for alpha-microglobulin by Western blotting.

Table 1. Comparison of binding of radiolabeled alpha-microglobulin in different species. The binding was determined in the absence of antiserum. The immune precipitate was washed, and the pellet was then washed again with 200 volumes of saline until no further precipitation occurred. The pellet was then dissolved in 0.2 ml of 0.2 M Tris-HCl, pH 7.5, and the supernatant was analyzed for alpha-microglobulin by Western blotting.

**References**


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The proteins in the pooled fractions from the gel-chromatographies of the seven different sera were separated by SDS-polyacrylamide gel electrophoresis. Fig. 4A shows the result when the proteins were stained with Coomassie Brilliant Blue. All pools contained proteins with molecular weights fairly evenly distributed between 10,000 and 70,000. Fig. 4B illustrates a separation on SDS-polyacrylamide gel electrophoresis of the same pools, radiolabeled with 125I. A pool of human serum contained radioactive protein bands. These bands represent several proteins. Two examples of this are a 27,000 Da-protein in human serum (lane 1) and a 18,000 Da-protein in mouse serum (lane 4). Fig. 4B also illustrates the almost complete absence of proteins larger than approximately 70,000 Da in the pools. No protein band appears as strikingly dominant or re-occurring in the pools.

![Image of gel electrophoresis](image.png)

*Figure 4* - SDS-polyacrylamide gel electrophoresis of the pooled fractions from gel-chromatography of sera from five different species: horse (1), monkey (2), rabbit (3), cat (4), mouse (5), dog (6), and man (7). Each well contained 2 µg of protein, and the gel was stained with Coomassie Brilliant Blue. In B, the proteins had been radiolabeled with 125I prior to the electrophoresis. 5.5-1 µg proteins were applied to each well, and after the electrophoresis, the gel was dried and subjected to autoradiography. The total concentration of acrylamide and bis was 12.7% and the degree of cross-linking was 3.7%. The start of the separation gel is denoted with an 0, and molecular weights of markers are given as Mₐ x 10⁵.