ALLOTYPY IN RABBIT 19S PROTEIN

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The allotypic specificities (intraspecies antigenic specificities) of rabbit γ-globulin have been shown to be controlled by two genetic loci, one of which controls the group comprising A1, A2 and A3,* while the second controls the group comprising A4, A5 and A6 (Oudin, 1960). In this paper evidence is presented for the presence of allotypic specificities of both groups in rabbit 19S protein, as well as in 7S γ-globulin.

METHODS

Gel filtration in Sephadex G-200 columns was performed at 5° C essentially as described by Flodin and Killander (1962) using physiologic saline buffered with 0.05 M phosphate at pH7 as the eluting medium. Two columns (56 x 4cm, 27 gm G-200, bed volume 705 ml, A ; and 107 x 5cm, 88 gm G-200, bed volume 2100 ml, B) were used. Four peaks were obtained in the eluate with crests reproducibly at 36, 49, 64, and 100 % of bed volume.

Allotypy in the fractions was determined by ring tests using sera prepared by Dr. J. Oudin (Oudin, 1960). The dilutions in 4% polyvinyl pyrrolidone of the antisera used were A1, 1/12; A3, 1/6; A4, 1/8; A5, 1/16; and A6, 1/12. The maximum twofold dilutions of sera from normal rabbits (heterozygous for the allotypic specificity in question) capable of giving a positive

* The notation for allotypy proposed by Dray et al (1962) is used in this paper.

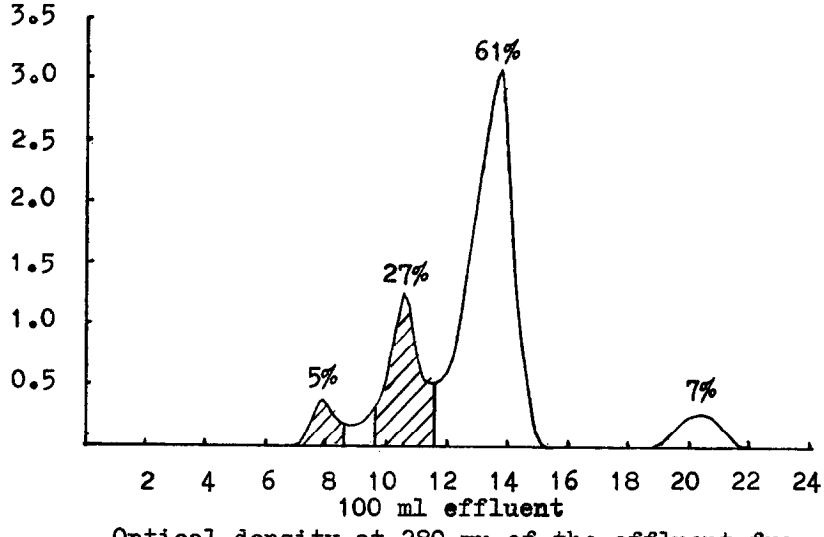
test with these diluted sera were : A1, 1/160; A3, 1/20; A4, 1/160; A5, 1/160; A6, 1/640. Sera from apparently homozygous rabbits usually react at one twofold dilution more. Gel filtration with A2 sera has not yet been attempted. Goat anti-III serum (Porter, 1959) was obtained from Dr. M. Cohn.

Centrifugation in a sodium chloride gradient from 3.5 to 19% concentration was performed at 30$\pm$ 1° C in a Spinco Model LHT preparative centrifuge provided with a heating unit using head SW39L at 39,000 rpm. The time of centrifugation was either 5 hrs 5 mins or 5 hrs 30 mins. Calculations indicated that in 5 hrs 30 mins the centers of the 7S and 19S bands should be located 65% and 18% of the distance from the bottom to the top of the tube.

RESULTS

Twelve and one-half ml of a mixture of equal parts of three rabbit sera : A1,4, A1,6, and A1,4,6, was filtered using column A . Analytical centrifugation of the fraction at the center of the first peak (OD at 280 mu, 1.760) showed but one peak with a sedimentation constant of 17.8 S_w^{20} (not extrapolated to infinite dilution). Ultracentrifugation in 20% aqueous potassium bromide (density 1.25 gm/ml) showed an accumulation of material at the inner(upper) surface, presumably the expected beta-lipoprotein. Ring tests showed all three specificities (A1, A4 and A6) to be present in the fractions of the first and second peaks. For each specificity the fraction at the center of the first peak was positive at one twofold dilution more than the fraction in the valley between the two peaks. This suggests that the positive tests in the first peak were not due to peak overlap.

Further evidence that the allotypy found in the first peak was not due to a contaminant from the second peak was obtained by G-200 filtration of 15 ml of A1,4 serum on a larger column (B). The results are summarized in Figure 1. Specificities A1 and A4 were found in both the first and second peaks, but several fractions giving negative reactions for both allotypic specificities were present in the valley between the two peaks. Moreover, when the fractions of the first peak were combined, concentrated by ultra-filtration, and repassed through the column, the allotypic specificities were found to persist in the single peak which emerged at the same effluent volume as did the original first peak.



Optical density at 280 mμ of the effluent from the G-200 filtration of 15 ml of A1,4 serum on column B, bed volume 2100 ml. Shaded areas indicate fractions which gave positive ring tests for both allotypic specificities. Percentages at the top of each peak represent the percent of the total optical density units (OD x Volume) found in each peak. The fraction at the center of the first peak gave a positive test for A1 at a dilution of 1/4 and for A4 at a dilution of 1/8. It was negative for III specificity. The fraction at the center of the second peak gave a positive test for A1 and A4 at a dilution of 1/64 and for III specificity at a dilution of 1/256.

Figure 1

To exclude the possibility that the allotypy of the first peak might be due to a 7S γ-globulin aggregate, ring tests were run with goat anti-III serum diluted 1/64 in 4% polyvinyl pyrrolidone. Ring tests with fractions from the center of the first peak and the valley between the two peaks were negative, whereas the fraction at the center of the second peak was positive to a dilution of 1/256. The fraction from the center of the first peak was also negative with undiluted anti-III serum, indicating that the absence of a positive reaction with the diluted serum was not due to a narrowed specificity of the antiserum resulting from the dilution. It thus seems unlikely that the allotypic specificity detected in the first peak was the result of contamination with 7S γ-globulin.

Centrifugation in a sodium chloride gradient was employed to determine the sedimentation behavior of the proteins carrying the allotypic specificities. An A3,4,5 serum (25ml) was subjected to gel filtration on column B. Ring tests showed the presence of all three allotypic specificities in the fractions of the first peak. Perhaps as a result of the greater protein charge placed on the column, tests with anti-III were positive in the second half of the first peak and the valley between the two peaks, but the fractions of the first half were negative with anti-III serum and yet positive with the anti-allotype sera. The fractions of the first peak were combined and concentrated to the original serum volume, and 0.5 ml of the concentrate was centrifuged in a sodium chloride gradient (see Methods). After centrifugation for 5 hrs 5 mins at 39,000 rpm the tube was fractionated into 23 fractions of 4 drops each, which were dialyzed overnight against physiologic saline. Ring tests were positive for A3 in fractions 1 through

5 (counting from the bottom of the tube), for A4 in 1 through 13 and for A5 in 1 through 5 plus 11. These results are consistent with the interpretation that most of the allotypy is carried by molecules with a sedimentation constant of 19S, but that some 7S material is present.

Further information on the sedimentation behavior of the molecules carrying allotypy was obtained using fractions from the gel filtration of 50 ml of A1,3,4 serum on column B. Gradient centrifugation (5 hrs 5 mins) of the fraction at the center of the second peak revealed A1 in fractions 8 through 16, A3 in 11 through 13, and A4 in 7 through 16 of 16 fractions, as expected for a molecule with a sedimentation constant of 7S. Gradient centrifugation (5 1/2 hrs) of the unconcentrated fraction at the center of the first peak revealed the presence of A1 in fractions 2 and 3, and A4 in fractions 1 through 4 of 18 fractions, as expected for a molecule with a sedimentation constant of 19S. Tests for A3 were negative, probably because of the difficulty of detecting A3 at moderate dilution (see Methods).

Thus it appears that allotypic specificities of both allelic groups are present in 19S protein. Assignment of the allotypic specificities to the 19S γ-globulin molecule must await evidence on the electrophoretic mobilities of the protein species bearing them. The assumption that these allotypic markers uniquely identify the peptide chains bearing them leads to the conclusion that two peptide chains used in the formation of 7S γ-globulin are also used in the formation of 19S protein. If the absence of positive ring tests in the fractions of the first peaks with anti-III sera is indicative of the absence of the chain bearing the isotypic specificity of piece III, one is led to the conclusion that three different chains are present in

the 7S γ-globulin molecule, although as yet only two have been identified (Fleischman, Pain and Porter, 1962).

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