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Preparation of the Peptide Chains of Normal Human IgG Globulin

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1. A method is described for the preparation of samples of pure heavy and light chains from IgG by gel filtration on Sephadex G 150 in alkaline solution.

2. The two types of light chains, $\varkappa$ and λ, could be obtained separately from the mixture by absorbing one with an appropriate antiserum of type K or L, followed by gel filtration.

Antibodies in normal human serum are associated with 4 classes of immunoglobulins (Ig). These are now designated as IgG, IgA, IgD, and IgM. All have a similar structure and are made up of four (to six?) chains. When IgG globulin is reduced in alkaline aqueous solution and acidified to pH = 2.3 the molecule dissociates into two types of peptide chains, heavy chains (M = 50 000) and light chains (M = 20 000) which can be separated by gel filtration in acid or even better in alkaline media. The light chains from all classes of human immunoglobulins occur in two forms, known as $\varkappa$ and λ chains. These two forms of light chains, found on separate Ig molecules, can be further separated into a large number of electrophoretically distinct forms.

Several methods for the separation of heavy and light chains have been reported. They are mainly based on the methods of: a) Edelman [1] who performed the separation in buffers containing urea, and b) Porter [2] who performed the separation in acetic acid or propionic acid.

The drawback of Edelman's method is that after removal of the urea the proteins become insoluble and immunologically inactive. Although Porter *et al.* preserved the activity of the peptide chains they could not obtain completely pure heavy and light chain fractions with their separation method.

In this paper a method is described for the preparation of pure heavy and light chains, produced and separated in alkaline solution, and a method for the preparation of light chains of the K and L types, starting from a normal preparation of light chains obtained from pooled human IgG, by absorbing one of the two types with the appropriate antibody followed by gel filtration on Sephadex G 150 at pH = 8.2.

MATERIALS AND METHODS

IgG globulin. An immuno-electrophoretically pure IgG globulin was obtained from Kabi, Stockholm, Sweden.

Chemicals. The chemicals used were of analytical reagent grade. Monoiodo-acetamide was recrystallised from water.

Immuno-electrophoresis and gel diffusion were carried out as described earlier.[3]

Starch-gel electrophoresis. Starch-gel electrophoresis was carried out in vertical or horizontal trays by using:

1. 7 M urea and 0.05 M formic acid buffer pH = 3 as described by Edelman and Poulik,[1]
2. 7 M urea and 0.035 M glycine buffer pH = 8.8 as described by Cohen.[4] Electrode vessels contained 0.3 M boric acid—0.06 N NaOH, pH = 8.2. The electrophoresis was continued for 24—70 h at 4—5 V/cm.

Fractionation by gel filtration. Gel filtration was performed with the use of Sephadex G 150 and G 75 (bead form).

The buffers used were: 0.1 M Tris-HCl — 0.5 M NaCl — 0.002 M EDTA, pH = 8.2 (alkaline buffer) and 0.01 M HCl — 0.025 M NaCl, pH = 2.

Buffers and gel suspensions were degassed before they were used. Column dimensions throughout all the experiments 100 × 3.2 cm. The ascending techniques for gel filtration [9] was used.

Concentration. Concentration of protein solutions was carried out by negative pressure dialysis with the aid of Visking dialysis tubing 8/32.

Amino acid analyses. These were carried out in a Technicon Amino Acid Analyser on samples hydrolysed for 24 h and 48 h under conditions described earlier.[5] Corrections for losses during hydrolysis were made.

Preparation and isolation of antibodies. Antisera to human IgG globulin and Bence-Jones proteins of type K and L were obtained by immunising rabbits with subcutaneous injections of the purified antigens in Freund's complete adjuvant (8 mg protein/week for 6 weeks and longer). The animals were bled for the first time after 6 weeks. The "7S" globulin fraction was isolated from the sera by gel filtration on Sephadex G 150, analogous to the procedure described by Flodin and Killander [6] (see Fig. 1).

The eluting buffer was 0.1 M Tris-HCl — 0.5 M NaCl — 0.002 M EDTA pH = 8.2.

EXPERIMENTS AND RESULTS

Reduction of IgG globulin and isolation of heavy and light chains. A 2 % solution of IgG globulin in 1 M Tris-HCl buffer, pH = 8 (degassed), was reduced at room temperature by adding 2-mercaptoethanol to a final concentration of 0.2 M. After 1 h the solution was cooled to 0° and an excess of MIAA * (0.4 M) was added to block the free SH groups. After stirring (magnetic) for 30 min the solution was dialysed against large volumes of 0.9 % NaCl for 20 h at 4°. The clear solution was then centrifuged for 30 min at 30 000 *g* and the supernatant acidified to pH = 2.3. After standing for 20 h at this pH and at 4°C the solution was neutralised and centrifuged as mentioned above.

* MIAA = monoiodo-acetamide.

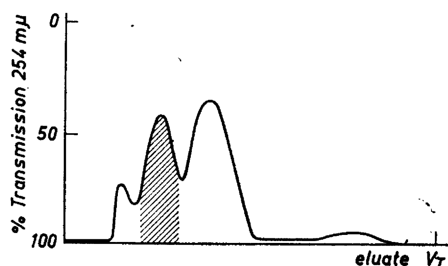

Fig. 1. Gel filtration of an antiserum on Sephadex G 150. Eluting buffer: 0.1 M Tris-HCl — 0.5 M NaCl — 0.002 M EDTA (pH = 8.2). Hatched area: fraction containing 7S globulins.

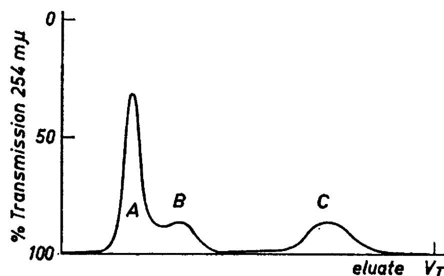

Fig. 2. Gel filtration of reduced IgG on Sephadex G 150. Eluting buffer as in Fig. 1.

The supernatant was next subjected to gel filtration on Sephadex G 150 in order to separate the heavy and light chains from each other with 0.1 M Tris — 0.5 N NaCl — 0.002 M EDTA, pH = 8.2, as eluting buffer. As Fig. 2 shows, three fractions were obtained.

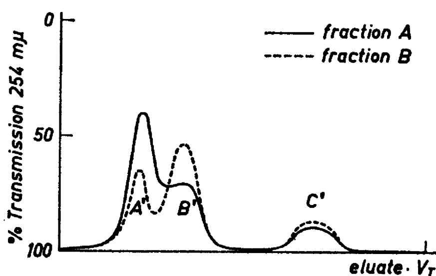

Fig. 3. Gel filtration of heavy chains (fractions A + B, Fig. 2) on Sephadex G 150 in 0.01 M HCl — 0.025 M NaCl.
A^1: heavy chains; B^1: heavy chains; C^1: light chains.

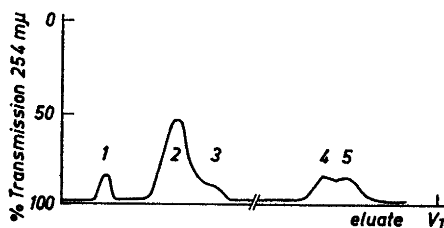

Fig. 4. Gel filtration of light chains (fraction, C, Fig. 2) on Sephadex G 75. Fraction 1 comprises heavy and light chains (judged from starch-gel electrophoresis); fractions 2 + 3 comprise di- and monomers of light chains. Fraction 4 comprises adsorbed heavy chains as judged from starch-gel electrophoresis, amino acid analyses, and immuno-electrophoresis. Fraction 5 comprises lower molecular weight material with a high glucide content.

Fractions A, B, and C were, after concentrating, again subjected to gel filtration; fractions A and B (presumably containing heavy chains) on Sephadex G 150 in acid medium (Fig. 3); fraction C (presumably containing light chains) on Sephadex G 75 in alkaline medium (Fig. 4).

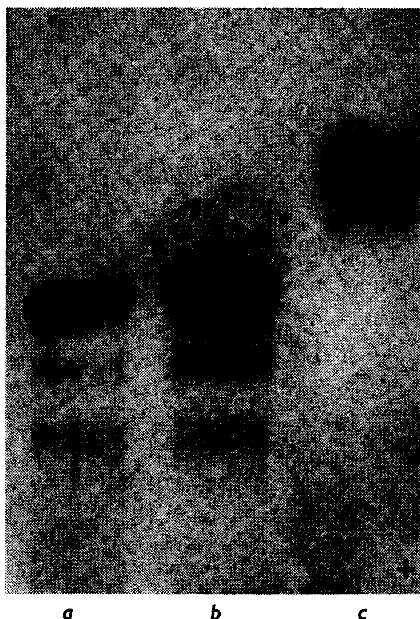

Fig. 5. Starch-gel electrophoresis at pH = 3.0 of H and L chains; a. fraction B^1 of Fig. 3; b. fraction A^1 of Fig. 3; c. fraction 2 of Fig. 4.

The fractions A′ and B′ (Fig. 3) and fractions 2 and 3 (Fig. 4) were subjected to starch-gel electrophoresis at pH = 3.0. As Fig. 5 shows, fractions A′ and B′ represent heavy chains, (presumably) contaminated with some polymers, but free of light chains; fractions 2 and 3 represent pure light chains.

Electrophoresis at pH = 8.8. has shown that fractions 2 and 3 are still electrophoretically very heterogeneous at that pH, as was first observed by Cohen.[4]

ISOLATION OF $\varkappa$ AND λ CHAINS

The fraction of normal light chains after gel filtration on Sephadex G 75 (fraction 2 of Fig. 4) is still a mixture of $\varkappa$ and λ chains in a ratio of about 2:1. These could be obtained separately by absorbing one of the two types with the appropriate antibody (λ chains with the "7S" globulin fraction of anti-K; $\varkappa$ chains with that of anti-L serum) and then passing the mixture over Sephadex G 150 at pH = 8.2 to separate the free chains from those bound to antibody and from excess antibody.

The procedure was as follows: The mixture of light chains (fraction 2, Fig. 4) was incubated with an excess of the "7S" fraction of an antiserum for 1 h at 37° and 20 h at 4°. That an excess was really present was checked by reacting the mixture with Bence-Jones proteins of the types K or L in immunoelectrophoresis and gel diffusion experiments. After centrifuging (30 min, 30 000 *g*) the supernatant was applied to a Sephadex G 150 column in alkaline buffer. The result is shown in Fig. 6.

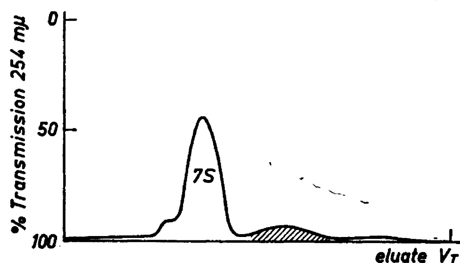

Fig. 6. Gel filtration of the incubation mixture of light chains + antiserum on Sephadex G 150. Eluting buffer as in Fig. 1. Hatched area: free light chains.

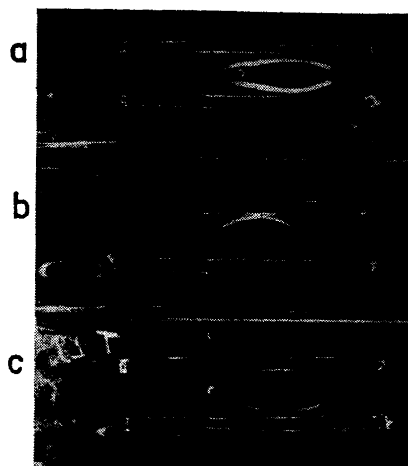

Fig. 7. Immuno-electrophoresis of light chains; a. mixture of light chains, ϰ and λ (fraction 2 of Fig. 4); b. after treatment with anti-L: ϰ chains; c. after treatment with anti-K: λ chains. The upper well contains anti-K serum; the lower well contains anti-L serum.

The shaded fraction was examined by immuno-electrophoresis and appeared to represent light chains of either type K or type L, depending on which type of antiserum was used for absorption (Fig. 7). Both types of light chains, ϰ and λ, showed the same electrophoretical heterogeneity as the total light chain mixture in a glycine-urea starchgel (pH = 8.8).

Table 1. Amino acid composition of IgG and peptide chains of IgG globulin.

Amino acid	IgG	Heavy	ϰ	λ
Asp	16.5	16.0	17.9	16.7
Thr	17.0	16.8	18.8	18.4
Ser	25.6	23.9	28.4	29.7
Glu	21.2	19.9	22.3	23.0
Pro	16.1	16.3	13.3	15.4
Gly	14.4	14.0	15.4	16.0
Ala	11.3	10.9	14.0	15.6
Val	18.8	18.4	14.2	16.1
Met	1.7	0.4	1.10	0.85
Ile	4.7	4.1	6.4	6.4
Leu	16.0	16.0	16.0	16.0
Tyr	8.6	6.5	6.2	6.9
Phe	6.7	6.6	7.7	7.2
Lys	13.9	13.0	12.6	12.3
His	4.4	4.0	3.9	3.9
Arg	6.3	6.4	6.6	6.5

Finally Table 1 gives the amino acid composition of the IgG globulin and its components: heavy, $\varkappa$, and λ chains, all calculated on an arbitrarily chosen basis of 16.0 leucine residues. The differences in overall composition of $\varkappa$ and λ chains are in line with the differences between these classes reported earlier for Bence-Jones proteins [7] and for the light chains obtained from myeloma proteins by Cohen.[8]

DISCUSSION

With the method, described in this paper, which differs from others reported previously in that separation takes place in alkaline solution, we could obtain pure heavy and light chains. A drawback is that the recovery for the light chains is low: about 40 % of the theoretical recovery. As regards the further separation of the light chains in types K and L, Cohen [8] reported a partial fractionation of $\varkappa$ and λ chains, taking advantage of the fact that λ chains dissociate from reduced and alkylated IgG at higher pH than do $\varkappa$ chains.

We have tried to apply Cohen's procedure, performing the dissociation at a range of pH values between 4.0 and 2.0, going down in steps of 0.2 unit. We were not able to obtain samples of light chains which contained more than 70 % of one of the two types. For this reason we have reverted to the immunochemical method described in this paper, which yielded us the two types in pure form, although with a considerable loss of protein. As mentioned earlier, the two types of light chains show many distinct bands in starch-gel electrophoresis at pH = 8.8 (with 7 M urea). This heterogeneity may result from the synthesis of chemically distinct light chains by different cell types.

It will be of great interest to have the disposal of all of the separate fractions (bands) of the $\varkappa$ and λ chains, besides many Bence-Jones proteins of type K and L with an electrophoretically different mobility, for a comparative study of the antigenic determinants of these proteins. Preliminary experiments with "whole" light chain preparations to isolate each of the electrophoretically distinct fractions from the mixture — a method consisting of a combination of gel filtration, ion-exchange chromatography and column electrophoresis — have given promising results. We could isolate fractions each containing only 2 bands. The amino acid composition of these fractions shows that small but significant mutual differences exist. It is our intention to further fractionate the $\varkappa$ and λ chain preparations in this manner.

A more convenient procedure related to the one described in this paper can presumably be developed by preparing an immunoadsorbent to either $\varkappa$ or λ chains. The recovery in the specific adsorption step is then likely to be improved. Furthermore both types of light chains should easily be recovered from an experiment involving the use of only one kind of antiserum. We are extending the present study to the use of immunosorbents of this kind employing Sephadex or cellulose as insoluble matrix.

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