Subtypes of Rabbit κ Light Polypeptide Chains Associated with the *b* Locus*

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ABSTRACT: Two distinct populations of light chains (L_I and L_{II}) were obtained by oxidative sulfitolysis and gel filtration of IgG from normal rabbits homozygous for each of the four known *b*-locus allotypes. Essentially all the λ-type light chains originally present in the IgG were found in the L_I fractions together with a distinct subtype of κ light chains (κ_A). The L_{II} fractions contained essentially only κ-type light chains κ_B).

The ratio of κ_B to κ_A was in b4 > b5 ≥ b6 > b9. This is also the order of the relative quantitative expression of these alleles in heterozygotes. In addition, an increase in κ_A was correlated with an increase in expression of λ-type chains. The same allotypic specificities were found on both κ_A- and κ_B-type chains and both subtypes from each *b*-locus allotype had alanine as well as some aspartic acid at the N terminal. The two subtypes from b5 light chains could be clearly distinguished since κ_B contained seven half-cystine residues while κ_A contained five half-cystine residues.

Immunoglobulins are made up of light and heavy polypeptide chains which are generally held together by covalent disulfide bridges as well as noncovalent bonds (Fleischman, 1966; Cohen and Milstein, 1967). The light chains isolated from the serum of an individual rabbit consist of structurally related subpopulations. Some of the structural differences such as those between κ and λ antigenic types result from the fact that more than one cistron in the genome is responsible for the production of a light-chain gene product. Furthermore, at a given cistron, genetic polymorphism can result in the production of structurally different allelic forms, *i.e.*, allotypes.

In rabbits, the majority of light-chain populations (70–90%) are designated *b positive* (b^+) since they carry allotypic antigenic determinants which seem to be controlled by simple Mendelian alleles at the *b* locus (Oudin, 1966). At present, there are four known alleles designated b^4, b^5, b^6, and b^9.[1] There are structural homologies in the carboxyl-terminal region between the b^+ light chains of rabbits and the major κ-type light chains of man and mouse (Doolittle and Astrin, 1967; Appella and Perham, 1967). The allelic allotypic forms also differ in sequence in this region (Appella *et al.*, 1969). In addition, there is chemical and immunological (Appella *et al.*, 1968; Rüde and Givol, 1968) evidence that light chains homologous to the λ type of man and mouse exist in rabbits. The λ type usually represents from 10 to 30% of the total population of rabbit light chains (Rejnek *et al.*, 1969; Carbonara and Mancini, 1968). Since these light chains do not carry antigenic determinants controlled by the *b* locus, they are termed *b negative* (b^-) (Oudin, 1966). Some of these b^- chains carry an antigenic determinant, c7, which appears to be controlled by a distinct locus, *c* (Mage *et al.*, 1968).

In this study, we performed oxidative sulfitolysis on IgG from normal rabbits homozygous for each of the four known *b*-locus allotypes. By gel filtration we separated the resultant *S*-sulfo derivatives of IgG into heavy chains and two populations of light chains (L_I and L_{II}) (Rejnek *et al.*, 1969). Essentially all the b^- light chains originally present in the IgG were found together with some b^+ populations in the L_I fraction, whereas the L_{II} fraction was comprised entirely of b^+ light chains. Previous studies (Rejnek *et al.*, 1969) indicated that approximately one-fifth of the total light chains was obtained as an L_I fraction from b4 or b5 IgG. The aims of this study were to determine whether the other *b*-locus allotypes had the same proportions of L_I and L_{II} chains and to compare and characterize the b^+ light chains within these two populations. It was of particular interest to determine the chemical and genetic basis for the separation of two distinct light-chain populations both carrying the same allotypic specificity.

Materials and Methods

Rabbits. Rabbits used to obtain serum pools were homozygous b^4b^4, b^5b^5, and b^6b^6 progeny of parents with known genotypes from colonies bred at the National Institutes of Health. Rabbits which were b^9b^9 homozygotes as well as serum pools from b^9b^9 rabbits were generously supplied by Dr. S. Dubiski. Sera from all of the above animals contained the c7 antigen. Rabbits of the ACEP strain were purchased from The Jackson Laboratory, Bar Harbor, Maine. They were homozygous b^4b^4 but lacked c^7.

Preparation of IgG and Light Polypeptide Chains. The IgG was prepared from rabbit sera by the rivanol method (Hořejší and Smetana, 1954) followed by ammonium sulfate precipitation (35% saturation) and chromatography of the dissolved and dialyzed precipitate on DEAE-cellulose. IgG was eluted successively with 0.0175 M (pH 6.9) [I] and 0.035 M (pH 7.5) [II] sodium phosphate buffers until in either case absorption measured at 280 mμ was 0.2. These IgG [I + II] eluates were shown to be free of contaminating serum proteins detectable by disc electrophoresis on polyacrylamide gels. By this two-step

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[1] In this paper allotypic specificities Ab4, Ab5, Ab6, and Ab9 as well as the genes Ab^4, Ab^5, Ab^6, and Ab^9 are abbreviated by omitting the capital "A" (Dray *et al.*, 1962).

elution procedure essentially 90% of the protein applied to the column was recovered whenever b4, b5, or b6 IgG was isolated. The remainder of the applied protein was eluted with 0.05 M phosphate buffer (pH 7.5); however, this eluate was heavily contaminated with serum proteins and consequently was not used to prepare light chains. In the preparation of b9 IgG, however, about 15–30% of the protein could be eluted with 0.05 M phosphate buffer (pH 7.5). Light chains were isolated from IgG [I + II] by gel filtration on Sephadex G-100 in 6 M urea containing 0.05 M formic acid after oxidative sulfitolysis of interchain disulfide bonds as described previously (Rejnek *et al.*, 1969).

Reagents. All solvents used in chromatography were reagent grade. Acrylamide and methylenebisacrylamide were recrystallized twice from acetone. Phenyl isothiocyanate (Eastman Organic Chemicals) was purified by distillation *in vacuo*. Dioxane was distilled over metallic sodium and stored frozen. Urea was deionized just prior to use.

Antisera. Anti-b4, -b5, and -b6 allotype antisera were prepared as described previously (Dray *et al.*, 1963). Anti-b9 allotype antiserum was a pool from rabbits immunized by Dr. S. Dubiski (Dubiski *et al.*, 1961) and was generously supplied by him.

Double immunodiffusion tests were performed in 1.2% agar in physiological saline (Ouchterlony, 1949).

Quantitative Precipitin Studies. Light chains labeled with ^{125}I were tested for precipitability (Gilman *et al.*, 1964). Measurements of radioactivity were made with a heavily shielded well-type scintillation spectrometer having a sodium iodide crystal. The average background count was approximately 60 cpm and a minimum of 100 times the background count was recorded for each sample. The total per cent of label precipitated by goat antiserum was determined by an initial direct precipitation with an excess of antiserum, followed by a second precipitation using unlabeled carrier and additional antiserum; with rabbit antisera, after initial direct precipitations, possible soluble complexes in supernatants were precipitated by adding to a portion of the supernatant, a second antiallotype antiserum specific for an allotype only present on the antibody portion of the complexes. Controls for nonspecific precipitation utilized normal rabbit sera in the first step.

Disc electrophoresis was carried out in 7.5% acrylamide gels at pH 9.4 (Reisfeld and Small, 1966).

Labeling of IgG and light chains with ^{125}I was carried out by the method of McFarlane (1958); 1–2 atoms/mole of protein was added and specific activities ranged from 2 to 4×10^5 cpm per mg. Each preparation was shown to be more than 99% precipitable in 10% trichloroacetic acid.

Specific immunoabsorbents were prepared by treatment of Sepharose 2B with cyanogen bromide using the method of Axén *et al.* (1967) with the modifications described previously (Rejnek *et al.*, 1969). In addition to immunoabsorbents with bound light chains, an immunoabsorbent with antibody was prepared as follows. The modified Sepharose suspension (50 ml) was mixed with 20 ml of antibody solution (containing 25 mg of purified antibody), stirred 2 hr at room temperature, and then held at 4° overnight. After this procedure 67% of the antibodies were bound to the absorbent to which now was added 5 ml of a 2% myoglobin solution following which the suspension was stirred for 2 hr at room temperature and then held at 4° overnight. The myoglobin solution was added to cover those reactive sites on the absorbent which did not react with the relatively large molecular weight IgG antibody. These sites, if not covered, seem to bind the much smaller light chains nonspecifically resulting in low yield of purified antigen. This procedure increased the yield of light chains by about 50%. The immunoabsorbent was then packed into a column (0.9×30 cm) and washed extensively and successively with 0.3 M borate buffer (pH 8.0), 0.05 M glycine–HCl buffer (pH 2.4), and again with the original borate buffer. The protein concentration of the eluate was estimated by measuring absorption at 280 mμ.

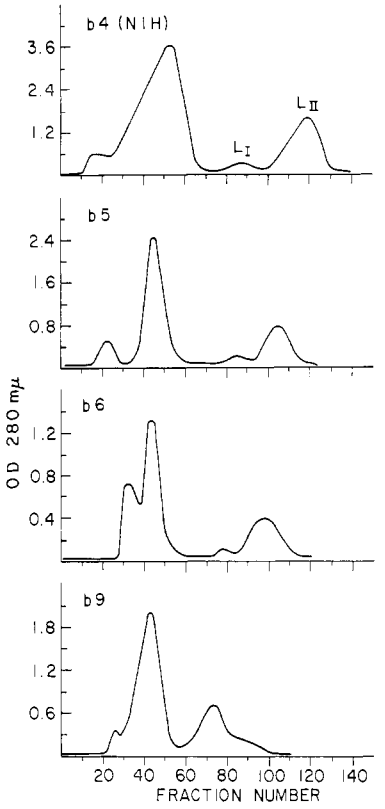

FIGURE 1: The elution patterns obtained from Sephadex G-100 columns following partial sulfitolysis of purified IgG (I + II) of different allotypes. The solvent used was 0.05 M formic acid containing 6 M urea. Column loads were: b4, 2.0 g (10×150 cm column); b5, 0.35 g (6×150 cm column); b6, 0.3 g (6×180 cm column); and b9, 0.49 g (6×180 cm column).

Amino Acid Analyses. Samples of approximately 0.3 mg each were lyophilized to dryness in hydrolysis tubes. Constant-boiling HCl (1 ml) was added to each tube, the samples were frozen, evacuated twice, and sealed under vacuum. Following hydrolysis at 110° for 24 and 72 hr, respectively, the samples were dried under vacuum and analyzed on a Beckman amino acid analyzer, Model SP 644, which was equipped for accelerated, sensitive analysis (Hubbard, 1965). Performic acid oxidation was performed according to the method of Moore (1963) but without the use of HBr as a reducing agent.

Amino-terminal amino acids were determined by a modification of the Sanger method (Fraenkel-Conrat *et al.*, 1955), employing [^{14}C]fluorodinitrobenzene. Amino-terminal end groups were also detected by the [^{35}S]phenyl isothiocyanate method (Laver, 1961).

Carboxyl-terminal amino acids were detected by hydrazinolysis (Appella and Tomkins, 1966) after performic acid oxidation of the protein samples.

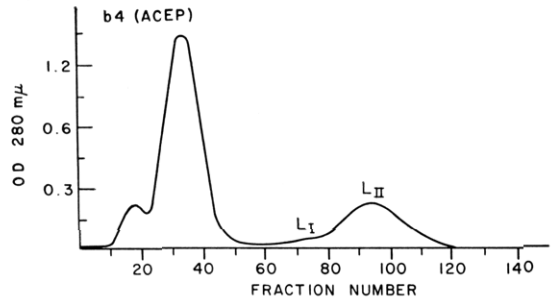

FIGURE 2: The elution pattern obtained from a Sephadex G-100 column following partial sulfitolysis of purified IgG (I + II) from the ACEP strain of b4 rabbits. The column load was 0.25 g on a 6 × 150 cm column.

Tryptic digestion was performed with an enzyme:substrate ratio of 1:100 in 0.5% NH_4HCO_3 using fully carboxymethylated protein derivatives.

Peptide mapping was carried out by two-dimensional paper chromatography and electrophoresis (Katz *et al.*, 1959). Spots were located by dipping the sheets in ninhydrin–cadmium reagent (Liu *et al.*, 1965).

Results

Initially we attempted to determine whether all the *b*-locus allotypes had the same proportions of L_I and L_{II} populations. Figure 1 illustrates the elution patterns obtained following the oxidation sulfitolysis of rabbit IgG bearing the four known allotypic specificities. It was of particular interest to prepare the light chains also from b4 ACEP rabbits since this IgG is devoid of molecules with c7 specificity. Figure 2 shows the elution pattern of sulfitolyzed IgG from the ACEP strain. This ACEP IgG which lacks detectable b^- populations carrying c7 determinants was found to contain only 6% L_I with 94% L_{II}. We compared the relative proportions of L_I and L_{II} populations from preparations of IgG with the c7 determinants and each of the four *b*-locus allotypes by cutting out the peaks and

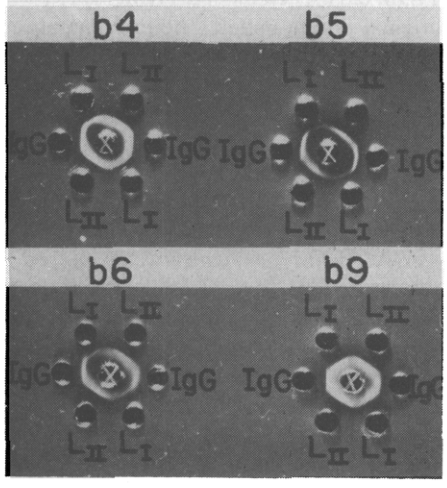

FIGURE 3: The gel diffusion patterns obtained when L_I, L_{II}, and IgG of each allotype were reacted with the homologous antiallotype antiserum⊗. Light-chain concentrations were approximately 1 mg/ml; IgG, 2 mg/ml.

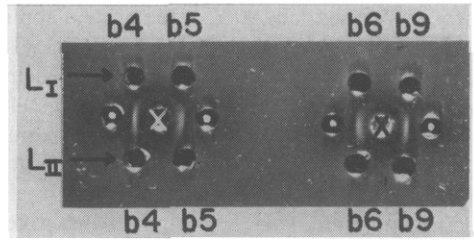

Figure 4: The gel diffusion patterns obtained when L_I and L_{II} of each allotype were reacted with anti-c7 antiserum (⊗). Light chain concentrations were approximately 1 mg/ml. Control serum containing c7 (⊙).

weighing them. Two experiments were done with different preparations of IgG from each allotype. The b9 light chains are markedly different, in that 65 and 78% was obtained as L_I and only 35 and 22% as L_{II} from two different preparations of IgG. In contrast, the b4, b5, and b6 preparations yielded 12–20% L_I. Although the differences in proportions of L_I and L_{II} populations between these allotypes were less pronounced, there was a definite trend indicating a greater proportion of L_I in b6 (20–21%) and b5 (15–18%) compared with b4 (10–12%). The L_I and L_{II} fractions were compared with IgG of each allotype using rabbit antiallotype antisera. In Figure 3, the gel diffusion patterns show that L_I and L_{II} preparations react identically in agar gel tests in which appropriate antiallotype antisera were used. Since the antigens used to prepare these antisera were whole IgG, it is not surprising that some of the sera appear to precipitate better with these antigens than with the free light chains. Although the anti-*b* allotype antisera

TABLE I: Quantitative Precipitation of ^{125}I-Labeled Light-Chain Fractions

		% ^{125}I Precipitated by	
Allotype	Fraction	Goat Anti-b^{-a}	Rabbit Anti-*b* Allotype[b]
b4	L_I	61.6	28.5
b4	L_{II}	2.0	86.8
b5	L_I	54.0	21.3
b5	L_{II}	0.8	93.9
b6	L_I	47.7	29.7
b6	L_{II}	1.4	85.3
b9	L_I	60.8	43.1
b9	L_{II}	2.9	84.9

[a] The results with the goat antiserum are the sums of the per cent ^{125}I label directly precipitated with antiserum and the increment precipitated on addition of unlabeled carrier and additional antiserum to portions of the initial supernatants. [b] The results with rabbit antisera are the sums of the per cent ^{125}I label directly precipitated with antiserum and the increment precipitated on addition of a second antiallotype antiserum to a portion of the initial supernatant to precipitate soluble complexes. The maximum per cent of label precipitated from a preparation is given. Nonspecific control values ranging from 0.2 to 3% have been substracted.

TABLE II: Amino Acids Composition of Unfractionated Light Chains and Purified b^+ (L_I b5) and b^+ (L_{II} b5) Expressed as Residues.[a]

Amino Acid	Unfractionated L-Chain b5[b]	b^+ (L_I b5)	b^+ (L_{II} b5)
Lys	9.6	9.00	9.19
His	1.4	1.21	1.19
Arg	3.1	2.94	2.92
Asp	17.9	16.80	16.93
Thr	25.3	23.70	25.74
Ser	23.1	22.70	23.80
Glu	19.8	19.90	20.05
Pro	13.4	13.00	13.36
Gly	18.1	17.00	17.60
Ala	16.4	15.60	15.80
Val	18.5	16.43	17.79
Met	0.7	0.76	0.87
Ile	7.3	7.14	7.11
Leu	13.5	13.54	13.56
Tyr	9.6	9.74	11.21
Phe	6.3	6.59	6.55
$CySO_3H$	6.4	4.80	6.80

[a] Calculated on the basis of 204 residues for all amino acids except tryptophan and half-cystine. [b] Data and standard errors taken from Reisfeld and Inman (1968). This is a preparation which contains both b^- (λ) and b^+ (κ) light chains.

do not distinguish between the L_I and L_{II} fractions in gel diffusion tests, anti-c7 antiserum reacts only with the L_I fractions (Figure 4). This correlates with two of our observations: (1) b^- light chains occur only in the L_I fraction (Rejnek *et al.*, 1969); (2) some of these b^- light chains carry c7 antigenic determinants (Mage *et al.*, 1968).

In the next series of experiments we tried to ascertain whether the varying amounts of L_I fractions found in IgG of different allotypic specificity, especially b9 and b4 (ACEP), were simply due to the presence of varying amounts of b^- light chains. To answer this question, we estimated by quantitative precipitin techniques the relative amounts of b^+ and b^- light-chain populations present in L_I and L_{II} fractions obtained from IgG with b4, b5, b6, b4 (ACEP), and b9 allotypic specificities. The data shown in Table I confirmed that b^- light chains are only detectable in L_I fractions and that the total population of b^- molecules remained about the same (50–60%) in the L_I fractions of all *b*-locus allotypes studied irrespective of the over-all proportion of this fraction. For example, 12% of the light chains from NIH b4 rabbits was in an L_I fraction and of these 61.6% was b^-. Therefore, within the total population there was approximately 7% b^- light chains. In contrast, 65% of the light chains from one b9 IgG preparation was contained within the L_I fraction and of these 60.8% was b^-, *i.e.*, approximately 40% of the b9 light chains was b^-. In the b4 (ACEP) preparation which lacks detectable c7 determinants and has the smallest L_I fraction (6%), both b^+ and b^- populations were detected in approximately equal amounts (35.1% of b^-, 37.9% of b4). In this strain only about 2% (35% of 6%) of the light chains reacted with the goat antiserum to b^- light chains. A similar proportion of b^+ populations was detected in all the L_I fractions (20–40%).

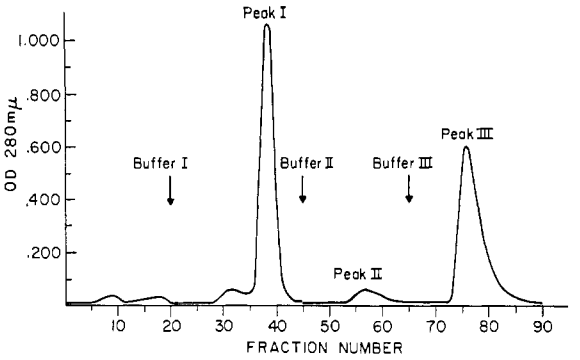

FIGURE 5: The elution pattern of L_I b5 from a Sepharose immunoabsorbent on which anti-b5 antibodies were bound. Buffer I, pH 3.4 (0.036 M glycine, 0.03 M HCl, and 0.15 M NaCl); buffer II, pH 2.4 (0.023 M glycine, 0.018 M HCl, and 0.15 M NaCl); and buffer III, pH 2.4 (0.023 M glycine and 0.018 M HCl).

The results obtained clearly indicate that two distinct populations of b^+ light chains can be separated from the IgG of all four *b*-locus allotypes. Since two b^+ populations which share common allotypic determinants were found in the L_I and L_{II} fractions, we wanted to ascertain whether they were chemically distinct. To separate b^+ and b^- light chains of the L_I fraction, rabbit anti-b5 antiserum was passed through a column of Sepharose which had bound to it L_{II} b5 light chains. The column was initially equilibrated with 0.3 M borate buffer (pH 8.0) and then excess serum proteins resulting from the passage of antiserum through the column were removed by thorough washing with this buffer. The absorbed antibodies were eluted with 0.05 M glycine–HCl buffer (pH 2.4) and the eluate was immediately neutralized to pH 7.0. The antibodies which eluted as a sharp, single peak were dialyzed against 0.9% NaCl and then bound to Sepharose which had been treated with CNBr as described previously (Rejnek *et al.*, 1969); 20 mg of L_I b5 light chain was dissolved in the borate buffer and the solution was passed through the immunoabsorbent. The elution was performed stepwise as shown in Figure 5. Fractions corresponding to single elution peaks were pooled, dialyzed against 0.5% NH_4HCO_3, lyophilized, and tested for the presence of b^+ and b^- light chains by immunodiffusion employing goat anti-b^- and rabbit anti-b^+ antisera. The results shown in Figure 6 demonstrate that peak I contains only b^- light chains while peak II contains b^+ light chains slightly contaminated with b^- light chains. Peak III was found to contain only b^+ light chains with no detectable b^- molecules. The results of amino acid analyses of unfractionated light chains and b^+ obtained from L_I b5 (peak III) and L_{II} b5 are shown in Table II. The data clearly indicate that the b^+ population of light chains in the L_I fraction contains essentially five half-cystine residues whereas the b^+ populations of the L_{II} fraction contain essentially seven half-cystine residues. In regard to the rest of the amino acids, the two fractions show significant differences only in threonine, valine, and tyrosine. For serine, proline,

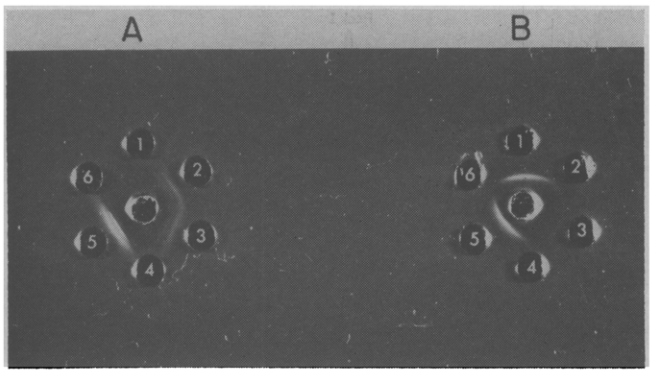

FIGURE 6: The gel diffusion patterns obtained when the fractions shown in Figure 5 were tested with rabbit anti-b5 antiserum (A) and goat antiserum to b-negative light chains (B). The center wells of each pattern contain antiserum and the outer wells were filled as follows: (1) peak I, 0.45 mg/ml; (2) peak II, 0.33 mg/ml; (3) peak III, 0.25 mg/ml; (4 and 6) the material eluted before addition of buffer I, 0.2 mg/ml; and (5) unfractionated L_Ib5, 1 mg/ml.

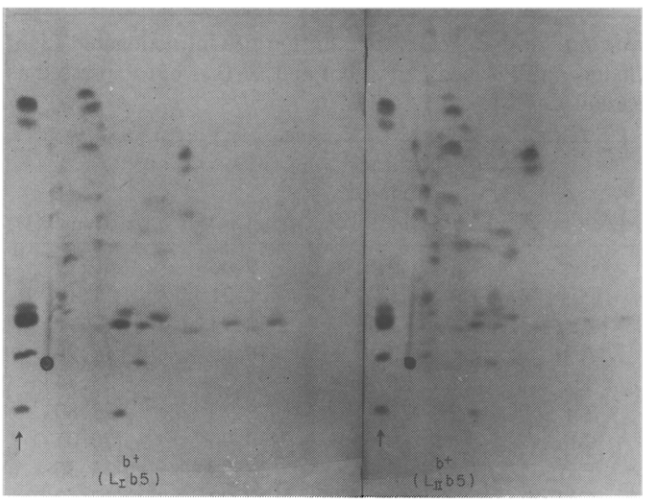

FIGURE 7: Peptide maps of b^+ (L_I b5) and b^+ (L_{II} b5) fractions. The amount used was approximately 2.5 mg for each of the samples. The arrow to the left indicates the standard amino acid mixture as separated by electrophoresis at pH 3.6.

and glycine, the differences do not exceed the standard error. Carboxyl-terminal analyses of the two b^+ populations showed that both contained cysteic acid after performic acid oxidation and hydrazinolysis. Both b^+ light-chain populations of each allotype contained amino-terminal alanyl and aspartyl residues. Estimations of the phenylthiohydantoin derivatives of alanine and aspartic acid were carried out by spectroscopy and radioactivity counting. It was found that there were approximately 0.75 (b9), 0.85 (b5 and b6), and 0.95 (b4) moles of amino-terminal alanine per 23,000 g of L_{II} light chains. The phenylthiohydantion derivative of aspartic acid accounted for the rest of the amino-terminal residues present. In the L_I light chains approximately 50% of the amino-terminals was unavailable. The yields of alanine and aspartic acid when properly corrected for this presumed contribution of λ chains were approximately the same as for the L_{II} light chains. Figure 7 shows the peptide maps of b^+ (L_I b5) and b^+ (L_{II} b5) fractions. The maps of these components are strikingly similar and only very minor differences can be found. The heterogeneity of the two components, however, is still clearly evident since the number of spots is about three times the number of peptides expected on the basis of the arginine and lysine content.

Discussion

Rabbit IgG was isolated from serum pools each with one of the four allelic allotypes of the *b* locus. Following oxidative sulfitolysis of IgG and subsequent gel filtration, two distinct populations of light chains were obtained. The first population, L_I, contained essentially all the b^- light chains, that is, those chains lacking determinants controlled by the *b* locus but sharing structural features at the amino and carboxyl termini with λ-type chains of man and mouse. In addition, L_I contained a distinct subpopulation of b^+ light chains carrying *b*-locus allotypic determinants very similar to those of the second population, L_{II}, which is largely or entirely composed of b^+ light chains. The carboxyl-terminal amino acid sequences of b4, b5, and b6 L_{II} light chains are homologous to those of the κ type in man and mouse (Doolittle and Astrin, 1967; Appella and Perham, 1967; Appella *et al.*, 1969). Since two b^+ populations are separable on Sephadex, chemically distinct, and yet share allotypic determinants, it suggests to us that they could represent two subtypes of the κ chain, κ_A and κ_B. Table III summarizes the types and subtypes of rabbit light chains which became evident from our studies.

The two subtypes from b5 light chains could clearly be distinguished since κ_A contained five half-cystine residues whereas κ_B was found to have seven half-cystines. In the native IgG molecule these probably contribute to one interchain and two or three intrachain disulfide bonds, respectively. In contrast to human and mouse κ chains (which contain two intrachain disulfide bridges and frequently have aspartic acid at the amino terminal), both κ_A and κ_B light chains of all *b*-locus allotypes contain predominantly alanine as well as some aspartic acid at the N terminal.

The most striking difference between κ_A and κ_B remains at the moment the number of half-cystine residues since the two subgroups have relatively similar amino acid compositions and peptide maps (Table II and Figure 7). The differences in half-cystine residues could well induce sufficient conformational changes to account for their distinct separation on Sephadex G-100 especially since the molecular weights of L_I and L_{II} appear to be the same (Zikán *et al.*, 1967). Further support for this hypothesis comes from the observation that λ (b^-) and κ_A chains, containing equal numbers of disulfide bonds elute in a single peak from Sephadex in spite of pronounced differences in amino acid composition, *i.e.*, primary structure, while κ_B with greater similarity to κ_A in primary structure (except for half-cystine residues) is separable. In addition, only slight conformational differences have been observed between human myeloma κ- and λ-type chains, both of which contain two disulfide bonds but differ significantly from each other in amino acid sequence (Jirgenson *et al.*, 1966; Ikeda *et al.*, 1968). Those *b* allotypic determinants which are still detectable on isolated L_I and L_{II} light chains appear to be unaffected by the configurational differences due to different half-cystine contents.

Another aspect of our results is the difference in the amount of κ_A and κ_B that we found in different allotypes.

TABLE III: A Summary of Types and Subtypes of Rabbit Light Chains.

Light-Chain Designation	Homologous Chain in Man and Mouse	Known Allotypic Markers	Half-cystines	Amino Terminal	Carboxyl Terminal
L_I b^-	λ	c7	5	Glp[a]	Ser
L_I b^- κ_A	κ	b4, b5, b6, b9	5[b]	Ala, Asp	Cys
L_{II} b^+ κ_B	κ	b4, b5, b6, b9	7	Ala, Asp	Cys

[a] Glp is an abbreviation for pyrrolidonecarboxylic acid. [b] The presence of five cysteic acid residues per mole in purified κ_A subtype has so far been shown for b5 allotype only.

Carbonara and Mancini (1968) recently reported that a preparation of b9 IgG was 32% b^-. We can calculate that one of our b9 IgG preparations was about 40% b^-, 28% κ_A, and 35% κ_B. Thus, contributing to the pronounced increase in the L_I fraction from b9 IgG were both b^- and b9 κ_A-type light chains. In contrast, from b4 (ACEP) IgG, we obtained approximately 2% b^-, 2% κ_A, and 94% κ_B light chains. It thus appears that an increased proportion of λ is correlated with an increased proportion of κ_A while a decreased proportion of λ is correlated with a decreased proportion of the κ_A type of b^+ light chain. Our previous studies (Appella *et al.*, 1968) raised the question whether the ACEP strain of rabbits contained any b^- IgG. In this study we detected this small population (2%) of b^- light chains lacking c7 determinants because they were concentrated entirely within the L_I fraction. The low concentration of b^- light chains in this strain suggests that synthesis of another type of λ chain is unable to compensate for the absence of the c7 type of chain.

In rabbits heterozygous at the *b* locus, allelic allotypes are not equally expressed (Mage, 1967). In this study, we found the ratio of κ_B to κ_A in b4 > b5 ≥ b6 > b9. This is also the order of the relative quantitative expression of these alleles in heterozygotes (Mage, 1967; Chou *et al.*, 1967). In addition, there is a corollary increase in κ_A when there is an increase in expression of λ-type chains. Many competing factors may influence the relative expression of these types of chains, which represent products of cells differentiated to express only one of alternative alleles or cistrons (Pernis *et al.*, 1965; Cebra and Bernier, 1967). The preferential utilization of different types of light chains in rabbits of various strains and allotypes may reflect selection pressure on antibody forming cell populations during differentiation in individual rabbits. On the other hand, marked differences in κ to λ ratios are known between species, *e.g.*, mice produce more than 95% κ chains, whereas horses produce largely λ chains (K. R. McIntire, manuscript in preparation, 1969; Hood *et al.*, 1967). Our rabbits express only a small quantity (~1–2%) of a type of light chain with amino-terminal aspartic acid and two disulfide bonds, which may be homologous to one or more of the major κ-light-chain types expressed in man and mouse. Conversely, the rabbit might express to a large extent, a type of light chain which although present in minute quantities in some other species, would not ordinarily be detected.

Several hypotheses have been advanced to provide a genetic and evolutionary explanation for data obtained from numerous sequence analyses. Studies of human and murine myeloma light chains show that each antigenic type is made up of a highly variable amino-terminal portion and a nearly constant carboxyl-terminal portion of about equal length (103–110 amino acids). Although there is as yet insufficient data, it is probable that normal human, murine, and rabbit light chains are also composed of variable and constant regions. Since human Inv types and rabbit *b* allotypes seem to be inherited in simple Mendelian fashion, the possibility that a large number of genes code for the entire length of the light chain is considered less likely. This had led to the idea that a single gene with several possible allelic forms codes for constant regions of light chains and a special mechanism permits fusion of this genetic information with that for the variable portions. Sequences of variable regions of human myeloma κ and λ chains have been subdivided into three or four basic types (Milstein, 1967; Langer *et al.*, 1968). Within each type, a greatly diminished variability is observed. The three or four basic sequences could reflect three or four structural genes coding for the amino-terminal half of the molecule and somatic events including crossing-over, specific insertions, and translational mechanisms could explain the additional diversity. Alternatively, a very large number of genes for the N-terminal halves could be present in the germ line. It had been proposed that the mechanism responsible for variable regions of light chains developed at an early stage in the evolution of vertebrates. The marked similarity of variable region sequences of human and murine myeloma light chains suggested that those features which were important for antibody complementarity and/or stabilization of the combining site, have been preserved over a relatively long period of evolutionary time with little species specificity (Kabat, 1967). Among mammals which have been studied in detail, the rabbit may be unique in having a major light-chain population with three disulfide bridges. If the additional disulfide is entirely in the variable region, it could stabilize the conformation of the antibody combining site. However, the question still remains whether the κ_A and κ_B subtypes are the products of distinct structural cistrons or are a novel expression of the variability of the amino-terminal half of the κ chain.

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