PROTEIN-PROTEIN INTERACTIONS AMONG L POLYPEPTIDE CHAINS OF BENCE-JONES PROTEINS AND HUMAN γ-GLOBULINS*

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L polypeptide chains are common structural elements among all known classes of γ-globulins. In each of the classes they are combined with a different type of H chain designated H_γ for 7S γ-globulins, $H_{\gamma_{1A}}$ for 7S γ_{1A}-globulins, and $H_{\gamma_{1M}}$ for 19S γ-globulins (1–3). L chains have molecular weights of 20,000, associate to form dimers in aqueous solutions, and have the physicochemical properties of Bence-Jones proteins (4). Individual Bence-Jones proteins consist of relatively homogeneous populations of L chains, and like heterogeneous normal L chains, they are able to interact with H chains or with themselves.

The present studies are concerned with the interaction of L chain monomers to form dimers and the interaction of L chain monomers with H_γ chains of normal human 7S γ-globulin (5). These interactions were studied by the methods of starch gel electrophoresis, gel filtration, equilibrium ultracentrifugation, and ultracentrifugation in sucrose density gradients. In addition, spectrofluorometric methods were employed to detect differences in the conformational stability of L chains in the free state and when combined with H chains.

Materials and Methods

Proteins.—Bence-Jones proteins were isolated either by dialyzing urine samples of patients with multiple myeloma against running water, or by half saturating the urine with $(NH_4)_2SO_4$ and storing at 4°C for 24 hours. The proteins were then dialyzed against distilled water and lyophilized. They were classified into either group I or group II by immunodiffusion against antisera to Bence-Jones proteins of known antigenic types (6, 7). Human 7S γ-globulin was obtained as fraction II of Cohn from Lederle Laboratories (Pearl River, New York).

Reduction and Alkylation.—The proteins were reduced by dissolving them in 0.05 tris[1] buffer, pH 8.0, which was made 0.1 N in 2-mercaptoethanol. This procedure produces soluble products (8). Reduction was stopped after 2 hours by placing the protein solutions onto a sephadex column in 0.5 N propionic acid, into the origin of a starch gel at acid pH, or by making the solution 0.2 N in iodoacetamide to alkylate free sulfhydryl groups. In some starch gel electrophoretic experiments the proteins were reduced in 8 M urea that was 0.1 in 2-mercaptoethanol.

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[1] Tris, tris(hydroxymethyl)aminomethane.

Separation of Polypeptide Chains.—Reduced alkylated L chains were separated by gel filtration on sephadex G-100 in 0.5 N propionic acid as described by Fleischman *et al.* (9).

Starch Gel Electrophoresis in Urea.—The method has been described elsewhere (8). Reduced material was not alkylated prior to being placed at the origin of the gels.

Spectrofluorometry and Nephelometry. These procedures have been described in detail elsewhere (4, 10).

Ultracentrifugation.—This was performed in a Spinco model E ultracentrifuge equipped with automatic temperature control and schlieren and interference optics. Molecular weights were measured by means of equilibrium centrifugation in short columns (11). When the molecular weights were measured in 0.5 N propionic acid, the solvent contained 1 per cent glucose to counteract possible convective disturbances.

Reoxidation.—The solution of reduced unalkylated material in 0.5 N propionic acid was neutralized with concentrated ammonium hydroxide. The solution was then concentrated by ultrafiltration to a protein concentration of about 15 mg/ml and dialyzed into 0.05 N tris buffer, pH 8.0, for 48 hours at room temperature. This procedure favors oxidation of free sulfhydryl groups to disulfide bonds (12).

Reconstitution of 7S Protein.—The methods of iodination of the different proteins, the separation of polypeptide chains by gel filtration, the conditions of mixing and dialysis, and the analysis by centrifugation in sucrose gradients were the same as those described by Olins and Edelman (5), except that the dialyzed samples were added directly to the sucrose gradient without being concentrated by ultrafiltration.

Enzymatic Treatment of Reconstituted Protein.—The hydrolysis with papain (13, 14) and immunoelectrophoresis of the products were carried out as previously described (14). The electrophoresis on cellulose acetate strips was performed as described by Olins and Edelman (5).

RESULTS

In Fig. 1 are shown the starch urea gel electrophoretic patterns of six individual Bence-Jones proteins and of reduced alkylated L chains obtained from normal human 7S γ-globulin. With the exception of samples Th and H, which showed contamination with higher molecular weight components, the patterns consisted of a small number of sharply delineated bands and are typical for Bence-Jones proteins (15). Samples B and P were group II proteins and showed only single bands with mobilities lower than those of reduced alkylated L chains. Samples H, Th, Z, and T belonged to antigenic group I and showed a band which migrated in the same region as the reduced alkylated normal L chains. In addition, each showed a band which had similar mobility to those of the group II proteins.

Since electrophoresis in starch gels can resolve proteins according to size (16), the results found for the group I proteins suggested that the slowly migrating band was a dimer of the faster migrating material. To investigate this possibility, 15 mg of Bence-Jones protein T were passed through a column of sephadex G-100 equilibrated with 0.2 N NaCl buffered to pH 8.0 with 0.1 N tris. Under these conditions, all of the protein emerged in a single peak at an elution volume compatible with a molecular weight of 40,000 to 50,000. If the same material was passed over a column of sephadex G-100 in a dissociating solvent consisting of 0.5 N propionic acid, however, the protein emerged in two

peaks containing approximately equal amounts of material (Fig. 2). The elution volume of the second peak corresponded closely to that obtained for normal L chains isolated under the same conditions from reduced alkylated γ-globulin.

The protein contained in each peak was dialyzed against distilled water, lyophilized, and electrophorized on a starch urea gel (Fig. 3). Material from the first peak corresponded in position to the slower band of the original Bence-

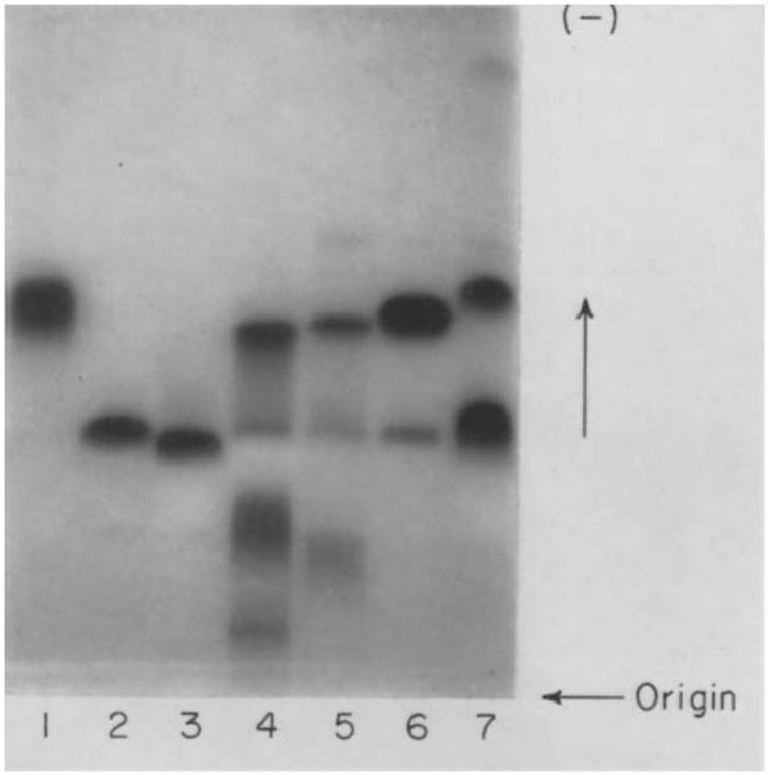


FIG. 1. Starch urea gel electrophoretic comparison of Bence-Jones proteins. *1*, Reduced alkylated L chains of normal γ-globulin; *2*, Bence-Jones protein B; *3*, Bence-Jones protein P; *4*, Bence-Jones protein H; *5*, Bence-Jones protein Th; *6*, Bence-Jones protein Z; *7*, Bence-Jones protein T.

Jones protein; that from the second peak had the same mobility as the faster band. When material from either peak was reduced in 8 M urea prior to electrophoresis, a single band appeared with a mobility intermediate between the two bands. This single band also was seen after the reduction of the unseparated Bence-Jones protein in 8 M urea. If the Bence-Jones protein was reduced in the absence of urea, the pattern on the gel consisted of a single band in the same position as the faster band in the unreduced protein.

In a non-dissociating solvent consisting of 0.2 N KCl, the weight average

molecular weight of the protein in the first peak was 40,000. Most of the protein in the second peak also had a molecular weight of 40,000 in this solvent; about 10 per cent of the protein had a molecular weight of 20,000.

In contrast to Bence-Jones protein T which was of antigenic group I, protein B of antigenic group II showed only one peak after gel filtration of sephadex G-100 in 0.5 N propionic acid. This peak emerged at the same position as did the first peak of protein T under the same conditions. After reduction in the absence of urea, however, the majority of protein B emerged in the same

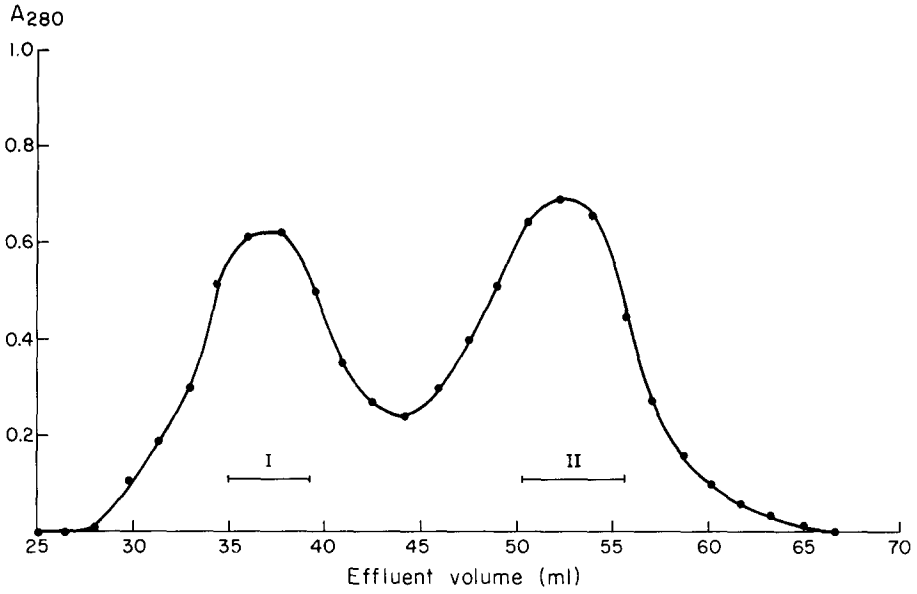


FIG. 2. Gel filtration on sephadex G-100 in 0.5 N propionic acid of Bence-Jones protein T. *I* and *II* are protein fractions pooled for further analysis. Column dimensions: 100 x 1 cm. A_{280}, absorbancy at 280 mμ.

position as the second peak of Bence-Jones protein T. When examined by starch gel electrophoresis in urea, reduced protein B migrated faster than the unreduced material. A similar result was obtained when Bence-Jones protein P was examined by this method.

The foregoing results suggested that the L chains of some Bence-Jones proteins exist as dimers in a non-dissociating solvent; in certain cases some of the dimers were not dissociable by 0.5 N propionic acid or by formate buffer that was 8 M in urea. After treatment with 0.1 N mercaptoethanol or 0.1 mercaptoethanol in urea, these resistant dimers could be dissociated, and it seemed probable that they were linked by disulfide bonds, as well as non-covalent interactions. An attempt was therefore made to form dimers that were stable in

dissociating solvents by reoxidation of reduced normal L chains. These were chosen because they resemble Bence-Jones proteins in all physical and chemical properties except homogeneity (4).

Human 7S γ-globulin was reduced in the absence of urea in order to cleave interchain disulfide bonds, and the polypeptide chains were separated by gel

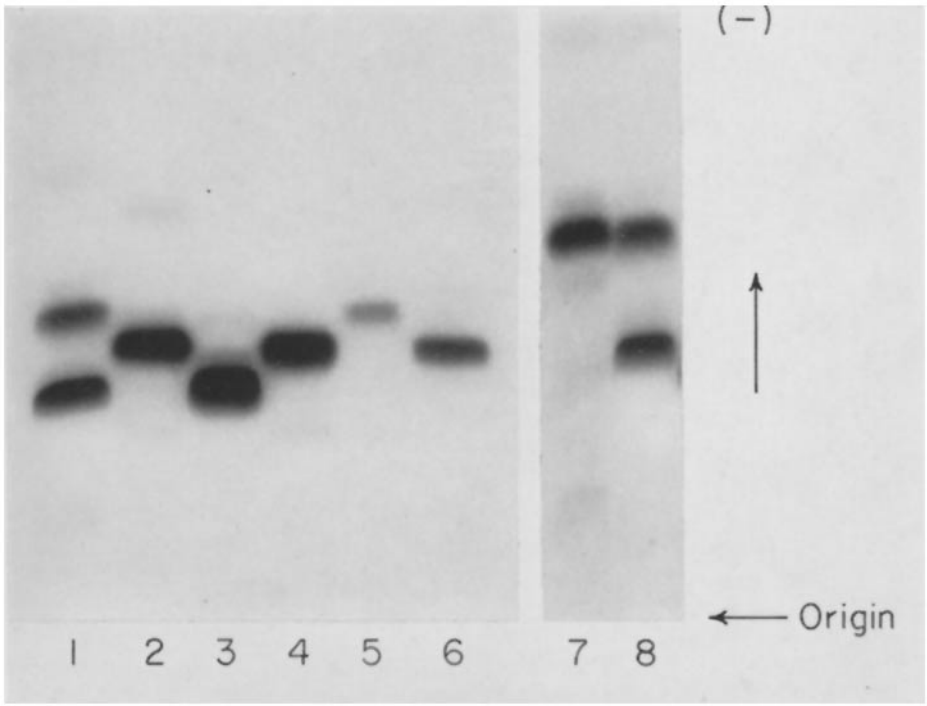


FIG. 3. Starch gel electrophoretic comparison of different fractions of Bence-Jones protein T. *1*, unseparated protein; *2*, unseparated protein, reduced in 8 M urea; *3*, fraction I; *4*, fraction I, reduced in 8 M urea; *5*, fraction II; *6*, fraction II, reduced in 8 M urea; *7*, unseparated protein, reduced in pH 8.0 tris buffer; *8*, unseparated protein. Fractions I and II were obtained by gel filtration (see Fig. 2).

filtration in 0.5 N propionic acid. The L chain fraction was brought to neutral pH by addition of concentrated ammonium hydroxide, concentrated to approximately 15 mg/ml by ultrafiltration, and then dialyzed for 48 hours against 0.05 N tris buffer, pH 8.0. These conditions would favor oxidation of free sulfhydryl groups to disulfide bonds by oxygen in the solution (12).

When reduced L chains that had been allowed to reoxidize were passed over a column of sephadex G-100 in 0.5 N propionic acid, the elution pattern consisted of two peaks (Fig. 4) and was similar to that of Bence-Jones protein T (compare

with Fig. 2). The second peak emerged at an elution volume similar to that of reduced alkylated L chains, and the first peak emerged at a volume expected for a protein of approximately twice the molecular weight of L chains. On the same column, L chains from γ-globulin which had been alkylated after reduc-

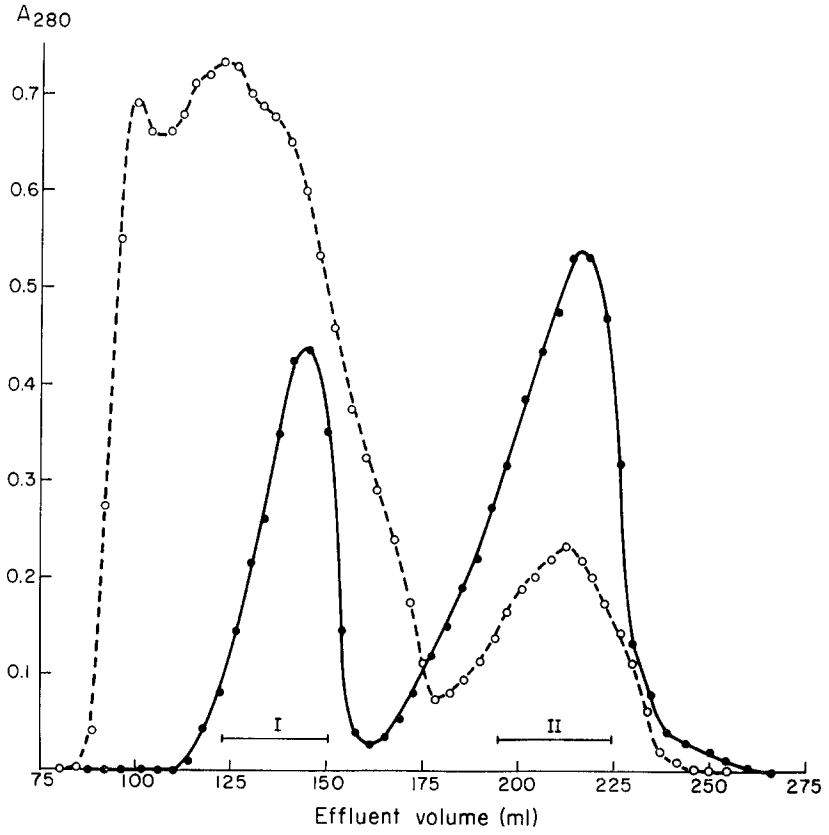


FIG. 4. Gel filtration on sephadex G-100 in 0.5 N propionic acid of reduced normal L chains after exposure to oxidizing conditions. ●——● Reduced L polypeptide chains which have been allowed to oxidize. *I* and *II* are fractions of unalkylated L chains pooled for further analysis. O-----O Reduced alkylated human γ-globulin separated for comparative purposes. (The first peak contains H_γ chains with molecular weight of approximately 60,000.) A_{280}, absorbancy at 280 mμ.

tion and reduced L chains which had not been allowed to reoxidize both emerged as single peaks in the same position as the second peak shown in Fig. 4.

The starch gel electrophoretic pattern of the material from each peak of reduced and "oxidized" L chains is presented in Fig. 5, along with the patterns of reduced alkylated human γ-globulin and reduced alkylated L chains. Material from the first peak migrated in a sharp band only slightly faster than the H_γ

chain band of normal γ-globulin and in a region corresponding to that of the slower band in the patterns of Bence-Jones proteins shown in Fig. 1. Material from the second peak had the same mobility as reduced alkylated L chains. After reduction and alkylation, material from the first peak could not be

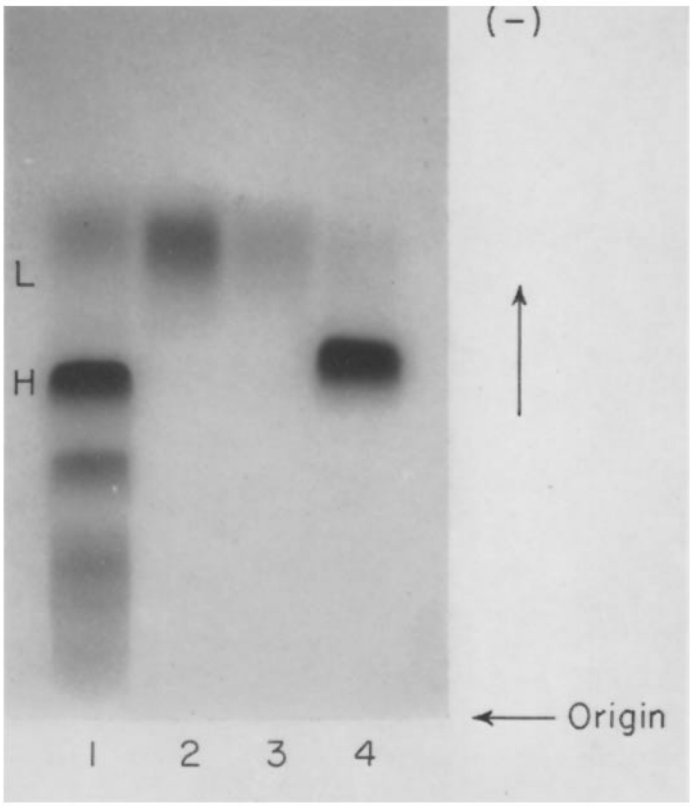
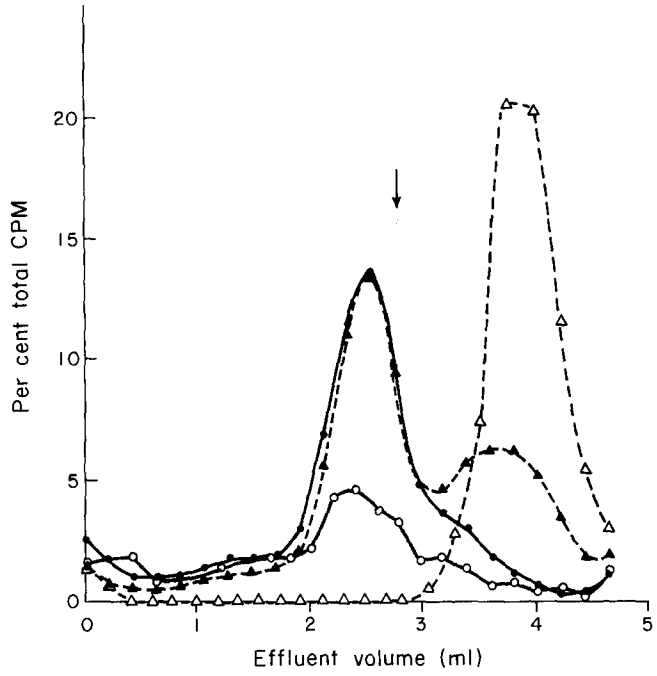


FIG. 5. Starch urea gel electrophoretic comparisons of different forms of L polypeptide chains of normal γ-globulin. *1*, reduced alkylated human γ-globulin; *2*, reduced alkylated L chains; *3*, L chains, reduced and allowed to oxidize (fraction II, Fig. 4); *4*, L chains, reduced and allowed to oxidize (fraction I, Fig. 4). *H*, H chain band. *L*, L chain band.

distinguished from that in the second peak either by starch gel electrophoresis or gel filtration.

In 0.2 N KCl the proteins in both peaks had weight average molecular weights of 50,000, although a small amount of the protein in the second peak had a molecular weight of 20,000. In 0.5 N propionic acid, the material in the first peak had a molecular weight of 46,000 and that in the second peak had a molecular weight of 24,000. An immunologic comparison of the two L chain fractions by the method of double diffusion using antisera to group I or group II Bence-Jones proteins failed to distinguish the two fractions.


Per cent total CPM
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15
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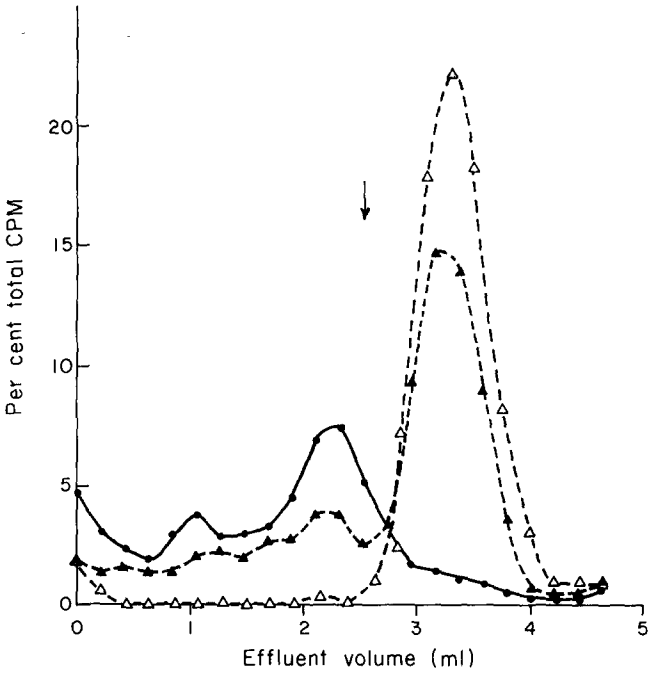
5
Effluent volume (ml)

FIG. 6 *a*


Per cent total CPM
20
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Effluent volume (ml)

FIG. 6 *b*

Olins and Edelman (5) have shown that when reduced alkylated L chains of normal 7S γ-globulin are mixed with normal H_γ chain fractions in 0.5 N propionic acid, and then dialyzed into tris buffer, pH 7.2, a molecular species resembling normal γ-globulin is formed. The same approach has been used in the present study to determine whether the L chain dimers stable in 0.5 N propionic acid would also bind to H_γ chains. L chains from reduced human γ-globulin were allowed to oxidize and were then iodinated with I^{131}. The iodinated protein was separated into monomeric and dimeric forms on sephadex G-100 in 0.5 N propionic acid. Each fraction was separately mixed with an H_γ chain fraction labeled with I^{125} in a ratio of one part L chains to six parts H_γ chains as determined by absorbancy at 280 mμ, and the mixtures, as well as the separate chain fractions, were dialyzed against 0.05 M tris buffer, pH 7.2. Samples of the dialyzed materials were then analyzed by ultracentrifugation in sucrose density gradients.

Under these conditions, the two L chain fractions had a similar distribution in the gradient (Figs. 6 *a* and 6 *b*). A large proportion of L chains from the second peak of a gel filtration pattern similar to that pattern shown in Fig. 4 sedimented with the H_γ chains (Fig. 6 *a*). This result is in accord with those of Olins and Edelman (5) who demonstrated binding between reduced alkylated L chains and H_γ chains. In contrast to the results obtained with dissociable L chain dimers, the L chain dimers emerging in the first peak after gel filtration in 0.5 N propionic acid bound only slightly to the H_γ chain fraction (Fig. 6 *b*).

The similarity of normal L chains and L chains of Bence-Jones proteins prompted an attempt to combine a Bence-Jones protein with normal H_γ chains in the fashion described above. Bence-Jones protein S of antigenic group II (occurring naturally in dimeric form) was chosen. When reduced, this protein behaved as a monomer on starch gel electrophoresis in urea and gel filtration in propionic acid, and after reoxidation most of the protein returned to the dimeric

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FIGS. 6 *a* and 6 *b*. Degree of interaction with H_γ chains of dissociable and stable dimers of normal L chains as shown by ultracentrifugation in sucrose density gradients. Solid symbols represent data obtained from mixtures of L and H chains, open symbols represent data on L chain fractions or H_γ chain fractions examined separately. I^{131} or I^{125} were counted separately in all experiments, and the data are expressed as per cent total counts per minute. The total counts per minute is the sum of the counts in the soluble material and in the pellet. ↓, position of alkaline phosphatase marker (sedimentation coefficient, approximately 6.0S). Sedimentation proceeded from right to left.

FIG. 6 *a*. ●——●, I^{125}-labeled H_γ chains mixed in 0.5 N propionic acid with I^{131}-labeled normal L chains in monomeric form. ▲------▲, I^{131}-labeled normal L chain monomers present in mixture with I^{125}-labeled H_γ chains. ○——○, I^{125}-labeled H_γ chains. △------△, I^{131}-labeled normal L chain dimers, dissociable in 0.5 N propionic acid.

FIG. 6 *b*. ●——●, I^{125}-labeled H_γ chains mixed in 0.5 N propionic acid with I^{131}-labeled stable L chain dimers. ▲------▲, I^{131}-labeled stable L chain dimers present in mixture with I^{125}-labeled H_γ chains. △------△, I^{131}-labeled stable L chain dimers.

form (Fig. 7). After iodination with I^{131}, an aliquot of the protein was reduced and alkylated. The reduced alkylated protein and the unreduced protein were purified by passage through identical columns of sephadex G-100 in 0.5 N propionic acid. The materials were then mixed with a normal H_γ chain fraction (labeled with I^{125}) in a ratio of one part Bence-Jones protein to six parts H_γ chain fraction as measured by absorbancy at 280 mμ. After extensive dialysis against 0.05 tris buffer, pH 7.2, the protein solutions were analyzed by density gradient centrifugation.

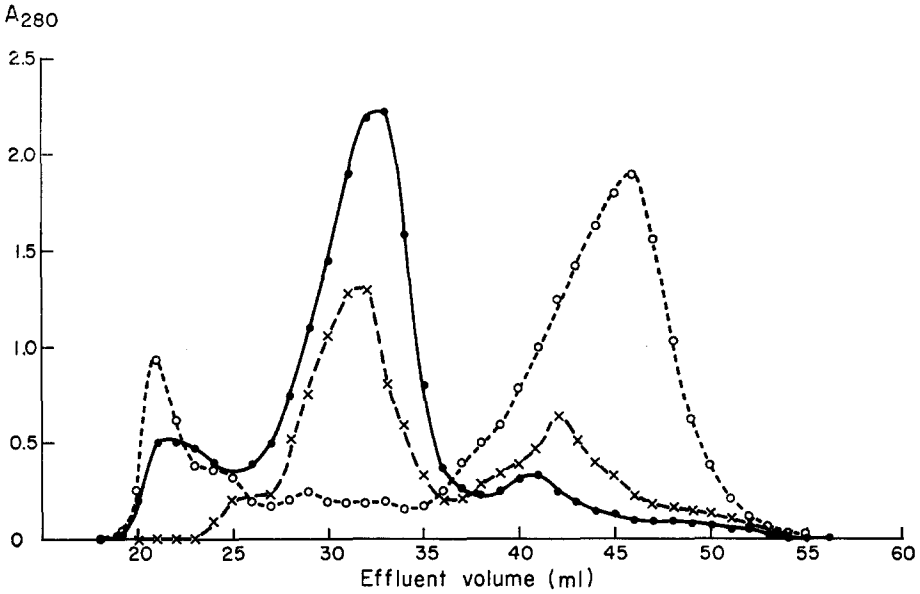
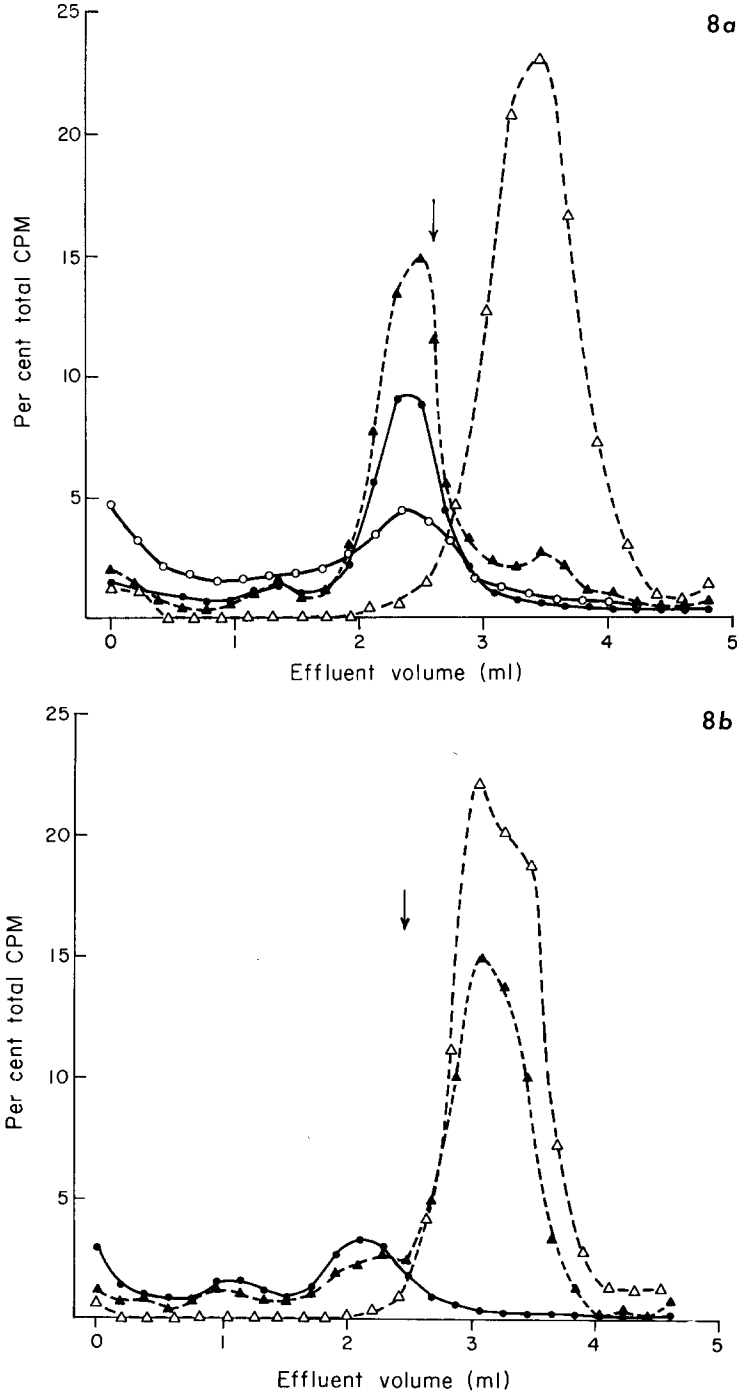


FIG. 7. Gel filtration of various forms of a group II Bence-Jones protein on sephadex G-100 in 0.5 N propionic acid. Column dimensions: 100 x 1 cm. ●——●, Bence-Jones protein S; O------O, reduced Bence-Jones protein S; x------x, material from major peak of the reduced protein after reoxidation. A_{280}, absorbancy at 280 mμ.

The results, shown in Figs. 8 *a* and 8 *b*, resemble those obtained with L chains of normal γ-globulin. The monomeric form was incorporated with H_γ chains in the 7S peak (Fig. 8 *a*), whereas the dimer of the Bence-Jones protein was not appreciably incorporated into material sedimenting in the 7S region (Fig. 8 *b*). From the measured specific activity of the labeled Bence-Jones protein, it was calculated that 20 per cent of the material which sedimented in the region of 7S γ-globulin (Fig. 8 *a*) was derived from the Bence-Jones protein.

If the material containing Bence-Jones protein and H_γ chains resembles 7S γ-globulin, hydrolysis with papain should yield S and F fragments similar to those of γ-globulin (14), and the Bence-Jones protein (L chain material) should



FIGS. 8 *a* and 8 *b*. Degree of interaction of dissociable and stable dimers of L chains of Bence-Jones protein S with normal H_γ chains as shown by ultracentrifugation in sucrose density gradients. The data are expressed as in Fig. 6. ↓, position of alkaline phosphatase marker (sedimentation coefficient, approximately 6.0S).

FIG. 8 *a*. ●——●, I^{125}-labeled H_γ chains mixed in 0.5 N propionic acid with I^{131}-labeled L chains of Bence-Jones protein in monomeric form. ▲------▲, I^{131}-labeled L chain monomers present in mixture with I^{125}-labeled H_γ chains. ○——○, I^{125}-labeled H_γ chains. △------△, I^{131}-labeled L chain dimers, dissociable in 0.5 N propionic acid.

FIG. 8 *b*. ●——●, I^{125}-labeled H_γ chains mixed in 0.5 N propionic acid with I^{131}-labeled stable L chain dimers. ▲------▲, I^{131}-labeled stable L chain dimers present in mixture with I^{125}-labeled H_γ chains. △------△, I^{131}-labeled stable L chain dimers.

be present in the S fragment (17). After dialysis into phosphate buffer, ($\Gamma/2$ = 0.1, pH 7.0), and ultrafiltration to a protein concentration of 0.5 per cent, the material in the 7S peak (Fig. 8 *a*) was mixed with papain at a substrate/enzyme ratio of 100/1. Samples were taken for immunoelectrophoresis after 4 and 24 hours of incubation at 37°C.

Immunoelectrophoresis showed the presence of non-cross reacting arcs similar to those of S and F fragments (Figs. 9 *a* and 9 *b*), whereas the material that was not exposed to papain gave a pattern similar to that of normal γ-globulin (Fig. 9 *a*). To localize the L chains of the Bence-Jones protein, the hydrolyzed

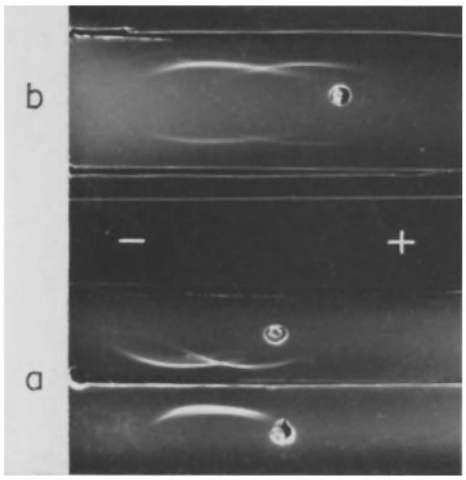


FIG. 9. Comparison of immunoelectrophoretic patterns of whole and papain-treated reconstituted protein. (*a*) upper origin, reconstituted protein after 16 hours of hydrolysis with papain; lower origin, reconstituted protein before hydrolysis. (*b*) reconstituted protein after 24 hours of hydrolysis with papain. Antisera, rabbit anti-human 7S γ-globulin.

material was also separated by electrophoresis on cellulose acetate. After electrophoresis was completed, the strip was cut into small segments which were assayed for I^{131} and I^{125}. The I^{131} label originally present on L chains was found in the slowly migrating fragments; *i.e.*, in the material closest to the cathodal end of the strip (Fig. 10). Electrophoresis of the unsplit 7S material showed that both labels migrated together in a single peak.

The failure of L chains in the stable dimerized form to interact with H_γ chains raises the question of whether L chains in the dimerized state have a different conformation than L chains bound to H_γ chains. The technique of spectrofluorometry was chosen to study this problem, since previous investigations have shown that individual Bence-Jones proteins and normal L chains undergo characteristic thermally induced transitions in molecular conformation

which are accompanied by increases in fluorescence intensity (4). As shown in Fig. 11, the two fractions of Bence-Jones protein T had the same transition temperature as the unseparated protein. There was a slight difference in the transition temperatures of dissociable and stable dimers of L chains of normal γ-globulin.

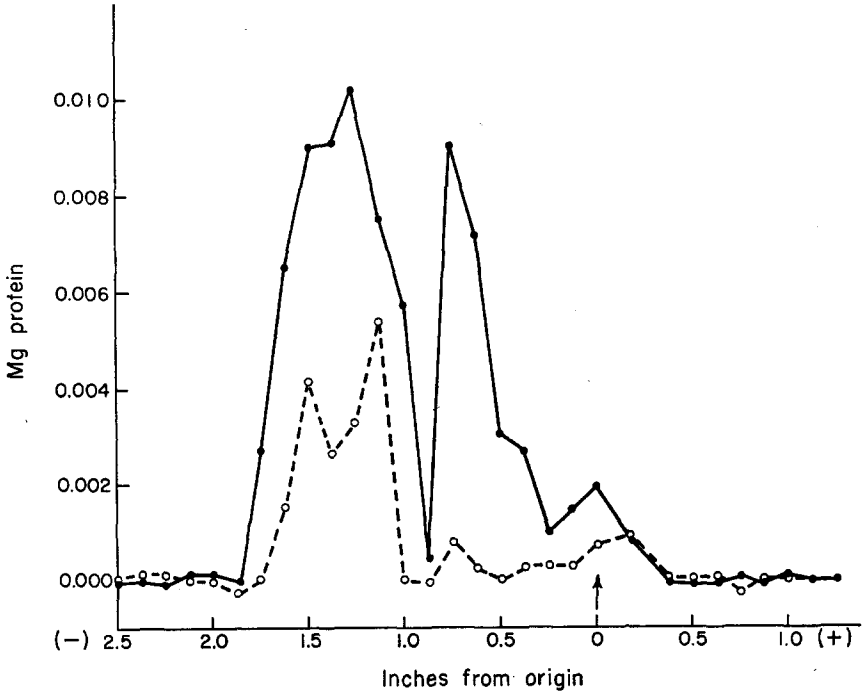


FIG. 10. Zone electrophoresis on cellulose acetate of reconstituted protein after 24 hour hydrolysis with papain. I^{131} label on L chains and I^{125} label on H_γ chain fraction were counted separately, and the amount of protein associated with this radioactivity was calculated from the measured specific activities of the chain fractions. ↑, origin.
●——●, I^{125}-labeled material; O------O, I^{131}-labeled material.

The heating curves of normal γ-globulin and its fragments differed from the curves of L chains and showed no evidence of a structural transition at 50°C (Fig. 12). Instead, a sharp increase in fluorescence intensity occurred at temperatures above 65°C, accompanied by increased scattering of the exciting light which resulted from aggregation of the protein in the neighborhood of the transition temperature. All of the protein was precipitated in this region. Reduced alkylated 7S γ-globulin and reduced alkylated S fragments had heating curves that were identical with those of the unreduced proteins (Fig. 12) but above the transition temperature approximately half of the reduced alkylated

S fragment and one-fifth of the reduced alkylated γ-globulin remained in solution. When the soluble material was reheated, heating curves resembling those of normal L chains were obtained (compare Figs. 11 and 12). Moreover, this soluble material migrated like L chains upon starch urea gel electrophoresis. The data indicate that L chains bound to H chains or to a fragment of H

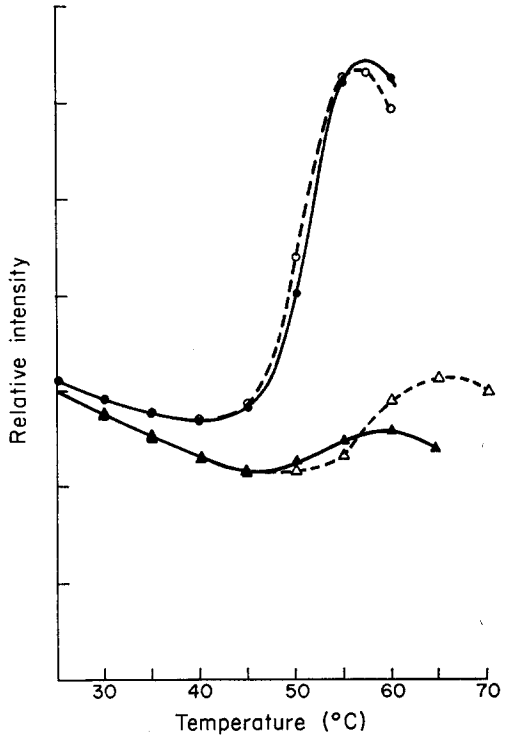


FIG. 11. Dependence on temperature of the fluorescence intensity of L chains in phosphate buffer (pH 7.0, Γ/2 = 0.2 at 25°C). Exciting wavelength: 280 mμ. Fluorescence measured at the wavelength of maximal emission. Protein concentration: 0.25 mg/ml.

O------O, Bence-Jones protein T, fraction I, Fig. 2; ●——●, Bence-Jones protein T, fraction II, Fig. 2; △------△, L chains of normal γ-globulin, fraction I, Fig. 4; ▲——▲, L chains of normal γ-globulin, fraction II, Fig. 4.

chains in the S fragment were prevented from undergoing the characteristic thermal transition at 50°C. When γ-globulin and S fragments which had been reduced and alkylated were heated to their aggregation temperature, free L chains were released into solution.

DISCUSSION

Previous studies have indicated that L chains obtained from reduced alkylated normal γ-globulin have molecular weights of 20,000 in 6 M urea and of

40,000 in aqueous buffers (4). The results of the present investigation suggest that L chains exist in three forms in non-dissociating solvents: monomers, dissociable dimers, and stable dimers. Dissociable dimers may be converted into monomers in solvents such as 8 M urea and 0.5 N propionic acid and appear to be linked by non-covalent interactions. Stable dimers are dissociable in these solvents only after prior reduction by mercaptans suggesting that they are

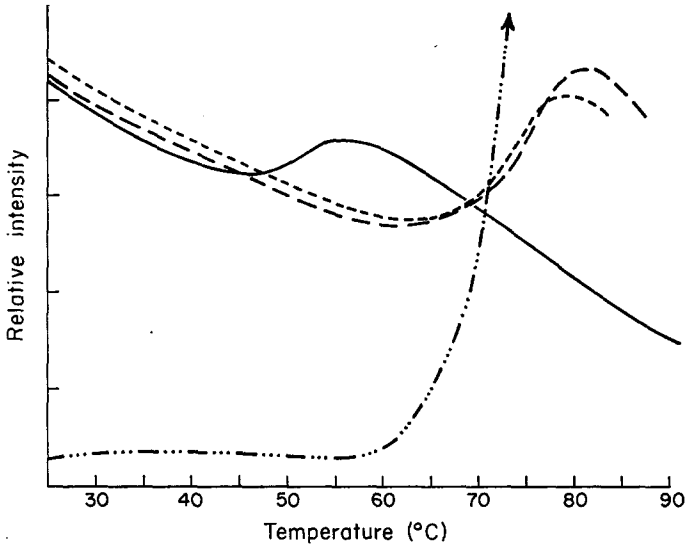


FIG. 12. Dependence on temperature of the intensity of the fluorescence at 335 mμ and the right angle scattering at 280 mμ of γ-globulin and its fragments. The solvent was 0.8 per cent NaCl, 0.01 M phosphate, pH 7.0 at 25°C. The wavelength of the exciting light was 280 mμ. — — —, relative fluorescence intensity of reduced, alkylated γ-globulin, concentration: 0.25 mg/ml. ----------, relative fluorescence intensity of reduced alkylated S fragment of γ-globulin, concentration: 0.25 mg/ml. —···—···, relative intensity of exciting light scattered at right angles from solutions of the reduced alkylated S fragment and γ-globulin. ————, relative fluorescence intensity of material remaining in solution after reduced, alkylated S fragment had been heated to 90°C and recooled. Concentration: 0.1 mg/ml. For purposes of comparison, the relative intensity of this sample has been increased by a factor of two.

linked by disulfide bonds. This is also indicated by the fact that a fraction of the dissociable dimers of normal unalkylated L chains are converted to stable dimers when allowed to oxidize. Equilibrium centrifugation in 0.5 N propionic acid showed that these stable dimers had molecular weights of 46,000, as compared to 24,000 for dimers that had dissociated in this solvent.

The Bence-Jones proteins of antigenic group I examined in this study appeared to contain all three forms of L chains: monomers, dissociable dimers, and stable dimers, apparently linked by disulfide bonds. These data are consistent with recently reported observations of Bernier and Putnam (18) that certain

Bence-Jones proteins of group I may be separated into two fractions in non-dissociating solvents, one fraction consisting of dimers of material in the other. The monomeric form of group I proteins can occur naturally and without prior dissociating treatment. This is indicated by the fact that certain Bence-Jones proteins in this group have been reported to have molecular weights of about 20,000 (19, 20). Moreover, Berggård and Edelman (21) have reported isolation of L chains in normal urine with molecular weights of approximately 25,000, and Berggård (22) has found material of this type to belong to antigenic group I. Although the L chains are usually found in the dimeric form in Bence-Jones proteins, there may be circumstances in which polymers of higher molecular weight are present. Bence-Jones proteins having molecular weights of up to 90,000 have been described (23), and it would seem likely that molecules of this type consist of more than two L chains.

van Eijk *et al.* (24) have found that three highly purified Bence-Jones proteins (two of group I and one of group II) had molecular weights of approximately 22,500 in the dissociating solvent 6 M guanidine. Two of these, including the group II protein, had a sedimentation velocity of 3.5S in a non-dissociating buffer, which would suggest the presence of dissociable dimers. In addition these authors studied two group I proteins which had molecular weights of 45,000 even in dissociating solvents. After treatment with mercaptoethanol, all of these proteins migrated in more than one band upon starch gel electrophoresis in 8 M urea, and van Eijk *et al.* concluded that Bence-Jones proteins might contain disulfide-linked polypeptide chains.

Disulfide bonds appear to play an important role in the maintenance of the stability of the stable dimers of normal L chains and Bence-Jones proteins. This is indicated by the dissociability of stable dimers following reduction, the reconstitution of stable dimers from reduced, dissociated L chains when oxidation is permitted, and the lack of such reconstitution when L chains are alkylated or otherwise not permitted to oxidize. Preliminary experiments (25) showed that stable dimers of normal L chains reacted with 0.32 moles of C^{14}-iodoacetic acid per 20,000 gm of protein; after reduction of the stable dimers, 1.16 moles of C^{14}-iodoacetic acid reacted per 20,000 gm of protein. If a single disulfide bond links two L chains to form a stable dimer, it would be expected that after reduction 1.0 moles of iodoacetic acid would react with each L chain. Amperometric titrations with silver, performed as previously described (8), yielded values of 0.71 sulfhydryl groups for the monomer and 0.06 sulfhydryl groups for the dimer. These results and those of the experiments on the dissociation of stable dimers after reduction suggest that the two L chains are linked by a single disulfide bond.

The two methods of separation used in the present studies easily distinguish between dissociable and stable dimers. After starch gel electrophoresis in urea, stable dimers migrated in a narrow region just preceding the band containing

the H_γ chains of normal 7S γ-globulin. Dissociable dimers were converted to monomers and migrated as a faster broad region indistinguishable from that of reduced alkylated L chains. Since the slow sharp band is composed of dimers of this faster moving material, it appears that the materials of higher molecular weight are not resolved as well as are those of lower molecular weight. This may explain why reduced alkylated guinea pig antibodies can be distinguished by differences in their L chain bands and yet no large differences can be found in the migration of their H chains (26).

Gel filtration was found to be a convenient means for isolating stable dimers from dissociable dimers and monomers. The dimers are eluted from the column of sephadex G-100 in 0.5 N propionic acid in the same position as the H_γ chains of normal γ-globulin. If stable dimers of L chains are formed when normal γ-globulin is reduced, this might explain why H chain material separated by gel filtration continued to contain contaminating antigenic determinants of L chains (17, 27). Another reason would be the presence of L-H interaction products or half molecules (5, 28).

Since stable dimers of L chains are readily formed, it might be expected that L chains are in this form when incorporated into the whole γ-globulin molecule. There are data to suggest that this is not the case, however. L chains are present in the S fragment of the γ-globulin molecule. Since the available evidence suggests that there are two S fragments and two L chains in the 7S molecule (5, 13), it is unlikely that the L chains are dimerized in the S fragment. Moreover, hydrolysis of 7S γ-globulin with pepsin yields a fragment which appears to consist of two fragments similar to S fragments linked by a single disulfide bond (29). It is probable that this bond is between the portions of the H chains in the S fragment. Using the method of Olins and Edelman (5) in the present study, it has been shown that only the dissociable dimers or monomers of L chains would combine with H_γ chains to reconstitute a molecule similar to 7S γ-globulin. Normal L chains that were reduced but not alkylated, and reduced alkylated L chains from a Bence-Jones protein were both incorporated into 7S protein. Artificially formed stable dimers of the normal L chains and naturally occurring stable dimers of a Bence-Jones protein of group II were incorporated to a negligible extent.

The incorporation of L chains of a Bence-Jones protein with normal H_γ chains into a 7S product is in accordance with the evidence that L chains of Bence-Jones proteins are present in the myeloma protein of the same patient (4). The reconstituted 7S material was hydrolyzed with papain to form fragments similar to S and F fragments, and it was shown that the L chain of the Bence-Jones protein was present in the slow fraction. This is consistent with data indicating that S fragments contain L chains (17, 30). Olins and Edelman (5) have obtained similar results using a reconstituted product of normal L and H_γ chains.

The failure to incorporate stable dimers of L chains into 7S molecules cannot be attributed to unavailability of free —SH groups in the dimers, since it has been shown by Olins and Edelman (5) and in the present experiments that alkylated L chains in the monomeric form are readily incorporated. A more probable explanation would be that the conformation of L chains interacting with H chains differs from that of L chains interacting with each other. Some support is lent to this notion by spectrofluorometric experiments. When L chains are incorporated into the γ-globulin molecule, their characteristic thermal transition is not seen at all, even after the disulfide bonds linking them to the rest of the molecule are broken by reduction and alkylation. When reduced alkylated γ-globulins or S fragments are heated to their higher transition temperature, they aggregate and precipitate, at the same time releasing L chains into solution. A solution of L polypeptide chains may be obtained by heating reduced alkylated γ-globulin to 75°C for several minutes, cooling, and removing the precipitate by centrifugation.

Although the spectrofluorometric method readily distinguishes different Bence-Jones proteins, no differences were found in the thermal transitions of stable dimers and dissociable dimers of a single Bence-Jones protein. This suggests that the thermally induced conformational transition does not reflect the dissociation of the dimer to the monomer, even through such a process may occur for dissociable dimers above the transition temperature. The slight difference in the transitions of stable dimers and dissociable dimers of normal L chains may result from differences in pairing among the heterogeneous L chains in the two forms, or from differences in the proportion of chains of the different antigenic classes in the two preparations.

SUMMARY

The L polypeptide chains of certain Bence-Jones proteins of group I have been found in three forms: monomers of molecular weight of about 20,000, dimers which monomerize in dissociating solvents, and dimers which are stable in such solvents. The L polypeptide chains of some Bence-Jones proteins of group II were found to occur naturally only as stable dimers. The L chains of normal human γ-globulin have been obtained in a reduced unalkylated form, and a fraction of these chains was found to form stable dimers under oxidizing conditions. It is suggested that a single disulfide bond is involved in stabilization of the dimer.

In experiments on the reconstitution of 7S γ-globulin, it was found that stable dimers of L polypeptide chains did not associate appreciably with H_γ chains to form a soluble product. L chains in the monomeric form, both of a reduced alkylated Bence-Jones protein and of reduced unalkylated γ-globulin, combined with H_γ chains to form a 7S product. After hydrolysis with papain, the 7S material containing the Bence-Jones L chains yielded fragments comparable to the fragments of papain-treated myeloma proteins.

As indicated by spectrofluorometric measurements, dissociable dimers and stable dimers of the L chains of a Bence-Jones protein both underwent identical thermally induced transitions in the temperature range 48–58°C. When L polypeptide chains were present in reduced alkylated γ-globulin or reduced alkylated S fragments, no transition occurred until 65°C, the coagulation temperature of γ-globulin and S fragments. Above this temperature, L chains were released into solution. These experiments suggested that free L chains and L chains bound to H_γ chains have different conformational stabilities.

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