Studies on the Structure of Mouse γA Myeloma Proteins*

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ABSTRACT: Mouse γA myeloma proteins were studied with respect to the interchain disulfide linkage of the light and heavy chains. Starch gel electrophoresis in urea–formate buffer (pH 3) and G-100 gel filtration in 1 M acetic acid indicated that in six of six γA myeloma proteins studied, the light chains were not bound to the heavy chains by disulfide bonds. Instead, light chains were present in the γA molecule as monomers as well as L–L disulfide-bridged dimers which were noncovalently linked to the heavy chains. Both monomer and polymer γA proteins had their light chains present in this form. Starch gel electrophoresis and gel filtration experiments led to the conclusion that interheavy-chain disulfide bridges were present in the monomer and polymer γA proteins.

Also, acid–urea starch gel electrophoresis of native polymer γA indicated that the interchain disulfide bridges involved in the polymer formation are located in the heavy chain. *In vitro* reduction and reoxidation experiments were performed and it was found that only after partial reduction was monomer γA capable of undergoing significant polymerization, suggesting the presence of a blocked sulfhydryl group in the monomer which is necessary for polymer formation. In short-term tissue culture experiments it was possible to demonstrate that there is some polymer γA produced within plasma cells making γA protein, but that the relative amount of polymer compared with the amount of monomer present is much lower in the intracellular than in the extracellular fluid.

Structural studies on γG-immunoglobulins of several mammalian species clearly indicate that proteins of this immunoglobulin class consist of two pairs of polypeptide chains, heavy and light, and that the four peptide chains are linked together by disulfide bonds (Edelman and Poulik, 1961; Fleischman *et al.*, 1962). Although it is also clear that γA proteins are made up of heavy and light chains, the nature of the interchain disulfide bonds has not been delineated (Carbonara and Heremans, 1963; Fahey, 1963a,b; Cohen, 1963). This is of particular interest in this class of immunoglobulins since γA proteins exist as monomeric proteins similar in size to γG, as well as in the form of disulfide-linked polymers of two or more monomeric subunits.

The present study was undertaken to investigate the structural requirements and subunit localization of the disulfide bonds responsible for polymerization. In the course of this study it was found that mouse γA myeloma proteins have a unique polypeptide chain structure in that the L–H interchain disulfide bonds are absent. The experiments to be described indicate that the light chains are bound to the heavy chains solely by noncovalent interactions and exist in the γA molecule as monomers or as disulfide-bound light-chain dimers.

Materials and Methods

Myeloma Proteins. Mouse γA proteins were obtained from the sera of BALB/C mice with plasma cell tumors (Potter and Boyce, 1962), and human γA proteins from the sera of patients with multiple myeloma. Mouse sera with high concentrations of both polymer and monomer, as judged by starch gel electrophoresis, were selected when possible. Proteins were isolated by starch block electrophoresis at pH 8.6 (Kunkel, 1954). Mouse γ-A polymer and monomer were separated by gel filtration through Sephadex G-200 (column size 2.5 × 100 cm) in phosphate-buffered saline (pH 7.2). In some instances proteins were labeled with ^{125}I or ^{131}I by a modification of the chloramine-T method (McConahey and Dixon, 1966).

Starch Gel Electrophoresis. Vertical starch gel electrophoresis (Smithies, 1959) was performed utilizing a glycine buffer (pH 8.8) (Fahey, 1963a,b) or an 8 M urea–formate buffer (pH 3) (Poulik, 1960). Glycine gels were subjected to electrophoresis for 5 hr at 450 V in the cold, and acid–urea gels for 16 hr at 170 V at room temperature.

Antigenic Analysis. Immunological studies of γA fragments and peptide chains were done by Ouchterlony immunodiffusion using hyperimmune rabbit antisera directed against mouse γA myeloma proteins or mouse κ chains. These antisera were rendered specific by absorption with other purified mouse proteins or, in the case of the anti-γA antiserum, by absorption with whole newborn mouse serum.

Separation of Heavy and Light Chains. The isolated myeloma proteins were partially reduced with 0.2 M 2-mercaptoethanol in 0.55 M Tris buffer (pH 8.2) for 1 hr at room temperature and alkylated with a 20% molar

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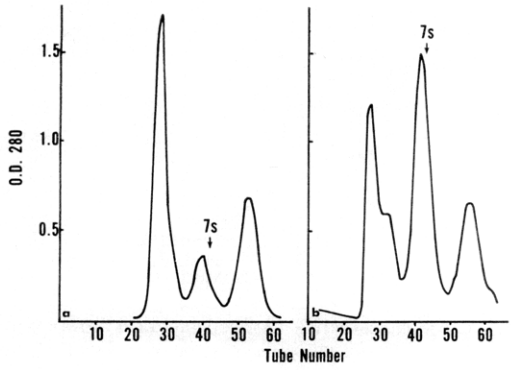

FIGURE 1: Purification of mouse γA myeloma proteins. Gel filtration (G-200) in phosphate-buffered saline (pH 7.0) of two γA myeloma proteins (1a, S32; 1b, S121) isolated by starch block electrophoresis. The first peak (polymer) emerged from the columns with the exclusion volume; the second peak (monomer) was eluted slightly ahead of [^{131}I]HGG used as a marker; and the third peak was mainly composed of transferrin.

excess of iodoacetamide for 1 hr at 4°. The partially reduced and alkylated proteins were separated into heavy and light chains by gel filtration through Sephadex G-100 in 1 M acetic acid. Distribution of protein to heavy and light chains was determined on a Technicon automatic nitrogen analyzer.

In Vitro Polymerization of γA Myeloma Proteins. Purified human and mouse γA polymer and monomer were labeled with ^{131}I or ^{125}I, reduced with 0.2 M 2-mercaptoethanol in phosphate-buffered saline (pH 7.0), and reoxidized by exhaustive dialysis against 0.1 M acetate buffer (pH 5.0) at room temperature for 48 hr with continuous stirring as recommended by Deutsch (1963). Unreduced proteins were subjected to a similar treatment. In some instances following partial reduction, proteins were alkylated with a 20% molar excess of iodoacetamide and dialyzed as described above. The degree of polymerization was evaluated by gel filtration through Sephadex G-200. Carrier human γ-globulin,[1] prepared by DEAE chromatography of Cohn fraction II was mixed with all labeled proteins subjected to oxidation at the end of the 48-hr period, and simultaneously subjected to gel filtration.

Cellular Synthesis of Murine γA Polymer and Monomer. Mouse plasma cell tumor types S32 and S116 were used for short-term tissue culture. The tumors (0.5–1.0-cm diameter) were excised, minced, suspended, and washed in minimal Eagle's medium. Labeling of the newly synthesized myeloma proteins with [1-^{14}C]leucine and separation of the extra- and intracellular fluid were done as previously described (Notani *et al.*, 1966). To both the extracellular and intracellular ^{14}C-labeled proteins, an equal volume of carrier myeloma serum from mice bearing the same tumor that was used for the ^{14}C incorporation was added. The γA proteins were isolated by starch block electrophoresis, and the polymer and monomer were separated by gel filtration on Sephadex G-200. The size distribution of the ^{14}C-labeled

[1] Abbreviation used that is not listed in *Biochemistry 5*, 1445 (1966), is: HGG, human γ-globulin.

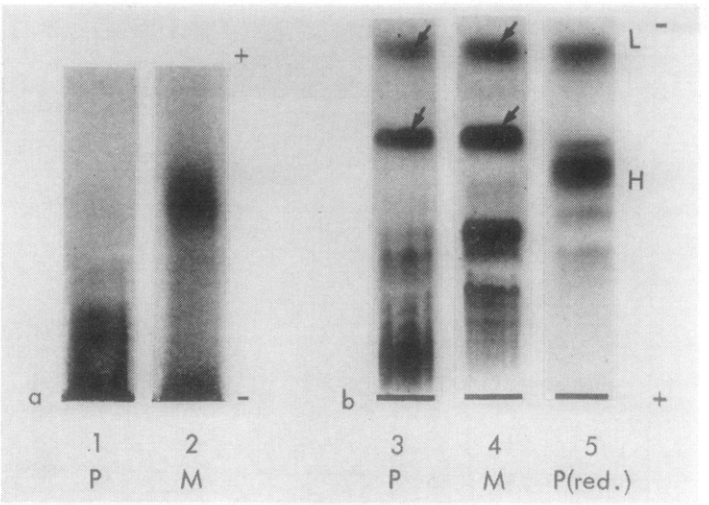

FIGURE 2: Starch gel electrophoresis of unreduced mouse γA monomer and polymer. 2a = glycine–starch gel (pH 8.8); 2b = formate–urea starch gel (pH 3.0); slots 1 and 3, polymer; slots 2 and 4, monomer; slot 5, reduced and alkylated polymer. Arrows indicate the two rapidly migrating bands common to both monomer and polymer γA which appear when electrophoresis is done in acid–urea buffer. The most rapidly migrating band corresponds in position to the light-chain band of the reduced and alkylated γA.

γA was determined by counting aliquots of each fraction in a Packard Tri-Carb liquid scintillation counter using Bray's solution as solvent (Bray, 1960). Quantitation of the polymer:monomer ratio in intra- and extracellular fluids was obtained by determining the per cent of ^{14}C counts that was precipitable from monomer and polymer peaks by a specific rabbit antimouse γA antiserum.

Results

Studies with Unreduced γA Proteins. Mouse γA myeloma proteins were isolated by starch block electrophoresis and further purified by gel filtration on Sephadex G-200. Figure 1 illustrates the elution pattern obtained from the sera of animals bearing two different tumors. The first peak was eluted immediately following the void volume of the column. The second peak was eluted slightly before ^{131}I-labeled HGG which was added as a marker (indicated by vertical arrow). The third peak consisted of transferrin and other nonimmunoglobulin β-globulins. The ratios of the first, polymer γA peak, and the second, monomer peak, differed from one tumor to the next. However, the ratio of polymer to monomer in a given tumor remained quite constant over a period of 15–20 generations of serial transfer of the tumor (during a 2-year period) despite marked alterations in growth rate and myeloma protein production over the same period of time.

In the process of evaluating the purity of the γA preparations isolated by this procedure starch gel electrophoresis was performed. Figure 2 illustrates the results obtained. In nondissociating solvents such as 0.05 M glycine buffer (pH 8.8), a single major band was observed for the monomer γA and several poorly resolved, slower

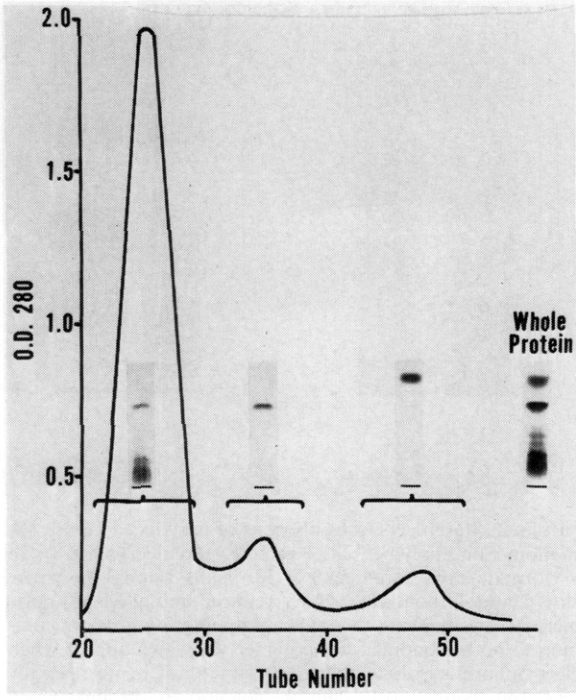

FIGURE 3: Sephadex G-100 gel filtration in 1 M acetic acid of unreduced mouse γA myeloma protein (S116, polymer). Patterns obtained from analysis of each of the three peaks in acid–urea starch gel electrophoresis (pH 3.0) are shown and compared to that of the isolated whole γA protein. The first peak was eluted from the column with its exclusion volume and is made up predominantly of the most slowly migrating material on starch gel electrophoresis along with a smaller amount of the first of the two cathodal bands. The second peak was composed only of the slower of the two cathodal bands. Dimer human Bence–Jones proteins were eluted at the same position as peak two. The third peak contained the fastest migrating starch gel band. The elution volume of this peak corresponded to that of monomer human Bence–Jones protein.

moving bands were observed for the polymer γA depending on the degree of polydispersity of the myeloma protein (Figure 2a). In contrast to these results starch gel electrophoresis in 8 M urea–formate buffer (pH 3.0) of monomer and polymer γA yielded a more complex pattern (Figure 2b). In addition to the bands related to the monomeric and polymeric forms of γA, two faster moving bands were observed which were present in both the monomer and polymer preparations (arrows Figure 2b). These results suggested that both monomer and polymer γA proteins contained moieties that were dissociated from the parent molecule under the conditions used for electrophoresis. The most cathodal migrating band occupied the same position in the gel as the light-chain band obtained with the partially reduced and alkylated γA polymer (Figure 2b, slot 5). No band corresponding to the reduced and alkylated heavy chains was observed in the unreduced proteins. To investigate this further, gel filtration of the native proteins was performed in 1 M acetic acid. Gel filtration of both the untreated γA polymer and monomer gave similar results and indicated that the γA protein was separable by this method into three fractions (Figure 3). The first peak emerged from the column with its exclusion volume and

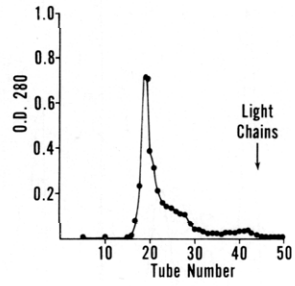

FIGURE 4: Sephadex G-100–1 M acetic acid gel filtration of the partially reduced and alkylated first peak isolated as shown in Figure 3. A single heavy-chain peak was obtained without any observable light-chain peak. Arrow indicates the elution volume for monomer light chains on this column.

corresponded to the slowest moving fraction in the acid–urea gel pattern. This first peak was incompletely separated from the second peak and contained some material which migrated in the same position as the material isolated from the second peak. The second peak emerged with an elution volume identical with that of a human dimer Bence–Jones protein examined separately on the same column. The third peak was eluted with the same elution volume as that of monomeric light chains and corresponded to the most rapidly migrating band on acid–urea starch gel electrophoresis. When the material in the second peak was reduced with 0.1 M 2-mercaptoethanol and analyzed by acid–urea gel it migrated in the same position as the third peak. Antigenic analysis with specific rabbit anti-κ and anti-γA antisera indicated that peak one was the only peak that contained γA specific determinants. Peaks two and three, on the other hand, reacted strongly with the anti-κ antiserum whereas peak one reacted only weakly with this antiserum. These data indicated that a considerable amount of the light chains present in this mouse γA myeloma protein were noncovalently bound to the heavy chain and existed in the molecule both as monomeric light chains (peak three, Figure 3) and as disulfide-bound dimer light chains (peak two, Figure 3). Protein determination on the three peaks indicated that 72% of the total protein was in peak one, 21% in peak two, and 7% in peak three. In total, 28% of the γA protein was therefore present in the form of light chains which were noncovalently bound to heavy chains. This is very close to the 32% of light chains found upon gel filtration of the reduced and alkylated γA protein and suggested that most, and probably all, of the light chains in the mouse γA were present in this form. To confirm this point, peak one obtained after G-100 gel filtration of the unreduced protein was reduced and alkylated and reapplied to a G-100 1 M acetic acid column. As is shown in Figure 4 no discernible light chain peak was observed, again indicating that most or all of the light chains in this γA protein were not disulfide bound to the heavy chains.

In all, six mouse γA myeloma proteins were available for study (Adj PC6A, S21, S32, S116, S117, and S121). By acid–urea starch gel electrophoresis, all six proteins showed noncovalently bound monomer and dimer light-chain bands very similar to the protein

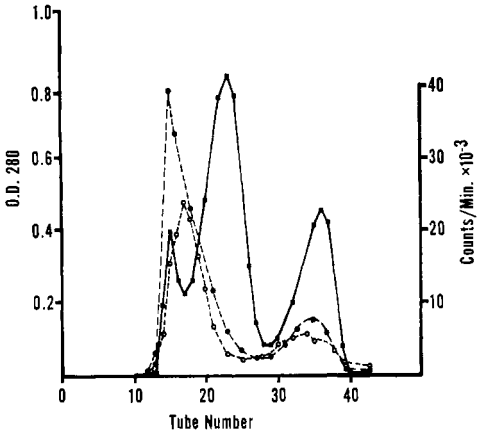

FIGURE 5: Sephadex G-100 gel filtration of reduced and alkylated mouse γA (S116). [^{125}I]HGG and [^{131}I]human γA2 were added to the mouse γA protein prior to reduction and were applied to the column together with the mouse γA protein; (■——■) mouse γA (OD_{280}), (O- - -O) [I^{125}]HGG (counts per minute), and (●- - ●) [I^{131}]human γA2 (counts per minute). Mouse α chains were eluted after human γ or α2 chains; all three light-chain preparations were eluted at close to the same volume.

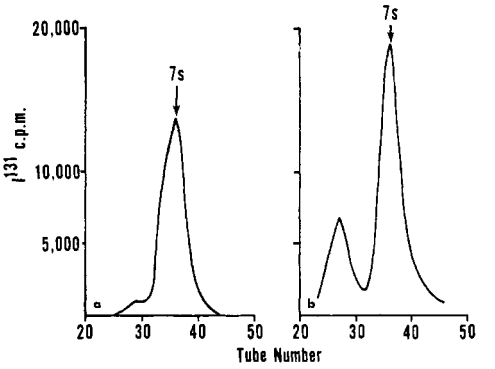

FIGURE 6: *In vitro* polymerization of mouse γA monomer. ^{131}I-labeled monomer, unreduced and oxidized, and subjected to Sephadex G-200 gel filtration is shown in Figure 6a. Elution pattern of the reduced and oxidized monomer is shown in Figure 6b. No significant polymer peak was observed in the unreduced protein; 30% of the counts were eluted from the column as polymer (first peak) after reduction and reoxidation.

shown in Figure 2. Three proteins (S32, S116, and S121) were further studied by G-100 gel filtration in 1 M acetic acid with subsequent antigenic analysis of the eluted fractions. All three showed the near-quantitative release of monomer and dimer light chains without prior reduction. These results would indicate that the great majority of mouse γA myeloma proteins have their light chains present in the γA molecule as monomers or disulfide bound dimers which are noncovalently bound to the heavy chains. In contrast to these results, similar studies with four mouse γG myeloma proteins gave no indication of the presence of noncovalently bound light chains in these proteins.

In order to minimize the possibility that disulfide exchange reactions caused some of the observed results, the myeloma sera were alkylated by overnight dialysis in the cold against 0.02 M iodoacetamide prior to the isolation of the myeloma protein. Starch gel electrophoretic analysis of the unreduced protein gave results identical with those shown in Figure 2, suggesting that disulfide exchange is not an important factor in the production of these results.

Comparison of the acid–urea starch gel electrophoresis pattern of monomer and polymer forms of the same myeloma protein as well as the patterns obtained with the reduced and alkylated myeloma protein suggest two other points of interest with regard to the interchain disulfide bonds of mouse γA proteins. First, since probably all the light chains were dissociated from the γA under the conditions used for electrophoresis the slowest migrating band of the unreduced monomer most likely represents the heavy chains of this protein. This band was distinctly more slowly migrating than the major heavy-chain band of the reduced and alkylated protein (Figure 2). This suggests that the H–H interchain disulfide bond(s) were still intact in the unreduced protein and the slower mobility of the heavy chains compared with those found in the reduced and alkylated protein was due to their being present in the form of disulfide-bound dimers in the unreduced protein. This was confirmed by comparing the elution volume of the heavy-chain peak of the unreduced monomer with that of the reduced protein on Sephadex G-100 in 1 M acetic acid. The unreduced heavy chains emerged with the void volume whereas the reduced heavy chains emerged at a volume close to that of the dimer light-chain peak shown in Figure 3. The second point of interest is that the polymer γA in acid–urea gel gave a complex series of slowly migrating bands in the region extending cathodally from the origin when compared to the monomer γA. This suggests that polymeric units were present. Since the light chains were dissociated from the molecule under the conditions of electrophoresis, it can be concluded that the disulfide bonds which link monomeric units of γA to form the polymer are H–H interchain disulfide bonds rather than L–L or L–H bonds.

Gel Filtration of Reduced and Alkylated γA Protein. Another characteristic which distinguished the mouse γA myeloma proteins from other mammalian immunoglobulins studied was the pattern the reduced and alkylated protein showed upon G-100 gel filtration in 1 M acetic acid (Figure 5). An ^{131}I-labeled human γA2 myeloma protein and ^{125}I-labeled HGG were added to the mouse γA myeloma protein prior to partial reduction with 0.2 M 2-mercaptoethanol. The light chains of all three proteins were eluted with almost identical volumes. The heavy-chain peaks were eluted at distinctly different volumes, however. The human γA human chain was eluted slightly ahead of the human γG heavy chain. The mouse γA heavy chain gave two peaks, an initial minor peak which was eluted with the void volume and a second major peak which was eluted well after either human α or γ chains and peaked in the trough region between the light and heavy chains of the two human proteins. Nitrogen determination of the light- and heavy-chain peaks indicated that 32% of the total protein was in the mouse γA light-chain peak and 68% in the heavy-chain region. Gel filtration of two other partially re-

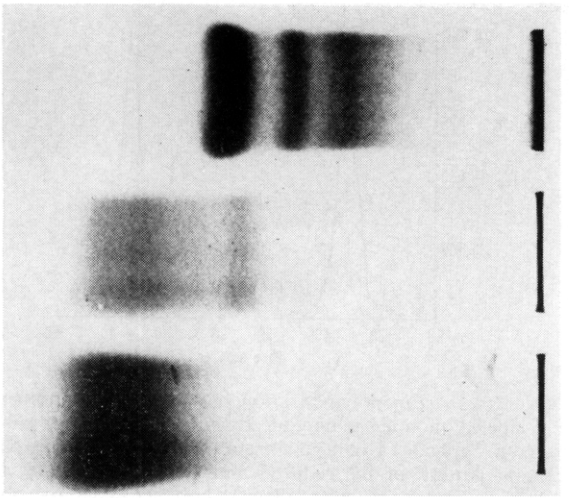

FIGURE 7: Starch gel electrophoresis in acid–urea of a reduced and reoxidized human γA monomer. Lower slot, unreduced monomer; middle slot, reduced and oxidized monomer; and upper slot, unreduced polymer. Both monomer and polymer were isolated from the same serum.

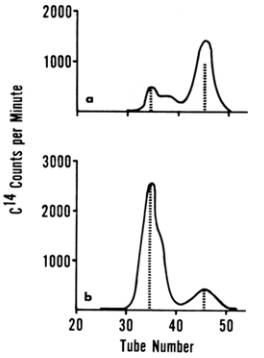

FIGURE 8: Sephadex G-200 gel filtration of newly synthesized ^{14}C-labeled intracellular (Figure 8a) and extracellular (Figure 8b) mouse γA myeloma protein (S116). First and second peaks are polymer and monomer, respectively. The polymer was eluted from the column with its exclusion volume, the monomer with a volume slightly smaller than that required to elute HGG. Vertical dotted lines represent amounts of radioactivity specifically precipitable by a rabbit antimouse γA antiserum in the region of antibody excess.

duced and alkylated mouse γA proteins gave similar results to that shown in Figure 5.

In Vitro Polymerization of γA Myeloma Proteins. In an attempt to determine whether monomer γA myeloma proteins were capable of undergoing polymerization, or whether there was a critical structural feature which was present in the polymer subunit which was not present in the native monomer γA protein, *in vitro* polymerization experiments were performed on mouse and human γA myeloma proteins. Oxidation was accomplished by exhaustive dialysis of the proteins using dialysis fluid through which air was constantly bubbled. Oxidation of the reduced and alkylated mouse γA monomer, or oxidation of the untreated monomer, by dialysis against 0.1 M acetate buffer (pH 5.0) did not result in a significant degree of polymerization. In contrast, reoxidation of the reduced, nonalkylated monomer resulted in the polymerization of 30–35% of the protein as measured by gel filtration on Sephadex G-200. Figure 6 illustrates these results using ^{131}I-labeled γA monomer. Unlabeled carrier HGG was added as a 7S marker and was eluted at the volume indicated by the arrow. Oxidation of the reduced, nonalkylated polymer resulted in a degree of polymerization comparable to that obtained with the reduced, nonalkylated monomer. When the polymer was reduced and alkylated, monomeric subunits resulted which did not polymerize after oxidation in acetate buffer. The results of these experiments are summarized in Table I.

TABLE I: *In Vitro* Polymerization of Mouse γA.

Protein	Treatment	% Polymer
Polymer	Red alkylation	9
	Red oxidation	31
Monomer	Red oxidation	35
	Unred oxidation	6
	Red alkylation	4

Analysis by starch gel electrophoresis in glycine buffer of the products resulting from reduction and reoxidation of a human γA monomer showed its partial conversion into a protein migrating in a position similar to that of the most anodic band of the γA polymer isolated from the same serum (Figure 7). This band probably represents a dimer γA and indicates that the polymerization does not lead to random extensive aggregation, but rather to a well-defined polymer structure.

Control experiments with nonaggregated human γG-globulins isolated from Cohn fraction II by column chromatography on DEAE-cellulose columns and subsequent gel filtration through Sephadex G-200 showed that neither the untreated nor the reduced and reoxidized proteins polymerized to a significant degree.

Cellular Synthesis of Mouse γA Myeloma Proteins. γ A myeloma proteins were isolated from both intra- and extracellular fluids obtained following incubation of mouse myeloma cells with ^{14}C-labeled leucine. As shown in Figure 8, a strikingly different ratio of polymer to monomer existed in the two fluids. The majority of the intracellular γA protein was present as monomer, while only a minor intracellular polymer peak was obtained. Analysis of the extracellular fluid revealed the polymer and monomer to be present in concentrations closely resembling that found in the sera of mice bearing the same tumor from which the cells were obtained. The prevalence of the polymer in the extracellular fluid and of the monomer in the intracellular fluid was found in experiments performed with two different tumor types (S32 and S116).

Discussion

The present studies on the subunit structure of mouse γA myeloma proteins indicate a somewhat different

subunit structure than previously described for mammalian immunoglobulins. The finding that the light chains are quantitatively released from the γA protein without reduction has led us to the conclusion that there are no L–H interchain disulfide bonds present and that only noncovalent interactions hold the light and heavy chains together in this immunoglobulin. Secondly, the finding that 75% of the released light chains were present in the form of disulfide-bound dimers indicates that the light chains lie in close proximity to one another in the γA molecule. These data suggest that the polypeptide chains are arranged as illustrated in Figure 9a. Besides the absence of L–H interchain bonds, this model differs from that proposed by previous workers for γG-immunoglobulins, as illustrated in Figure 9b, in that the light chains are placed inside the two heavy chains in order to place them in close apposition to one another so that they may form an L–L disulfide dimer.

Neither mouse nor human γG myeloma proteins showed any indication of a similar lack of L–H disulfide bonds. However, the minor subclass of human γA (γA2) also lacks the L–H interchain disulfide bonds and the light chains are present in this γA subclass almost exclusively in the form of L–L disulfide dimers (Grey *et al.*, 1968). As yet no mouse γM proteins have been investigated, but four of four human γM paraproteins possessed L–H disulfide bonds. Despite the fact that evidence for the positioning of the light chains in close apposition to one another only exists for the mouse γA and human γA2 proteins it appears possible that this arrangement may also apply to the structure of immunoglobulin classes that possess L–H interchain bonds.

Why the mouse γA myeloma proteins lack the L–H interchain disulfide bridges is at present unknown. Since the light chains appear to be common to all immunoglobulin classes and since they are capable of forming L–L interchain bonds in the γA proteins studied, it is most probable that the structure of the heavy chain is the determining factor. Two possible structural changes in the heavy chain that could cause the loss of the L–H bridges are (i) through some genetic event such as point mutation or deletion, the mouse α chain has lost the cysteine residue on the heavy chain which is responsible for the L–H bridge; (ii) the proper cysteine residue is present on the α chain, but due to the tertiary folding of the α chain is not in the proper steric relationship with the light chain to form a disulfide linkage with it and therefore remains as a free SH group or enters into an intra- or interheavy-chain disulfide bond. No direct evidence for either of these mechanisms was obtained in the present studies. However, the gel filtration data obtained with the reduced and alkylated mouse γA suggests that the mouse α chain may be considerably shorter than other mammalian heavy chains. These two structural features, *i.e.*, a relatively short heavy chain and the absence of the L–H disulfide bond, would most readily be explained on the basis of a deletion involving the region of the heavy chain which contains the cysteine residue that participates in the L–H bridge. Studies on the molecular weight of the mouse α chain and quantitation of the number and location of the inter- and intraheavy-chain disulfide bridges are in progress. This will provide further evidence which may allow a more definite conclusion to be made regarding the nature of the structural variations present on the mouse α chains.

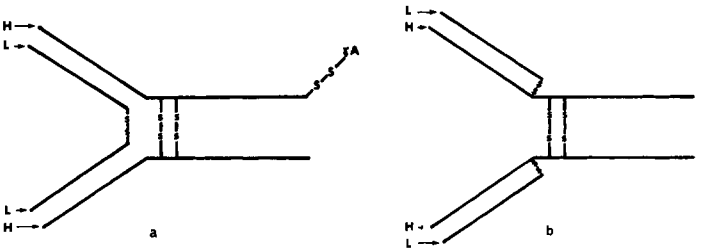

FIGURE 9: Proposed model for arrangement of polypeptide chains and interchain disulfide bridges in mouse γA-globulin (Figure 9a) compared with previously proposed model (Fleischman *et al.*, 1962) for mammalian γG-globulins (Figure 9b).

The remainder of the experiments reported above were designed to obtain information regarding the subunit location of, site of synthesis of, and structural requirements for the formation of the disulfide bonds which link γA monomers together to form a polymer. The experiments performed to compare the ability of γA polymer and monomer to undergo *in vitro* polymerization demonstrated the inability of the native unreduced γA monomer to polymerize. After partial reduction and reoxidation, however, the monomer polymerized to the same extent as reduced and reoxidized polymer. The inability of the unreduced monomer to polymerize suggests that the cysteine residue involved in the polymerization step is blocked in the native monomer and becomes unblocked subsequent to reduction. Disulfide-bond linkage to small molecules such as cysteine or glutathione could be a mechanism by which this residue would become blocked, in a manner similar to that described for monomeric albumin (King, 1961).

A previous study (Abel and Grey, 1967) indicated that γA proteins possess a cysteine residue penultimate to the carboxy-terminal tyrosine. No direct information is available as to the participation of this cysteine in the formation of the polymer. However, the present study has presented evidence that the intersubunit disulfide bond is located in the γA heavy chain. Studies with human γM proteins have further localized the intersubunit bonds to the Fc fragment of the γM (Onoue *et al.*, 1968; Mihaesco and Seligmann, 1968).

The studies performed to investigate the *in vivo* site of polymerization indicated the presence of small amounts of polymer intracellularly in plasma cells actively synthesizing myeloma proteins. The relative amount of intracellular polymer was much less than was present in the extracellular fluid. Two possible explanations for this discrepancy are: (i) polymer is more rapidly secreted from the plasma cell than is monomer, so that the intracellular pool of polymer would, as a result, be lower than that of monomer; (ii) polymerization takes place in both intra- and extracellular compartments, but occurs to a greater extent extracellularly. Experiments planned to distinguish between these two possibilities are in progress.

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Kinetics of Coupled Enzymes. Creatine Kinase and Myosin A*

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ABSTRACT: When adenosine triphosphate is added to a two-enzyme system containing myosin A, creatine kinase, and creatine phosphate, the adenosine diphosphate produced by the myosin-catalyzed hydrolysis of adenosine triphosphate can be rapidly rephosphorylated at the expense of creatine phosphate by the action of creatine kinase. If both enzymes in the two-enzyme system are able to function as they do individually, then, under appropriate conditions, the rate of creatine production should reflect the rate of adenosine triphosphate hydrolysis. It is observed, however, that the rate of creatine production in the two-enzyme system is less than the rate of inorganic phosphate production in the corresponding myosin system. Activities in the presence and absence of added magnesium and pyrophosphate also show this discrepancy. Evidence indicates that magnesium introduced with the kinase can account for only a part of the inhibition. The remaining difference in the activities of the two systems cannot be attributed to inhibition of myosin A by creatine phosphate, to a delay in establishing the steady-state rate of regenerating adenosine triphosphate, nor to a steady-state adenosine triphosphate concentration appreciably below the total nucleotide concentration. The inhibition of enzymatic activity in the two-enzyme system seems to be attributable to a direct inhibition of myosin by creatine kinase.

In the contraction–relaxation cycle of muscle, ATP is considered to be the immediate source of chemical energy; but a major reservoir of readily available chemical energy is creatine phosphate. Creatine kinase, which catalyzes the transphosphorylating reaction in which ATP is formed from ADP and creatine phosphate, can act in tandem with myosin ATPase to constitute a coupled system catalyzing the hydrolysis of creatine phosphate (reactions i–iii). Creatine kinase has also been

$$\text{ATP} + \text{H}_2\text{O} \rightleftarrows \text{ADP} + \text{P}_i \qquad \text{(i)}$$

$$\text{ADP} + \text{creatine phosphate} \rightleftarrows \text{ATP} + \text{creatine} \qquad \text{(ii)}$$

$$\text{creatine phosphate} + \text{H}_2\text{O} \rightleftarrows \text{creatine} + \text{P}_i \qquad \text{(iii)}$$

implicated in the relaxation process either as a direct participant or as an important means of regenerating ATP utilized in the process of calcium accumulation by the sarcoplasmic reticulum (Lorand *et al.*, 1965). Myosin and creatine kinase can be extracted from muscle in comparable amounts on a molar basis. But, since these enzymes are known to be compartmented within the

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