Human Myeloma IgG Half-Molecules. Structural and Antigenic Analyses†

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ABSTRACT: The structure and antigenic characteristics of a human κ, IgG myeloma protein that formed half-molecules were analyzed. Most of the myeloma protein found in the patient's serum and urine consisted of two chain 4.3S half-molecules. A small amount of four chain 7S myeloma protein was, however, found in the serum and was apparently formed by the same clone of tumor cells. Polyacrylamide gel electrophoresis in 8 *M* urea and 1% sodium dodecyl sulfate and analytical ultracentrifugation in 6 *M* guanidine of the fully reduced and alkylated half-molecule indicated that this myeloma protein had a heavy chain of a smaller molecular weight (approximately 45,000) than that of normal γ chains. Except for this apparent deletion, the heavy chain resembled γ_1 chains. The amino acid composition of the peptides containing the half-cysteine residues forming the interchain disulfide bonds, the glycopeptide of the Fc fragment and the COOH-terminal structure were similar if not identical with the analogous structures of γ_1 chains. No Fc fragment could be prepared because the Fc portion of the heavy chain of the myeloma protein was extremely susceptible to degradation with papain. After mild reduction and alkylation, the 7S myeloma protein dissociated into half-molecules, indicating a lack of noncovalent interactions in the Fc fragment that are present in all classes of human immunoglobulins and are responsible for the formation of Fc dimers. The half-molecule was antigenically deficient in the Fc fragment. It failed to precipitate with anti-Fc fragment antisera in double gel diffusion tests and inhibited a Fc-anti-Fc fragment binding reaction weakly and incompletely. The half-molecule and the 7S protein had the same genetic markers on the first and second homology region of the γ chain. The half-molecule lacked, however, the corresponding markers on the third homology region. These findings suggest that this myeloma protein had a deletion in the γ chain which was probably located in the third homology region and was likely the structural abnormality responsible for the lack of noncovalent interaction in the Fc fragment and absence of most of the antigenic determinants characteristic of γ chains.

Immunoglobulin half-molecules consisting of one heavy and one light chain appear to be a rare type of immunoglobulin. They were first described as occurring in low concentrations in the sera of colostrum deprived piglets (Franek and Rika, 1964). Subsequently, several lines of mineral oil induced murine plasmacytomas were detected which produced IgA half-molecules (Lieberman et al., 1968). Recently, immunoglobulin half-molecules have also been detected in patients with plasma cell tumors. Hobbs and Jacobs (1969; Hobbs, 1971) reported the first three cases, all suffering from extramedullary soft-tissue plasmacytoma. Two similar patients have since been observed, one having plasma cell leukemia (Spiegelberg and Heath, 1973; Spiegelberg et al., 1975) and the other classical multiple myeloma (Seligmann et al., 1973, and personal communication). All patients excreted into the urine relatively large amounts of κ, IgG half-molecules which consisted of one heavy and one light chain. This report describes structural and antigenic features of the IgG half-molecule found in our patient in whose serum four chain myeloma protein was also detected, besides the half-molecules.

Materials and Methods

Myeloma Protein. The myeloma protein was isolated from the plasma of patient KN obtained before treatment and stored at −20° and from serum obtained 3–5 months after chemotherapy. Both specimens contained about three times more half-molecules than 7S IgG, although only about half the quantity of myeloma protein (5–10 mg/ml) was present after treatment. The urine available for isolation of the half-molecule was obtained 3–4 months after treatment. It contained approximately 15 mg of half-molecules/100 ml as compared to 300 mg/100 ml before treatment. The urine was dialyzed against 0.005 *M* phosphate buffer (pH 8.0) containing toluene as a preservative and lyophilized. The IgG fractions of both plasma and urine were isolated by DEAE-cellulose chromatography employing 0.015 *M* phosphate buffer (pH 8.0). The protein eluted with this buffer was concentrated by pressure dialysis and applied to Sephadex G-200 columns equilibrated with phosphate buffered 0.15 *M* NaCl (pH 7.0) (saline) at 4°. Two protein peaks were eluted when the serum IgG fraction was applied but only one major peak when the urinary protein was applied. The first peak of the serum protein eluted at a position similar to normal IgG.

Heavy and Light Chains. The proteins dissolved in 0.5 *M* Tris buffer (pH 8.2) were mildly reduced by incubation with 0.02 *M* dithiothreitol for 2 hr at room temperature and alkylated by addition of 0.05 *M* twice recrystallized iodoacetamide containing 1 μCi of ^{14}C-labeled iodoacetamide/mg of protein for 30 min at 4°. Complete reduction and alkylation were performed in the same manner except that either 8 *M* urea or 5 *M* guanidine was substituted for the Tris buffer. In order to isolate heavy and light chains, the reduced and alkylated proteins were dialyzed against 1 *N*

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acetic acid and applied to a Sephadex G-100 column equilibrated with the same solvent. A trace amount of ^{13}I-labeled normal IgG was added in two experiments before reduction and alkylation in order to monitor the elution of normal IgG heavy and light chains. The protein peaks eluted from the column were dialyzed against water and concentrated by pressure dialysis. Protein nitrogen analyses were performed by using a Technicon automated microKjeldahl apparatus. The proteins were radioiodinated by a modified method of the chloramine-T procedure (McConahey and Dixon, 1966).

Enzymatic Digestions. The protein was digested with twice recrystallized papain (Worthington Biochemicals, Freehold, N.J.) according to the method described by Porter (1959) employing digestion periods of 30 min and 18 hr. The digestion was terminated by addition of twice molar excess of recrystallized iodoacetamide.

Digestion with neuraminidase was performed by addition of 50 units of neuraminidase (Behringwerke, Germany)/10 mg of protein dissolved in 0.05 *M* acetate buffer (pH 5.5) containing 0.01 *M* calcium chloride and toluene as a preservative. The mixture was incubated for 18 hr at 37° and then dialyzed against saline at 4°.

Cyanogen Bromide (CNBr) Cleavage. Fifty milligrams of heavy chain isolated from the urinary half-molecule was dialyzed against water, lyophilized, and dissolved in 5 ml of 70% formic acid. Ten times the protein weight of CNBr was then added and the mixture incubated at room temperature overnight in the dark. The mixture was diluted with 50 ml of water and lyophilized; 2 ml of water was added to extract the water soluble peptides, which were relyophilized and applied in a small volume on a Sephadex G-50 column equilibrated with 0.1 *N* NH_4OH. The elution of the peptides was monitored at 220 nm. Heavy chains from an IgG_1 myeloma protein were similarly treated as a control for the elution position and approximate recovery of the carboxy (COOH)-terminal octadecapeptide typical for γ_1 chains (Prahl, 1967). The peptide peaks eluted from the column were lyophilized and a portion was hydrolyzed for amino acid analysis.

Amino Acid Analysis and Peptide Mapping. Proteins or peptides were hydrolyzed under vacuum for 20 hr at 110° with 1 ml of double distilled 6 *N* HCl. An automated Beckman Model 120 amino acid analyzer was employed; no corrections were made for loss of amino acids. The number of "labile" half-cysteine residues was determined as carboxymethylcysteine after reduction and alkylation in Tris buffer and the total number after reduction and alkylation in 8 *M* urea. The yields of carboxymethylcysteine from normal light and myeloma γ_1 chains used as a control were 88–95% of the expected value. Glucosamine and galactosamine were differentiated using a 12-cm column of Aminex A5 as previously described (Spiegelberg et al., 1970).

Radio-peptide maps, elution of peptides and analysis of the amino acid composition of the peptides were carried out as described previously (Grey and Kunkel, 1967; Spiegelberg et al., 1970).

Carboxy Terminal Sequence Analyses. Digestion of isolated heavy chain suspended in 0.1 *M* NH_4HCO_3 with carboxypeptidase A (Worthington Biochemicals, Freehold, N.J.) was performed for 2 hr at room temperature (Abel and Grey, 1967). For digestion with carboxypeptidase Y (Hayashi et al., 1973) the heavy chain was dialyzed first against 1.0 *M* NaCl and then against 0.05 *M* acetate buffer (pH 5.5). Enzyme/protein ratio on a weight basis w/w was 1:20. The digestion was terminated by addition of 50% sulfosalicylic acid. Zero time digestion controls were performed by addition of the acid before the enzyme and by subtracting the quantities of amino acids of the supernatant from the experimental value. Heavy chains of an IgG_1 myeloma protein served as a control.

Starch Gel Electrophoresis. Vertical starch gel electrophoresis was performed either by employing a 0.05 *M* glycine buffer (pH 8.8) (Fahey, 1963) or an acid urea formate buffer (pH 3.5) (Poulik, 1960).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed in 8 *M* urea and 1% sodium dodecyl sulfate (SDS)[1] according to the procedure described by Weber and Osborn (1969). The half-molecule was labeled before reduction with ^{125}I, normal human IgG and a IgM macroglobulin were labeled with ^{131}I, and the three preparations were mixed for reduction and the following procedures in order to have an internal marker for μ, γ, and light chains. The gels were sliced and the radioactivity was determined using a Nuclear Chicago γ-scintillation counter.

Ultracentrifugation. Analytical ultracentrifugation was performed by using a Beckman Model E analytical ultracentrifuge equipped with Schlieren optics. The sedimentation rates were determined for at least two different protein preparations and calculated by the standard procedure (Schachman, 1959).

Antigenic Analyses. Antisera to normal human IgG were prepared in two goats and in rabbits by multiple injections of purified Cohn Fraction II incorporated into complete Freunds adjuvant. The antisera were rendered specific for the Fc fragment by absorption with Fab fragments and κ and λ Bence-Jones proteins. A commercial anti-Fc fragment antiserum was obtained from Hyland Laboratories, Costa Mesa, Calif. Rabbit antisera to κ and λ light chains and the different classes of immunoglobulins were prepared as described by Fahey and McLaughlin (1963). Antisera to IgG subclasses were prepared in rabbits as previously described (Spiegelberg and Weigle, 1968). Antisera to the urinary half-molecule were prepared in two rabbits and one rhesus monkey by multiple injections of the isolated protein incorporated into complete Freunds adjuvant. For analysis of idiotypic determinants, the antisera were absorbed with excess normal IgG Cohn Fraction II. In order to determine how much of the 7S IgG of the patient could be precipitated with anti-idiotypic antisera, the 7S fraction was radioiodinated with ^{125}I. Different quantities ranging from 5 to 50 μg of N proteins were added to 0.5 ml of undiluted and 1:5 and 1:10 dilutions of the anti-idiotypic antiserum. A rabbit anti-normal human IgG Fab fragment antiserum was used as a control for complete precipitation of the IgG.

Quantitative inhibition studies were performed by a modified ammonium sulfate method described by Cerottini (1968). Normal human Fc fragments were used as the antigen. A goat anti-Fc fragment antiserum was diluted to give 50–60% binding of the labeled Fc fragment. The antiserum was first incubated for 2 hr at 4° with varying concentrations of inhibitor protein, followed by addition of the labeled Fc fragment (0.1 μg of protein nitrogen) and incubation for 1 hr at 4° before 40% saturated $(NH_4)_2SO_4$ was added.

[1] Abbreviation used is: SDS, sodium dodecyl sulfate.

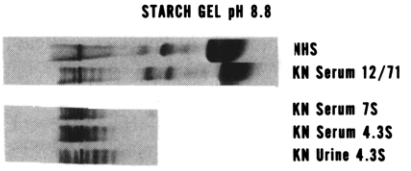

FIGURE 1: Starch gel electrophoretic analysis employing a glycine buffer (pH 8.8) of normal human serum (NHS), serum of patient KN obtained in December, 1971, and the isolated myeloma protein fractions from the serum having a sedimentation rate of 7.0 S (KN serum 7 S) and 4.3 S (KN serum 4.3 S) and the urinary myeloma protein (KN urine 4.3 S).

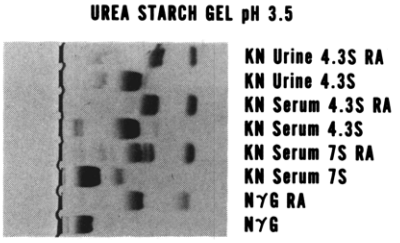

FIGURE 2: Starch gel electrophoretic analysis employing an 8 *M* urea acid buffer (pH 3.5) of normal (NγG) and reduced and alkylated normal IgG (NγG RA) and the native and reduced and alkylated (RA) serum and urinary 7 S and 4.3 S myeloma protein fraction.

Results

Ultracentrifugal and Electrophoretic Analysis of Myeloma Protein KN. The IgG fractions of the serum and urine were isolated by a standard DEAE-cellulose procedure and the proteins analyzed by analytical ultracentrifugation and starch gel electrophoresis. As shown previously (Spiegelberg et al., 1975) the urinary IgG fraction consisted of one major protein peak and the serum IgG fraction of two peaks. The urinary protein and the major serum peak had a corrected sedimentation rate of 4.3 S. The minor serum protein peak had a sedimentation rate of 7.0 S.

The analyses of the serum and isolated 7S and 4.3 S myeloma protein preparations by starch gel electrophoresis are shown in Figure 1. All three preparations showed multiple bands of almost identical electrophoretic mobility, suggesting that the same clone of tumor cells produced both the 4.3S and 7S myeloma protein.

Heavy and Light Chains. The isolated serum 7S, 4.3S, and the urinary 4.3S myeloma protein preparations were analyzed by urea–starch gel electrophoresis in both native and reduced and alkylated forms (Figure 2). The native proteins showed a single major band. The 7S myeloma protein had a mobility similar to normal IgG, whereas the 4.3S proteins migrated further into the gel toward the cathode. The minor bands seen in these preparations probably represented trace contaminants and aggregates that are usually seen when proteins are analyzed by this method at a relatively high protein concentration of 5–10 mg/ml. Significantly, however, no bands were observed in the position where light chain dimers or monomers would appear, indicating that both types of myeloma protein consisted of covalently linked polypeptide chains. Following either mild or complete reduction and alkylation, the proteins separated into two major sets of bands, one having a mobility slightly faster than normal γ chains and the other similar to normal light chains. The double band of the myeloma heavy chains was most likely the result of heterogeneity in sialic acid content which will be shown below for the Fab fragment. The

FIGURE 3: Polyacrylamide gel analysis of reduced and alkylated urinary KN half-molecule (KN 1/2 M) labeled with ^{125}I and normal IgG and a IgM macroglobulin (IgG and IgM) labeled with ^{131}I. A 6% gel was used and 8 *M* urea and 1% SDS. The labeled half-molecule, IgG, and IgM were mixed before reduction and electrophoresis in order to have internal control protein peaks.

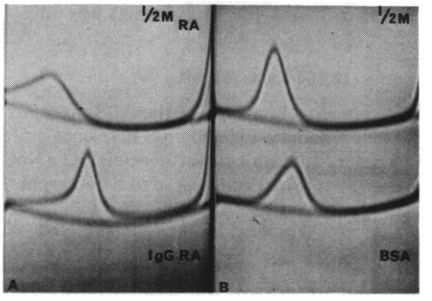

FIGURE 4: Ultracentrifugal analysis and uncorrected sedimentation rate of: (A) reduced and alkylated urinary half-molecule (1/2 M RA; 1.0 S) and normal IgG (IgG RA; 1.4 S) in 5 *M* guanidine; and (B) unreduced half-molecule (KN; 0.8 S) and bovine serum albumin (BSA; 1.1 S) in 6 *M* guanidine. Pictures were taken 384 min after reaching two-thirds of maximum speed (52,600 rpm) using a double sector cell.

reduced and alkylated urinary half-molecule was also analyzed by polyacrylamide gel electrophoresis in 8 *M* urea and 1% SDS (Figure 3). The heavy chain peak migrated significantly further into the gel than normal γ chains, suggesting that the γ chain of protein KN had a smaller molecular weight (approximately 45,000 as determined by this method) than normal γ chains. In contrast, the light chain peak of protein KN migrated in the position of the broad peak of normal light chains. In order to further determine if the heavy chain of the myeloma protein had a smaller molecular weight than normal γ chain, the fully reduced and alkylated urinary half-molecule was analyzed by analytical ultracentrifugation in 5 *M* guanidine (Figure 4A). The heavy chain peak of the half-molecule sedimented significantly slower than that of normal IgG indicating that the heavy chain of the the myeloma protein was of smaller molecular weight. The intact half-molecule was also analyzed in 6 *M* guanidine as compared to bovine serum albumin having a molecular weight of about 68,000. As can be seen in Figure 4B, the half-molecule sedimented more slowly than bovine serum albumin in 6 *M* guanidine. The heavy and light chains were isolated by Sephadex G-100 gel filtration in 1 *N* acetic acid. The heavy chain of the protein KN eluted only slightly behind normal γ chains and the light chain identical with normal light chains.

Papain Digestion. The urinary half-molecule was digested with papain in two experiments, first for 18 hr and sec-

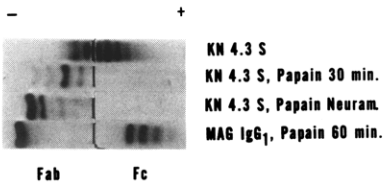

FIGURE 5: Starch gel electrophoresis analysis employing a glycine buffer (pH 8.8) of native urinary protein KN (KN 4.3 S), papain digested protein KN (KN 4.3 S, papain, 30 min), and the neuraminidase treated papain digested protein (KN 4.3 S, papain Neuramin). The papain digest of a control IgG 1 myeloma protein (MAG) is shown for positions of Fab fragments (Fab) and Fc fragments (Fc) on gel.

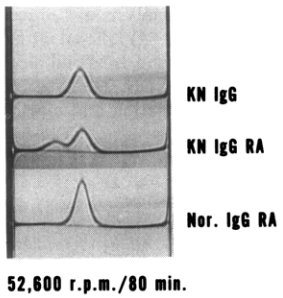

FIGURE 6: Ultracentrifugal analysis of the 7.0S myeloma protein KN (KN IgG), the reduced and alkylated 7.0S myeloma protein KN (KN IgG, RA), and reduced and alkylated normal IgG (Nor. Ig G RA). Picture taken after 80 min at a rotor speed of 52,600 rpm.

ond for 30 min. The preparations were dialyzed against saline and analyzed by starch gel electrophoresis (Figure 5). In both experiments, a set of protein bands more cathodal than those of the undigested protein was seen but no other bands appeared, particularly not in the area where normal Fc fragments were found. In order to determine if sialic acid was attached to the Fab fragment, the preparations were treated with neuraminidase and its electrophoretic mobility was compared with untreated Fab fragments. Following neuraminidase treatment, the multiple bands changed to a major protein band which had a slower electrophoretic mobility than most of the untreated Fab fragment. This indicates that sialic acid was removed from the Fab fragment. The protein obtained following 30-min digestion was applied on a Sephadex G-100 column equilibrated with saline. Only one protein peak was eluted from the column which consisted of Fab fragments as determined by antigenic analyses. The Fc position of the half-molecule heavy chain was apparently digested into dialyzable peptides even after the brief digestion period of 30 min.

Reduction and Alkylation of the 7S Myeloma Protein. Because the 7S myeloma protein had an almost identical electrophoretic mobility in starch gels to the half-molecules, it appeared likely that the 7S myeloma protein represented the covalently linked form of the half-molecule myeloma protein. In order to determine if the 7S protein would dissociate following reduction of the covalent interchain disulfide bonds, the protein was mildly reduced, alkylated, and examined by ultracentrifugation (Figure 6). Indeed, the 7S IgG peak dissociated under these conditions into two protein peaks in contrast to normal IgG which remains a 6.6S protein when treated under the same conditions. The faster moving protein peak had a sedimentation rate of 7.0 S and the slower peak 4.3 S, like the urinary or serum half-molecule. The area of the newly formed 4.3S peak comprised 31% of the total 7S peak that was reduced and alkylated.

Table I: Amino Acid Composition of Peptides of Heavy Chain KN Compared to Analogous Peptides of γ_1 Chains.[a]

	H–L KN	H–L γ_1	H–H KN	H–H γ_1	G-I KN	G-I Fc γ_1	G-II KN
Lys	0.8	1	2.0	2			1.0
His			0.9	1			
Arg					.7	1	
Asp	1.0	1	.5		1.0	1	4.1
Thr			1.9	2	1.2	1	1.8
Ser	0.9		1.3	1	.9	1	0.9
Glu			1.5	1	2.7	3	1.5
Pro			6.8	8			0.9
Gly			2.3	2			1.3
Ala			1.1	1			1.0
Val			1.3	1			2.2
Met							
Ile							1.9
Leu			3.2	3			1.3
Tyr					1.8	2	0.9
Phe			1.7	2			0.8
CM-Cys	0.9	1	1.9	2			0.8
GluN					+	+	+

[a] H–L peptide containing half-cysteine residue involved in inter-heavy-light and H–H in inter-heavy–heavy chain disulfide bond. G-I, glycopeptide analogous to Fc γ_1 chain glycopeptide (Fc, γ_1); G-II, glycopeptide of γ chain KN.

Amino Acid and Peptide Analysis. The amino acid analysis of the heavy chain of the urinary protein KN was unremarkable except for a content of 3.1 labile and a total of 9.3 mol/mol of carboxymethylcysteine per 45,000 mol wt KN γ chain. No carboxymethylcysteine was detected when the native urinary protein was alkylated without reduction, either by uptake of [^{14}C]iodoacetamide or amino acid analysis. The heavy chain of patient KN contained about twice the quantity of glucosamine than that seen with normal γ chains.

Heavy chains, which had been alkylated with [^{14}C]iodoacetamide after mild reduction and alkylation, were analyzed by radio-peptide mapping. Only two radioactively labeled peptides were observed in positions on the map similar to those of two radioactive peptides of γ_1 chains used as a control. Furthermore, the amino acid compositions of the two peptides were strikingly similar to those of the tryptic hinge peptides of γ_1 chains (Table I). The peptide involved in the formation of the inter-heavy–light disulfide bond is small and was recovered in relatively pure form. The other peptide is large and was contaminated to some extent by overlapping peptides on the map; its amino acid composition resembled, however, the peptide of γ_1 chains involved in the formation of the inter-heavy–heavy chain disulfide bonds. Two glycopeptides, which are characteristically localized in the line of application on the peptide maps, were also eluted (Table I). One had a composition similar to the glycopeptide of γ_1 chains localized in the Fc fragment. A second glycopeptide that appeared as multiple spots had an amino acid composition which did not allow positioning it in the constant region of the γ_1 chain. It contained 1 mol/mol of carboxymethylcysteine and two isoleucine residues suggesting that it could have been derived from the variable region either near the half-cystine residues 22, or 88 (Gally and Edelman, 1972). It has previously been shown that some myeloma proteins have sugar attached in the variable region of the heavy chain (Spiegelberg et al., 1970). Both glycopeptides contained glucosamine as the sole amino sugar.

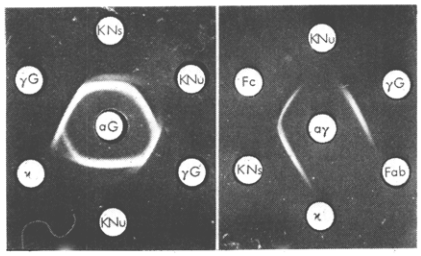

FIGURE 7: Double gel diffusion analysis of serum IgG fraction of patient KN (KNs) containing both 7S and 4.3S myeloma protein, isolated urinary myeloma protein (KNu), normal IgG (γG), Fc and Fab fragments and Bence-Jones κ chain (κ) employing a goat antiserum specific to IgG (aG) and to the IgG Fc fragment (aγ).

Table II: Amino Acids Released from 10 n*M* Heavy Chain KN and 20 n*M* γ_1 Chains after Incubation with Carboxypeptidase Y for 5 min at 37°.[a]

γ_1EU	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly-COOH
KN*	TR	1.2	1.8	3.6	3.6	3.6	4.0	3.2
γ_1*	0.8	1.4	6.6	8.0	8.0	8.0	7.4	8.8

[a] The amino acid yields are arranged according to the carboxy-terminal sequence of the γ_1 chain EU (Edelman et al., 1969).

Carboxy Terminal Sequence Analyses. Following CNBr fragmentation of γ chains, an octadecapeptide is recovered that is characteristic for the different γ chains (Prahl, 1967). When the heavy chain from the urinary half-molecule was fragmented with CNBr, three small peptides eluted from G-50 Sephadex columns; the one which contained the least amount of homoserine had an amino acid composition similar to the COOH-terminal octadecapeptide from γ_1 chains. Further evidence suggesting that the heavy chain of protein KN had a COOH-terminus similar to γ_1 chains was obtained by digestion of heavy chains with carboxypeptidases A and Y. No amino acids were released with carboxypeptidase A as in the γ_1 chain control. In contrast, carboxypeptidase Y released amino acids in quantities and quality similar to amino acids released from a myeloma γ_1 chain used as control (Table II).

Antigenic Analysis. The isolated serum IgG fraction containing both 7S and 4.3S myeloma protein and the urinary 4.3S protein were analyzed by double gel diffusion with antisera to normal IgG having antibodies to determinants on both the Fab and Fc fragment and with antisera specific for the Fc fragment (Figure 7). When analyzed with an antiserum to the whole IgG molecule, the serum fraction showed a double precipitin line and the urine a single line, forming a line of identity with the major precipitin line of the serum fraction. Normal IgG formed a spur over the urinary protein which merged into the second weaker precipitin line of the serum fraction. The urinary protein formed a spur over free κ chain (Bence-Jones protein), indicating that it contained antigenic determinants in addition to the light chain determinants. In contrast, when antisera specific for the IgG Fc fragment were used, only a single precipitin line was seen with the serum fraction forming a line of identity with normal Fc fragments or IgG, but no precipitin line was obtained with the urinary protein. The urinary protein inhibited, however, the precipitin line formed between the serum fraction and normal IgG or Fc fragments from extending toward the antigen well, suggesting that some of the determinants on the Fc fragment were expressed on the half-molecule. When antisera specific to κ chains were used, a single line was seen with the serum and urinary myeloma protein fraction, both forming a line of identity with normal IgG or κ chain.

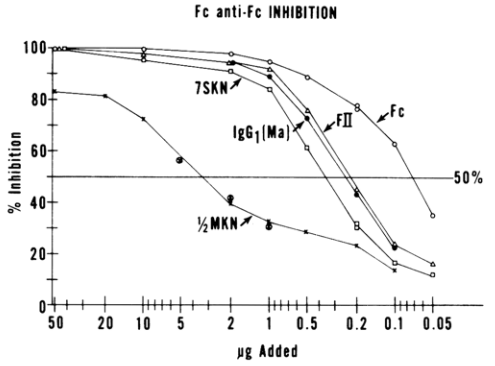

FIGURE 8: Quantitative inhibition of a Fc fragment antigen binding reaction, Fc fragment (Fc), by using 0.1 μg of protein nitrogen fragment, normal human IgG (FII), a myeloma IgG 1 protein (Ma), the isolated 7S myeloma protein KN (7S KN), and the 4.3S serum (O) and urinary (x) myeloma protein KN (1/2 M KN); abscissa, μg of protein nitrogen added.

Because of the absence of a precipitin line between the half-molecule and anti-Fc fragment antisera, the antigenic properties of the half-molecule were further investigated by quantitative inhibition analysis of a Fc anti-Fc fragment system (Figure 8), as compared to different 7S IgG preparations. More of the isolated 7S protein peak was necessary to inhibit 50% of the reaction than of normal IgG (purified Cohn Fraction II) or of an IgG 1 myeloma protein. The slope of the inhibition curve, however, was identical with that of other IgG preparations. In contrast, the half-molecule isolated either from the serum or from the urine showed an entirely different slope and 30–40 times more half-molecule was necessary to inhibit 50% of the reaction. Furthermore, even in relatively large antigen excess, the reaction was not completely inhibited. In order to determine the percentage of myeloma protein in the 7.0S serum IgG fraction, the quantity of ^{125}I-labeled protein that could be precipitated with a rabbit anti-idiotype antiserum was determined. Assuming that both the myeloma protein and the normal IgG in this preparation labeled equally well with ^{125}I, a maximum of 37% of the labeled protein could be precipitated with the anti-idiotype antiserum whereas an anti-Fab fragment antiserum precipitated 97–99% of this preparation. In contrast to the 7S myeloma protein preparation, the anti-idiotypic antiserum precipitated 92% of the labeled 4.3S half-molecule preparation.

Genetic Markers and Idiotypic Determinants. Antigenic markers characteristic for the three homology regions CH_1, CH_2, and CH_3 of the constant portion of γ chain (Natvig and Kunkel, 1973) were determined by Dr. H. G. Kunkel for both the isolated 7S protein peak and the urinary half-molecule. The marker Gm (f), localized on CH_1, was strongly positive for both proteins, and similarly a marker on the CH_2 region, IgG 1-3-4a, was positive on both proteins. In contrast, two markers on the CH_3 region, IgG 1-2-3 and Gm (non a) were absent on the half-molecule. The 7S protein peak was positive for these two markers.

The 7S myeloma protein shared the same idiotypic determinants with the half-molecule. When two rabbits and one monkey antisera to the half-molecule were made specific for idiotypic determinants by absorption with normal IgG,

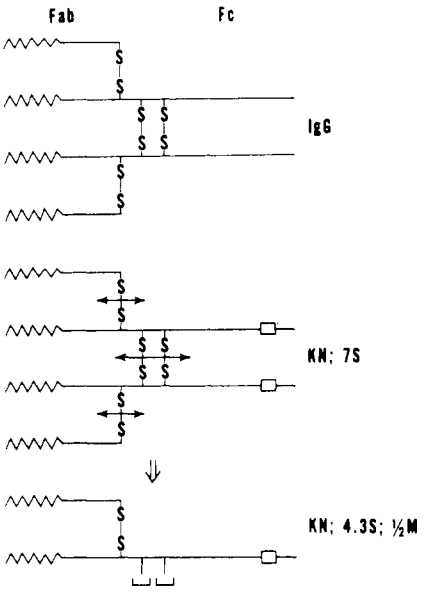

FIGURE 9: Schematic model of normal IgG and 7S and 4.3S myeloma proteins KN. The shaded areas represent the localization of noncovalent bonds between the polypeptide chains. After mild reduction of the interchain disulfide bonds, the 7S myeloma protein dissociated into 4.3S half-molecules. No free sulfhydryl groups were detected on the half-molecule isolated from the serum or urine. The open square in the γ chain symbolizes the probable location of the deletion in the γ chain.

they still formed precipitin lines with the half-molecule and 7S protein showing a line of identity and no reaction with normal IgG. The rabbit and the monkey antisera formed a stronger precipitin line with IgG 1 myeloma proteins than with proteins of the other subclasses and IgG 1 proteins formed spurs over IgG 3 and IgG 4 myeloma proteins but not IgG 2 proteins when analyzed with these antisera.

Discussion

The structural features of this rare type of human IgG myeloma protein forming half-molecules are summarized in the scheme of Figure 9. Most of the myeloma protein found in the serum and urine consisted of two chain half-molecules but the serum also contained 7S four chain molecules that were apparently produced by the same clone of tumor cells. The "hinge" region of the heavy chain that contained the half-cysteine residues forming the interchain disulfide bonds appeared to be normal in both the 7 S and half-molecule myeloma protein and similar if not identical with that of γ_1 chains. In contrast, the noncovalent interactions present between the Fc portions of the heavy chains were very weak or absent since the 7S protein dissociated into half-molecules after mild reduction and alkylation in aqueous solution, a condition that does not cause half-molecule formation in any known class or subclass of immunoglobulins. The heavy chain of the myeloma protein was found to have a significantly smaller molecular weight than that of normal γ chains. The apparent deletion was probably localized in the third homology region and was most likely the cause of the lack of noncovalent interactions and antigenic deficiency in the Fc fragment. We do not know whether the myeloma plasma cells synthesized four chain molecules that were reduced in vitro and dissociated into half-molecules or whether the lack of noncovalent bonds between the heavy chains impaired the four chain molecule synthesis. The latter appears to be more likely since the 7S protein was stable in vitro, and observations suggesting that immunoglobulins become partially reduced in vivo have not been reported. Obviously, if studies similar to those performed in vitro by Baumal et al. (1971) and other investigators could have been done on the synthesis of myeloma protein by KN's plasma cells, they would have helped to answer this question. The possibility existed that the 7S myeloma protein represented a different tumor cell clone than the 4.3S myeloma protein. However, this is very unlikely, since (1) the electrophoretic mobilities of the two proteins in starch gels were indistinguishable, (2) the four chain molecule dissociated into half-molecules, and (3) most importantly, the 7S and 4.3S proteins had the same idiotypic and genetic markers on the Fab fragment.

The deletion in the γ chain could not be localized in this study. However, it appears to be most likely that it was in the third homology region. The protein lacked the genetic markers of that region and was antigenically deficient in the Fc fragment and not Fab fragment. Structural analyses of a murine IgA half-molecule have demonstrated a large deletion at the COOH-terminus of the α chain and another deletion possibly in the inter-Fd-Fc region (Seki et al., 1968). In vitro studies of such tumor cell lines producing half-molecules revealed synthesis of small quantities of four chain IgA (Bevan, 1971), a phenomenon analogous to KN's myeloma protein. Although not emphasized by Seki et al. (1968), the IgA half-molecules probably also lacked the noncovalent interactions in the Fc fragment since no noncovalent four chain molecules were found in the serum and urine. In contrast to these murine IgA half-molecules, the myeloma KN did not appear to have a COOH-terminal deletion. The classical COOH-terminal octadecapeptide was recovered in relatively pure form following CNBr fragmentation of the γ_1 chains and the COOH-terminal amino acid analyses performed with carboxypeptidases A and Y further suggested that the COOH-terminus of the γ chain was probably intact. The total content of nine half-cysteine residues/45,000 dalton γ chain is significantly lower than the 11 residues characteristic for γ_1 chains and suggests that the deletion might have involved half-cysteine residues forming an intrachain disulfide bond, may be that localized in the CH_3 region. Besides the "hinge" peptides two relatively pure glycopeptides were obtained from peptide maps of the heavy chain. One resembled the tryptic glycopeptide of γ_1 chains localized in the CH_2 region (Edelman et al., 1969). The other was probably derived from the variable region of the heavy chain and was therefore unlikely related to the structural abnormality responsible for the half-molecule formation. Attachment of sugar to the variable regions of γ chains has also been observed in "normal" IgG myeloma proteins (Spiegelberg et al., 1970).

The half-molecule was antigenically deficient in the Fc fragment. In double gel diffusion experiments, the protein failed to form a precipitin line with anti-Fc fragment antisera and in a quantitative antigen binding system the half-molecule inhibited a Fc anti-Fc reaction poorly and incompletely. It appears most likely that a deletion in the Fc fragment was responsible for this lack of reaction. Most antigenic determinants characteristic for the Fc fragment are localized in the Fc′ fragment which represents mostly the CH_3 region (Natvig and Kunkel, 1973) and it has been shown that the genetic markers of this region are also missing in the half-molecule. The 7S protein preparations contained 60–70% of normal IgG and it was therefore not possible to determine if the 7S myeloma protein was also antigenically deficient. The fact that more of this preparation

was necessary to inhibit an Fc-anti-Fc binding reaction than normal IgG suggests that the 7S myeloma might have also been antigenically deficient.

The information available on the other known human myeloma half-molecules (Hobbs and Jacobs, 1969; Seligmann, 1973, and personal communications) is relatively scarce. The heavy and light chains were linked covalently in all cases. No evidence of a deletion was reported; however, the proteins were not studied as extensively as ours. The protein described by Seligmann et al. (1973) had genetic markers and hinge peptides similar to IgG 1 proteins and no COOH-terminal deletion. This same protein was also antigenically deficient in the Fc fragment. Hobbs and Jacobs (1969) reported that their patient's half-molecule precipitated with anti-γ chain antisera. This does not contrast with our findings since the antiserum employed by Hobbs and Jacobs (1969) was not absorbed with Fab fragments and therefore not specific for the Fc fragment.

Whether this myeloma protein is an abnormal protein formed only by plasma tumor cells or represents a monoclonal correlate of a normally occurring type of immunoglobulin is unknown. To our knowledge two chain antibodies have not been reported in man, but a careful search has not been made. In rabbits nonprecipitating anti-ovalbumin antibodies having a sedimentation rate of 4.3 S have been described by Sutherland and Campbell (1958) and it is possible that these antibodies represent half-molecules. Two chain immunoglobulin molecules have also been found in low concentrations in the serum of colostrum deprived piglets (Franek and Rika, 1964; Prokesova and Rejnek, 1973). Therefore, in the future, IgG half-molecules could possibly be found in normal human serum as rare members of the immunoglobulin family.

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