Molecular Size and Shape of the J Chain from Polymeric Immunoglobulins

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ABSTRACT: J chain was isolated from IgA polymers by two different methods and the purity of the preparations was established by both immunological and chemical criteria. In sedimentation analyses of these preparations, J was found to be an elongated molecule with a minimum axial ratio of 17.9, an $s_{20,w}$ of 1.28, a $D_{20,w}$ of 6.96, and a mol wt of 15,000 ± 500. These measurements showed that the mol wt of 25,000–27,000 obtained by polyacrylamide gel electrophoresis and gel filtration is incorrect. From analyses of the parameters involved in the electrophoretic method the anomalous behavior of J chain on gels could be explained by its elongated conformation. Using the correct molecular weight, the half-cystine content of J chain was found to be 7–8 residues/mol. This number makes it impossible for one J chain to be disulfide bonded to each heavy chain in the higher immunoglobulin polymers and leads to the suggestion of two alternative linkage models.

Recent studies have shown that polymeric immunoglobulins contain a third polypeptide, the J chain, in addition to the basic units of heavy and light chains (Halpern and Koshland, 1970; Mestecky *et al.*, 1971b). Considerable evidence has accumulated suggesting that J chain plays an important structural role in the assembly of these polymers. First, J is absent from all monomeric forms of immunoglobulin (Halpern and Koshland, 1970). Second, J has been found in the immunoglobulin polymers of essentially all chordates including one of the most primitive, the shark (Klaus *et al.*, 1971). Third, the same J is present in the various dimers, trimers, and tetramers characteristic of IgA and in the pentamers characteristic of IgM (Mestecky *et al.*, 1971b; Morrison and Koshland, 1972). Finally, J chain has an unusually high half-cystine content and is linked by disulfide bond(s) to the heavy chain constant regions in the respective IgA and IgM polymers (Meinke and Spiegelberg, 1971; Mestecky *et al.*, 1971a).

Two different mechanisms can be postulated to explain the role of J chain. J may provide the backbone to which monomer units are disulfide bonded, or, alternatively, J may bind to only one monomer unit and induce direct S–S bonding between the other monomer units. To help distinguish between these mechanisms, it was necessary to know the number of half-cystine residues per molecule of J chain and the number of J chains per polymer. The present paper describes the determination of the molecular weight of J and thus its half-cystine content, while the subsequent papers in this series will present the stoichiometric measurements.

The human J chain was originally estimated to have a mol wt of 27,000 based on its mobility on polyacrylamide gels and its elution volume on sizing columns (Halpern and Koshland, 1970). However, the validity of this estimate was questioned when subsequent studies showed that J was an elongated molecule (Morrison and Koshland, 1972) containing 7.6% carbohydrate (Tomana *et al.*, 1972). Furthermore, O'Daly and Cebra (1971) reported that isolated rabbit J chain had a mol wt of 15,000 in sedimentation equilibrium analysis. The aim of the present work was, therefore, to resolve these conflicting data by examining the human J chain in the ultracentrifuge and determining not only its molecular weight but also its other physical parameters.

Materials and Methods

Preparation of J Chain. 1. DIFFERENTIAL REDUCTION METHOD. Secretory IgA was isolated from normal human colostrum by the method of Tomasi and Bienenstock (1968). The heavy–light interchain bonds were cleaved by reducing the protein (15 mg/ml in 0.3 M Tris buffer, pH 8.2) with 2 mM dithiothreitol. The solution was stirred for 1 hr at room temperature and then made 5 mM in [^{3}H]iodoacetic acid. After 24-hr dialysis against 1 M acetic acid, the bulk of the light chain was removed by chromatography on a column of G-100 Sephadex equilibrated with 1 M acetic acid. The eluted heavy chain fractions were pooled, concentrated by Diaflo ultrafiltration with PM 10 membranes to 15 mg/ml, and dialyzed against 0.3 M Tris buffer, pH 8.2. The α–J bonds were then cleaved by reducing the heavy chain pool with 50 mM dithiothreitol and alkylating with 120 mM [^{3}H]iodoacetic acid under the conditions described above. The J chain was separated by repeat chromatography of the alkylated mixture on Sephadex G-100 equilibrated with 1 M acetic acid. For final purification, the J-containing fractions were passed through immunoabsorbent columns (Morrison and Koshland, 1972). Tritium counting was performed using a toluene base scintillation fluid containing 5% Bio-Solv BBS-3.

2. ELECTROPHORETIC METHOD. IgA myeloma polymers were isolated from human plasma by sodium sulfate precipitation and chromatography on Sephadex G-200 according to the method of Vyas and Fudenberg (1969). Ten-milliliter samples containing 20 mg/ml were reduced in 50 mM dithiothreitol and alkylated with a 2.4 molar excess of [^{3}H]iodoacetic acid. The free label was removed by filtration over Sephadex G-25 equilibrated with 0.01 M Tris buffer, pH 8.2. The protein was applied in 15 ml of 5 M urea to a 5.7-cm 4%

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polyacrylamide gel in a Buchler Polyprep 200 apparatus. The gel buffer was 0.375 M Tris-HCl, pH 8.79, the upper buffer was 0.0423 M Tris and 0.0464 M glycine, pH 8.88, the elution buffer was 0.120 M Tris-HCl, pH 8.23, and the lower buffer was 0.480 M Tris-HCl, pH 8.23. Electrophoresis was carried out at a constant current of 50 mA and the gel was cooled by a 0° constant temperature circulating water bath. The elution rate was 36 ml/hr and the effluent was monitored by absorbance at 278 nm and tritium counting. The J-containing fractions were pooled and further purified by passage through appropriate affinity columns (Morrison and Koshland, 1972).

Analyses of J-Chain Preparations. The amino acid compositions were determined on a Model 120c Beckman amino acid analyzer (Koshland *et al.*, 1966). Free amino-terminal groups were assayed by the dansylation method of Hartley (1970). Carbohydrate was determined by the periodic acid–Schiff staining technique of Zacharius *et al.* (1969) following electrophoresis of the J protein on acrylamide gels. The size homogeneity of the J preparations was analyzed by electrophoresis on 10% sodium dodecyl sulfate–polyacrylamide gels 7 cm in length run at 8 mA/gel (Shapiro *et al.*, 1967). Both the gels and the eluting buffer contained 0.1% sodium dodecyl sulfate and 0.1 M Tris, pH 8.0. The gels were either stained with 0.25% Coomassie Blue in 7% acetic acid and scanned in a Joyce-Loebl double beam microdensitometer, or frozen on Dry Ice, thawed slightly, and sliced into segments 1 mm in width. Before counting, the slices were incubated at 37° for 24 hr in 5 ml of toluene base scintillation fluid containing 10% NCS solubilizer.

Ultracentrifuge Analyses. All ultracentrifuge determinations were performed on a Spinco Model E ultracentrifuge equipped with monochromatic ultraviolet scanner and conventional optical systems. Before centrifugation the J-chain preparations were dialyzed exhaustively either in 0.5 M triethylammonium acetate buffer, 0.05 M Tris, and 0.001 M EDTA, pH 8.0, or 0.05 M Tris buffer, 0.5 M NaCl, and 0.001 M EDTA, pH 8.0. The viscosities of the buffers relative to water were determined using a capillary viscometer, and their densities relative to water using a pycnometer. For sedimentation equilibrium runs, double sector cells were used in the AnD or AnF rotors at a speed of 48,000 rpm and a temperature of 6°. Attainment of equilibrium was judged complete when no change was observed in the concentration distribution for a period of 3 hr. The concentration distribution throughout the cell was measured in units of optical density at 280 nm obtained directly from the uv scanner traces. In the velocity runs the sedimentation coefficient was determined using Schlieren optics to measure the distance of the boundary from the axis of rotation. The diffusion coefficient was calculated from the rate of spreading of the boundary formed in a capillary type synthetic boundary cell (Schachman, 1957). In the calculations of molecular weight from both the equilibrium and kinetic data, corrections were made for the density and viscosity of the buffers and the partial specific volume of the protein. A $\bar{v}$ of 0.710 was determined (Cohn and Edsall, 1943) from the amino acid and carbohydrate composition of completely reduced and alkylated J chain.

Results

Preparation and Purity of J Chain. The results obtained by two different methods of J chain isolation are illustrated in Figures 1 and 2. In both cases, the J-containing fractions were identified by measuring the [^{3}H]carboxymethylcysteine content of the eluates. Since the specific activity of the J chain in these experiments was at least eight times that of light chain and four times that of α chain, this assay provided a sensitive means for locating J.

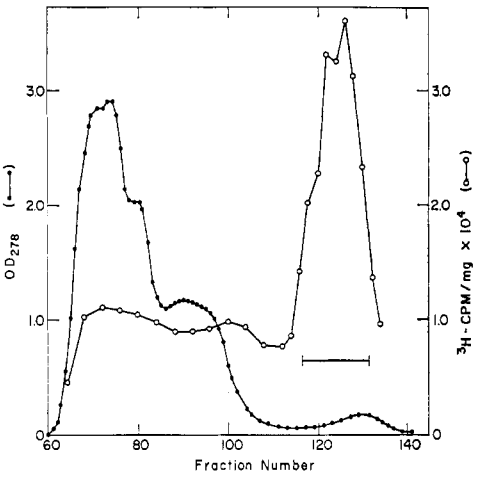

FIGURE 1: Separation of J and α chains on a 2.2 × 110 cm column of Sephadex G-100 equilibrated in 1 M acetic acid; fraction volume was 2 ml. The horizontal bar indicates pooled fractions.

In the first method, advantage was taken of a difference in lability between α–J and α–light bonds to isolate J on a sizing column with a minimum contamination by co-eluting light chain. The SIgA was first reduced and alkylated under very mild conditions which permitted selective cleavage of the heavy–light bonds. Over 90% of the light chain and less than 10% of the J chain were separated when the reduced mixture was chromatographed on a column of Sephadex G-100 equilibrated with 1 M acetic acid. The remaining heavy chain fraction was then reduced and alkylated under more stringent conditions required for quantitative cleavage of the α-J bonds. As shown in Figure 1, repeat chromatography on Sephadex G-100 yielded a small fraction containing 65% J chain, 30% light chain, and 5% material with α chain determinants. These contaminants were easily removed by successive passage of the mixture through immunoabsorbents, one prepared with equal quantities of anti-κ and anti-λ chain antibodies, and the other prepared with anti-α chain antibody. The final yield of

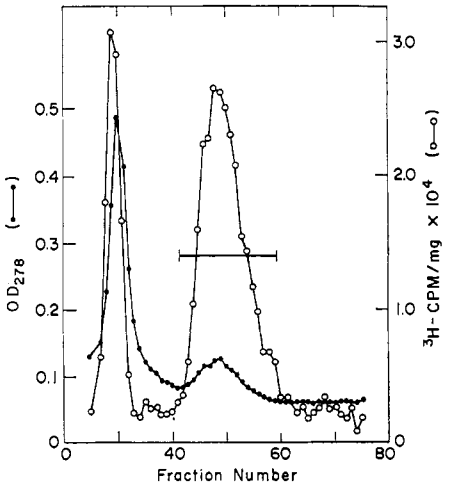

FIGURE 2: Separation of J chain from reduced and alkylated myeloma IgA by preparative polyacrylamide gel electrophoresis. Fractions of 4.5 ml were collected just prior to the elution of the tracking dye. The horizontal bar indicates pooled fractions.

TABLE I: Comparison of the Amino Acid Composition of α-Chain Contaminants with the Parent α Chain.

Amino Acid	Secretory IgA			Myeloma IgA		
	α Chain	α-Chain Contaminant[a]	Δ	α Chain	α-Chain Contaminant[a]	Δ
Lys	17.9	25.2	+7	17.4	23.1	+6
His	9.2	8.7		5.9	6.1	
Arg	19.1	19.8		17.7	16.9	
Asp	33.8	33.4		30.0	30.4	
Thr[b]	46.9	42.3	−6	48.7	42.2	−6
Ser[b]	48.4	58.6	+10	54.7	62.2	+8
Glu	43.7	49.1	+5	44.8	51.3	+6
Pro	40.2	32.6	−8	42.8	33.6	−9
Gly	35.6	31.4	−4	35.6	33.5	−2
Ala	32.0	29.5	−3	27.4	23.0	−4
Val	36.8	33.2	−4	36.7	32.9	−4
Met	3.8	2.4	−1	1.1	0.3	−1
Ile	7.6	10.6	+3	9.2	13.1	+4
Leu	45.7	39.7	−6	47.2	40.0	−7
Tyr[b]	14.8	17.4	+3	15.5	19.1	+4
Phe	14.9	16.6	+2	15.0	17.7	+3
Total residues	450	450		450	450	

[a] Expressed as residues/450 residues for comparison with parent α chain. [b] Corrected for loss during hydrolysis.

purified J chain was calculated to range from 1.5 to 2.0 mg/100 mg of SIgA, using extinction coefficients, $E_{278}^{1\%}$, of 7.0 for J and 15.0 for SIgA.

In the second method, advantage was taken of the high net negative charge of the J chain at alkaline pH. Myeloma or secretory IgA was reduced with 50 mM dithiothreitol, alkylated with 0.12 M [^{3}H]iodoacetic acid, and then electrophoresed on a preparative polyacrylamide gel buffered at pH 8.9. The J chain was found to be concentrated in a fraction eluting directly behind the dye marker and ahead of the light and α chains (Figure 2). However, analyses showed that this fraction also contained material with α-chain determinants (25% of the total fraction) and trace amounts of light chains. After purification by the appropriate affinity chromatography the yield of J chain averaged 1.4 mg/100 mg of starting material.

TABLE II: Amino Acid Composition of J-Chain Preparations.

Amino Acid	Residues/1000			Residues/mol
	Electrophoretic Method	2-Step Reduction Method	Standard Method[a]	
Lys	43.4	43.3	42.8	5.1
His	7.2	7.1	7.4	0.9
Arg	72.8	72.7	72.0	8.7
Asp	168.5	167.1	168.2	20.2
Thr[b]	93.8	95.4	96.1	11.6
Ser[b]	58.7	57.7	57.7	6.9
Glu	114.2	113.3	112.0	13.4
Pro	60.0	59.0	60.1	7.2
Gly	16.7	16.9	16.0	1.9
Ala	42.4	43.4	43.4	5.2
Val	75.7	75.5	75.6	9.1
Met	4.7	5.7	5.6	0.7
Ile	66.6	66.1	66.9	8.0
Leu	61.4	60.9	60.4	7.2
Tyr[b]	45.0	44.9	45.2	5.4
Phe	10.2	10.4	10.1	1.2
CM-Cys	59.0	59.5	60.2	7.2
Total residues	1000.2	999.3	999.7	119
Amino acid wt				13,790
Amino acid + CHO wt				14,920

[a] Average of analyses from seven different preparations (Morrison and Koshland, 1972). [b] Corrected for losses during hydrolysis.

The contaminants with α-chain reactivity were further characterized after elution from the immunoabsorbents. Amino acid analyses showed that their compositions were clearly distinct from those of intact α chains (Table I). At the same time, the composition of each contaminant reflected the characteristic amino acid content of the α chain from which it was derived, either the heterogeneous mixture from normal SIgA or the homogeneous myeloma heavy chain. Moreover, the fragments were found to bind light chain at physiological pH. On the basis of these data, the α-chain contaminants were identified as Fd fragments with sieving and charge properties similar to those of J chain. These results explain the observation that J-chain preparations often elicit anti-α chain as well as anti-J chain antibodies (Kehoe *et al.*, 1972; Morrison and Koshland, 1972). The residual α-chain fragment, present in too small amounts to be detected by the usual immunological methods, induced a measurable anti-α chain response.

The purity of the J-chain preparations was established by chemical as well as immunological criteria. Amino acid analyses showed that the preparations had compositions typical for pure J chain (Mestecky *et al.*, 1971a; Morrison and Koshland, 1972) with large amounts of arginine, aspartic acid, and isoleucine, and small amounts of serine, glycine, and phenylalanine (Table II). The contents of these amino acids are so unusual in J chain that as little as 5% of α-chain or light-chain contaminants could be recognized. For example, if the J prep-

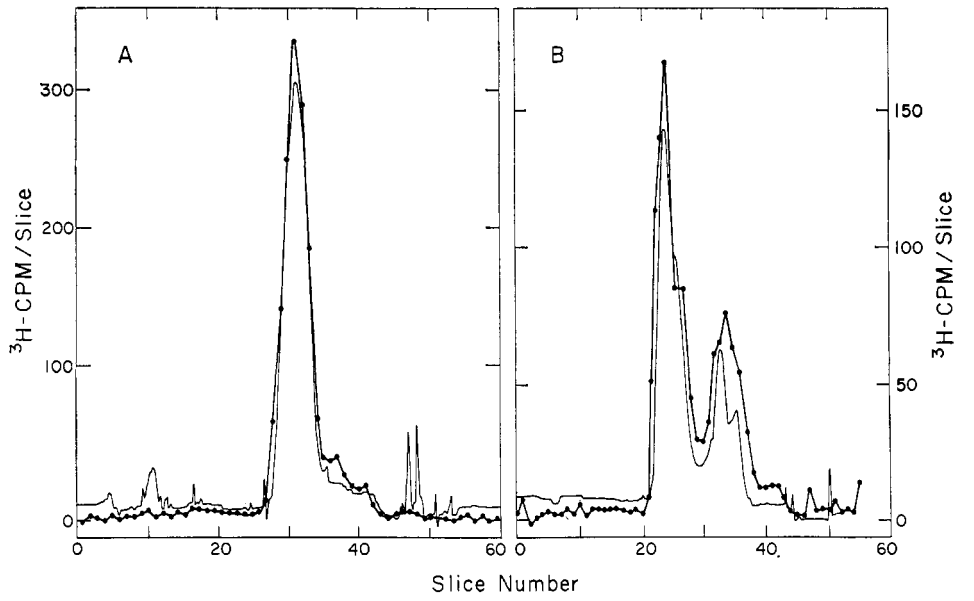

FIGURE 3: Electrophoresis of J chain on 10% sodium dodecyl sulfate–polyacrylamide gels. Samples were taken before (A) and after (B) equilibrium ultracentrifugation at 22°. Each of two duplicate gels was loaded with 25 μg of J chain. One gel was stained for protein (—) and one was sliced and counted (●–●).

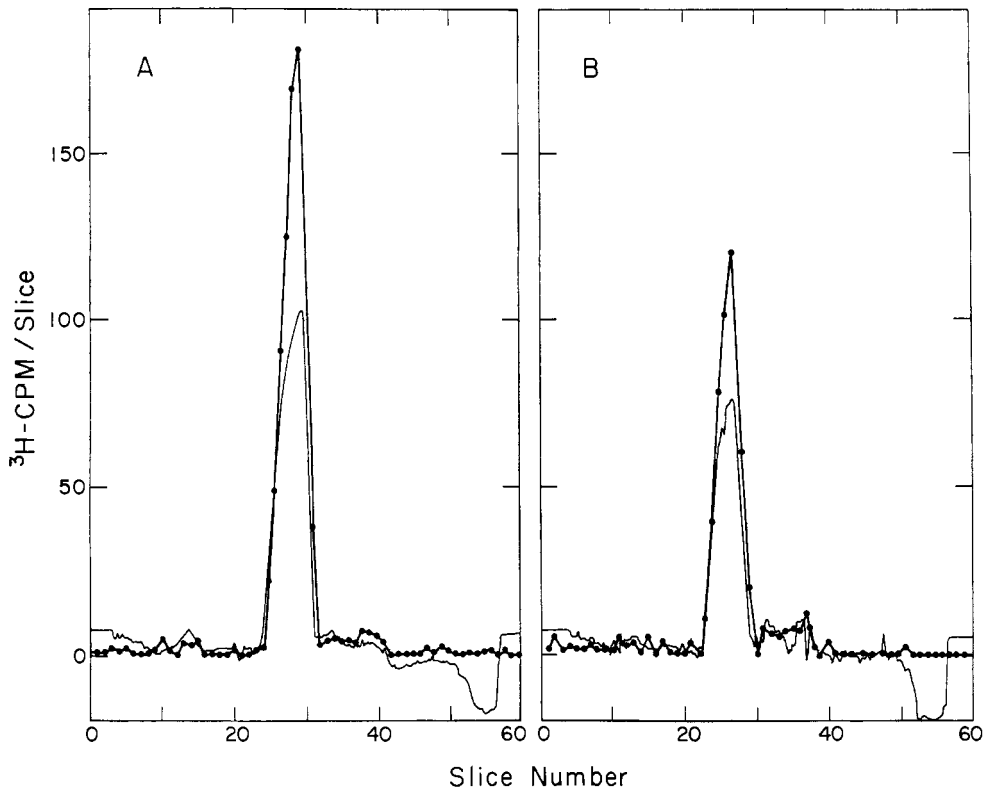

FIGURE 4: Electrophoresis of J chain on 10% sodium dodecyl sulfate–polyacrylamide gels. Samples were taken before (A) and after (B) equilibrium ultracentrifugation at 6°. Each of two duplicate gels was loaded with 25 μg of J chain. One gel was stained for protein (—) and one was sliced and counted (●–●).

arations contained 5% α-chain fragment or light chain, the glycine content of the mixture would be 19.5 residues/1000 instead of the 16.5/1000 observed.

Assays for free amino-terminal groups in the J preparations gave negative results. No dansylated derivatives, other than the expected ε-lysine and *O*-tyrosine, were detected even when the amount of J tested was 15 times that required for a strong positive and 500 times that required for detection. Since the amino-terminal residue of J chain has been identified as pyrrolidonecarboxylic acid (Meinke and Spiegelberg, 1972), these results provided further evidence for the absence of protein and peptide contaminants.

Analyses by sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed that the J chain migrated in a single major band. The patterns for duplicate gels of the J preparations, one sliced for determination of the [^{3}H]carboxymethylcysteine content and the other stained for protein, are given in Figures 3A and 4A. No small radioactive contaminants were

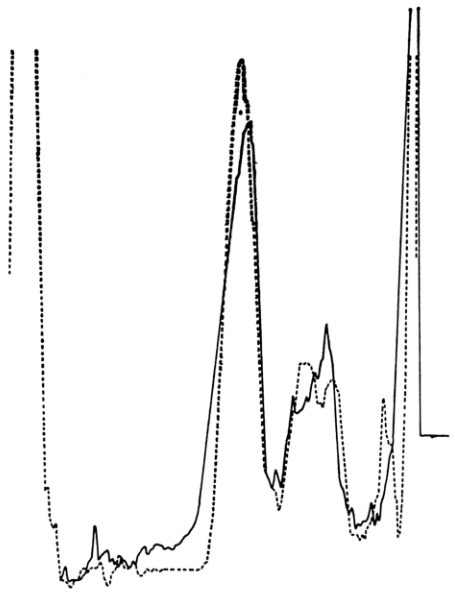

FIGURE 5: Electrophoresis of J chain on 10% sodium dodecyl sulfate–polyacrylamide gels. Duplicate gels were run; one gel was stained for protein (—) and one was stained for glycoprotein (- -).

detected on the counted gels nor unlabeled protein contaminants on the stained gels. The preparation of J isolated by electrophoresis (Figure 3A) had small diffuse bands migrating faster than the major peak. This minor fraction exhibited the same specific activity as the major band and thus was assumed to represent intact J chain with altered electrophoretic properties.

Molecular Weight Determinations. The mobility of J chain in sodium dodecyl sulfate–polyacrylamide gels was compared with that of a series of markers. The J chain, which was completely reduced and alkylated (Table II), migrated significantly behind mildly reduced and alkylated light chain, only slightly behind completely reduced and alkylated light chain, and at the same rate as native chymotrypsinogen. Since the molecular weights of light chain and chymotrypsinogen have been established as 23,500 and 25,500 daltons, respectively, these data indicated that the J chain was of a comparable size

Very different results were obtained when the J chain was examined in the ultracentrifuge. Sedimentation equilibrium analysis of the J preparation isolated by electrophoresis gave an average mol wt of 14,900 ± 300. However, the plot of the data for the lowest J concentration showed a small but significant curvature which indicated that the preparation was not completely homogeneous. To evaluate these results, it was necessary to determine whether the observed heterogeneity reflected a breakdown of J chain during the prolonged centrifugation. Since the method of sedimentation equilibrium has a bias toward the measurement of the smaller size components in a sample, small amounts of breakdown products could result in a falsely low value for the molecular weight.

The centrifuged samples were, therefore, analyzed by gel electrophoresis and the patterns were compared with those obtained before centrifugation. A considerable difference was observed (*cf.* Figures 3A and 3B). The proportion of faster moving material increased from 10% in the starting material to 35% in the centrifuged sample. Moreover, the faster moving material resolved into two fractions with mobilities corresponding to molecular weights of 18,300 and 15,600, respectively. These changes were not effected specifically by centrifugation since similar shifts in mobility were found to occur at

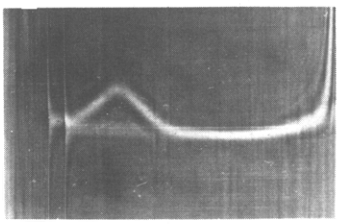

FIGURE 6: Schlieren pattern of J chain (1.2 mg/ml in 0.05 M Tris–0.5 M NaCl–0.001 M EDTA, pH 8), taken 164 min after reaching a speed of 68,000 rpm. The velocity sedimentation was carried out at 6° using an AnH rotor.

a slow rate in J preparations stored in the cold and to be accelerated by handling and exposure to room temperatures.

The fast moving components could not be explained as products of peptide-bond cleavage. No free amino-terminal residues were detected in the centrifuged samples. No corresponding small fragments were found by scanning the gels either for [^{3}H]carboxymethylcysteine-containing peptides or for unlabeled peptides large enough to be fixed and stained (Figure 3B). Finally, no significant amounts of amino acids were observed in the supernatant when the centrifuged samples were precipitated with alcohol, and the amino acid composition of the precipitate was identical with that of the starting material.

There was also no evidence for a change in the carbohydrate moiety of J chain. Duplicate samples of the centrifuged preparation were electrophoresed on polyacrylamide gels; one gel was stained for glycoprotein and the other for protein content. As the scans in Figure 5 show, the ratio of the two staining intensities remained constant in the three J bands.

On the basis of these data, the fast moving bands appeared to represent intact J molecules with altered charge and/or shape, and thus altered electrophoretic mobilities. Such changes would not be expected to have a significant effect on the measurement of molecular weight by sedimentation equilibrium. This interpretation was supported when the ultracentrifuge analysis was repeated on a preparation of J chain which was freshly isolated and carefully maintained at temperatures less than 10°. A value of 14,800 was again obtained for the molecular weight although the preparation contained no fast moving components either before or after centrifugation (Figures 4A and 4B).

The behavior of the J chain was also investigated by sedimentation velocity. The Schlieren pattern shown in Figure 6 was obtained after 164 min, the minimum time required for the J-chain boundary to clear the meniscus. Although the protein diffused considerably by this time, the peak appeared to be symmetrical. Furthermore, in Schlieren patterns taken at earlier times, no aggregated material could be detected. The $s_{20,w}$ calculated from the Schlieren patterns was 1.28×10^{-13} sec^{-1}.

To measure the molecular weight of J by an alternate method, the protein was centrifuged at low speed in a synthetic boundary cell and its diffusion coefficient was determined by plotting σ^2 against time. The slope of the line gave a $D_{20,w}$ value of 6.96×10^{-7} sec^{-1}. Using the observed sedimentation and diffusion coefficients, the mol wt of J was calculated to be 15,400 ± 1,000. This result was in good agreement with the value of 14,850 obtained from sedimentation equilibrium and provided additional evidence that the J preparations were homogeneous with respect to size.

Both the sedimentation and diffusion coefficients of J chain were low compared to those observed for globular proteins of

comparable molecular weight. The magnitude of the differences suggested that the shape of J deviated considerably from a sphere. To obtain an approximation of its relative dimensions, the axial ratio (a/b of a prolate ellipsoid) was calculated from the diffusion coefficient. Since the available data did not allow extrapolation to zero concentration, this calculation gave only the minimum ratio of length to width. The value of 17.9 obtained contrasted sharply with the ratios of 2.1, 4.3, and 3.0 reported (Tanford, 1967) for more typical globular proteins, ribonuclease, lysozyme, and chymotrypsinogen, respectively, and indicated that the reduced and alkylated J chain used in these measurements was an elongated molecule. Although it has not been possible to study J in its native state, indirect evidence has suggested that its conformation is not appreciably altered by reductive modification. For example, immunological analyses have shown that completely reduced and alkylated J and reoxidized and reformed J share most of the same antigenic determinants when tested against specific antisera (Morrison and Koshland, 1972). Thus, the physical parameters obtained in these experiments were considered to provide a reasonably valid description of the native molecule.

Discussion

In the studies reported here the human J chain, like its rabbit counterpart (O'Daly and Cebra, 1971), was found to have a mol wt of 15,000 by sedimentation analyses and mol wt of 25,000–27,000 by analyses in sodium dodecyl sulfate–polyacrylamide gels. Since the preparations used in these experiments contained no detectable smaller molecular weight breakdown products or larger molecular weight aggregates, the possibilities for errors in the sedimentation analyses were minimized. The value of 15,000 was, therefore, judged to be the correct molecular weight, and the problem remained to account for the large discrepancy in size observed in the electrophoretic method.

Other protein–sodium dodecyl sulfate complexes have been reported to move at anomalously slow rates in sodium dodecyl sulfate gels, and the deviation from the expected rates has been ascribed to (1) the presence of a carbohydrate moiety which increases the water of hydration and thus the effective size (Schubert, 1970), (2) intrinsic charges on the protein which inhibit the binding of sodium dodecyl sulfate micelles and thus reduce the overall negative charge on the protein–sodium dodecyl sulfate complex (Pitt-Rivers and Impiombato, 1968), and (3) unusual primary structures which resist transformation by sodium dodecyl sulfate into compact rodlike molecules (Reynolds and Tanford, 1970; Tung and Knight, 1972). In the case of the J chain, the carbohydrate does not appear to be a determining factor. In aged preparations, a fraction of the J molecules was found to migrate at a rate consistent with the correct molecular weight, and yet this fraction contained the normal complement of carbohydrate. Similarly, the high net negative charge on the J chain would not appear to be a significant factor. The migration of J in sodium dodecyl sulfate gels was unchanged whether its SH groups were alkylated with iodoacetamide which did not alter the charge or with iodoacetic acid which increased the negative charges by seven. Moreover, in gel filtration chromatography where charge effects are unimportant, the J chain eluted at a volume corresponding to a mol wt of 25,000–27,000. The most likely explanation for the anomalous electrophoretic behavior of the J chain is its conformation. Proteins with high axial ratios, for example, fibrinogen which has an axial ratio of 20 (Tanford, 1967), have been shown to migrate with slower than expected rates on sodium dodecyl sulfate gels (Andrews, 1970). In the sedimentation velocity measurements reported here, the J chain was found to be an elongated molecule with a minimum axial ratio of 18. This expanded structure would account for the larger apparent molecular weight obtained by both electrophoretic analysis and gel filtration. Furthermore, the shift to fast mobilities seen in aged and centrifuged preparations could be explained by the gradual refolding of the J chain into a more compact shape.

Once the correct molecular weight of the J chain was established, an estimate could be made of the efficiency of the isolation procedures. If the dimer secretory IgA is assumed to contain one molecule of J and one of secretory component, the J chain would comprise 3.6% of the complex on a weight basis. The actual yields of purified product ranged from 1.5 to 2.0 mg/100 mg of SIgA, 40–60% of the theoretical amount present. The estimation of yields from the myeloma IgA protein was more difficult because the preparations were found to be polydisperse in the ultracentrifuge and the J content of the higher polymers has not been ascertained. If a maximum value is assumed, *i.e.*, one J for every two monomer units, the myeloma IgA would contain 4.4% J by weight and the observed recoveries of 1.4 mg/100 mg of starting material would represent a minimum yield of 30%. In either case, the yields were sufficiently high so that the possibility of selecting a minor J population did not present a serious problem in interpreting the results of the sedimentation or electrophoretic analyses.

The determination of the correct molecular weight also permitted the data from amino acid analyses to be expressed as residues/mole (Table II). In general, the values obtained for stable residues present in limited quantities approached whole numbers, for example, 0.9 residue of histidine, 1.9 of glycine, 5.2 of alanine, 8.0 of isoleucine, and 7.2 of leucine. However, the phenylalanine content was consistently 1.2 residues/mol, even after 72 hr of hydrolysis when all phenylalanine peptide bonds should have been cleaved and any co-eluting glucosamine should have been degraded. Although the possibility of error in the phenylalanine determinations cannot be completely excluded until the sequence of J chain is completed, it is also possible that the fractional yield is real and reflects polymorphism in human J chain.

Finally, the determination of the correct size and half-cystine content of the J chain allowed an evaluation of its linkage in immunoglobulin polymers. Two types of structures have been postulated on the assumption that each polymer contains one J chain. In the "bracelet" model the J chain is extended around the polymer so that disulfide bonds can be formed between J and each heavy chain and the polymer is closed by an intra-J S–S bond. In the "clasp" model the J chain is linked only to two heavy chains in adjacent monomer units, its remaining half-cystines forming intra-J bonds, and the polymer is completed by direct S–S bonding between monomer units. These models are illustrated in Figure 7, using pentamer IgM as the most restrictive case.

It is not possible to distinguish between the models from the size of J chain alone. The "bracelet" model is not ruled out by the length of J since its sequence of 119 amino acids can be extended to approximately 450 Å, a distance more than twice the circumference of the IgM molecule (Metzger, 1970) in the region where the intersubunit bonds have been located (Putnam *et al.*, 1972). However, the finding that the J chain has a mol wt of 15,000 rather than 27,000 and contains 7–8 half-cystines rather than 12–13 puts a severe limitation on the "bracelet" model. The number of half-cystine residues is not

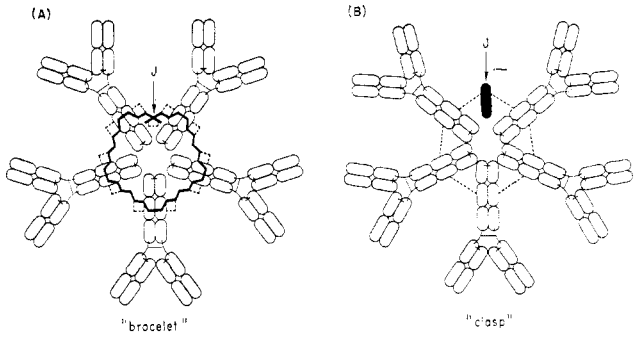

FIGURE 7: Schematic representation of pentameric IgM (after Metzger, 1970) showing postulated J-chain linkage. The dashed lines symbolize inter-monomer and J-monomer disulfide bonds.

sufficient to allow a bond to be formed with each heavy chain in pentamer IgM and tetramer IgA. The possibility that the J chain is linked to only one of the heavy chains in each monomer unit is unlikely because the remaining half-cystines on the alternate heavy chains cannot be arranged in intersubunit bonds which are compatible with the closed structure and the odd number of subunits in IgM. Thus, the "bracelet" model is feasible only if the higher polymers, pentamer IgM and tetramer IgA, contain two J chains, each linked by S–S bonds to alternate heavy chains in the polymer. Since the correct molecular weight of J chain has been established, stoichiometric measurements are now possible to obtain a definitive answer to the mode of J chain linkage.

Added in Proof

Since the submission of this report, Schrohenloher *et al.* (1973) have reported a molecular weight for a human J chain of 15,600 ± 200 obtained by equilibrium centrifugation in 5 M guanidine hydrochloride. This value is in close agreement with the value reported above.

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