The Molecular Weight of J Chains Derived from Human Immunoglobulin M

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J chain was isolated from sulphonated human immunoglobulin M molecules by electrophoresis on polyacrylamide gels. When determined by electrophoresis in sodium dodecyl sulphate–polyacrylamide gels, the molecular weight of the protein was about 27000. After suspension in 5M-guanidine hydrochloride solution for 21 days, two groups of three bands appeared on the gels. Most of the protein dissociated to components of molecular weight 15000. The molecular weight of purified J chain was also determined by ultracentrifugation. In borate–saline solution the average weight-average molecular weight was about 29000. The molecular weight slowly decreased upon prolonged exposure to guanidine hydrochloride, and after 14 days the minimum molecular weight was about 15000. Some association between chains still existed. These data suggest that J chain derived from the paraprotein exists in borate–saline solution as dimers held by strong non-covalent forces.

A polypeptide, termed J chain, recently was found to be a component of polymeric immunoglobulins. One J chain has been reported to be associated with dimeric secretory IgA‡ (Morrison & Koshland, 1972; Mestecky *et al.*, 1972) and with one IgM molecule (Mestecky *et al.*, 1971). In either case, the polypeptide is covalently attached to the immunoglobulins through disulphide bridges.

In most of their properties, such as electrophoretic mobility on polyacrylamide gels, peptide ‘maps’ and antigenic determinants, the J chains from secretory IgA and from IgM appear to be identical (Morrison & Koshland, 1972). The amino acid contents of J chains derived from human colostrum IgA, myeloma IgA, and IgM also are very similar if not identical (Morrison & Koshland, 1972). Assuming a molecular weight for the J chain of 24000, Morrison & Koshland (1972) reported a total of 12 half-cystine residues. J chains were released from IgA apparently after eight half-cystine residues were modified, but the modification of ten seemed to be required for release of the polypeptide from IgM.

The most striking characteristic of J chain about which the results are not consistent is its molecular weight. The reported values have covered the range from approximately 15000 (O’Daly &

Cebra, 1971; Schrohenloher *et al.*, 1973) to about 48000 (Kownatzki, 1971). The present paper indicates that J chains prepared from IgM may exist as dimers held together by non-covalent interactions that resist the dissociative effects of sodium dodecyl sulphate and guanidine hydrochloride.

Materials and Methods

Preparative methods

Preparation of IgM. Euglobulin IgM (λ type) was prepared from the plasma of a patient (Gra) afflicted with Waldenström’s macroglobulinemia (Morris & Inman, 1968). The protein was purified by passage through columns of Bio-Gel P-200. Purity of the IgM fraction was established by using immunoelectrophoresis and anti-(human serum) antiserum.

Preparation of J chains. IgM was treated with 0.25M- Na_2SO_3 as described by Edelman & Marchalonis (1967). The J-chain protein sometimes was isolated by electrophoresis of the sulphonated IgM on slabs of polyacrylamide gel (Ricardo & Inman, 1973). Alternatively, sulphonated IgM was subjected to polyacrylamide-gel electrophoresis and one gel was stained with Coomassie Blue (Ricardo & Inman, 1973). The stained gel was used as a marker for the J-chain position, and the corresponding regions were cut out of the unstained gels. The J chain was then extracted as described for isolation from slabs (Ricardo & Inman, 1973).

‡ Abbreviations: IgM, immunoglobulin M; IgA, immunoglobulin A. The symbols μ , λ and L denote the heavy chain, lambda light chain and light chain (lambda or kappa).

J chains also were prepared from IgM treated with 24mM-mercaptoethylamine for 30min at 30°C as described previously (Ricardo & Inman, 1973). J chains were isolated by polyacrylamide-gel electrophoresis.

General methods

Antigenic analysis of the purified J chains. The J-chain protein was analysed by immunoelectrophoresis or double diffusion in 0.85% agarose gels made with 0.1M-veronal, pH8.6. Anti-(J chain) antiserum was prepared as described before (Ricardo & Inman, 1973). Anti-(μ chain), anti-(λ chain) and anti-(human serum) antisera were commercial products.

Analytical ultracentrifugation. Sedimentation-velocity analyses were done with a Beckman Spinco model E analytical ultracentrifuge equipped with schlieren optics. An An-H rotor was operated at 60000rev./min and the temperature was maintained at 20°C. The proteins at 7mg/ml were suspended in borate-saline solution (0.16M-NaCl-1mM-sodium borate, pH8).

Determinations of molecular weights. An extinction coefficient $E_{280, 1\text{cm}}^{1\%} = 14.7$ was assumed for calculating the concentration of protein in preparations of J chain. Before determination of the molecular weight of the protein, most J-chain preparations were suspended in 5M-guanidine hydrochloride (Ultra Pure; Schwarz/Mann, Orangeburg, N.Y., U.S.A.) solutions for 2-28 days. The pH of the suspensions was 5.6, 6.1 or 8.0.

High-speed sedimentation-equilibrium measurements (Yphantis, 1964) were employed with an An-D rotor operated at 36000rev./min in the Beckman Spinco model E analytical ultracentrifuge at 20°C. The instrument was equipped with interference optics. The protein was in borate-saline solution, pH8, at a concentration of 0.26mg/ml. The column height was 3mm and interference patterns were photographed 24h after attaining the designated rotor speed. The values for molecular weight were obtained by using a computer program developed at the University of Georgia.

Low-speed sedimentation-equilibrium measurements were done with the ultracentrifuge equipped with a photoelectric scanner. The rotor speed was varied from 10000 to 24000rev./min, and the scan profile at 280nm was obtained 24h after reaching the indicated rotor speed. The protein concentration was varied from 0.170mg/ml to 0.311mg/ml and 0.1ml was used. The slopes of the plots of absorbance versus radial distance squared were determined by a least-squares treatment of the data.

Molecular weights were also determined by electrophoresis in polyacrylamide gels containing

sodium dodecyl sulphate. Basically, the method of Weber & Osborn (1969) was used. However, we used several modifications that gave better resolution and diminished non-specific binding of Coomassie Blue. The observed molecular weights were not changed by the modified procedure. The modifications were as follows: the 10cm clean glass tubes were dipped in 1% Kodak Photo-Flo for 1h and dried overnight at ambient temperature. An 8% (w/v) solution of polyacrylamide gel was used with 0.22% methylenebisacrylamide. The amount of ammonium persulphate used was 1.3ml of a solution containing 1.5mg/ml. The polymerized gels were subjected to a constant current of 3mA/tube for 4h before loading. The conducting buffer was that used by Weber & Osborn (1969) except that it was made 1M in urea (Ultra Pure; Schwarz/Mann).

The standard proteins of known molecular weight were pepsin (35000), carbonic anhydrase (29000), trypsin (23300) and lysozyme (14300). The amount of protein applied to each gel was 5-10 μ g in a volume of 30 μ l. Electrophoresis of these proteins or J-chain protein was done at 3mA/tube for 9h.

An attempt to determine if the J chains existed in a covalently associated polymer form was undertaken by suspending J chains (which had been isolated after sulphitolysis or reduction of IgM) in a solution of 5M-urea (Ultra Pure)-0.15M-sodium phosphate-1% sodium dodecyl sulphate at pH7 and then treating the suspension with 0.2M- β -mercaptoethanol for 2h at 37°C. The reaction was terminated with 0.40M-iodoacetamide. The protein was dialysed in the gel buffer of Weber & Osborn (1969) before electrophoresis.

Some J-chain preparations were dialysed for 21 days in 5M-guanidine hydrochloride solution, pH6.1. Before determination of molecular weight by electrophoresis, the protein was dialysed overnight in the buffer of Weber & Osborn (1969).

After electrophoresis the gels were stained for only 2h in a staining solution containing 0.1% Coomassie Blue. Destaining was done in 10% (w/v) acetic acid.

Partial specific volume. The partial specific volume ($\bar{v}$) of J chains was determined by the method of Edelstein & Schachman (1967). The protein samples were dialysed for 21 days in two changes of 5M-guanidine hydrochloride (Ultra Pure) solution made in water or in deuterium oxide (99.7%; Sigma Chemical Co., St. Louis, Mo., U.S.A.). The density of an aqueous solution of 5M-guanidine hydrochloride was determined pycnometrically to be 1.2166g/ml. Deuterium oxide solutions of 5M-guanidine hydrochloride had a density of 1.3216g/ml. The density of borate-saline solution was 1.006g/ml. The deuterium oxide solutions of 0.16M-NaCl had a density of 1.1050g/ml.

The apparent $\bar{v}$ also was calculated from the amino acid composition of J chain. For this purpose the carbohydrate content reported by Tomana *et al.* (1972) and the amino acid composition (M. J. Ricardo & F. P. Inman, unpublished work) were used. The $\bar{v}$ value was ascertained with the aid of a computer program developed at the University of Georgia by using the partial specific volumes of amino acids given by Cohn & Edsall (1943) and an average of 0.649 g/ml for the reciprocals of the densities of carbohydrates.

Results

Purity of J chains

J chains were separated from sulphonated IgM by electrophoresis on a block of polyacrylamide gel. In analysis by immunoelectrophoresis the purified protein gave a single precipitin line with anti-(J chain) antiserum. It failed to produce any precipitin line with anti-(whole human serum), anti-(μ chain), or anti-(λ chain) antisera. A similar analysis done by the Ouchterlony (1948) method showed that the only antigenic component detected was J-chain protein.

Preparations of J chains were also analysed by polyacrylamide-gel electrophoresis. A representative analysis is shown in Fig. 1. Only one band of stained protein was evident. Its rapid anodal migration is characteristic of J chain (Ricardo & Inman, 1973).

Fig. 1. Polyacrylamide-gel-electrophoretic analysis of J chain

The anode is at the bottom and the position of J chain is indicated by the letter J. Gel (a) was loaded with sulphonated IgM and (b) with purified J chain.

Our experience indicates that contamination with λ chain must be less than 1%.

In addition, a single symmetrical boundary was observed in sedimentation-velocity experiments.

Molecular weight determined by electrophoresis in sodium dodecyl sulphate-polyacrylamide gels

The apparent molecular weight of J chain determined from the plotted data shown in Fig. 2 was 27000. The protein migrated as a single component. Preparations of J chains which were reduced and alkylated in 5M-urea after their isolation also migrated as single components.

J chains that had been suspended in 5M-guanidine hydrochloride for 2 or 21 days were also analysed by this method. In the former case, only a single band was observed. After being in guanidine hydrochloride for 21 days, two groups of stained bands were observed. The three closely migrating bands of one group had relative mobilities slightly faster than carbonic anhydrase. Their apparent molecular weights were 28600, 26200 and 24000 (Fig. 2).

Fig. 2. Molecular weight of J chains

J chains were prepared from sulphonated IgM by preparative polyacrylamide-gel electrophoresis. The J-chain protein then was electrophoresed in polyacrylamide gels containing sodium dodecyl sulphate. Its positions, relative to the standard protein molecular-weight markers (A, pepsin, 35000; B, carbonic anhydrase, 29000; C, trypsin, 23300; D, lysozyme, 14300) are shown. Δ , Position of purified J chains; $\circ$, positions of components derived from J-chain protein exposed to 5M-guanidine hydrochloride for 21 days; $\bullet$, position of protein molecular-weight standards, as labelled.

Additionally, three faster-migrating bands comprising most of the J-chain material had mobilities in the lysozyme region. Their apparent molecular weights were 15400, 14900 and 14300 (Fig. 2).

Partial specific volume

The partial specific volume of J-chain protein determined by centrifugation in borate-saline solution (Edelstein & Schachman, 1967) was 0.719 ml/g.

The partial specific volume was also determined by centrifugation in a deuterium oxide solution of guanidine hydrochloride (Figs. 3a and 3b). At a rotor

speed of 16130 rev./min $\bar{v}$ was 0.711 ml/mg; at 20060 rev./min it was 0.708 ml/g, giving an average of 0.710 ml/g. The $\bar{v}$ calculated from the amino acid composition of J chains was 0.714 ml/g.

Molecular weight determined by ultracentrifugation

The apparent molecular weight of J chain in borate-saline solution was ascertained at both low and high rotor speeds. At 10000 rev./min the average weight-average molecular weight was 29800 (Table 1; Expt. 1). However, the data indicated that the material was not homogeneous as some aggregation was detected. When the centrifuge was operated at a higher speed the average weight-average molecular weight was about 28500 (Table 1; Expt. 2) and some aggregation was still evident.

The molecular weights of several preparations of J chain in guanidine hydrochloride solution were determined at rotor speeds of 10000 rev./min or higher. For protein suspended either 2 or 7 days in the dissociating agent, the trend was for the molecular weight to decrease as the rotor speed was increased (Table 1; Expts. 3-4). This reflects heterogeneity in particle size. As the speed increases there is preferential sedimentation of the particles of higher molecular weight. Thus at higher rotor speeds the calculated molecular weight will increasingly reflect that of the smaller entities (Table 1; Expts. 3-7).

An example of a sedimentation-equilibrium analysis of J chain is given in Fig. 3. The heterogeneity of the preparation is shown by the upward curvature of the plots. The minimum molecular weight of the protein in Fig. 3(a) was 15100 and that in Fig. 3(b) was 15700 (Table 1; Expts. 5-6).

The lowest values for the average weight-average molecular weight were calculated for samples in guanidine hydrochloride solution for 21 days or longer (Table 1; Expts. 6-7). The mean of these weights was 17400. In contrast, the lowest minimum molecular weights were first found in samples suspended in guanidine hydrochloride solution for 14 days (Table 1; Expt. 4). The mean of the lowest average minimum molecular weights (Table 1; Expts. 4-7) was 15200. For protein in guanidine hydrochloride solution from 14-28 days, the calculated lowest minimum molecular weights did not deviate significantly from the mean at rotor speeds of 14125-24350 rev./min. The pH of the guanidine hydrochloride solution within the range 5.6-8.0 had no effect on the molecular weight. The possibility that disulphide interchange could account for the changes in molecular weight is remote. Sulphitolysis was done in the presence of copper which converts both sulphur atoms of the disulphide into S-sulphocysteine, an entity which does not readily undergo the reverse reaction in the absence of a mercaptan.

Fig. 3. Determination of partial specific volume of J chain

The partial specific volume of purified J chain was measured in aqueous (●) and in deuterium oxide (○) solutions of guanidine hydrochloride (5M). Rotor speed was 16130 rev./min (a) or 20060 rev./min (b). Calculations were made at points up to $r^2 = 50.35 \text{ cm}^2$. The protein was suspended in guanidine hydrochloride for 14 days at pH 8 (a) or 21 days at pH 5.6 (b) before analysis.

Table 1. *Molecular weights of J chains as determined by ultracentrifugation*

J chains prepared from sulphonated human IgM were suspended for the periods indicated below in borate-saline solution (Expts. 1 and 2) or in 5M-guanidine hydrochloride (Expts. 3-7). All molecular-weight determinations of a given experiment were done with the same J-chain preparation and at the same rotor speed.

Experiment no.	Speed (rev./min)	pH of solvent	Period in solvent (days)	Minimum mol. wt.	Average weight-average mol. wt.
1	10000	8.0	2		29800
2	36000	8.0	2		28500
3	10000	6.1	2	27300	30300
		6.1	7	22500	26700
4	14125	6.1	2	25000	28300
		6.1	7	18400	22600
		6.1	14	15400	21300
5	16130	5.6	2	23100	26500
		5.6	14	14800	19900
		8.0	14	15100	19900
6	20060	5.6	2	22200	24600
		5.6	21	15700	17300
		8.0	2	21300	24000
		8.0	21	15100	17600
7	24350	5.6	21	15000	17200
		7.0	28	15300	17200

Discussion

The molecular weight of J chains from IgA and IgM have been determined by several methods. O'Daly & Cebra (1971) reduced rabbit colostral IgA and isolated the F component (apparently analogous to J chain) by gel filtration and ion-exchange chromatography. The molecular weight of F component was 22500 when measured by sodium dodecylsulphate-polyacrylamide-gel electrophoresis. However, when determined in the ultracentrifuge the number-average and weight-average molecular weights were about 15000 whereas the Z-average molecular weight was about 21000 in 5M-guanidine hydrochloride.

Recently, Schrohenloher *et al.* (1973) isolated J chains from human IgA myeloma protein. The average molecular weight of the polypeptide suspended in 5M-guanidine hydrochloride solution was 15600, in good agreement with the lower molecular weights obtained by O'Daly & Cebra (1971).

Halpern & Koshland (1970) completely reduced and alkylated rabbit and human colostral IgA. They found that the J-chain fraction eluted with light chains when the reduced IgA was filtered through Sephadex G-200 equilibrated with sodium dodecyl sulphate. A molecular weight of 23000 was estimated by this method. Subsequently a weight of approximately 24500 was reported (Morrison & Koshland, 1972).

Mestecky *et al.* (1971) obtained J chains from human secretory IgA and Waldenström's IgM after

oxidative sulphytolysis. From polyacrylamide-gel electrophoresis in sodium dodecyl sulphate a molecular weight of 26000 was estimated.

By using electrophoresis in sodium dodecyl sulphate-polyacrylamide gel we determined the molecular weight of J chain extracted from sulphonated IgM to be 27000. This value apparently was not affected by subsequent exposure of the isolated J chain to a mercaptan in urea. If J chain was allowed to remain in 5M-guanidine hydrochloride for 21 days most of the protein dissociated into a group of three rapidly and closely migrating components with apparent molecular weights of 14300-15400. The appearance of multiple bands might indicate microheterogeneity among J chains or proteolysis caused by some undefined enzyme or conditions.

Accurate determination of molecular weight by the electrophoretic method depends, in part, on the total disruption of disulphide bonds. Ricardo & Inman (1973) reported that 14mM-Na₂SO₃ would completely convert IgM (Gra) into IgMs and free J chain. In the present report, the concentration of Na₂SO₃ was increased about 18-fold over that required to free all J chain and the reaction was allowed to progress for 18h instead of 30min. Under these conditions the IgMs dissociated during polyacrylamide-gel electrophoresis into μ and L chains. Although we did not determine the magnitude of disulphide cleavage by sulphytolysis, one would expect it to be extensive.

The disruptive effect on disulphide bonds was

measured, however, on J chains isolated from IgM (Gra) previously reduced with 24mM-mercaptoethylamine and alkylated as described (Ricardo & Inman, 1973). This concentration of the mercaptan is just sufficient to dissociate all IgM (Gra) to IgMs and free J chain. The isolated J chains had five carboxymethylcysteine residues per 15000 molecular weight (M. J. Ricardo & F. P. Inman, unpublished work). Morrison & Koshland (1972) treated human IgM with 100mM-2-mercaptoethanol and found this to be the mildest reducing condition giving a good yield of J chain. Their alkylated J chain contained ten amidocarboxymethylcysteine residues per 24000 molecular weight. When the concentration of 2-mercaptoethanol was doubled, there were 12 such residues on the J chain. By using our value of 15000 for the molecular weight, their recalculated data would show about six labelled half-cystines after minimum reduction and seven after a greater reduction. The discrepancies between the two sets of data have not been resolved. The point to be made with respect to the present paper, however, is that the smallest number of disulphide bonds apparently were modified when J chains were freed from the IgM (Gra) molecules with 24mM-mercaptoethylamine, with an additional intra-chain disulphide bridge perhaps remaining intact in the J chain.

Molecular weights were also measured by ultracentrifugation methods. When J chain was suspended in borate-saline solution the calculated molecular weights were about 29000. A slow progressive dissociation took place in 5M-guanidine hydrochloride solution, however, so that the mean minimum molecular weight was about 15000.

The molecular weights determined both by electrophoresis in sodium dodecyl sulphate-polyacrylamide gel and ultracentrifugation agree. Although the J chains are not dissociated initially, the fact that we obtained the same average molecular weight by electrophoresis and centrifugation indicates that the error in molecular weight determined by the former method is small. This is consistent with the probability of extensive disulphide bond disruption in the J chains derived from IgM (Gra) by sulphitolysis. A simple explanation for the resistance to dissociation in sodium dodecyl sulphate or guanidine hydrochloride solution is not available. Nelson (1971) showed that some proteins, such as glucose oxidase, catalase, papain and pepsin, are resistant to denaturation by sodium dodecyl sulphate. It was reported recently that a soya-bean trypsin inhibitor is completely unfolded by 6M-guanidine hydrochloride only after 14 days of exposure (Fish & Leach, 1973). The J chain of IgM (Gra) apparently also falls into the category of proteins resistant to denaturation.

It seems clear from our results that the confusion in reported values for molecular weight is due to the

tendency for J chain to aggregate and to the very slow dissociation of the aggregate in sodium dodecyl sulphate and guanidine hydrochloride solutions. This produces a suspension of considerable heterogeneity in protein molecular weight. At faster rotor speeds the components of lowest molecular weight increasingly dominate the experimentally accessible region until they eventually may appear to be the only species present (Schrohenloher *et al.*, 1973).

The $\bar{v}$ of proteins in guanidine hydrochloride solutions was reported to be less than that calculated from their amino acid composition (Montgomery *et al.*, 1969). The $\bar{v}$ for J chain in 5M-guanidine hydrochloride was 0.710ml/g or 0.004ml/g less than the value determined from the composition. O'Daly & Cebra (1971) used 0.703ml/g and Schrohenloher *et al.* (1973) used 0.705ml/g. Recalculation of our molecular weight with their values for $\bar{v}$ would result in a 4-6% decrease.

These data indicate that J chain we prepared from human paraprotein IgM (Gra) exists as dimers when suspended in borate-saline solution. As the dimers resist dissociation in polyacrylamide gels containing sodium dodecyl sulphate and as more than 2 days in 5M-guanidine hydrochloride solution is necessary for dissociation to approach completion the union is resistant to these denaturants. The gradual dissociation of the dimers effected by guanidine hydrochloride probably is due to disruption of non-covalent interactions rather than cleavage of peptide bonds. We have not presented firm evidence to support this, but the fact that no peptides migrate electrophoretically between those three of molecular weight about 26000 and those of about 15000 lends support to this belief.

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