# VARIABLE J-CHAIN CONTENT IN HUMAN IgM

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## 1. Introduction

Human IgM consists of 10 heavy '$\mu$' chains and 10 light-chains interlinked by disulfide bridges and organized in 5 subunits (IgM's) each containing 2 '$\mu$'- and 2 light-chains. An additional polypeptide fragment, designated J-chain, has been found associated with polymeric immunoglobulins IgA and IgM [1, 2]. The structure of J-chain appears to be unrelated either to immunoglobulin 'homology' regions or to the 'secretory piece' which is associated with secretory IgA [3–7]. The present study was undertaken to examine if the stoichiometry of the J-chain in IgM was as rigid as that found for the heavy- and light-chains in these polymers.

## 2. Materials and methods

Monoclonal IgM globulins were isolated, from sera of six patients with Waldenstrom's macroglobulinemia, following previously published methods [8, 9]. The proteins were pure as judged by immunoelectrophoresis using protein concentrations of 20 mg/ml and a potent rabbit anti-human serum antiserum and all had K-type light chains. As judged by analytical ultracentrifugation the pentameric IgM's were substantially freed ($\leqslant$7%) of aggregates ('superpolymers') by chromatography on agarose (Sepharose 6B, Pharmacia). Fractions from the ascending portion of the absorbance peak were enriched in superpolymers, generally containing 40–45% of such material.

Freshly prepared IgM (10 mg/ml) was reduced at room temperature with 0.005 M dithiothreitol in a 0.2 M Tris–HCl buffer pH 8.6. After 1 hr, the pH was adjusted to 8.0 and the sulfhydryls alkylated with a 10% equivalent excess of [$^{14}$C] iodoacetic acid (0.91 Ci/mole) [10]. After 1 hr, 1 ml of the solution was applied to a 90 × 1.5 cm column of Sephadex G-100 equilibrated with 0.2 M ammonium bicarbonate. In the experiments described here the same column was used throughout. Fractions of 1.5 ml were collected, the absorbance at 280 nm determined and 0.250 ml removed for radioactivity measurement. Radioactivity counting was performed in 10 ml Soluene (Packard) scintillation fluid using a Packard Model 2420 Tricarb counter. The counting efficiency was 90% and each sample was counted at a constant 2% standard error.

In some experiments the appropriate fractions were pooled and the relative protein content determined using the Folin–Lowry method [11].

Polyacrylamide gel electrophoresis in 8 M urea at pH 9 was performed according to Anker's modification [12] of Reisfeld and Small's procedure [13] in order to obtain complete solubilization of the gel in 10 ml of Soluene (R) for radioactivity counting. Fifty microliter samples, containing 50–100 mg protein, were applied to duplicate gels. One gel was stained with Coomassie brilliant blue and the other was frozen, cut into 2 mm slices, and the slices counted.

Calculations were made as follows. For the gel filtration experiments, in which two major peaks were observed, all fractions under the first peak, including the fraction at the minimum between the peaks, were

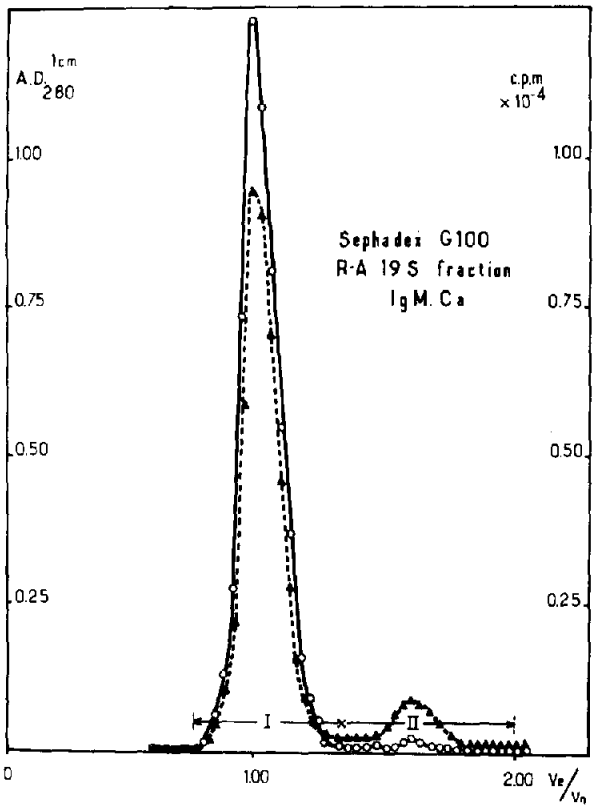


Fig. 1. Typical gel filtration pattern of reduced–alkylated IgM Ca: a) $A_{280}^{1\,cm}$ pattern (o-o-o); b) $^{14}$C-radioactivity pattern (-▲--▲-▲).

assigned to the first component. Subsequent fractions were assigned to the second component until background counts were again reached. The counts under each peak were expressed as a percentage of the total under both peaks. The percentage of absorbance at 750 nm as determined by the Folin–Lowry method was similarly calcultaed. The radioactivity in the respective bands observed by polyacrylamide electrophoresis was also calculated as a percentage of total recovered counts.

## 3. Results

Fig. 1 shows a typical Sephadex elution pattern of the reduced IgM preparations. Peak I, representing IgM's was eluted at the void volume ($Vo$) while the ratio of the elution volume ($Ve$) to $Vo$ was 1.3–1.5 for peak II, representing J-chain. The radioactivity and 280 nm absorbance were superimposed in peak I. The absorbance under peak II was too low to yield a reproducible pattern. Some IgM samples eg. Du and Col showed minor supplementary radioactive peaks between the major components. The counts in these fractions were assigned to peak II for purposes of calculation. On acrylamide electrophoresis a partially resolved slowly migrating complex was observed which was consistent with the expected positions for $\mu$- and light-chains. A rapidly migrating (anodic) component (often clearly consisting of 3–4 closely apposed bands) was consistent with the expected position of J-chain. The patterns observed with all the IgM preparations were very similar, though a few showed faintly stained additional bands of intermediate mobility. When material from Sephadex peak I was freeze dried, re-reduced with 0.02 M dithiothreitol, alkylated, and resubmitted to gel electrophoresis, no further J-chain bands were observed. Sephadex peak II material showed predominantly J-chain material with only a small contamination of light-chain components. The minor intermediate components seen on the Sephadex elution patterns of the IgM from Du and Col (above) on gel electrophoresis showed mainly J-chain components and minor amounts of material of $\mu$- and light-chain mobility.

The quantitative aspects of these results are collected in table 1. The following findings are noteworthy: 1) While the results for any one IgM were adequately reproducible the 6 different IgM proteins showed a spectrum of J-chain contents; 2) the variability observed by the gel filtration method closely paralled that observed by polyacrylamide gel electrophoresis; 3) the variability seen on the basis of the radioactivity measurements was paralleled by the results of the Folin–Lowry determinations; 4) the variability observed in the monomer, 19 S, IgM preparations was also seen in the superpolymer-enriched preparations and was quantitatively similar.

Before discussing these results a few other findings should be mentioned. When intact (unreduced) IgM preparations were examined by gel electrophoresis no J-chain material was observed when a 100 $\mu$g of protein per gel was applied. When, however, 500 $\mu$g of protein was used (fig. 2) free J-chain material was observed with proteins Lau and Col (high J-chain content) but not with Esp and Du (Low J-chain content). When the radioactivity per units absorbance of

Table 1
Summary of quantitative data on J-chain yields obtained by reduction–alkylation of 6 monoclonal IgM globulins.

| IgM globulin | '19 S' fraction | | | 'Superpolymer' fraction |
|---|---|---|---|---|
| | Sephadex peak II % $^{14}$C counts of total | PAG electrophoresis % $^{14}$C counts of total in J-chain bands | Sephadex peak II Folin–Lowry % O.D.U. of total | Sephadex peak II % $^{14}$C counts of total |
| Esp | 8.4 | – | 2.3 | 8.3 |
| | 8.5 | – | 2.5 | 8.0 |
| Du | 12.0 | – | 3.4 | 12.0 |
| | 11.6* | 10.2* | 3.6* | – |
| | 11.7* | 9.6* | – | – |
| | 13.6* | 13.4* | 4.2* | – |
| | 12.0* | 10.4* | 3.5* | – |
| Mar | 14.0 | – | – | 18.0 |
| | 14.0 | – | – | – |
| Ca | 15.0 | – | 4.5 | 19.0 |
| Lau | 17.0 | 24.9 | – | 20.0 |
| Col | 17.0 | – | 5.8 | 22.0 |
| | 17.0 | – | 5.9 | 22.0 |

* Obtained on the same freshly prepared sample.

peak I was translated into moles per mole IgM's, the expected value (≃10) was observed. J-chain material from protein Mar was purified and amino acid analyses performed. The composition agreed extremely well with the values given for J-chain by Morrison and Koshland [14].

## 4. Discussion

To interprete our results in molecular terms we need to know the number of easily reduced disulfides per mole of IgM and J-chain. While there is general agreement that IgM (free of J-chain) has 25 easily reduced disulfides per molecule [10, 15], the number per J-chain remains somewhat uncertain. Initial molecular weight estimates (based on gel filtration and/or gel electrophoresis [1] appear to have been grossly in error. Equilibrium sedimentation determinations now suggest a molecular weight of about 15 000 [3, 16] rather than the previous estimate of 24 000. The number of sulfhydryls released per mole J-chain would then be between 6 and 7. The percentage of the total SH in J after mild reduction of IgM would therefore be approximately $[6/(50+6)] \times 100 \simeq 11\%$. With the possible exception of IgM Esp, our results are therefore consistent with there being at least 1 molecule of J associated with each molecule of IgM.

What then accounts for the variability we have observed? It is unlikely that it is due to technical problems since individual proteins, even when isolated at different times and alternatively from fresh or stored serum, gave highly reproducible results. Inadequate reduction is unlikely since: a) the number of SH per mole IgM was similar and as expected in all cases examined carefully; b) re-reduction failed to increase the yield of J; c) even the initial reduction conditions should have been adequate to completely reduce all the interchain disulfide bridges [10, 15]. Unusual radioactivity (SH) ratios might occur if the structure of J-chain was variable. This again is unlikely since the electrophoretic and gel filtration mobilities of the J-chain showed no systematic or significant variations. Since the IgM's subunits in peak I have '$\mu$'- and light-chains held together by non-covalent bonds only, it is not surprising that a small amount of chain dissoci-

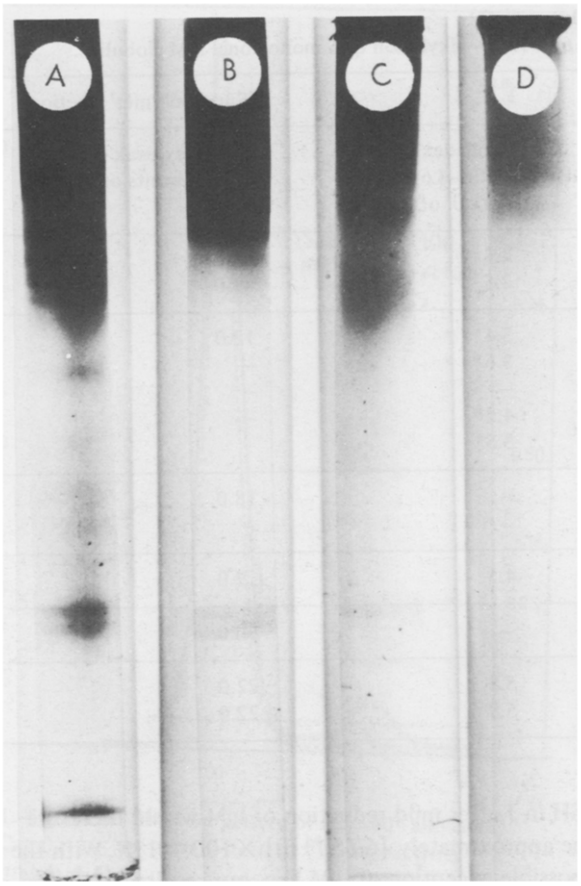


Fig. 2. Polyacrylamide gel electrophoresis in 8 M alkaline urea of: A, reduced alkylated γ M Col; B, native γ M Col; C, reduced alkylated γ M Esp; D, native γ M Esp. 500 μg protein were applied on each gel.

ation was observed. However, it can be readily calculated that the sulfhydryl content of these chains is much too low to have accounted for our findings. Finally, that similar variations in the relative yield of J-chains were observed by a completely independent type of analysis (Folin–Lowry), supports the idea that the variations are real.

Two of the IgM proteins with the highest apparent J-chain content showed some release of J-chain material even in the absence of added reducing agent while the two proteins with the lowest J-chain content did not. We are uncertain whether such non-covalently bound J-chain quantitatively accounts for the variations observed. Possibly the 'extra' J-chains are attached alternatively by disulfide bond or non-covalent interactions. It is noteworthy that non-covalent binding of J-chain to IgM and colostral IgA have been described recently [17, 18]. It is also well known [15] that a variety of proteins or other materials [19, 20] may attach thenselves to IgM either by covalent or non-covalent bonds.

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