Characterization of a Large Fragment Produced by Proteolysis of Human Immunoglobulin M with Papain*

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SUMMARY

The controlled digestion of human immunoglobulin M (IgM) (Patient Dau.) with papain in the presence of 0.002 M mercaptoethylamine resulted in the release of a basic subunit-like fragment which we designated IgM,. It resembled the subunit (IgM) produced by mild reduction of IgM in the following ways. The molecular weight (186,000) and sedimentation coefficient (6.2 to 6.5 S) of IgM, were similar to those of IgM. IgM, contained light chains and a large portion of each of its µ chains. IgM, was antigenically identical with IgM and IgM when tested with specific anti-IgM antiserum. The mobilities of IgM, and IgM in agarose seemed to be virtually identical. In contrast, IgM, did not possess the free sulfhydryl groups which were found on IgM,. It was concluded that IgM, was formed from IgM by proteolysis of the µ chains near the carboxyl terminus. The fragment retained intact its interchain disulfide bonds.

Extensive investigations of the structure of immunoglobulins have led to the belief that from an architectural point of view all classes of immunoglobulins are remarkably similar (1). These proteins are biologically important because they include both antibody and non-antibody immunoglobulins.

The basic structure of the three major immunoglobulins, IgG, IgA, and IgM, apparently incorporates units of four polypeptide chains held together by disulfide bonds. The structural architecture of IgA and IgM is modeled after that of the most prevalent immunoglobulin, IgG, in which each of two light polypeptide chains is bound to one of two, but not to the same, heavy polypeptide chain (1). In all cases, only one disulfide bond links a light chain to a heavy chain. In addition, the two heavy chains of rabbit IgG are bound covalently by a single disulfide bond (3–5), whereas in human IgG two disulfide bonds may be present (6).

It has been shown that IgM comprises five similar subunits, designated IgM (7, 8). Each heavy (µ) chain of a subunit seemed to be involved with four interchain disulfide bonds, one of which linked the µ chain to a light chain (9). Evidence recently was presented which indicated that each subunit is bound through a single disulfide bond to another subunit on either side (10). On the basis of these data, there must be two intrasubunit, interchain disulfide bonds which connect the two µ chains. Although this previously was postulated (11), it was not without question.

Onoue, Kishimoto, and Yamamura (12) subjected IgM and IgM, to digestion with papain. The subunit was degraded to fragments which resembled Fragment Fab and possibly Fragment Fc of IgG. We were attempting to confirm the above work, and specifically wanted to obtain the fragments with their disulfide bonds intact. Early in the course of our experiments, it was found that a large fragment, possibly similar to IgM, was released from IgM if conditions were suitable. This report describes the production and characterization of this IgM-like fragment which we have designated IgM,.

EXPERIMENTAL PROCEDURE

Preparation of IgM—When necessary, lipoproteins were removed from the plasma of a patient (Dau.) suffering from Waldenström’s macroglobulinemia by raising the density to 1.2 by addition of saturated NaBr solution. The plasma then was centrifuged for 30 min at 48,000 × g and 22°C. The lipoprotein which floated was removed by filtering the plasma through a plug of glass wool. Excess NaBr was removed by dialysis.

Crude IgM was precipitated by dialyzing about 40 ml of plasma into 10 to 14 times its volume of distilled water. After centrifugation at about 12,000 × g, the globulin precipitated was dissolved in not more than 50 ml of 0.32 M NaCl solution containing 1 mM sodium borate, pH 8.0. The precipitation process was repeated.

To whom inquiries should be made.

1 IgA, IgG, and IgM, symbols for immunoglobulin recommended by a conference on human immunoglobulins sponsored by the World Health Organization (2).
three additional times, and the final precipitate was taken up in about 30 ml of Buffer SB, 0.32 M. The crude IgM then was filtered through Sephadex G-200 equilibrated with Buffer SB, 0.32 M, at a ratio of 1 mg of protein per ml of packed column.

**Extinction Coefficients**—All quantitative determinations of protein concentration were based on an ultraviolet light absorption of 280 \( \text{nm} \). We used \( E_{1\text{cm}}^{1\text{cm}} = 12 \) for IgM and IgM\(_{\text{a}}\) (7); it was assumed to apply also to IgM\(_{\text{p}}\). For the \( \mu \) chain, \( E_{1\text{cm}}^{1\text{cm}} - 13.7 \) (13) was utilized, and it was assumed to be valid for the \( \mu \) chain fragment from IgM\(_{\text{a}}\). The extinction coefficient \( E_{1\text{cm}}^{1\text{cm}} \) applied to calculations of light chains was 11.8 (13).

**Immunoelectrophoresis and Gel Diffusion**—Glass plates (11.5 × 5.7 cm) were flooded with about 10 ml of 0.85% agarose prepared in 0.1 M barbital, pH 8.2. The thickness of the gelled agarose was about 1.5 mm. Wells 1 mm in diameter and troughs 1 mm wide were cut with a gel punch. The wells were filled with the experimental samples by use of small bore glass tubing pulled out to a fine thread. For electrophoresis, the conducting buffer was 0.05 M barbital. Current was passed through the system for 90 min at 8 ma per plate. After electrophoresis, the gel in the precast troughs was removed, and the troughs were flooded with antisera either to human serum or human IgM. The antisera were obtained from Hyland Laboratories (Los Angeles, California).

Ouchterlony type gel diffusion analyses were done similarly. Glass plates (6.3 × 6.3 cm) were flooded with about 6 ml of the agarose, giving a gel thickness of about 1.5 mm. Wells 2 mm in diameter were punched from the gel, and were filled with the antigen or antiserum. Anti-\( \kappa \)- and anti-\( \lambda \)-specific antisera were the gift of Dr. William Mandy.

**Enzymatic Digestion of IgM (Patient Dau.)**—The IgM was dialyzed overnight against 0.1 M sodium phosphate buffer, pH 7. The protein was always subjected to proteolysis at 37° with 1% by weight of papain (twice crystallized; Worthington Biochemical Corporation, Freehold, New Jersey) in an atmosphere of nitrogen. Several preliminary experiments were performed to determine a concentration of reducing agent which would activate the enzyme but not in itself reduce the IgM as determined by the appearance of IgM\(_{\text{a}}\) in schlieren patterns. A concentration of 0.002 M mercaptoethanol was found to be satisfactory. The final digestion mixture was made 0.02 M with respect to disodium ethylenediaminetetraacetate.

The length of the digestion period is given under "Results." At the end of the digestion period, neutralized iodoacetic acid was carefully added to a 10-fold molar excess over the MEA. This terminated enzyme action and alkylated any substrate sulfhydryl groups. The alkylated mixture was allowed to stand at room temperature for at least 1 hour before dialysis or further treatment.

**Preparation of IgM**—The preparation and partial characterization of IgM, has been reported previously (10). IgM (Patient Dau.) was reduced with 0.015 M MEA for 30 min at 30° in an atmosphere of \( \text{N}_2 \). The reduction mixtures were alkylated with a 2-fold excess of iodoacetic acid. IgM\(_{\text{a}}\) was separated from unreduced IgM by gel filtration through Bio-Gel P-200 with Buffer SB, 0.32 M, as solvent.

**Determination of \( 
\text{S}_{0.5} \) and \( 
\text{S}_{0.2} \)**—The product of digestion of IgM (Patient Dau.) by papain was dialyzed against Buffer SB, 0.32 M. For determination of \( 
\text{S}_{0.5} \) and \( 
\text{S}_{0.2} \), the protein was centrifuged in the Beckman model E analytical ultracentrifuge at 60,000 rpm and 20°. \( 
\text{S}_{0.5} \) and \( 
\text{S}_{0.2} \) were corrected to \( 
\text{S}_{0.5} \) and \( 
\text{S}_{0.2} \) according to the system of Schachman (14).

**Determination of Molecular Weights**—The molecular weights of the proteins were determined by the procedure of Yphantis (15) with samples ranging from 0.010 to 0.600 mg of protein per ml. The proteins were dialyzed against Buffer SB, 0.32 M, prior to centrifugation. No corrections for possible concentration dependence were made. For calculation of molecular weights, we assumed \( 
\text{S}_{0.5} \) of the digestion product, IgM\(_{\text{a}}\), to be 0.74 which is that previously found for IgM\(_{\text{a}}\) (7), and 0.722 for IgM (Patient Dau.) (7). The molecular weight of rabbit IgG was calculated with 0.745 for \( 
\text{S}_{0.5} \) (16).

**Amino Acid Analysis**—Protein samples were hydrolyzed for 18 hours at 110° in 6 N HCl in evacuated, sealed glass tubes. The analyses were performed with a Beckman model 120C amino acid analyzer. Carboxymethylcysteine was determined by slightly overloading the long column, essentially according to the procedure of Palmer and Nisonoff (3).

**Separation of Heavy (\( \omega \)) and Light Chains**—Prior to reduction with 0.2 M 2-mercaptoethanol, IgM and IgM\(_{\text{a}}\) were dialyzed against 0.05 M Tris-0.5 M NaCl buffer, pH 8.0. After reduction for 1 hour at 30°, the protein sulfhydryl groups were alkylated with 0.4 M neutralized iodoacetic acid for 30 min at room temperature. The reduced-alkylated protein was dialyzed overnight against 1 M propionic acid and subsequently gel-filtered through a column (2.5 × 34 cm) of Bio-Gel P-150 which had been equilibrated with 1 M propionic acid. Fractions of about 2 ml were collected, and their protein content was determined by their optical density at 280 \( \text{nm} \).

**RESULTS**

**Effect of Digestion Time**—Small amounts of IgM (Patient Dau.) which had been freed of non-IgM contaminants by gel filtration were digested with papain in the presence of 0.002 M MEA. Controls were treated similarly, but either papain or MEA was omitted. The experimental and control samples were incubated simultaneously, and portions of the same preparations of papain, MEA, and other reagents were used in each sample. At the end of the designated time, the digestion mixtures were removed from the water bath and immediately alkylated with iodoacetic acid. The samples then were allowed to stand overnight in the refrigerator.

A part of each mixture was diluted with phosphate buffer to a concentration appropriate for analysis by ultracentrifugation. The results may be seen in Fig. 1. The untreated IgM (Fig. 1A) contained a small amount of a faster component which is assumed to be an aggregate of IgM (17, 18). When the protein was examined by immunoelectrophoresis, a single precipitin line formed with antiserum against human serum. Also, a single precipitin are developed when the IgM was diffused opposite specific anti-\( \kappa \) antiserum; no line developed when anti-\( \lambda \) antiserum was used. Therefore, we believe that the faster component is not a protein species different from IgM.

In samples subjected to 5 min of digestion, the small amount of component sedimenting faster than IgM had disappeared (Fig. 1B). After digestion for 5 min or longer, a more slowly sedimenting constituent appeared (Fig. 1B). The amount of this constituent was at its maximum after 30 min of digestion (Fig. 1D). Components with sedimentation rates intermediate between IgM and the slower constituent observed in Fig. 1B were never evident (Fig. 1, B to F).
FIG. 1. Schlieren patterns of control and papain-digested human IgM. The experimental mixtures (B to F) consisted of IgM (Patient Dan.), papain, 0.002 M MEA, and 0.62 M EDTA in 0.1 M sodium phosphate buffer, pH 7. The control mixtures (G to J) were prepared similarly, except that the indicated reagents were omitted. Sample A was the untreated IgM centrifuged in phosphate buffer. The time of incubation at 37°C ranged from 0 to 80 min as indicated. The reactions were terminated by addition of iodoacetic acid. A portion of each mixture was adjusted to 5 mg per ml with phosphate buffer and then centrifuged at 60,000 rpm. Schlieren patterns A and G to J were photographed 16 min after reaching speed, and patterns B to F were photographed 32 min after reaching speed.

Concomitant with extension of the period of digestion, the amount of IgM decreased and a third schlieren peak was obvious on centrifugation (Fig. 1, C to F). It was apparent that this very slowly sedimenting peak was being formed at the expense of the intermediate component first noted in Fig. 1B. The three peaks observed in the schlieren patterns of Fig. 1, C to F seemed to be formed sequentially. It appeared that the IgM first is degraded to the intermediate component first seen in Fig. 1B. It is this component that is most important to the present report, and it will be referred to as IgM\textsubscript{p}. With a longer period of digestion, IgM\textsubscript{p} evidently was broken into smaller material which constitutes the most slowly sedimenting peak first evident in Fig. 1C.

The controls in this experiment were particularly important as an aid in defining the mechanism by which IgM\textsubscript{p} was produced. Neither IgM\textsubscript{p} nor smaller degradation products were observed in schlieren patterns of IgM which had been incubated with papain in the absence of MEA (Fig. 1, G and H). Similarly, when IgM was incubated with MEA, but in the absence of papain, smaller products were not seen (Fig. 1, I and J). Evidently, papain was inactive in the absence of reducing agent. Since the concentration of MEA used was insufficient to reduce IgM to the subunit called IgM\textsubscript{s} (7), the use of the name IgM\textsubscript{p} for the first product of digestion seems justified at this stage.

Isolation of IgM\textsubscript{p}—Various amounts of IgM (Patient Dan.) were digested for 15 to 25 min, and then alkylated with iodoacetic acid. The alkylation period varied from 30 min at room temperature to overnight in the refrigerator. Some digests were applied to columns of Bio-Gel P-200 without prior dialysis, and others were first dialyzed against Buffer SB, 0.32 M. A typical elution pattern of a digestion mixture gel filtered without prior dialysis is shown in Fig. 2. The ratio of packed column volume to milligrams of protein applied usually was 3 or 4 to 1.

Undigested IgM comprised Fraction A. IgM\textsubscript{s} constituted Fraction B and it usually was eluted as a single symmetrical component, clearly separated from Fractions A and C. The material of Fraction C corresponded to the slowest sedimenting component seen in Fig. 1F. The nature of the material in this fraction currently is under investigation and will be reported in a separate paper. Fraction D comprised at least two components and also is being studied further; it probably consists of dialyzable material. A partial characterization of IgM\textsubscript{p} is given below.

Physicochemical Characteristics of IgM\textsubscript{p}—The material in the tube which constituted the major part of Fraction B (Fig. 2) was pooled and concentrated by ultrafiltration with a Diaflo Ultrafiltration Cell (Amicon Corporation, Cambridge, Massachusetts) equipped with a UM-1 membrane. The concentrated protein then was diluted for analysis by ultracentrifugation (Fig. 3). The schlieren pattern for this particular preparation of IgM\textsubscript{p}...
was typical, and the protein appeared homogeneous. IgM<sub>p</sub> had a sedimentation coefficient (s<sub>20, w</sub>) of 6.2 at 7 mg per ml and, by comparison, IgM<sub>s</sub> sediments at 6.2 S at a concentration of 6.2 mg per ml (10).

The molecular weights of IgM, IgM<sub>p</sub>, IgM<sub>s</sub>, and rabbit IgG were ascertained, and the averages of several determinations for each protein are given in Table I. Although the data from most experiments resulted in a straight line when plotted, an occasional sample exhibited heterogeneity. Only plots showing homogeneity were used for calculating the values reported in the table.

The molecular weight of IgM (Patient Dau.) was 1,080,000. It compared favorably with the values reported by others (7, 19, 20). The s<sub>20, w</sub> of this particular IgM was 17.4 S (10). IgM<sub>s</sub> had a weight of 186,000, and the value for IgM<sub>p</sub> was 177,000. The weights of IgM<sub>p</sub> and our IgM<sub>s</sub> were similar to that reported for IgM<sub>s</sub> (185,000) by Miller and Metzger (7). However, the values recorded by the two different groups may not be directly comparable, especially since our estimation for IgM was greater than that reported previously (890,000) (7). The molecular weight of IgG was determined as a control for our technique, and it lies within the currently acceptable range of values (7, 21).

On the basis of the similarity of their sedimentation coefficients and of their molecular weights, one may conclude that proteolysis of IgM with papain under the described conditions results in the formation of a large fragment, IgM<sub>p</sub>, which resembles in size the basic subunit, IgM<sub>s</sub>.

Immunological Analyses—IgM (Patient Dau.) was shown previously to be κ type (10). Further tests of IgM<sub>s</sub>, included analyses by double diffusion in agarose, and immunoelectrophoresis. When IgM and IgM<sub>s</sub> were diffused against anti-IgM antiserum in an Ouchterlony-type analysis, a single precipitin line formed for both proteins (Fig. 4A). The lines fused and no spurring was observed even after prolonged development. In another test, IgM<sub>p</sub> formed a line of identity with IgM<sub>s</sub> also. Evidently, the enzymatic production of IgM<sub>p</sub> did not result in the loss of the antigenic sites against which the anti-μ chain antibodies were directed.

Similarly, a single precipitin line was formed when the diffusing antiserum was against whole serum. This antiserum was known to contain antibodies against two different parts of the IgM under investigation. Spur formation was not noted.

IgM<sub>p</sub> was also examined by immunoelectrophoresis. In accordance with the Ouchterlony type analysis, only one precipitin line was observed for each protein preparation when anti-IgM antiserum was used to develop the plate (Fig. 4B). The mobility of the two proteins was very similar, and thus IgM<sub>p</sub> apparently has a net charge resembling that of its parent IgM at pH 8.2.

Upon immunoelectrophoresis with the polyvalent anti-human serum antiserum for development of the plate, a single precipitin line was formed with either IgM or IgM<sub>p</sub>. This clearly indicated that the IgM<sub>p</sub> preparation constituted a single species of protein.

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>Molecular weight (× 10&lt;sup&gt;6&lt;/sup&gt;)</th>
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<tbody>
<tr>
<td>IgM (Patient Dau.)</td>
<td>1,080 ± 90&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgM&lt;sub&gt;s&lt;/sub&gt;</td>
<td>186 ± 8</td>
</tr>
<tr>
<td>IgM&lt;sub&gt;p&lt;/sub&gt;</td>
<td>177 ± 6</td>
</tr>
<tr>
<td>IgG&lt;sup&gt;##&lt;/sup&gt;</td>
<td>157 ± 2</td>
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<sup>*</sup> Standard deviation.

<sup>##</sup> Rabbit IgG was determined as a control for technique.
IgM, IgM<sub>p</sub>, and IgM<sub>α</sub> were compared on the same immunoelectrophoresis plate (Fig. 4C). The mobilities of IgM<sub>p</sub> and IgM<sub>α</sub> appeared to be virtually identical, and except for having broader spreading arcs, their mobilities were very similar to that of IgM. Thus, the resemblance of IgM<sub>p</sub> to IgM<sub>α</sub> is reflected in the apparent similarity of their net charges.

**Determination of Carboxymethylcysteine**—Although it seemed that IgM<sub>p</sub> was released from IgM by proteolysis, the possibility remained that the combination of mercaptan and the sulhydryl-containing enzyme was sufficient to reduce the disulfide bonds linking the monomeric subunits. If this were the case, then we actually would be working with IgM<sub>p</sub>, and IgM<sub>α</sub> would be a misnomer. Since the enzyme reaction mixture was alkylated with iodoacetic acid, freed sulhydryl groups on IgM<sub>p</sub> could be determined by amino acid analysis for carboxymethylcysteine. The results ranged from negligible to about 0.1 carboxymethylcysteine group per molecule of IgM<sub>p</sub>. On the other hand, IgM<sub>p</sub> was shown to contain approximately two residues of carboxymethylcysteine per molecule (10). Therefore, the possibility that IgM<sub>p</sub> was produced by reduction of IgM was negated.

**Dissociation of IgM**—The experimental results already described strongly suggest that IgM<sub>p</sub> is very similar to IgM<sub>α</sub> and therefore should comprise light chains and almost complete μ chains. Furthermore, IgM<sub>p</sub> should have retained intact its interchain disulfide bonds. The following experiment was performed to confirm the latter of these suppositions.

A sample of IgM (Patient Dau.) was reduced with 0.2 M 2-mercaptoethanol and subsequently alkylated with iodoacetic acid. The reduced and alkylated IgM was dialyzed against 1 M propionic acid, and a small quantity (about 20 mg) was applied to a column of Bio-Gel P-150 equilibrated with 1 M propionic acid. The elution profile is shown in Fig. 5 (Reduced IgM) and is similar to that obtained by others who treated IgG (22, 23) or IgM (7, 24) similarly. The identity of the protein comprising each peak was established by gel diffusion with anti-IgM antiserum and anti-α antiserum. The first peak corresponded to μ chains and the second to light chains. The light chain material comprised 22 to 29% of the total protein recovered in the two peaks.

IgM<sub>p</sub> was dialyzed against 1 M propionic acid without subsequent reduction, and about 12 mg were applied to the same P-150 column. There was no peak (Fig. 5, IgM<sub>p</sub>) corresponding in position to the light chain peak of IgM. The interchain disulfide bond between the μ and light chains evidently remained intact in IgM<sub>p</sub>.

Another portion of the same IgM<sub>p</sub> preparation was reduced, alkylated, and dialyzed in 1 M propionic acid just as the IgM was treated. The reduced-alkylated IgM<sub>p</sub> (about 10 mg) then was filtered through the same column of P-150, and its profile also is shown in Fig. 5 (reduced IgM<sub>p</sub>). There are two peaks, and the second corresponded to the light chain peak from reduced IgM. The yield of light chains varied from 24 to 31% of the total recovered protein. This was additional proof that IgM<sub>p</sub> does contain light chains which are covalently bound to μ chains.

**DISCUSSION**

The determination of the structure of a complex macromolecule can best be done if one has as starting material the purified substance in which the original integrity is maintained. Macroglobulin can be obtained in this form, but it is too complex to study. By moderate reduction, the basic subunits of IgM, IgM<sub>α</sub>, can be released from the parent molecule. IgM<sub>α</sub> has been reported to comprise two μ chains and two light chains (7, 8, 24). On the basis of chemical determinations, one light chain is bound through a single disulfide bond to one μ chain (13, 24). Because each μ chain appears to be involved with four interchain disulfide bonds (9), one must account for the other three.

Onoue et al. (12) digested IgM and IgM<sub>α</sub> with papain in the
presence of 0.01 M cysteine. A fragment corresponding to Fragment Fcα was isolated, and one which possibly was Fragment Fcβ or a part of it was observed but could not be purified in quantities sufficient for study. Similar results were obtained by Ungar-Waron, Jatson, and Sela (25). In each case, the authors concluded that the Fragment Fcα region of IgM was much more susceptible to hydrolysis by papain than is Fragment Fcβ.

When rabbit IgM was digested with papain, the concentration of cysteine determined the extent of reduction of the inter-γ chain disulfide bond (26). Furthermore, the disulfide bond area was slowly removed from Fragment Fe as the length of digestion was extended. With this in mind, it was considered possible that the digestion of IgM with papain in the presence of a low quantity of reducing agent might result in less degradation of the Fragment Fcα region, which then possibly could be isolated and studied.

Morris and Inman (10) demonstrated that incubation of IgM with 0.004 M mercaptoethylamine resulted in very little liberation of IgMα. When IgG was digested with papain in the presence of 0.001 M cysteine, the enzyme still was activated, and Fragment Fe contained intact the inter-γ chain disulfide bond (26). Thus, in the present study we used a very low concentration of reducing agent (0.002 M mercaptoethylamine) to activate the enzyme. This amount of mercaptoan did not liberate IgMα from IgM (Patient Dau.), although it was sufficient to make the enzyme active (Fig. 1).

The first product to form because of the proteolytic process was similar to IgMα in size and is referred to as IgMα to distinguish it from the subunit liberated by mild reduction. Upon continued digestion, IgMα was fragmented into smaller parts which probably comprise Fragment Fabα and perhaps Fragment Fcα.

IgMα was isolated easily by filtration through Bio-Gel P-200, and strongly resembled IgMα in the following ways: (a) it had a sedimentation coefficient of 6.2 to 6.5 S which is concentrationdependent; (b) its molecular weight of about 186,000 was very close to that of IgMα (177,000 and Reference 7); (c) upon reduction, IgMα was found to comprise μ chains (or very large pieces of the μ chains) and light chains; (d) IgMα contained the specific antigenic sites on the μ chain which also were present on untreated IgM and on IgMα; (e) by immunoelectrophoresis, the net charge on IgMα appeared to be virtually identical with that on IgMα. One striking difference between IgMα and IgMα was that the latter possessed no free sulfhydryl groups. In contrast, IgMα produced by minimal reduction of IgM (Patient Dau.) contained two sulfhydryl groups (10), presumably one on or near the carboxyl terminus of each μ chain (27, 28).

The sedimentation coefficient of IgMα indicated the protein was similar in size to IgMα, which has a constant $s_{20,w}$ of 7.07 (7). The molecular weight of IgMα (186,000) was very close to that reported by Miller and Metzger for their IgMα (185,000) (7), and to our value of about 177,000. These values clearly supported the premise that the fragment is approximately the size of the subunit. Actually, IgMα should have had a molecular weight less than IgMα depending on the point of hydrolysis of the subunit by the enzyme. These data indicated that proteolysis occurs very near the carboxyl terminus of the μ chain.

The release from IgM of a fragment or subunit which comprises μ and light chains could occur either by proteolysis of the μ chains or reduction of intersubunit disulfide bonds, respectively. IgMα did not appear in the schlieren patterns (Fig. 1) when IgM was incubated with either papain or MFA alone. However, it might be argued that the combination of mercaptoan and enzyme was capable of reducing the intersubunit disulfide bonds. That this was not the mode of release of IgMα is indicated by the absence of carboxymethylcysteine on amino acid analysis of IgMα.

Consequently, the most logical explanation of the production of IgMα is that papain attacks each monomeric subunit in a single molecule of IgM on the μ chain just below the inter-monomer interchain disulfide bond.

Since fragments intermediate in size between IgM and IgMα were not observed, and since the hydrolysis of all the IgM did not necessarily occur, it would appear that proteolysis of a single IgM molecule is an all-or-none situation. There seems to be no reason, a priori, why proteolysis should occur this way. It has been suggested that the covalently bound subunits arrange themselves in a stable pentameric ring in intact IgM (9). This hypothesis seems to be supported by the electron micrographs of human and rabbit IgM taken by Svebag, Chesbro, and Holt (29). Perhaps when the closed IgM molecule initially is ruptured, the ellipsoidal structure falls open creating a situation in which the remaining subunits are particularly susceptible to hydrolysis.

The controlled digestion of IgM by papain seems to be a way of obtaining a basic subunit-like fragment which still maintains most of its original architecture. Since IgMα contains no free sulfhydryl groups, and because one disulfide bond covalently binds a light chain to a μ chain (13, 24), one has an excellent tool which can be used to determine conclusively that there are two inter-μ chain disulfide bonds in each subunit, IgMα. Because IgMα has never been reduced, it may be most useful for further investigations concerning the extra light chain reported recently to be present in the subunits of IgM (30).

Acknowledgments The plasma from which we isolated IgM was the gift of Dr. J. Claude Bennett. Mrs. Sara Clett performed the amino acid analyses, and Mr. Terry Spencer determined the molecular weights.

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Characterization of a Large Fragment Produced by Proteolysis of Human Immunoglobulin M with Papain
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