Proteolytic Degradation of IgD and Its Relation to Molecular Conformation*

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SUMMARY

“Spontaneous” degradation of IgD into Fc and Fab fragments frequently occurs during purification and storage. Because proteolysis is the likely mechanism for cleavage of IgD under these conditions, the susceptibility of IgD and other immunoglobulins to digestion with trypsin, plasmin, and papain was analyzed. IgD was much more susceptible to proteolysis than the other immunoglobulins studied. The fast fragments, Fc, obtained after spontaneous degradation and after proteolysis were immunologically identical and this was also true of the slow fragments, Fab. The sedimentation coefficients and Stokes radii of the intact protein and fragments were determined and the frictional coefficient ratios and molecular weights calculated. Both the intact IgD and the Fc fragment appear to be less compact than other intact immunoglobulins or the Fc fragment of IgG. We propose that this apparent difference in conformation may account in part for the increased susceptibility of IgD to proteolysis. Finally, attention is drawn to the potential contamination of IgD preparations with the fifth component of complement (C5).

IgD possesses two light and two heavy chains, yields Fab and Fc fragments on digestion with papain, has a high carbohydrate content (approximately 11 to 14%), and has a sedimentation coefficient of approximately 6 to 7 S. The molecular weight estimated on different myeloma proteins and by different techniques has varied from 160,000 to 200,000. IgD has two inter-heavy-light and a single inter-heavy-heavy chain disulfide bonds (2-7). Antigen-combining activity has been demonstrated for IgD (8, 9) but, as yet, no biological function attributable to the Fc portion of the molecule has been found.

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† The nomenclature for immunoglobulins is from Reference 1.

IgD has been relatively difficult to study because its serum concentration is low, about 30 µg per ml, because IgD myeloma proteins are infrequent, and especially because of a remarkable susceptibility to degradation which appears to be unique among immunoglobulins. In serum the protein may degrade to fragments similar to Fc and Fab almost completely in several days, even at 4°C. Several workers have mentioned this problem, and it has been tentatively attributed to proteolysis (5, 6, 10-12). During the course of efforts to purify IgD from myeloma serum, we observed that the protein was degraded into several fragments. In this study, we have compared the susceptibility of IgD and other immunoglobulins to cleavage by proteolytic enzymes and have demonstrated an apparent conformational difference among these proteins.

METHODS AND MATERIALS

Myeloma Proteins - Plasma from two patients with IgD multiple myeloma (L.11. and C.J.) were collected by plasmapheresis. Fibrinogen was removed by dialysis against 0.01 M CaCl2, 0.15 M NaCl at 4°C, and the serums were stored at -20°C. The IgD was obtained from sera by chromatography on DEAE-cellulose. IgM was obtained as a fibrinogen precipitate from serum of a patient with macroglobulinemia and further purified by zone electrophoresis. When these purified proteins at concentrations of approximately 10 mg per ml were analyzed by immunoelectrophoresis with potent antiserum to whole human serum and to the given immunoglobulin, they reacted strongly with homologous antisera and only IgA showed a trace contamination (with IgG). The IgA preparation also contained trace amounts of plasmin as shown by micro-Ouchterlony immunodiffusion analysis with antiserum to plasmin.

Chemicals—Papain twice recrystallized was obtained from both Mann and Worthington Laboratories. Plasmin in 50% glycerol and containing 10.1 caseinolytic units per ml was obtained as a gift from Dr. J. T. Sgouros of the Michigan State Laboratories, Lansing, Michigan. Trypsin (twice recrystallized, containing 50% MgSO4), chymotrypsinogen, cytochrome c, horse spleen ferritin twice crystallized, lysozyme thrice crystallized, and Coomassie brilliant blue stain were obtained from...
Mann Chemicals. Bovine serum albumin was obtained re-
crystallized from Miles Labs. Bovine liver catalase, crystalline,
was obtained from Sigma Chemical Company. DEAE-cellulose
was Whatman DE-32. Sephadex gels and blue dextran
were obtained from Pharmacia. ^125I was obtained
from Cambridge Nuclear Corporation.

Antiserums—Anti-IgD antiserum was prepared by subcu-
taneous immunization of a goat with IgD myeloma protein T.H.
followed by absorption with normal human serum as described
previously (9). This antiserum contained antibodies reactive
with IgD class specific determinants as well as idiotypic Fab
determinants. Other antiserums to immunoglobulins were pro-
duced by immunization of goats and rabbits with purified im-
munoglobulins from normal serum and were absorbed to render
them Fe specific. Anti whole human antiserum was obtained
from Hyland Labs. Anti-plasminogen-plasmin antiserum was
obtained from Behringwerke and was found to react with plasmin
and to give a single band in the α region on immunoelectro-
phoresis with normal human serum.

Purification—IgD was purified from serum by (a) dialysis
of serum for 18 hours against 0.02 M potassium phosphate
buffer, pH 5.4, containing 0.02 M EDTA and, and subsequent removal of
the euglobulin precipitate by centrifugation, (b) precipitation
of supernatant proteins in 50% ammonium sulfate, and subse-
quent desalting by Sephadex G-25 gel filtration, (c) DEAE-
chromatography with 0.02 M Tris-HCl buffer, pH 8.1, and elu-
tion of the protein with a linear NaCl gradient from 0 to 0.1 M
and 0.01 M NaCl. IgD fragments produced by enzyme digestion
were carried out at 4°C. At concentrations of approximately
10 mg per ml the IgD preparations thus obtained sedimented
as a single sharp peak in a Beckman model E ultracentrifuge,
showed only one band when they underwent electrophoresis
on cellulose acetate and were developed with Coomassie brilliant
blue, and showed no reaction on immunoelectrophoresis with
the use of antiserum to the whole human serum while reacting
strongly with antiserum to IgD. However, in several of the
preparations trace amounts of IgG and α-2-macroglobulin could
be demonstrated by Ouchterlony analysis.

IgD Fragments—Naturally occurring degradation fragments
and fragments produced by enzyme digestion were predominantly
of two species electrophoretically, one of fast and the other of
slow mobility, and were separated by preparative electrophoresis
in agarose gel or potato starch with the use of 0.05 M barbital
buffer, pH 8.6.

Enzyme Digestion—Papain was activated in 0.01 M cysteine
and 0.01 M EDTA. Digestion was carried out at 23-25°C with
5 to 10 mg per ml of IgD solutions in 0.01 M Tris-HCl buffer,
pH 8.0, containing 0.2 M NaCl. Papain concentrations were
adjusted to 0.12 to 0.17 unit per ml (1 to 2 μg of papain per mg
of IgD), and the reaction was allowed to proceed up to 15 min
before it was stopped with 1 mM p-chloromercuribenzoate or 2
mM iodoacetamide. Trypsin digestion was carried out at 23-25°C
with 5 to 6 mg of IgD per ml in 0.02 M Tris-HCl, pH 8.0, con-
taining 0.02 M NaCl and 0.01 M CaCl₂. Trypsin concentration
was 0.05 mg per ml (6 μg of trypsin per mg of IgD). The
reaction was allowed to proceed up to 1 hour. Plasmin diges-
tions were carried out at 37°C with 0.25 caseinolytic unit of enzyme
per ml and 5 to 10 mg of IgD per ml in 0.2 M NaCl buffered with
0.02 M Tris-HCl, pH 8.0. Reactions were allowed to proceed
up to several hours. Both trypsin and plasmin digestions were
stopped by quick freezing in dry ice and acetone.

Protein Quantitation—IgD concentrations were estimated
by ultraviolet absorption at 280 nm. An extinction coefficient,
ε₂₈₀ 16.2 was determined for IgD (L.H.) by micro-Kjeldahl
analysis, assuming 15% nitrogen content (6). This value is
higher than the value of 14.5 reported by Spiegelberg et al. (6);
no explanation for their difference is readily apparent

Gel Diffusion Techniques—Immunoelectrophoresis and micro-
Ouchterlony immunodiffusion were performed as described pre-
viously (13).

Electrophoresis—Analytical electrophoresis was performed on
cellulose acetate strips (Gelman Instrument Company, Ann
Arbor, Michigan) in a Beckman Microzone cell with the use of
0.05 M barbital buffer, pH 8.6, μ = 0.075 at 25°C for approximately
20 min at 250 volts. Strips were developed with Coomassie
brilliant blue stain as described by St. Groth (14).

Stokes Radius—Stokes radii were determined by gel filtration
at 6°C with Sephadex G-200 for the intact protein and Fe frag-
ments and G-100 for the Fab fragments. Column dimensions
were 2.5 × 90 to 95 cm. The gels were suspended in either 0.02
M Tris-HCl or 0.01 M sodium phosphate buffers, pH 8.0, con-
taining 0.2 M NaCl. Blue dextran, ferritin, catalase, and bovine
serum albumin were used as markers on the G-200 column. Blue
dextran, bovine serum albumin, chymotrypsinogen, and cyto-
chrome c served as markers on the G-100 column. Stokes radii
of the markers were taken from published values (15) except for
catalase, 50.1 Å, which was calculated from more recently re-
obtained physical data (10) and ferritin which was experimen-
tally determined (vide infra). Test proteins were labeled with
^125I using the chloramine-T method as described by Greenwood
et al. (17). Each marker (3 to 8 mg) and approximately 0.1 μg
of labeled test protein (specific activity 8 to 25 mCi per mg)
were combined in a total volume of 1.2 ml. One ml of this
mixture was applied to the bottom of the column and eluted by
upward flow. Flow rates were 8 to 10 ml per hour, and fractions
were collected by drop counting in volumes of 2.0 to 2.3 ml with
a variance of approximately 0.1 ml. Radioactivity was mea-
sured in a well type scintillation counter. The markers were
detected by their absorbance at 225 nm. The results were ana-
lyzed by the method of Ackers (18). Only the markers catalase
and bovine serum albumin were used in the calculations of the
Stokes radius for the intact IgD and the Fe fragment. With
these same markers the Stokes radius of ferritin was calculated
as 69.1 Å. IgG was studied unlabeled at the same concentra-
tion as the marker proteins. Because catalase and IgG have similar
elution positions, the Stokes radius of IgG was calculated with
the markers bovine serum albumin and ferritin, assigning the
experimentally determined value of 69.1 Å to the Stokes radius
of ferritin. This procedure was employed because the physical
parameters of ferritin are not entirely clear as discussed by Radola
et al. (19), and it assured that the values for the intact IgD and IgG
could be fairly compared because the markers, though they dif-
fered, established the same slope.

Sedimentation Coefficients—The sedimentation coefficients of
the fragments were determined in sucrose gradients by a modifi-
cation of the method of Martin and Ames as described by Ryan
et al. (20). Lysozyme and bovine serum albumin were used as
markers at concentrations of 10 mg per ml; 8.5%, of 1.75 and
3.90, respectively, were used for these standards in calculations
of s for the fragments (20). One-tenth ml containing 1 mg of
the marker protein and 0.3 to 0.4 μg of the 131-I-labeled test protein were layered on top of 4.9 ml of 5 to 20% sucrose gradients in 0.05 m Tris-HCl buffer, pH 7.5. The radiolabeled test proteins had specific activities of 40 to 60 mCi per mg.

Calculations—The molecular weights were calculated by the equation

\[ M = \frac{6πNρa}{(1 - \bar{V})} \]

where \( M \) is the molecular weight, \( N \) is Avogadro’s number, \( ρ \) is viscosity of medium, \( a \) is Stokes radius, \( s \) is sedimentation coefficients, \( \bar{V} \) is partial specific volume, and \( ρ \) is density of medium.

The frictional coefficient ratios (\( f/f_{\text{min}} \)) were calculated from the equation

\[ f/f_{\text{min}} = \frac{a}{3\bar{V}M} \left( \frac{4πN}{3} \right)^{1/3} \]

Alternatively \( f/f_{\text{min}} \) was calculated from the molecular weight and the sedimentation coefficient (21).

If the molecular weight derived from Equation 1 is combined with the sedimentation coefficient to calculate \( f/f_{\text{min}} \) by the second method (21), the only two experimental values in either method of calculating \( f/f_{\text{min}} \) are the Stokes radius and the sedimentation coefficient. This assumes that \( f/f_{\text{min}} \) is the same by the two methods of calculation. If a test of agreement is desired between \( f/f_{\text{min}} \) values obtained by the two different procedures, it is necessary to use an independently determined value for the molecular weight in the second method.

The reported value of 0.71 is used for the partial specific volume of the intact IgD (6). A value of 0.730 was assumed for the Fab fragments since it approximates the values of the light chain and the peptide portion of the heavy chain that can be calculated from amino acid analysis data on another IgD myeloma protein (6). A value of 0.690 was calculated for the Fe region from the relationship \( \bar{V} \) IgD = \( \frac{4}{3} \) \( \bar{V} \) Fab + \( \frac{4}{3} \) \( \bar{V} \) Fc, since the contributions of the various regions should be additive (22). However, in this computation the molecular weight of the Fe region is taken to be 94,000 (176,000 - 2 × 41,000) whereas the isolated Fe fragment only had a molecular weight of 58,000. We have had to assume, therefore, that the \( \bar{V} \) calculated for the Fe region approximates the true value of the \( \bar{V} \) for the Fe fragment.

RESULTS

Purification—The euglobulin precipitation step at pH 5.4, the only unique aspect of the purification scheme, was incorporation to separate a protein that was only partially removed by the other procedures. This contaminant was identified as the fifth component of complement (C5) by reaction in Ouchterlony analysis with specific anti-C5 antiserum (a gift from Dr. F. C. McDuffie) and subsequent removal by a procedure known to precipitate C5. Its presence was initially recognized by the observation that our unabsorbed anti-IgD antisera contained idiotypic specificities for the Fab fragment. The anode is to the left. The upper well contains largely intact IgD and the cathodic band extending from the major IgD arc is the first evidence of fragmentation. The middle well demonstrates intermediate and the bottom well complete “spontaneous” fragmentation.

Fig. 1. Analysis of intact and fragmented IgD (L.H.) by immunoelectrophoresis using unabsorbed anti-IgD antisera containing idiotypic specificities for the Fab fragment. The anode is to the left. The upper well contains largely intact IgD and the cathodic band extending from the major IgD arc is the first evidence of fragmentation. The middle well demonstrates intermediate and the bottom well complete “spontaneous” fragmentation.

Susceptibility to Proteolysis—In the above experiment, “spontaneous” degradation occurred readily in some IgD preparations, but very slowly in others. In these latter preparations, an inhibitor of plasmin, α-2-macroglobulin, was often detected. This observation, along with the finding of maximum degradation at pH 6 to 8, suggested the possibility of enzymatic proteolysis due to serum enzymes such as plasmin. Because this is not readily observed with other immunoglobulins similarly handled, it raised the possibility of an increased susceptibility of IgD to proteolysis. To test this, samples of IgA, IgM, the first two subgroups of IgG, and IgD were subjected to proteolysis by plasmin, papain, and trypsin. Digestions of the several immunoglobulin substrates by any one enzyme were carried out simultaneously with identical conditions and enzyme to substrate ratios. After digestion was initiated, samples were removed at intervals and the reactions stopped by the procedures noted under “Methods and Materials.” The samples from any one digestion were evaluated for extent of proteolysis by undergoing electrophoresis simultaneously on cellulose acetate. To evaluate the amount of degradation that occurred between thawing and electrophoresis of the samples (plasmin and trypsin digests), similar ratios of enzyme and immunoglobulin were mixed and immediately quick frozen. These were then thawed and underwent electrophoresis along with the other samples and are labeled “ice control” in the photographs of the strips. IgD was the most rapidly degraded of the proteins studied while IgG1 was the next most susceptible. IgG2 and IgM were relatively resistant to digestion, and IgA did not fragment under these conditions. The results of the experiments with IgD
FIG. 2. Digestion of IgD and IgG1 with three proteolytic enzymes. The samples, removed at the time intervals shown, were subjected to electrophoresis on cellulose acetate, the anode being to the left. a, plasmin; b, trypsin. The sample labeled control, ice indicates the amount of digestion that occurred between thawing the samples and electrophoresis. IgD was completely digested by trypsin during this step. c, papain. Two IgD myeloma proteins plus IgG1 are shown. The second band from the bottom is a control in which p-chloromercuribenzoate (PCMB) is added before papain. Detectable splitting of the IgG1 occurred in the 20-min sample.

FIG. 3. Immunodiffusion analysis of IgD fragments. A, by double diffusion (left): 1, top well is intact IgD. Other wells contain: 2, plasmin-produced Fe; 3, trypsin-produced Fe with trace Fab; 4, papain-produced Fe and Fab; 5, naturally occurring Fab with trace Fe; and 6, plasmin-produced Fab. The center well contains unabsorbed anti-IgD. Immunological identity is seen among all fragments possessing the same general mobility, i.e. fast or slow. Both slow and fast fragments give reactions of partial identity with intact IgD, but reactions of non-identity with each other. B, by immunoelectrophoresis of plasmin digested IgD (right). Anode is at the left. The upper trough was filled with Fe-specific anti-IgD, while the lower trough was filled with anti-IgD which was unabsorbed and contained antibodies to both Fe and idiotype Fab determinants.

and IgG1 are shown in Fig. 2. The digestion with plasmin was continued beyond that shown in Fig. 2a, and after five hours of digestion no intact IgD remained. IgG1 was intact at 14 hours but showed slight splitting when analyzed after 24 hours of digestion. IgD was rapidly degraded by trypsin as shown in Fig. 2b and after only 5 min no intact IgD remained. In the "control ice," IgD also degraded, presumably during the period between thawing and subsequent electrophoresis. With papain obvious splitting of IgD occurred after 1 min of digestion as shown in Fig. 2c. A second myeloma IgD protein (C.J.) also degraded rapidly during papain digestion, as shown in Fig. 2c.

Because it was possible that the immunoglobulin preparations contained unknown contaminants which could either inhibit or activate the enzymes used for digestion, a mixture of IgD and IgG1 was digested and compared to simultaneous digestions of the individual immunoglobulins. Analysis was made by visual comparison of the protein bands after electrophoresis on cellulose acetate and staining with Coomassie brilliant blue. With each of the three enzymes used the IgD-IgG1 mixture demonstrated the expected IgD fragmentation and no splitting of the IgG1.

Immunologic Comparison of Isolated Fragments—With further digestion most samples eventually yielded single fast and slow fragments, and this also occurred naturally. As shown in Fig. 3A, all of the slow fragments demonstrated reactions of identity in Ouchterlony analysis. Likewise, all of the fast fragments were immunologically identical. Both the slow and the fast fragments gave reactions of partial identity with the intact IgD but reactions of non-identity with each other. The fast fragments reacted in Ouchterlony analysis with antisera to Fe-IgD (a gift from Dr. John Fahey); the slow fragments did not react with this antisera. Both types of fragments reacted with unabsorbed antisera to IgD as shown in Fig. 3B. These findings suggest that the fragments, either enzyme produced or naturally occurring, represent Fe and Fab.

FIG. 4. Sephadex G-200 gel filtration of 125I-IgD and marker proteins. Solid line peaks represent blue dextran, ferritin, catalase, and bovine serum albumin from left to right. Broken line represents 125I-labeled IgD. The small trailing peak of radioactivity is thought to represent an early fragmentation product.
The $f/f_{\text{min}}$ values in parentheses under IgD L.H. and IgG were calculated from the sedimentation coefficients and independent molecular weights to afford a comparison of the value for this parameter when arrived at by two independent calculations (see discussion of calculations under "Methods and Materials"). In this case a molecular weight of 145,000 was used for IgG (23) and 172,000 for IgD. Stokes radii of IgD, IgD fragments, and pooled IgG were experimentally determined as described under "Methods and Materials". Where shown, $\bar{X} \pm S.E.$ refers to a minimum of three determinations and up to five in the case of $S$ for Fab and Fc from IgD. Stokes radii of IgM, IgE, and IgG fragments were calculated from the referenced data. Sedimentation coefficients of IgD fragments were determined as described under "Methods and Materials." Molecular weights of IgD, IgD fragments, and pooled IgG were calculated from Stokes radii and the sedimentation coefficients.

### Table I

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<th>Fab</th>
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<td>6.7$^c$</td>
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<td>1.47 (1.43)</td>
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<td>$f/f_{\text{min}}$</td>
<td>1.80 (1.78)</td>
<td>1.47 (1.43)</td>
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### Physical Parameters

Because the increased susceptibility of IgD to proteolysis might reflect a configurational difference from the other immunoglobulins, the Stokes radii and the sedimentation coefficients of the intact IgD protein and the fragments produced by proteolysis were determined. The result of a typical gel filtration experiment is shown in Fig. 4, and in Table I the Stokes radii obtained for IgD, the IgD fragments, and pooled IgG are listed. With the use of the analytical ultracentrifuge the extrapolated value for $s_{20,w}$ of the intact IgD (L.H.) protein was 6.6. The sedimentation coefficients for the fragments as determined on sucrose gradients are also listed in Table I along with the calculated frictional coefficient ratios and molecular weight values.

### Discussion

Spontaneous degradation of IgD has been noted in several laboratories and has tentatively been attributed to proteolysis. Several investigators have found that they were able to circumvent this problem almost entirely by incorporating $5 \text{mM}$ e-aminocaproic acid into all of the buffers except that used for the DEAE-chromatography (5, 6). This is a concentration of e-aminocaproic acid which inhibits the activation of plasminogen but does not affect plasmin activity. Unfortunately, with serum L.H. this did not appreciably affect the rate of spontaneous degradation. The reason for the difference is not clear, but it could obviously be related to (a) differences in the IgD proteins, as in the IgG subtypes, (b) differences in activities of other serum enzymes or inhibitors (or both), perhaps related to differences in the disease processes of the patients from whom the proteins were obtained, or (c) a combination of these two. We were unable to demonstrate plasmin activity on fibrin plates or the presence of plasminogen-plasmin by immunodiffusion analysis with antisera in any of our preparations of IgD. Neither did prior treatment of the L.H. sera with urokinase, a potent plasminogen activator, followed by exposure to $0.01 \text{M}$ diisopropyl fluorophosphate result in a clear-cut difference in degradation of IgD during subsequent purification and handling, though this treatment may have resulted in a slight increase in yield.

Of interest is the observation that very little, if any, degradation of L.H. IgD occurred spontaneously at pH 5.0. Though we did not attempt to devise a purification scheme at this pH, it may well have proved fruitful.

The increased susceptibility of IgD to proteolysis was marked and evident with all three proteolytic enzymes used in this study. This susceptibility was also demonstrated with a second IgD myeloma protein which, combined with the experience of other investigators, makes unlikely the possibility that IgD (L.H.) was in some way unusual and therefore not representative of the class. Presumably, this represents some unique difference in the structure of IgD. Because of this possibility we determined the Stokes radius and sedimentation coefficients on both the intact protein and on the fragments produced by digestion. These findings allow calculation of one of the hydrodynamic properties of macromolecules, the frictional coefficient ratio, as well as the molecular weight. As can be seen in Table I, the $f/f_{\text{min}}$ for both the intact protein and the Fc fragment are significantly larger than that of IgG, and the $f/f_{\text{min}}$ of IgG is significantly larger than that of the more typical globular proteins. Average values of $f/f_{\text{min}}$ for most proteins range from 1.10 to 1.25 (21). Glycoproteins hydrate extensively at the carbohydrate moieties, the effect of which is to increase the value of the $f/f_{\text{min}}$. Because IgD contains more carbohydrate than IgG, approximately 11 to 14% compared to 3% (28), a comparison of the frictional coefficient ratios of IgD and IgE and the reduction monomer of IgM (IgM$_b$) might be more valid as a reflection of protein structural differences. These latter two molecules are similar to IgD in both molecular weight and carbohydrate content, IgM$_b$, containing approximately 10 to 12% carbohydrate and IgE containing 11 to 14% carbohydrate (24, 26, 27, 29). For this reason literature values of the same physical parameters of IgM and IgE are included in Table I. Though the differences are not as striking as the comparison with IgG, the $f/f_{\text{min}}$ of IgD is larger than that of either IgE or IgM$_b$. The $f/f_{\text{min}}$ value calculated for the IgD Fab fragment indicates a

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$^a$ E. S. Rowe, R. W. Griffiths, and C. Tanford, unpublished data.

$^b$ Reference 24.

$^c$ Reference 25.

$^d$ Reference 26.

$^e$ Reference 27.
more compact globular structure and is quite comparable to the same parameter for both IgG Fab and Fc.

These results suggest that intact IgD is less compact than either IgG, IgE, or IgM, and that the IgD Fc fragment is less compact than the IgG Fc fragment. In contrast the \( f_{\text{max}} / f_{\text{min}} \) for Fab from both IgD and IgG are comparable to those of globular proteins. We propose that the apparent difference in conformation of IgD may account in part for its marked susceptibility to proteolysis. A high \( f_{\text{max}} / f_{\text{min}} \) does not differentiate between an unusually elongate (or flat) structure and a more globular structure with a high degree of hydration (hydration in this context includes hydrodynamically trapped solvent) (30). However, since the latter consideration implies loosely coiled polypeptide chains, in either event there would presumably be an increased amount of exposed peptide chain which could provide sites for proteolytic attack. Because proteolysis with several enzymes results in the production of discrete fragments, Fc and Fab, it is possible that IgD has a relatively exposed area (“hinge” region) (25) which is especially susceptible to proteolysis. It will be noted that the sum of the molecular weights of the fragments (58,000 + 2 \( \times \) 41,000) is 36,000 daltons less than the intact IgD. Though we did not measure the amount of small peptide released during digestion to produce Fab and Fc fragments, the high calculated value is in keeping with this hypothesis, as is the observation by Spiegelberg et al. (6) that the sites of early cleavage by papain and trypsin are separated by at least 15 amino acid residues. The high \( f_{\text{max}} / f_{\text{min}} \) for the remaining Fc fragment is not inconsistent since this parameter is a reflection of hydration and conformation but not of mass. The high \( f_{\text{max}} / f_{\text{min}} \) for the intact molecule could thus be a reflection of both an increased “hinge” region and a less compact Fc fragment. With prolonged exposure to the enzymes used we noted that the Fc fragment would quite readily degrade completely.

The calculated molecular weight for the intact protein is larger than the recently reported value of Spiegelberg et al. (6), but somewhat lower than the reported value of Saha et al. (3). The discrepancies in these values seem larger than might be expected from the molecular weight studies done on other immunoglobulins, and an explanation is not readily apparent. However, the sedimentation coefficients of three different IgD myeloma proteins in one study were found to vary outside of the experimental error (4). Perhaps, then, there is a wider variance in IgD molecules than is presently appreciated.

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