Noncovalent Interactions between Immunoglobulin Polypeptide Chains. Stability to Dissociation by Denaturants*

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ABSTRACT: The stability of mildly reduced immunoglobulins in varying amounts of denaturant was evaluated by electrophoresis on starch gels equilibrated at pH 3.1 with increasing molarities of urea. The results indicated that in the immunoglobulin G (IgG) class, λ light chains were more readily dissociated than κ chains. However, dissociation segregating according to light-chain type did not occur with IgM nor IgA. Light chains were separated relatively easily from IgM regardless of L-chain type while examination of the IgA subclasses revealed that the light chains of IgA_2 proteins were relatively resistant to dissociation compared to those of IgA_1 proteins. Other data pertaining to the stability of inter-heavy-chain noncovalent bonding were obtained by column chromatography and starch gel electrophoresis. The results indicated that the inter-heavy-chain bonds in the IgA_2 subclass were easily disrupted by mild denaturing conditions creating half-molecules while those of IgG_1 were relatively resistant, thereby creating heavy-chain dimers following light-chain release.

Immunoglobulins, irrespective of class, subclass, or phylogenetic origin, are characterized by a basic four-chain structure consisting of two heavy and two light chains. The integrity of this multichain unit is maintained by interchain disulfide bridges acting in concert with weaker cooperative noncovalent forces. It has been previously established (Cohen and Gordon, 1965) that there are significant differences between the stability of IgG[1] κ proteins and IgG λ proteins in that λ light chains are more readily released from human IgG than are κ chains.

The finding that certain immunoglobulins (IgA_2) lacked L–H disulfide bridges (Abel and Grey, 1968, 1971; Grey *et al.*, 1968) led us to consider the noncovalent interaction that holds polypeptide chains to one another in a variety of immunoglobulin classes. Our attention was naturally concentrated upon differences that might exist in the stability of the noncovalent forces between light and heavy chains of IgA_2 proteins that lacked L–H disulfide bridges and IgA_1 proteins that contained them. In order to obtain comparative data it was considered of interest to study the noncovalent interaction between polypeptide chains of as many immunoglobulin classes as possible including H–H as well as H–L interactions. We have also examined some lower vertebrate immunoglobulins for these properties since fragmentary data in the literature would suggest that there may be considerable differences in the amounts of denaturant required to separate heavy and light chains from immunoglobulins of different species (Dreesman and Benedict, 1965; Marchalonis and Schofeld, 1970).

Materials and Methods

Isolation and Isotopic Labeling of Protein. Myeloma proteins were isolated from whole serum by starch block electrophoresis followed by Sephadex G-200 column chromatography in PBS. Normal IgA was isolated as previously described (Vaerman *et al.*, 1963), and normal IgM was isolated by euglobulin precipitation followed by starch block electrophoresis and G-200 gel filtration. Purity of these preparations was monitored by immunoelectrophoresis (Scheidegger, 1955), after which aliquots of the purified proteins were trace labeled with ^{125}I by a modification of the chloramine T method (McConahey and Dixon, 1966).

Reductive Cleavage and Enzymatic Digestion of Immunoglobulins. Mild reduction was done on 8–10 mg of uniodinated protein together with 5 μCi of radioiodinated protein in 0.5 ml of 0.55 M Tris (pH 8.2). Reduction was performed with 0.02 M DTT for 1 hr at room temperature followed by alkylation with 0.06 M recrystallized iodoacetamide for 1 hr at 4°. In a number of experiments the reduction was done with 0.005 M DTT or 0.2 M 2-mercaptoethanol to rule out dependency of the results obtained on the amount and type of reducing agent. The lower molarity of reducing agent resulted in a portion of immunoglobulin remaining unreduced but otherwise the findings were essentially the same. Papain digestion was performed as described previously (Zimmerman *et al.*, 1971).

Vertical Starch Gel Electrophoresis. The method was a modification of the technique described by Smithies (1959) and Poulik (1960). The formate buffers for gel and vessels were adjusted to pH 3.1 ± 0.1 with concentrated NaOH. Hydrolyzed starch (batch 279-1, Connaught Laboratories, Toronto, Can.) in amounts varying from 60 to 70 g, depending on urea concentration, was equilibrated with 500 ml of formate buffer–0.05 M, pH 3.1 containing final molarities of urea varying from 0 to 8 M. No attempt was made to determine or change the pH after addition of urea. The suspension was gellified either over an open flame or at 60° in a water bath (4–8 M urea starch gels). The reduced proteins were electrophoresed for 16–18 hr at room temperature at voltages varying from 100 to 180 V according to the molarity of urea and migration of an albumin marker. Amperage never exceeded 30 mA. The voltage chosen was such that the colored marker, albumin stained with bromophenol blue dye, migrated 3 in. from the origin in 16–18 hr. Following electrophoresis the gels were sliced

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[1] Abbreviations used are: IgG, immunoglobulin G; PBS, phosphate-buffered saline; DTT, dithiothreitol.

into a top and bottom half, both of which were stained with Amido-Schwarz and decolorized overnight. The top portion of the gel was examined by autoradiography to ensure correspondence between isotope bands and stained protein. The lower half of the gel was cut lengthwise into ten strips, each corresponding in width to one of the ten slots, and then the individual strips were further fractionated widthwise into 3-mm slices which were counted in a well-type crystal counter.

Reproducibility of the results obtained by electrophoresis was assessed by repeated examinations of the same material isolated and labeled on different occasions and reduced with varying molarities of reducing agent. The application of free light chain, free heavy chain, and unreduced protein served to monitor band positions. On each starch gel series a standard reduced myeloma protein was applied to ensure that reproducible electrophoresis was occurring. Reproducibility was generally within ±7% depending to some extent on the degree to which the light chains of different proteins labeled with ^{125}I, and the relative mobility of the dissociated light chains in starch gel electrophoresis. To rule out artifacts caused by electrophoresis on a starch gel medium, some of the reduced myeloma proteins were also studied by column chromatography on Sephadex G-200 equilibrated with 0.05 M formate buffer (pH 3.1). These results supported the findings obtained by starch gel electrophoresis.

Calculation of Per Cent Release of Light Chain. By summing the counts under the free light-chain peak and dividing by the total number of counts, the fraction of released L chains was calculated, *i.e.*, (counts under L-chain peak/counts under L-chain peak + counts under other peaks) × 100. The release resulting from the 8 M urea gel was assumed to be maximal and by taking all other fractions as a per cent of the 8 M gel figure, the per cent of maximal light chain released could be standardized and expressed graphically as a function of urea molarity.

Column Chromatography. Sephadex G-200 (230–270 mesh, Pharmacia) equilibrated with PBS (pH 7.0) or 0.05 M formate buffer (pH 3.1 ± 0.1) was packed in columns (100 × 2.5 cm) adapted for upward flow. Forty to fifty milligrams of protein equilibrated by overnight dialysis against the eluting buffer was applied to the columns and chromatographed at a constant rate of 11 ml/hr at 4°. Apparent molecular weights (M_w) were calculated by comparing the distribution coefficients (K_d) of the proteins of unknown molecular weight with the K_d of marker molecules of known M_w. $K_d = (V_e - V_0)/V_i$. Where V_e is the elution volume of the protein under study, V_0 is the column excluded volume and V_i is the column included volume. A graph of $(K_d)^{1/2}$ *vs.* $(M_w)^{1/2}$ was created for the marker molecules and the $(K_d)^{1/2}$ of the unknown placed on the curve (Andrews, 1964, 1965).

Results

Dissociation of Light Chains. Figure 1a–c illustrates the results obtained using the starch gel system. The electrophoretic pattern of reduced IgG_1 λ, IgG_1 κ, and IgM κ proteins in 8 M urea is displayed in comparison with the pattern of these same preparations in 0.5 M urea at the same pH. Significant amounts of free light chain were released from the IgM κ and IgG_1 λ at 0.5 M urea while little release had occurred from the IgG_1 κ. As the molarity of urea was increased, a corresponding increase in release of light chains from the reduced IgG_1 κ took place.

Following electrophoresis the gels were stained, sectioned into 3-mm portions, and counted for radioisotope. Plots such as the one shown for IgG_1 κ in Figure 1d were obtained. The total counts under the free light-chain peak was calculated as a fraction of the total counts. This fraction was then used together with the data obtained from the 8 M urea gel to calculate the percentage of light chain released.

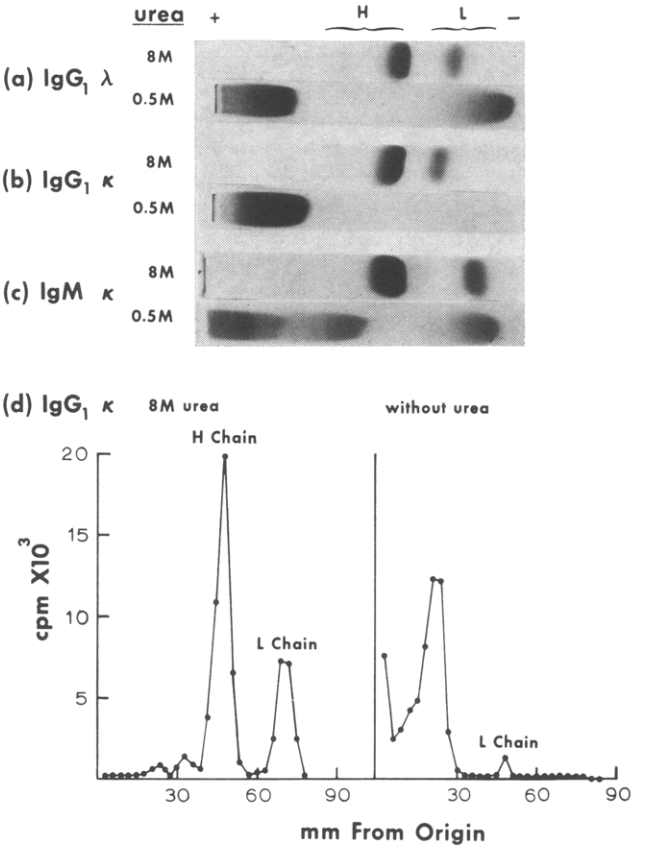

FIGURE 1: Quantitation of L-chain release from partially reduced and alkylated IgG and IgM by acid–urea starch gel electrophoresis. (a–c) Compare electrophoresis of a reduced IgG_1 λ, IgG_1 κ, and IgM κ, respectively, on formate starch gels, pH 3.1 plus 8 or 0.5 M urea. (d) Compares radioisotope peaks obtained for IgG_1 κ on formate starch gel, pH 3.1 with 8 M or pH 3.1 without urea.

Figure 2 illustrates the results obtained for normal human IgG and two IgG_1 myeloma proteins. IgG myeloma proteins were found to be heterogeneous with respect to the quantities of denaturant needed to dissociate their light chains. Although some exceptions were noted these differences generally segregated between κ and λ IgG (Figure 2; Table I) such that λ chains dissociated under milder denaturing conditions than κ chains. At 1 M urea, a concentration of urea resulting in roughly 50–60% release of light chain from normal IgG, IgG_1 κ proteins demonstrated an average release of 40% of their light chains compared to 100% release of the light chains from IgG_1 λ proteins. The correlation of light-chain dissociability with light-chain type appeared to exist for IgG_2 and IgG_4 proteins as well. However, again, exceptions were noted, since an IgG κ protein from each subclass examined tended to release large quantities of light chain at low molarities of urea in a manner resembling that of λ-chain release (Boa IgG_1, Saw IgG_2, and Rey IgG_4, Table I). Proteins of the IgG_3 subclass were not available for this study.

Light-chain dissociation from IgA was next examined. Figure 3 illustrates representative curves for an IgA_1 λ protein, normal IgA, and two IgA_2 λ proteins. The data on several IgA_1 and IgA_2 proteins are summarized in Table II and

TABLE I: Light-Chain Dissociation from IgG Proteins; Per Cent Released on Formate Starch Gel, pH 3.1, 0–2 M Urea.

IgG Subclass		κ Chains Urea Concn 0	κ Chains Urea Concn 1 M	κ Chains Urea Concn 2 M		λ Chains Urea Concn 0	λ Chains Urea Concn 1 M	λ Chains Urea Concn 2 M
IgG_1								
	Mur	6	35	80	Qua	76	100	100
	Cut	5	25	67	Chu	74	100	100
	Wil	5	30	70	Way	83	100	100
	Boa	*a*	79	100				
	Av %	5	42	79		78	100	100
IgG_2								
	Til	2	21	41	Dah	78	100	100
	Saw	51	71	100	New	*a*	74	100
	Av %	27	46	71			87	100
IgG_4								
	Ger	6	35	*a*	Heb	84	100	100
	Fer	3	16	47				
	Rey	*a*	69	76				
	Av %	5	40	62				
Normal IgG		35	57	90				

[a] Not determined.

indicate that differences in susceptibility to denaturant dissociation existed within the IgA class but that this variation did not correlate with light-chain type, but rather with the IgA heavy-chain subclass. IgA_1 proteins, regardless of light-chain type, released 70–80% of their light chains at 1 M urea compared to only 20–30% for IgA_2 proteins (Table II). As might be expected, normal IgA gave data intermediate to that obtained with the IgA myeloma proteins of the two different subclasses.

Since most proteins of the IgA_2 subclass lack disulfide bridges between light and heavy chains, dissociation of L and H chains could also be studied in the unreduced state. Because of difficulties resulting from poor migration of the IgA polymer into the lower molarity urea gels only fragmentary data were obtained. The results suggested a slightly greater stability of the unreduced protein compared to that of the mildly reduced protein since less than 100% release of light chains had occurred by 4 M urea, an amount of denaturant which resulted in complete dissociation of the reduced IgA_2. However, with a further 1 M increment complete dissociation did occur.

Several IgM myeloma proteins (Table III) and a preparation of normal IgM (Figure 4) were also studied. It was ap-

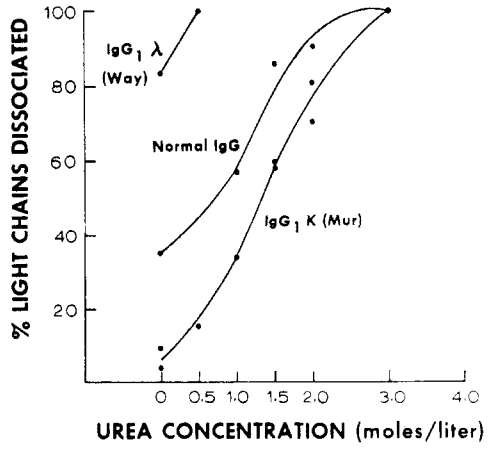

FIGURE 2: Effect of urea concentration on dissociation of L chains from partially reduced and alkylated IgG_1 myeloma proteins and normal human IgG, electrophoresed on starch gels, pH 3.1, urea concentrations varying from 0 to 8 M.

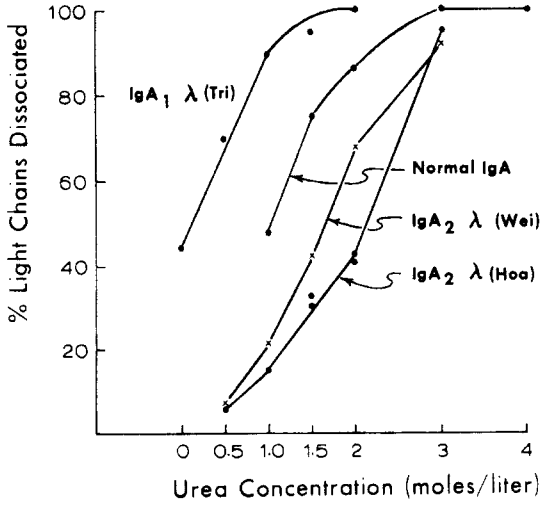

FIGURE 3: Effect of urea concentration on dissociation of L chains from partially reduced and alkylated IgA myeloma proteins and normal IgA, electrophoresed on starch gels, pH 3.1, urea concentrations varying from 0 to 8 M.

TABLE II: Light-Chain Dissociation from IgA Proteins; Per Cent Released on Formate Starch Gel, pH 3.1, 0–2 M Urea.

		κ Chains Urea Concn				λ Chains Urea Concn		
IgA Subclass		0	1 M	2 M		0	1 M	2 M
IgA_1								
	Mun	26	80	100	Tri	37	85	100
	Mau	23	75	100	Bro	*a*	50	91
	Van	6	60	100				
	Gle	27	97	100				
	Rie	28	61	100				
	Kwa	58	100	100				
	Her	*a*	73	100				
	Zap	25	61	100				
	Av %	28	76	100			68	96
IgA_2								
	Cla	10	40	84	Hoa	5	15	42
	Kee	5	15	62	Wei	7	22	68
	Hay	17	34	94				
	Av %	11	30	80		6	19	55
Normal IgA		*a*	48	86				

[a] Not determined.

TABLE III: Light-Chain Dissociation from IgM Proteins; Per Cent Released on Formate Starch Gel, pH 3.1, 0–2 M Urea.

		κ Chains Urea Concn				λ Chains Urea Concn		
		0	1 M	2 M		0	1 M	2 M
IgM proteins	Vdl	76	100	100	Mo	100	100	100
	Crd	79	100	100	Vis	49	100	100
					Kat	100	100	100
					Sal	60	100	100
	Av %	78	100	100		77	100	100
Normal IgM		86	100	100				

parent that IgM released free light chains relatively easily with 100% dissociation occurring at 1 M urea in all samples studied. No differences were found between light-chain type and no indications were found to suggest the existence of a subpopulation of IgM with more stable light- to heavy-chain bonding.

The finding that immunoglobulin H-chain and L-chain classes differed in their structural stability in the presence of denaturants suggested that the class-specific portion of the L chains and Fd fragment (*i.e.*, the C-terminal half) may be very important in determining the degree of stability that a given L–H pair has. This speculation was strengthened considerably by the study of two proteins, an IgM λ and IgA_2 λ (IgA_2 Wei, Table II and Figure 3) which were products of a biclonal myeloma. The light chains of both these proteins appeared to be identical or almost identical, by virtue of identical migration on gel electrophoresis and shared idiotypic specificity (G. Penn, 1971, personal communication). Similarly, the Wei μ and Wei α_2 chains shared variable-region antigens and are therefore probably very closely related to one another in the primary sequence of their Fd-variable regions. Most of the light chains from the IgM preparation (50–75%) were released on formate starch gels equilibrated with 0 or 0.5 M urea. This represents a minimal figure fo the dissociability of the IgM since there was always a significant contamination of the IgM preparation with polymer IgA (15–30%). These same gels demonstrated release of only 7–10% of light chains from the IgA_2, thereby adding further evidence of the importance of the constant region of the Fd fragment in H–L chain interactions.

Because IgM has been postulated from phylogenetic studies on shark and other lower order vertebrates to be the earliest evolving mammalian immunoglobulin (Grey, 1969), it was of interest to study the 16–19S immunoglobulins from some

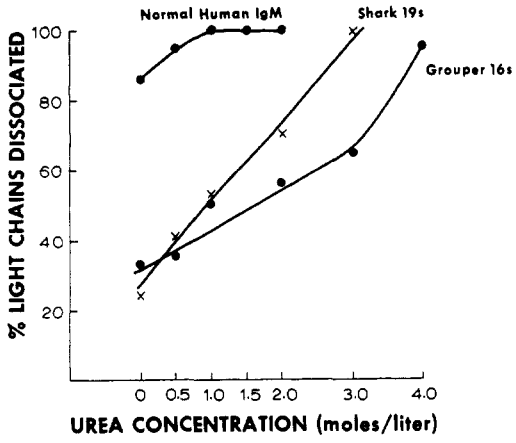

FIGURE 4: Effect of urea concentration on dissociation of L chains from partially reduced and alkylated IgM from human, shark, and grouper, electrophoresed on starch gels, pH 3.1, urea concentrations varying from 0 to 8 M.

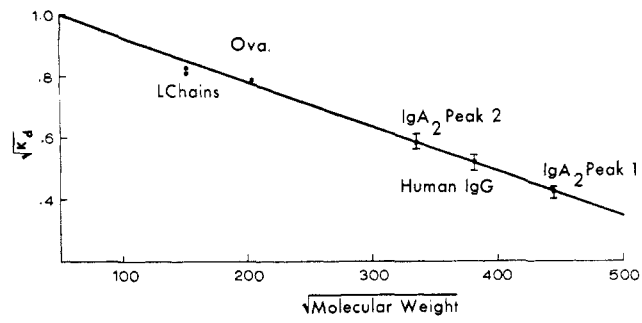

FIGURE 6: Plot of $(K_d)^{1/2}$ *vs.* $(M_w)^{1/2}$ for partially reduced and alkylated IgA_2 after gel filtration on G-200 Sephadex, formate buffer, pH 3.1 (as shown in Figure 5c). The molecular weights of the markers were 23,000 for light chains, 42,000 for ovalbumin, 150,000 for human IgG. $K_d = (V_e - V_0)/V_i$, where V_e = elution volume, V_0 = excluded volume, and V_i = included volume. The apparent molecular weights for the partially reduced IgA_2 peak 1 = 195,000; IgA_2 peak 2 = 111,000.

primitive vertebrate species. Light-chain dissociation curves are plotted in Figure 4 for reduced shark and grouper polymer immunoglobulins compared to normal human IgM. It was apparent that the two fish immunoglobulins were more resistant to urea–acid dissociation than human IgM. At 1 M urea the former had released 50% or less of their total light chains while human IgM had reached maximum release at this point.

Dissociation of Inter-Heavy-Chain Noncovalent Bonds. Examination of starch gel electrophoreses in low molarities of urea revealed that reduced IgA_2, while demonstrating minimal light-chain release, exhibited a relatively fast mobility. Thus, reduced IgA_2 could be shown to be faster than intact monomer but slower than either light chain or free α_2 chains. These observations suggested that the reduced IgA_2 was dissociating into symmetrical half-molecules under mild denaturing conditions. On the other hand, the slow mobility of μ and γ chains at the low molarity urea gels (Figure 1) compared to their mobility in 8 M urea suggested that the heavy chains of these two classes were present as dimers, or higher aggregates, under these conditions.

Further evidence for the suggested generation of half-molecules from partially reduced and alkylated IgA_2 under mild denaturing conditions was obtained from Sephadex gel filtration; while the suggestion of γ-chain dimers being formed after total light-chain release was pursued by studying the dissociation of mildly reduced Fc and Fab fragments of IgG_1 on starch gels.

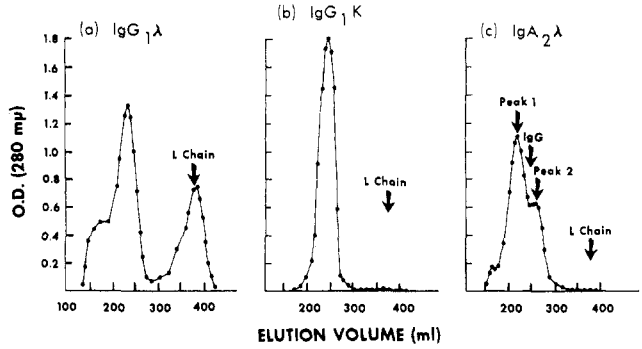

FIGURE 5: Gel filtration of partially reduced and alkylated (a) IgG_1 λ, (b) IgG_1 κ, and (c) IgA_2 λ. Gel filtration was performed using G-200 Sephadex column (2.5 × 100 cm) equilibrated with 0.05 M formate buffer (pH 3.1 ± 0.1). With each run trace amounts of ^{125}I normal IgG were included as an internal standard. Excluded and included volumes were also calculated by determining the elution volume of Dextran Blue and sucrose.

Figure 5 compares the elution profiles of mildly reduced IgG_1 λ, IgG_1 κ, and IgA_2 λ proteins on a Sephadex G-200 column equilibrated with 0.05 M formate buffer (pH 3.1). The results agree very well with the data obtained by starch gel electrophoresis. The IgG_1 λ protein released most if not all of its light chain. On the other hand, both the IgG_1 κ and the IgA_2 λ proteins released none of their light chains. The IgA_2 did, however, demonstrate a complex profile consisting of two poorly separated peaks, the first of which eluted just ahead of an unreduced IgG marker and the second peak emerged later than the IgG marker. Chromatography of a number of different reduced IgA_2 proteins resulted in essentially the same elution pattern.

The apparent molecular weights of the two peaks were calculated on a calibrated formate pH 3.1 Sephadex column (Figure 6). Molecular weights of 195,000 for the first peak and 111,000 for the second peak were obtained. The molecular weight of 195,000 was greater than the 162,000 molecular weight of IgA_2 obtained by sedimentation equilibrium (Abel and Grey, 1971); however, it was consistent with a molecular weight of 180,000 estimated by chromatography of reduced IgA_2 on a Sephadex G-200 column equilibrated with PBS and calibrated with the same marker proteins. This dissociation of reduced IgA_2 by acid pH was reversed by dialyzing the proteins back into PBS. Gel filtration of this material on a G-200 column equilibrated in PBS gave a singly symmetrical peak that eluted in the same position as unreduced IgA_2 monomer.

The above data were in contrast to those obtained with IgG λ and IgM proteins where interpretation of the starch gel patterns led to the suggestion that the heavy chains did not electrophorese as monomer bands until application of high molarity urea (2–3 M). This was further investigated by examining the dissociation of several reduced Fc and Fab fragments of IgG_1 κ and λ proteins on starch gel electrophoresis using unreduced Fc and Fab as markers for the undissociated fragments. Figure 7 shows the results obtained with an IgG_1 λ protein in which free monomer light chain migrated faster and could be distinguished from the free monomer Fd fragment. This allowed separate quantitation of light-chain re-

TABLE IV: Relative Stability of Noncovalent Bonding between Immunoglobulin Polypeptide Chains.[a]

Class		H–L κ	H–L λ	H–H
IgG		S	L	S
IgA	A_1	I	I	?I
	A_2	S	S	L
IgM		L	L	S

[a] S = stable noncovalent interactions, *i.e.*, less than 40% dissociation at pH 3.1, 1.0 M urea; L = labile noncovalent interactions, *i.e.*, 90–100% dissociation at pH 3.1, 1.0 M urea. I = noncovalent interactions intermediate in stability to the above two categories.

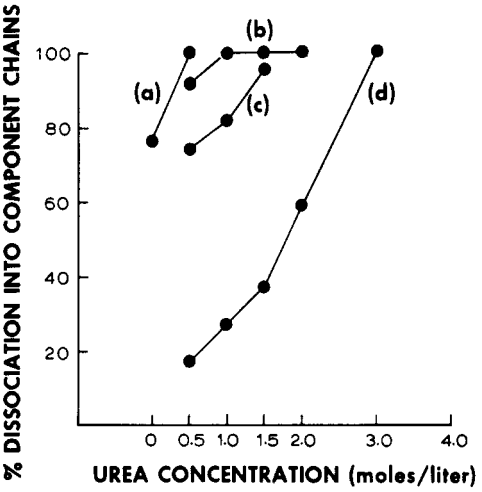

FIGURE 7: Effect of urea concentration on the dissociation of partially reduced and alkylated Fab and Fc fragments into component polypeptide chains. Fab and Fc fragments were obtained from an IgG_1 λ myeloma protein (Qua). Plots a and b represent light-chain dissociation from Qua whole molecule and from Qua Fab, respectively. Plot c is the appearance of Fd monomer. Following light-chain release, at low molarities of urea, Fd fragments tended to polymerize. Plot d is the dissociation of Fc fragment into monomeric Fc piece.

lease as well as the amount of aggregated and monomer Fd present. The light chain reached 100% dissociation at roughly the same molarity of urea (0.5–1 M) from both whole molecule and Fab fragment (Figure 7a,b). At 1 M urea and less the Fd fragment that was dissociated from light chains had a tendency to aggregate but by 1.5 M urea the Fd migrated as a single monomer band (Figure 7c). The Fc fragment of the IgG_1 λ protein was reduced and the dissociation into monomer Fc pieces was also quantitated (Figure 7d). The monomer Fc piece represented less than 30% of the total Fc at 1 M urea, a concentration which resulted in 100% dissociation of light chain from both the whole molecule and the Fab fragment. By 3 M urea, however, all the Fc was migrating ahead of the unreduced Fc marker as monomer Fc piece.

The dissociation of four IgG_1 Fc fragments (two from IgG_1 λ proteins and two from IgG_1 κ proteins) was studied with very similar results which demonstrated an average dissociation of less than 40% into monomer Fc piece at 2.0 M urea. This compares to an average 79% release of κ chains from IgG_1 proteins (Table I). These results indicate that, in general, in IgG_1, dissociation of either κ or λ light chains occurs before disruption of the inter-heavy-chain noncovalent binding.

Discussion

The present paper has surveyed the various human immunoglobulin classes and subclasses to determine the relative stability of the noncovalent interactions that maintain the basic four chain immunoglobulin structure. The release of polypeptide chains from partially reduced immunoglobulins and their fragments was measured under conditions of varying denaturants, *i.e.*, mild acid pH with increasing molarities of urea. The findings indicated significant differences in the degree to which the noncovalent forces were subject to disruption by the same quantities of urea.

A summary of the data classified according to a simplified scheme is shown in Table IV. While there are individual exceptions to this classification, the noncovalent interactions of the myeloma proteins studied can be grouped into two major categories: those that are labile (L) to mild denaturing conditions (*i.e.*, 90–100% dissociation at 1 M urea, pH 3.1) and those that are stable (S) (on average <40% dissociation at 1 M urea, pH 3.1). The labile category includes the association of: λ with γ chains; κ and λ with μ chains; and the binding between heavy chains of IgA_2. The stable group includes the κ-light-chain binding to γ heavy chains; both κ and λ binding to α_2 heavy chains; and the inter-heavy-chain binding of IgG_1 and IgM. A third category consisting of interactions of intermediate stability includes the light- to heavy-chain and heavy- to heavy-chain binding of IgA_1 as well as the light- to heavy-chain binding of grouper and shark 16–19S immunoglobulins. The biologic importance, if any, of this considerable heterogeneity in conformational stability is unknown. One possible important role of heterogeneity in the L–H interaction would be to modulate the conformation of the antigen-binding site and thereby influence the affinity and possibly specificity of an antibody molecule for a given antigen.

The association showing the greatest stability in urea was found between the polypeptide chain moieties of the Fc fragment of mμ and γ_1 chains while the most labile interactions were between the light and heavy chains of IgM and between λ light chains and IgG heavy chains. Acidic conditions (pH 3.1) resulted in the release of the great bulk of these light chains. These latter findings confirm and extend the studies of Cohen and Gordon (1965) on IgG. Unfortunately, the electrophoretic technique used did not allow examination of the release of light chains at higher pH values since it was found that resolution of the L- and H-chain migration patterns was poor at higher pH.

The major purpose of the present investigation was the examination and comparison of proteins in the IgA class. A striking difference exists between the IgA_1 and IgA_2 subclass in that most proteins of the IgA_2 subclass lack any L–H disulfide bridges while this covalent bond is uniformly present in the IgA_1 subclass. This observation raised questions about the stability of L–H binding in IgA_2. The current study demonstrated significant differences in the stability of the noncovalent interactions between heavy and light chains of IgA_1 and IgA_2 as measured by light-chain release in different molarities of urea. (IgA_1 proteins released 50% of their light chains at 0.5 M urea whereas 1–2 M urea was required to accomplish a comparable release in IgA_2.) This reciprocal association between absence of L–H disulfide bridge and presence of stable noncovalent binding, on the one hand, and the presence of the

L–H disulfide bond with more labile noncovalent binding, on the other, suggests the possibility that proteins lacking the L–H disulfide bridge may be under selective pressures to have a concomitant increase in noncovalent interactions compared to proteins with similar structure which contain the L–H covalent bond. In this respect, it would be of interest to examine the IgA_2 Am 2– proteins which represent the minor allotype of the IgA_2 subclass and which contain the L–H disulfide bridge.

Regarding the structure(s) of importance in stabilizing the L–H interactions, the opportunity to study an IgA_2 and an IgM protein from a multiple myeloma patient with a biclonal gammopathy were quite revealing. These two proteins contained the same λ light chain and the variable region on the light chains as well as Fd regions of the α and mμ chains were very similar, if not identical (G. Penn, 1971, personal communication). However, despite these variable-region similarities there were striking differences in the noncovalent interactions between the light and heavy chains which must therefore be attributable to the differences in primary sequence between the constant regions of the Fd fragment in α_2 and μ chains. However, the fact that some individual myeloma proteins did not conform to the classification outlined in Table IV (*e.g.*, proteins Boa, Saw, and Rey in Table I) obviously indicate that the variable region of the Fd and light chain may modify (at times extensively) the degree of noncovalent interactions.

Similar class-specific differences appeared to exist in the stability of Fc region noncovalent binding. This interaction is very labile in reduced IgA_2 proteins where, due to H–L stability and H–H instability, mild denaturing conditions resulted in the formation of half-molecules. This phenomenon was less readily evaluated in the IgA_1 proteins since significant light-chain release also occurred at low molarities of urea. However, both the IgG and IgM class of proteins appeared to have quite stable noncovalent interactions in that the Fc fragments of IgG dissociated only when the urea concentration was increased to 2–3 M urea. These findings would appear to be in contrast to the findings that have been published with rabbit IgG where the H–H interactions are more labile than the H–L (Hong and Nisonoff, 1965). However, we have some data from mouse myeloma IgG that indicate certain reduced mouse IgG (MOPC 21 and S19) demonstrate similar lability in their inter-heavy-chain noncovalent binding, forming half-molecules at pH 3.1, a pH which does not cause significant light-chain release. These data imply that as well as differences between immunoglobulin classes and subclasses, there is considerable species variation also. We attempted to evaluate some of the species variation by examining two lower order vertebrate (shark and grouper) polymeric immunoglobulins which are probably counterparts of mammalian IgM (Clem and Small, 1967; Clem, 1971). When compared to human IgM the high molecular weight immunoglobulins of the shark and grouper were considerably more resistant to disruption of their L–H noncovalent bonds by urea. We also examined the L–H interactions in the chicken and duck 7S immunoglobulins. As previously described (Dreesman and Benedict, 1965), some light chains (30–35%) dissociate at neutral pH in the absence of urea. However, complete light-chain release was effected only on the addition of 1 M urea to the pH 3.1 buffer.

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