Differential Reduction of Interchain Disulphide Bonds of Mouse Immunoglobulin G

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The relative lability of the interchain disulphide bonds of mouse G_{2a}-myeloma protein 5563 was studied as a function of 2-mercaptoethanol concentration. Analysis of partial-reduction mixtures by polyacrylamide-gel electrophoresis and microdensitometry showed that the disulphide bonds between light and heavy chains are much more susceptible to reduction than the bonds between heavy chains. At a low concentration of 2-mercaptoethanol (10mM) the major dissociable products of mouse immunoglobulin G are heavy-chain dimers and free light chains. These findings contrast with the reported behaviour of rabbit immunoglobulin G, for which the lability of inter-heavy-chain bonds was found to exceed that of the bonds linking light and heavy chains (Hong & Nisonoff, 1965); the relative stability of rabbit immunoglobulin G interchain bonds was confirmed in the present study. Examination of human immunoglobulin G and an immunoglobulin G (γ_2) of guinea pig showed that at least in the majority of molecules, as with mouse immunoglobulin G, the disulphide bonds between light and heavy chains are more susceptible to reduction than the inter-heavy-chain bonds.

In this paper we have where possible followed the nomenclature recommended by the World Health Organisation (1964, 1965).

The four-polypeptide-chain structure originally proposed for rabbit immunoglobulin G (IgG) (Edelman & Poulik, 1961; Porter, 1962) is apparently correct in its essential features for IgG from a wide range of species (see the review by Cohen & Porter, 1964). The IgG molecule, as currently envisaged, consists of two identical heavy (H) chains, linked by one or more disulphide bonds, and two identical light (L) chains, each attached to one of the heavy chains by a single disulphide bond. Treatment with 0.1M-2-mercaptoethanol splits IgG into the heavy and light chains in all species examined (human, mouse, guinea pig, rabbit etc.). Hong & Nisonoff (1965) have shown that the stabilities of the two types of interchain disulphide bonds (L-H and H-H) in rabbit IgG differ such that conditions of reduction can be defined for the preferential reduction of the H-H bond with minimal cleavage of L-H disulphide bonds; at low concentrations of reducing agent, rabbit IgG can be dissociated into half-molecules, each comprising one light chain joined to one heavy chain.

In the present study we have found that in mouse IgG the relative stability of L-H and H-H disulphide bonds is opposite to that found in rabbit

IgG. We have utilized electrophoresis on polyacrylamide gels to analyse partial-reduction mixtures of IgG, and, coupled with microdensitometry, this technique has enabled all the possible intermediate reduction products to be determined. A low concentration of mercaptoethanol that cleaved rabbit IgG into half-molecules without the liberation of detectable free light chains resulted in preferential reduction of the L-H bonds in mouse IgG and G_{2a}-myeloma protein, so that the molecules could be dissociated mainly into free light chains and unreduced heavy-chain dimers. Examination of IgG of man and an IgG (γ_2) of guinea pig has shown that at least in the majority of molecules, as with mouse IgG, the L-H disulphide bonds are more susceptible to reduction than the H-H bonds; in rabbit IgG, on the other hand, we have confirmed the previous report (Hong & Nisonoff, 1965) that the disulphide bond between the two heavy chains is susceptible to preferential reduction.

MATERIALS AND METHODS

Chemicals. 2-Mercaptoethanol was purchased from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.) and redistilled before use. 2-Mercaptoethylamine hydrochloride and thioglycolic acid were obtained from British Drug

Houses Ltd. (Poole, Dorset). Aqueous solutions of these reducing agents were prepared immediately before use.

Iodoacetamide was purchased from British Drug Houses Ltd. and recrystallized from ethanol.

Immunoglobulins. Mouse G_{2a}-myeloma protein formed by plasma-cell tumour 5563 was purified from serum or ascitic fluid by chromatography on DEAE-cellulose (Askonas, 1961) followed by fractionation on Sephadex G-200 columns (90 cm. × 2.5 cm.; 0.3 M-NaCl-10 mM-phosphate buffer, pH 8) to free the sample from transferrin. G_{2a}-myeloma protein from the AdjPC-5 tumour (Potter, Appella & Geisser, 1965) was partly purified by two precipitations with 35%-saturated (NH₄)₂SO₄ at room temperature. Mouse IgG was isolated from sera of mice hyperimmunized with haemocyanin as described by Fahey & Askonas (1962); the preparation contained some transferrin. Light and heavy chains produced by reduction and alkylation of 5563 myeloma protein were isolated by gel filtration by the method of Fleischman, Pain & Porter (1962).

Rabbit IgG purified on DEAE-cellulose in 20 mM-phosphate buffer, pH 7.2, was kindly supplied by Dr S. Utsumi. This protein contained traces of transferrin.

Guinea-pig IgG (γ₂) was purified by elution from DEAE-cellulose with 20 mM-sodium phosphate, pH 7.5 (Askonas, White & Wilkinson, 1965).

Human IgG was obtained from the Dutch Red Cross. A₂ cryoglobulin was a kind gift from Dr A. S. MacFarlane, and three purified G-myeloma proteins (IgG_{2b}, κ; IgG_{2c}, λ; IgG_{2e}, κ) were kindly sent to us by Professor S. Cohen.

Reduction of IgG. 2-Mercaptoethanol. Reduction was performed in 0.2 M-tris-HCl, pH 8.2, for 1 hr. at room temperature by adding sufficient 2-mercaptoethanol to give the required final concentration; reduction was terminated with a 1.5-fold molar excess of iodoacetamide. Reaction mixtures were stored at 4° before analysis.

2-Mercaptoethylamine. The solvent was 0.1 M-sodium acetate buffer, pH 5.5; reaction was for 1 hr. at 37° and was stopped by alkylation as described above.

Thioglycolate. Thioglycollic acid was treated with methylamine to remove contaminating polythioglycolides by the method of White (1960). Reduction was carried out in 0.1 M-phosphate buffer, pH 6.5, for 1 hr. at 37° and was stopped by alkylation as described above.

Polyacrylamide-gel electrophoresis. Electrophoresis was performed in 8 cm. columns of polyacrylamide gel supported in glass tubes (0.6 cm. internal diameter). Gels, 7.0% (w/v) with respect to acrylamide, containing 0.1% (w/v) of SDS,* 0.5 M-urea and 0.1 M-phosphate buffer, pH 7.2, were prepared by the procedure of Summers, Maizel & Darnell (1965) and were run at 10 mA/column. The same buffer without urea was used for the electrode vessels.

The acid-urea gels [7.5% (w/v) of acrylamide] were prepared in a manner similar to the neutral SDS gels but contained 0.1 M-sodium formate, pH 2.5, and 5.5 M-urea. The electrode vessels contained 0.1 M-sodium formate, pH 2.5. Electrophoresis was carried out at a constant current (5 mA/column) for the times indicated. In the acid-urea gels the current was applied to the gel for 30 min. before application of the sample to remove ionic impurities from the urea.

After electrophoresis protein components were stained with Amido Black. The relative intensities of the stained

protein bands were measured with a double-beam recording microdensitometer (Joyce-Loebl Ltd., Gateshead, Co. Durham).

RESULTS

Analysis of mouse IgG partially reduced with 2-mercaptoethanol

G-myeloma protein 5563. Myeloma protein 5563 shows four or five discrete bands on electrophoresis at pH 8.6. For the present studies, however, conditions of electrophoresis were chosen under which the charge differences between the bands were suppressed. The extent of reduction of the two types of interchain disulphide bonds (between the heavy chains or heavy and light chains) was measured by electrophoresis on polyacrylamide gel under two different sets of conditions that dissociate non-covalently linked sub-units: (a) at neutral pH: phosphate buffer, pH 7.2, containing 0.1% SDS and 0.5 M-urea in the gel; (b) at acidic pH: formate buffer, pH 2.5, with 5.5 M-urea in the gel. The electrophoretic patterns obtained after reduction of G-myeloma protein 5563 with various concentrations of mercaptoethanol are illustrated in Plate 1. The positions on polyacrylamide-gel electrophoresis of the original myeloma protein and its sub-units (heavy and light chains) were identified by comparison with the original purified myeloma protein and heavy and light chains separated according to the method of Fleischman *et al.* (1962). In neutral SDS the heavy chains show some banding (Plate 1a); the reasons for this are unclear, but may be related to the tendency of heavy chains to denature, aggregate and possibly bind differing ratios of SDS because of altered folding. Both heavy and light chains show slight contamination with each other. At a given concentration of mercaptoethanol, the patterns resulting from electrophoretic analysis under the two different sets of dissociating conditions, (a) and (b), were remarkably similar; they show two important points: (i) at low concentrations of mercaptoethanol (5–10 mM) appreciable release of free light chains occurs without the corresponding release of free heavy chains; (ii) accompanying this release of light chains, there is the appearance of two new stained protein components that run just ahead of myeloma protein, but not as far as half-molecules from 47A-myeloma tumour (Potter & Kuff, 1964) (see the position of half-molecules on Plate 3); these components are designated as L-H-H (myeloma protein from which one light chain has been removed) and H-H (heavy-chain dimer), on the basis of the evidence discussed below.

The extent of light-chain release and the nature of the new components thereby generated were clarified by quantitative measurement of the stained protein bands on the gels by microdensito-

* Abbreviation: SDS, sodium dodecyl sulphate.

EXPLANATION OF PLATE I

Electrophoretic analysis of partial-reduction products of mouse G-myeloma globulin 5563 on polyacrylamide gels. Protein was reduced at the concentrations of 2-mercaptoethanol (ME) indicated, and alkylated as described in the Materials and Methods section. *A*, G-myeloma protein 5563 (IgG); IgG reduced by: *B*, 1 mM-ME; *C* and *I*, 5 mM-ME; *D* and *J*, 10 mM-ME; *E* and *K*, 0.1 M-ME. Purified IgG chains: *F* heavy (H), chain; *G*, light (L) chain. (a) For samples *A-G* 7.0% gels were used in SDS-urea-phosphate, pH 7.2; (b) for samples *I-K* 7.5% gels were used in urea-formate, pH 2.5. In all cases electrophoresis was for 5 hr.

EXPLANATION OF PLATE 2

Electrophoretic analysis of partial-reduction products of (a) pooled mouse IgG (samples *A*, *B* and *C*) and (b) human cryoglobulin G (samples *D*, *E* and *F*). (a) Heterogeneous mouse IgG: *A*, unreduced IgG; IgG reduced by: *B*, 10mM-ME; *C*, 0.1M-ME. (b) Human IgG; *D*, unreduced IgG; IgG reduced by: *E*, 10mM-ME; *F*, 0.1M-ME. For all samples 7.0% gels were used in SDS-urea-phosphate, pH 7.2, and the current was 10mA/tube for 5hr. ME, 2-Mercaptoethanol; for details see the Materials and Methods section.

EXPLANATION OF PLATE 3

Electrophoretic analysis of partial-reduction products of rabbit IgG. *A* and *D*, Unreduced rabbit IgG; rabbit IgG reduced by: *B* and *E*, 1mM-ME; *C* and *F*, 10mM-ME; *G*, 0.2M-ME. Markers: *I*, purified protein from mouse myeloma MOPC 47A (half-molecules, L-H) containing some aggregated material (slower band); *J* heavy (H) and light (L) chains of mouse myeloma protein 5563. (a) For samples *A-C* 7.5% gels were used in urea-formate, pH2.5; (b) for samples *D-J* 7.0% gels were used in SDS-urea-phosphate, pH7.2. In all cases electrophoresis was for 5hr. ME, 2-mercaptoethanol; for details see the Materials and Methods section.

metry. The resultant traces are illustrated by the examples shown in Fig. 1; gels run in the acid-urea were scanned because of the multiple banding of alkylated heavy chains in SDS at neutral pH (see above). The amount of each protein component (Table 1) was estimated by summation of the area under the peaks. Use of this method of quantitative measurement is justified by the fact that the uptake of dye by myeloma protein, light chains and heavy chains is of a similar order; reduction with 0.1M-mercaptoethanol yielded 31% light chain, 65% heavy chain and 4% heavy-chain dimer (Table 1), and thus gave results in agreement with a theoretical calculation based on the four-chain model of IgG and approximate molecular weights of heavy and light chains (50 000 and 20 000

respectively) (see Cohen & Porter, 1964). The two new protein components were identified by taking into consideration the relative release of light chains with 1-10mM-mercaptoethanol (Table 1). Thus 5mM-mercaptoethanol attacked almost 90% of the myeloma protein and released 52% of the light chains but only 7% of the heavy chains; with 10mM-mercaptoethanol 86% of the light chains were released and only 14% of the heavy chains, and the faster of the two new protein bands was present in higher concentration (Table 1 and Plate 1).

The ready susceptibility to reduction of L-H disulphide bonds of G-myeloma protein 5563 is apparent in these analyses. Removal of one or both of the light chains from IgG would (according to the four-chain model) result in the structures L-H-H and H-H respectively. That the two new components resulting from release of light chain correspond to these two structures is supported by their relative electrophoretic mobilities, their yields relative to free light chains and the order and mode of their appearance and disappearance; these points are expanded in the Discussion section.

Other mouse IgG molecules. To find out whether G-myeloma protein 5563 represented a special case or whether its properties were characteristic of mouse IgG in general, we applied the same techniques for analysis of the stability of the interchain disulphide bonds to low concentrations of reducing agent to another G_{2a}-myeloma protein (AdjPC-5 tumour line) (not illustrated) and to heterogeneous IgG isolated from mouse serum (Plate 2a). In both instances most of the light chain was released in 10mM-mercaptoethanol as described above for the 5563 myeloma protein. Owing to the heterogeneity of mouse IgG from pooled immune sera the heavy-chain products could not be analysed as clearly. The banding of light chain previously reported at neutral pH (Cohen & Gordon, 1965) was suppressed by the presence of SDS. The lability of the L-H disulphide bond to low concentrations of mercaptoethanol thus appears to be characteristic of mouse IgG in contrast with rabbit IgG as described below.

Fig. 1. Microdensitometry traces of stained protein bands on polyacrylamide gels. (a) Gel J (Plate 1), 10mM-mercaptoethanol, and (b) gel K (Plate 1), 0.1M-mercaptoethanol, are illustrated. Origin and anode are on the right.

Table 1. Analysis of reduction products of 5563 G-myeloma protein

Concn. of 2-mercaptoethanol (mm)	Percentage of IgG as					Percentage of total light chain dissociated as free L*	Percentage of total heavy chain dissociated as free H*
	L	H	H-H	L-H-H	(L-H) ₂ (i.e. IgG)		
1	10	1.5	14.5	38	36	32	2
5	16	5	46.5	20	12.5	52	7
10	26.5	9.5	42	11.5	10.5	86	14
100	31	65	4	0	0	100	94

* Calculated on the basis that IgG contains 31% light chains and 69% heavy chains.

Table 2. *Analysis of reduction products of rabbit IgG*

Analysis was as described in Table 1 except that the sample treated with 0.2M-mercaptoethanol was analysed by electrophoresis in SDS-urea-phosphate, pH 7.2.

Concn. of 2-mercaptoethanol (mM)	Percentage of IgG as			
	L	H	L-H	(L-H) ₂ (i.e. IgG)
1	Not detectable		34	66
10	Not detectable		87	13
200	27	63	10	0

Partial reduction of rabbit IgG

The electrophoretic patterns of a series of reduction mixtures of rabbit IgG run under either neutral conditions in the presence of SDS or acidic conditions in the presence of urea are shown in Plate 3. There is a marked difference in the extent to which partially reduced IgG dissociates under the two sets of conditions, and this suggests that the 0.1% SDS-0.5M-urea is not always sufficient to dissociate chains linked only by non-covalent bonds. After reduction with 1mM- or 10mM-mercaptoethanol rabbit IgG dissociated only slightly in SDS at neutral pH, to give one new minor component only. This component, however, had a mobility corresponding closely with that of the protein product of mouse myeloma MOPC 47A (Potter & Kuff, 1964), which has been characterized as having a sedimentation coefficient of 4.7s (at neutral pH) and consisting of one light and one heavy chain, i.e. 'half-molecules'; although this marker protein was from mouse, the size of half-molecules produced from rabbit IgG should be similar. When the same reduction mixtures were run in acid-urea much more extensive dissociation of non-covalent linked sub-units was observed; again only one new band was produced, having a mobility of the order of that expected for half-molecules, and no free light or heavy chains were detectable. After reduction with 10mM-mercaptoethanol 87% of rabbit IgG was dissociated by acid-urea into half-molecules (L-H) (Table 2). The quantitative results are in good agreement with the findings obtained by analytical ultracentrifugation (Hong & Nisonoff, 1965). Nisonoff and co-workers (Palmer, Nisonoff & Van Holde, 1963; Hong & Nisonoff, 1965) showed that dissociation into half-molecules occurs at pH 2.5 but not at neutral pH. It can be seen in Plate 3 that the addition of 0.1% of SDS at neutral pH did not result in quantitative dissociation into half-molecules, but 5.5M-urea in 0.1M-formate buffer, pH 2.5, did cause complete dissociation. However, after extensive reduction of interchain disulphide bonds with 0.2M-mercaptoethanol, dissociation into free heavy and light chains was effected by SDS at neutral pH (Plate 3 and Table 2). Electrophoresis in

acid-urea failed to resolve heavy and light chains of fully reduced rabbit IgG, a single band being obtained moving with approximately twice the mobility of half-molecules.

IgG of other species

The techniques of partial reduction with mercaptoethanol followed by gel-electrophoretic analysis were applied to IgG from man and guinea pig to compare the relative stabilities of their interchain disulphide bridges to reducing agents with those of the two widely different species described above (mouse and rabbit).

Normal human IgG, a human cryoglobulin G and three human G-myeloma proteins were examined. Extensive release of free light chains after reduction with 10mM-mercaptoethanol and alkylation was apparent in all these cases. A characteristic pattern obtained with human IgG is illustrated in Plate 2(b).

With purified guinea-pig IgG (γ_2), a similar lability of the L-H disulphide bond was evident in 10mM-mercaptoethanol reduction mixtures. The relative intensity of the free heavy-chain protein band, however, suggests that the H-H bond is more susceptible to reduction in guinea-pig IgG than in mouse or human IgG. In heterogeneous mixtures of IgG molecules, heavy-chain analysis is not as clear-cut as with a homogeneous myeloma protein and one has to keep in mind the possibility that there is some variation in H-H bond stability between different molecules within a given species, class or subclass of IgG molecules.

Reduction of mouse IgG with 2-mercaptoethylamine or thioglycollate

To test whether the charge on the reducing agent affected the preferential reduction of certain bonds, mouse G-myeloma protein 5563 was reduced with various concentrations of either 2-mercaptoethylamine or thioglycollate. The reduction mixtures were analysed by electrophoresis in the acid-urea gels; the amounts of each protein component obtained after reduction at two different concentrations of each reagent are given in Table 3. The pattern of dissociation giving predominantly free

Table 3. Action of mercaptoethylamine and thioglycollate on G-myeloma protein 5563

The reduction products were analysed as described in Table 1.

Concn. of 2-mercaptoethylamine (mM)	Percentage of IgG as				
	L	H	H-H	L-H-H	(L-H) ₂ (i.e. IgG)
10	24	6	46	15	9
50	26	33	30	6.5	4.5
Concn. of thioglycollate (mM)					
10	26	7	47	12	8
50	31	63	6	0	0

light chains and heavy-chain dimers was remarkably similar at a concentration of 10mM-2-mercaptoethylamine, 10mM-2-mercaptoethanol or 10mM-thioglycollate. There does, however, appear to be a difference in the susceptibility of the H-H bond in heavy-chain dimers depending on the reducing agent: thus an increase in concentration of thioglycollate from 10mM to 50mM led to almost quantitative reduction of heavy-chain dimer to free heavy chain, whereas 50mM-2-mercaptoethylamine leaves a considerable proportion of the heavy-chain dimer unreduced (Table 3).

DISCUSSION

The present studies on mouse G_{2a}-myeloma protein 5563 have shown that the two types of interchain disulphide bond differ markedly in their lability to reduction; the bonds between light and heavy chains are much more susceptible to reduction than the bonds between the heavy chains. This is in contrast with the behaviour of rabbit IgG, for which it has previously been shown that the inter-heavy-chain disulphide bonds are preferentially reduced (Palmer *et al.* 1963; Hong & Nisonoff, 1965).

The observed preferential release of light chains from mouse IgG would, as mentioned above, result in the formation of heavy-chain dimers, with L-H-H as an intermediate. Two new components were indeed observed in partial reduction mixtures and are assigned the structures L-H-H and H-H (see Plate 1); the properties of these two components support those designations.

(i) The electrophoretic mobilities of the two new components are only slightly greater than that of myeloma globulin G, and are not as great as would be expected for half-molecules; this is especially meaningful in SDS at neutral pH, where the sieving effect of polyacrylamide gel orders protein components primarily according to molecular size.

(ii) The determination of the partial reduction products (Table 1) allows us to estimate the com-

position of the two new components. In the 10mM-mercaptoethanol reduction mixture, 86% of the light chains of the original IgG were present as free light chains and a further 10% of light chains remained in unreduced IgG; only 14% of total heavy chains were found free, so the two new components together contained approximately 80% of the heavy chains of the original IgG (7% of heavy chains remained in unreduced IgG) and only 4% of the total light chains.

(iii) As the concentration of mercaptoethanol is increased the relative amounts of the two new components are consistent with their assigned structures; thus with only one-third of the total light chain released (at 1mM-mercaptoethanol) the slower component (L-H-H) predominated, whereas at higher concentrations of mercaptoethanol, when increasing amounts of free light chains were present, the faster component (H-H) predominated.

With rabbit IgG the same method of analysis confirmed the results of Hong & Nisonoff (1965) (see Plate 3 and Table 2); at low concentrations of mercaptoethanol, rabbit IgG was split into half-molecules, showing the lability of the inter-heavy-chain disulphide bonds relative to the L-H bonds. Mouse G-myeloma protein 5563 and rabbit IgG appear to represent extreme alternative possibilities of stepwise reduction thus:

Both in our experiments and in those of Hong & Nisonoff (1965) the labilities of interchain bonds were measured as a function of concentration of reducing agent, with other parameters of time, temperature etc. kept constant. At a concentration of 2-mercaptoethanol (10mM) with which the major reduction products of the mouse 5563 myeloma protein were heavy-chain dimers and free light chains, the ratio of the extent of reduction of bonds between light and heavy chains to that of bonds between the two heavy chains was greater

than 6:1; from the data of Hong & Nisonoff (1965) one can calculate that in rabbit IgG under similar conditions the extent of reduction of the H-H interchain bonds is more than 5 times that of the L-H bonds.

In assessing the reasons for differential lability of interchain disulphide bonds one would expect the following two factors to be important: (i) the primary sequence in the vicinity of the disulphide bond; (ii) the overall folding of the polypeptide chains. The nature of the amino acid residues adjacent to the two half-cystine residues will influence the strength of the disulphide bond formed; on the other hand, the configuration of the whole protein may be such as to hinder the approach of reducing agents to the disulphide bond, or to increase the resistance of the bond to reduction by strong non-covalent interactions (see discussion by Cecil, 1963; Hong & Nisonoff, 1965).

Evidence having a bearing on these points comes from our comparison of the reduction patterns of (i) different molecular species of IgG from the same biological species and of (ii) pooled heterogeneous IgG, each pool from a different biological species. These data support the view that the pattern of relative labilities of the two types of interchain disulphide bond is characteristic of the species from which the IgG is derived and is relatively constant for different IgG molecules from the same animal. This generalization is consistent with the fact that the interchain disulphide bonds of IgG are located in regions of the polypeptide chains that have been shown to have constant amino acid sequences for a given biological species. However, it should be realised that the generalization is based on only a limited survey and it may be that there are subtle differences of lability between molecular species of different subclasses or even within a given subclass; it has, for instance, been suggested that in some molecules of pooled rabbit IgG the stability of the inter-heavy-chain disulphide bonds may be greater than in the majority of the molecules (Palmer & Nisonoff, 1964; Nisonoff & Dixon, 1964; Hong & Nisonoff, 1965).

The use of different reducing agents with different charges revealed additional differences in the relative stability of interchain disulphide bonds. One difference reported here is the greater susceptibility of the H-H interchain bond of mouse 5563 G-myeloma protein to reduction by thioglycollate than to reduction by mercaptoethylamine; this comparison was made at similar pH values. Differences in the reduction pattern of rabbit IgG when either mercaptoethanol or mercaptoethylamine was being used were noted by Hong & Nisonoff (1965), but these authors attributed the differences, at least in part, to the different pH values at which the reagents were used.

The use of polyacrylamide-gel electrophoresis for the separation and quantitative measurement of IgG partial-reduction mixtures greatly facilitated the present studies. By selecting the appropriate pH and dissociating agent all the possible intermediates can be separated. This method provides a rapid way of analysing the relative stability of the interchain disulphide bonds in any IgG without measurement of the extent of disulphide bond cleavage, which is complicated at higher concentrations of reducing agent by partial splitting of intrachain disulphides.

The conditions described here for the production of heavy-chain dimers from mouse IgG, and also from IgG of other species, open the possibility of studying the chemical and biological properties of heavy chain in a more native form. We have reported preliminary evidence that heavy-chain dimers may be an intermediate in the biosynthetic assembly of G-myeloma globulin 5563 (Askonas & Williamson, 1967).

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