Biochem. J. (1980) **187**, 775–780
Printed in Great Britain

Dimeric, Trimeric and Tetrameric Complexes of Immunoglobulin G Fix Complement

J. Keith WRIGHT,* Jürg TSCHOPP,† Jean-Claude JATON‡ and Jürgen ENGEL†
**Max-Planck-Institute for Biology, D-7400 Tübingen, Federal Republic of Germany, †Department of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland, and ‡Department of Pathology, Faculty of Medicine, University of Geneva, CH-1211 Geneva, Switzerland*

(*Received 7 September 1979*)

The binding of pure dimers, trimers and tetramers of randomly cross-linked non-immune rabbit immunogloblin G to the first component and subcomponent of the complement system, C1 and C1q respectively, was studied. These oligomers possessed open linear structures. All three oligomers fixed complement with decreasing affinity in the order: tetramer, trimer, dimer. Complement fixation by dimeric immunoglubulin exhibited the strongest concentration-dependence. No clear distinction between a non-co-operative and a co-operative binding mechanism could be achieved, although the steepness of the complement-fixation curves for dimers and trimers was better reflected by the co-operative mechanism. Intrinsic binding constants were about $10^6 M^{-1}$ for dimers, $10^7 M^{-1}$ for trimers and $3 \times 10^9 M^{-1}$ for tetramers, assuming non-co-operative binding. The data are consistent with a maximum valency of complement component C1 for immunoglobulin G protomers in the range 6–18. The binding of dimers to purified complement subcomponent C1q was demonstrated by sedimentation-velocity ultracentrifugation. Mild reduction of the complexes by dithioerythritol caused the immunoglobulin to revert to the monomeric state ($s_{20,w} = 6.2$–6.5 S) with concomitant loss of complement-fixing ability.

In the absence of antigen, IgG interacts only weakly with the first complement component, C1 (Schumaker *et al.*, 1976). The binding of multivalent antigen to antibody leads to activation of the complement system. Antigen may function merely by aggregating antibody into an array, whose effective valence for interaction with multivalent partners is increased. Such complexes would bind with an increased overall affinity to complement component C1 or membrane receptors by virtue of multiple binding interactions. This aggregative model for the mode of antibody action is a tenable alternative to a putative allosteric model (Metzger, 1974, 1978). Experiments have indicated (*a*) that neither hapten nor antigen binding alters the conformation of the hinge peptides of IgG (Wright *et al.*, 1978*a*), (*b*) that the interactions of Fab- and Fc-specific ligand with antibody are independent (Wright *et al.*, 1978*b*), and (*c*) that the inter-heavy-chain and heavy–light-chain disulphide bonds are not indispensable for complement fixation (Wright, 1978). Such observations favour a passive, albeit specific, role for antigen in an aggregative model of antibody function.

Abbreviation used: IgG, immunoglobulin G.

An allosteric role for antigen has been favoured by certain spectroscopic studies (Schlessinger *et al.*, 1975; Jaton *et al.*, 1975; Lancet & Pecht, 1976), by complement-fixation data (Brown & Koshland, 1975) and the accessibility of the Fc-associated J-chain in immunoglobulin M to specific antibody (Brown & Koshland, 1977). A hypothesis was advanced on the basis of composite X-ray data depicting a putative allosteric change triggered by antigen binding (Huber *et al.*, 1976). More recent X-ray studies, however, have demonstrated that certain structural features of IgG hinder the transmission of a conformational change from the Fab to the Fc region (Silverton *et al.*, 1977) and that the relative arrangement of variable and constant modules is variable, suggesting a lack of strong interactions between adjacent domains that could form the basis of a relay system between the Fab and Fc domains (Matsushima *et al.*, 1978).

Hyslop *et al.* (1970), however, had shown that tetramers were the smallest species of antibody cross-linked by a bivalent hapten that fixed complement. Poorly defined non-covalently linked dimers and trimers isolated from heat-aggregated IgG (Barandun *et al.*, 1962) showed some comple-

0306-3275/80/060775-06$01.50/1

ment-fixation ability. Barring specific geometric requirements in the structure of IgG oligomers for binding to complement component C1, stable randomly cross-linked dimers and trimers of rabbit antibody should fix complement at concentrations consistent with their expected affinities for component C1. The experiments reported in the present paper demonstrate that antibody dimers fix complement and bind to complement subcomponent C1q.

Materials and Methods

Proteins

IgG and IgG oligomers cross-linked by dithiobis(succinimidylpropionate) were prepared by method B (without antigen) by Wright *et al.* (1980). The ratio of cross-linker to IgG and the concentration of IgG are critical parameters. For the preparations used in the present study, the molar ratio of cross-linker to IgG was 1.74–3.1 and the concentration of cross-linker was 0.47–0.62 mM. At a ratio of 4.7, oligomers were formed in excellent yields, but these oligomers showed very low binding affinity to component C1. Reduced and alkylated oligomers of IgG were prepared by treatment with 10 mM-dithioerythritol for 2 h and subsequent alkylation by iodoacetamide (50 mM). The reactants were removed by dialysis against complement-fixation buffer. Subcomponent C1q was purified from an euglobulin precipitate of human serum by the method of Assimeh *et al.* (1974).

Complement fixation

The complement-fixing abilities of purified IgG oligomers were tested by a modification of the micro-complement-fixation technique (Levine & van Vunakis, 1967). The diluent employed in these studies consisted of 2 parts of the standard diluent plus 1 part of a solution containing 0.15 M-saccharose, 10 mM-NaCl, 0.15 mM-$CaCl_2$ and 0.5 mM-$MgCl_2$ in 10 mM-Tris/HCl buffer, pH 7.4. To 0.05 ml samples of oligomer, 0.10 ml of a 1:20 dilution of guinea-pig serum was added, and the solutions were kept for 18 h at 4°C. Finally 0.50 ml of a suspension of 2×10^7 sensitized sheep erythrocytes/ml was added, and the samples were shaken for 30 min at 25°C. The samples were rapidly cooled in ice and centrifuged. The absorption of the supernatant solution at 413 nm was read.

Ultracentrifugation

Sedimentation-velocity experiments were performed in a Beckman model E analytical ultracentrifuge at 20°C with absorption optics. IgG oligomers and subcomponent C1q were dissolved in 10 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl. Reported sedimentation coefficients have been corrected to $s_{20,w}$ values.

Results

The dimers, trimers and tetramers of rabbit IgG fixed complement (Fig. 1). The data are presented in plots of $Y/(1-Y)$, where Y is the degree of lysis, versus logarithm of the total concentration of antibody oligomer after the addition of erythrocytes. The complement-fixation curves for dimer, trimer and tetramer had different profiles. If only an undetectable amount of tetramer in the samples of dimer or trimer were responsible for complement fixation, then the fixation curves for the smaller oligomers should have the same profiles as that for the tetramer and be merely shifted to the right.

Actually, the complement-fixation curves become steeper with decreasing size of the oligomer. This behaviour is typical of a system in which the valence of one partner (here the IgG oligomer) in the association is lowered. The greater number of molecules required to saturate the other partner (component C1 or subcomponent C1q) results in a more pronounced concentration-dependence of the binding. Thus the different appearance of the complement-fixation curves for dimeric, trimeric and tetrameric IgG aggregates may reflect the different valences and affinities of each of these oligomers for component C1. Estimates of these parameters may be derived from the complement-fixation data.

The binding of IgG oligomers (G) to component C1 (C1) may be described by the following scheme:

$$n\mathrm{G} + \mathrm{C1} \overset{K_1}{\rightleftharpoons} (n-1)\,\mathrm{G} + \mathrm{C1\text{–}G_1} \overset{K_2}{\rightleftharpoons} \cdots \overset{K_n}{\rightleftharpoons} \mathrm{C1\text{–}G_n} \qquad (1)$$

Here n is the maximum number of oligomers that can be accommodated at the component C1 molecule.

Two simple special cases of scheme (1) are used below to fit the experimental data.

(1) Isodesmic model, with all binding constants equal ($K_1 = K_2 = K_n = K$). For this model the ratio of free component C1 concentration ([C1]) to total component C1 concentration ($[\mathrm{C1}]_0$) is given by (Van Holde, 1971):

$$\frac{[\mathrm{C1}]}{[\mathrm{C1}]_0} = \frac{1}{(1+[\mathrm{G}]K)^n} \qquad (2)$$

with:

$$[\mathrm{G}] = \tfrac{1}{2}([\mathrm{G}]_0 - n[\mathrm{C1}]_0 - K^{-1} + \sqrt{(n[\mathrm{C1}]_0 + K^{-1} - [\mathrm{G}]_0)^2 + 4K^{-1}[\mathrm{G}]_0})$$

(2) All-or-none model, for which, owing to a high co-operativity, the concentrations of all complexes other than $C1–G_n$ are negligible. Then the equilibrium is described by a single apparent binding constant $K_{app.}$ (Van Holde, 1971):

$$nG + C1 \overset{K_{app.}}{\rightleftharpoons} C1G_n \qquad (3)$$

and:

$$\frac{[C1]}{[C1]_0} = \frac{1}{1 + K_{app.}[G]^n} \qquad (4)$$

where [G] is obtained from the relation:

$$[G]_0 = n[C1]_0 \frac{K_{app.}[G]^n}{1 + K_{app.}[G]^n} + [G]$$

For a better comparison of the two models it is assumed that $K_{app.} = K_{av.}{}^n$, where $K_{av.}$ is an average intrinsic binding constant of the single steps in scheme (1). It has to be remembered, however, that in order to satisfy the all-or-none condition a difficulty of nucleation has to be introduced for the first steps. This may be energetically compensated for by a more favourable binding in the last steps.

The total component C1 concentration in guinea-pig serum is only approximately known. It may be estimated from the subcomponent C1q concentration in human serum, which is about 70–180 μg/ml (Ziccardi & Cooper, 1977). Under our experimental conditions (120-fold dilution of the serum) a component C1 concentration of about 1 nM is estimated this way. When $n[C1]_0 < K^{-1}$, little or no dependence of $[C1]/[C1]_0$ on $[C1]_0$ is predicted by eqn. (2) or eqn. (4). In fact, for sufficiently small component C1 concentrations, the expression $[C1]/[C1]_0$ is independent of $[C1]_0$.

Isodesmic model:

$$\frac{[C1]}{[C1]_0} = \frac{1}{(1 + K[G]_0)^n}; \qquad [C1]_0 \ll K \qquad (5)$$

All-or-none model:

$$\frac{[C1]}{[C1]_0} = \frac{1}{1 + (K[G]_0)^n}; \qquad [C1]_0 \ll K \qquad (6)$$

In order to relate the calculated ratio $[C1]/[C1]_0$ to the experimentally observed degree of lysis, Y, the empirical van Krogh equation (Kabat & Mayer, 1971) was used:

$$\frac{Y}{1-Y} = \frac{Y_0}{1-Y_0}\left(\frac{[C1]}{[C1]_0}\right)^S \qquad (7)$$

Y_0 is the degree of lysis in the absence of IgG oligomers. The empirical parameter S (Kabat & Mayer, 1971) was determined to be 0.52 in a separate experiment. Note that eqn. (7) implies that only completely free component C1 is effective in lysis. This will lead to an overestimate of the effectiveness of complement fixation when imcompletely saturated component C1 complexes in scheme (1) can also participate in the lysis reaction.

Fig. 1 shows a comparison of the experimental results with theoretical curves calculated for the two model mechanisms and various values of n and K. The fitting of curves to the experimental results was performed with eqn. (2) or eqn. (4), assuming $[C1]_0 = 1$ nM. Since in most cases $[C1]_0 \ll K^{-1}$, the value of $[C1]_0$ is not critical and the simplified eqn. (5) or eqn. (6) could also be used. An exception is the inhibition curve of the tetramers, for which K^{-1} is of the same order of magnitude as $[C1]_0$. The parameters K (or $K_{av.}$) and n used for the fitting are summarized in Table 1.

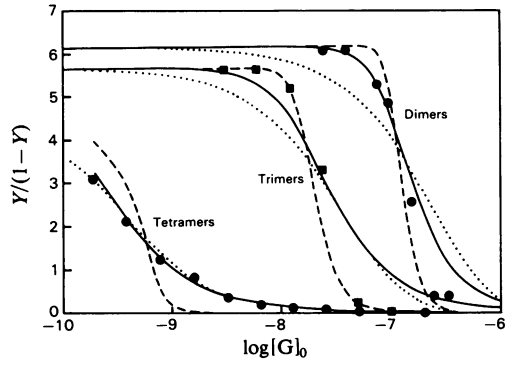

Fig. 1. *Complement fixation by dimers, trimers and tetramers of randomly cross-linked rabbit IgG*

To dilution series of tetramer, trimer and dimer in complement-fixation buffer (low ionic strength, without albumin), guinea-pig serum was added to a final dilution of 1:120 (total volume 0.60 ml). After an 18 h incubation at 4°C, 0.50 ml of sensitized erythrocytes (2×10^7 cells/ml) was added, and the suspensions were shaken at 25°C for 30 min. The results are presented in a plot of $Y/(1-Y)$, where Y is the fraction of lysis, versus $\log[G]_0$, where $[G]_0$ is the final molar concentration of antibody in the presence of the erythrocytes. Dimers, trimers and tetramers fixed complement with increasing affinity. Monomeric antibody isolated from the coupling mixture did not fix complement at a final concentration of 0.9 μM. Theoretical curves were calculated for the isodesmic model (all intrinsic binding constants equal) with eqns. (2) and (7) and $n = 9$ for dimers, $n = 6$ for trimers and $n = 2$ for tetramers (·····). Curves for this all-or-none model were calculated with eqn.s (4) and (7), assuming $n = 3$, 2 and 1.5 (——) and $n = 9$, 6 and 2.5 (– – – –) for dimers, trimers and tetramers respectively. The other fitting parameters are listed in Table 1.

Table 1. *Analysis of complement-fixation data assuming non-co-operative isodesmic or highly co-operative binding of IgG oligomers to complement component C1*

n is the maximum number of oligomers that can bind to a component C1 molecule and $n' = n \cdot m$ is the maximum number of IgG protomers that can be accommodated. The parameters listed correspond to the theoretical curves shown in Fig. 1. The values by which a reasonable fit to the experimental results were obtained are underlined. K is the intrinsic equilibrium constant for the independent binding of an IgG oligomer to component C1 and $K_{av.}$ is the average intrinsic binding constant of the individual binding steps in an all-or-none binding mechanism.

	Isodesmic binding (scheme 1)			All-or-none binding (scheme 3)		
IgG oligomers $(IgG)_m$	n	n'	K (M^{-1})	n	n'	$K_{av.}$ (M^{-1})
Dimer, $m = 2$	9	18	9×10^5	$\frac{3}{9}$	$\frac{6}{18}$	9×10^6
Trimer, $m = 3$	6	18	9×10^6	$\frac{2}{6}$	$\frac{6}{18}$	6×10^7
Tetramer, $m = 4$	2	8	3×10^9	$\frac{1.5}{2.5}$	$\frac{6}{10}$	7.5×10^9

As an unambiguous demonstration that immunoglobulin dimers bound complement, IgG dimer and subcomponent C1q were mixed and examined in the ultracentrifuge (Fig. 2). IgG dimer and subcomponent C1q possessed similar sedimentation coefficients [9.6S (Fig. 2*a*) and 9.4S (Fig. 2*b*) respectively]. A mixture containing 0.5 μM-IgG dimer and 0.7 μM-subcomponent C1q yielded a much broader sedimentation boundary (average $s_{20,w}$ = 13.9S). Very little material of 9.4–9.6S could be detected. From the complete or almost complete removal of free subcomponent C1q from the reaction mixture at the concentrations given above, a binding constant $K > 10^6 M^{-1}$ may be estimated for the binding of IgG dimers to subcomponent C1q.

To demonstrate that the complement-fixing ability of the IgG oligomers arose solely from the multivalency of the oligomers and was not due to some structural modification caused by the cross-linking procedure, the complexes were dissociated by mild reduction of the cross-linker (cf. Lomant & Fairbanks, 1976). Cleavage of the cross-linker led to the dissociation of the oligomers (Table 2), as manifested by the decreases in the sedimentation coefficients to values characteristic of monomeric IgG. Concomitant with the transformation of the oligomers into monomer was the loss of complement-fixing ability (Table 2). Since monomeric IgG isolated from the coupling mixture and monomeric IgG derived from the oligomers by reduction of the cross-linker did not fix complement, the complement-fixing ability of the oligomers was not ascribable to structural alterations in the protein occasioned by the cross-linking procedure. IgG inter-chain disulphide bonds are also reduced under the conditions employed to abolish the cross-linking (Press, 1975). However, reduced and alkylated

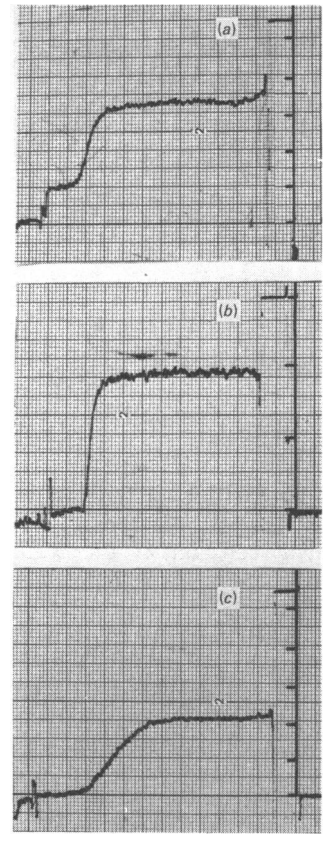

Fig. 2. *Demonstration of complement subcomponent C1q binding to dimeric antibody in the ultracentrifuge*

Sedimentation profiles of dimeric IgG (1 μM) (*a*), subcomponent C1q (1.4 μM) (*b*) and a mixture of 0.5 μM-IgG dimer and 0.7 μM-subcomponent C1q (*c*) were determined with the u.v. scanning optics. The binding of subcomponent C1q to IgG dimer was evidenced by the disappearance of the sharp boundary of subcomponent C1q (*b*) and the appearance of a distribution of more rapidly sedimenting material (*c*) with sedimentation coefficients between 11.6 and 17.2S, in contrast with the more slowly sedimenting IgG dimers (9.6S) and subcomponent C1q (9.4S). The solvent was 150mM-NaCl/10mM-Tris/HCl buffer, pH 7.4. Scans were recorded 16min after the rotor attained a speed of 50000rev./min. The temperature was 20°C. Divisions on the axes to the right correspond to 0.2 A_{280} unit.

Table 2. *Effect of reduction of cross-linking agent on IgG oligomers*

Samples of IgG were treated with dithioerythritol and alkylated with iodoacetamide as described in the Materials and Methods section. Sedimentation coefficients were determined at 20°C with the absorption optics. The observed sedimentation coefficients were corrected to $s_{20,w}$ values. Samples were tested for complement-fixing ability as described in the Materials and Methods section and in Fig. 1. The concentrations of samples at the end of the tests were: 0.8 μM-monomer, 0.2 μM-dimer, 0.1 μM-trimer and 0.02 μM-tetramer. Untreated and reduced and alkylated samples were present at the same concentration for each species. Complement-fixing ability is given by $100Y(1-Y)$, where Y is the fraction of indicator cells lysed.

	$s_{20,w}$ (S)		Complement-fixing ability	
Species	Unreduced	Reduced	Unreduced	Reduced
Monomer*	6.5	6.2	0	1
Dimer	9.6	6.4	67	3
Trimer	11.2	6.5	94	5
Tetramer	13.6	6.3	96	2

* Monomeric IgG isolated from the cross-linking reaction.

antibody fixes and activates complement under the conditions of the complement-fixation test (Wright, 1978), strongly suggesting that the inability of the IgG from dissociated complexes to fix complement was due to this particular modification.

Discussion

Dimers, trimers and tetramers of randomly cross-linked rabbit immunoglobulin fixed complement in the absence of antigen. These antibody oligomers probably possessed open linear geometries. Dimeric antibody containing no antigen was observed to bind to subcomponent C1q in the ultracentrifuge with a binding constant $K \geqslant 10^5 M^{-1}$. Dissociation of these oligomers by reduction of the cross-linking agent removed the complement-fixing ability of the IgG. Several features of this investigation may account for the discrepancy between the observation that dimers of antibody fix complement (present work) and the assertion that only tetrameric or larger complexes fix complement (Hyslop *et al.*, 1970; Segel *et al.*, 1979). The antibody oligomers, whose preparation was described in the accompanying paper (Wright *et al.*, 1980), were covalently cross-linked and possessed open structures (Wright *et al.*, 1980). The long incubation and low concentration of antibody used in the micro-complement-fixation test would favour dissociation of IgG oligomer–component C1 complexes. This is of importance, since most oligomers used before were cross-linked by dissociable bivalent haptens (Hyslop *et al.*, 1970; Jaton *et al.*, 1976). The concentrations of complement and antibody employed to obtain the complement-fixation results reported in the present paper were higher than those normally employed (Segal *et al.*, 1979), as the expected weaker binding of component C1 to dimeric antibody on pure thermodynamic grounds required. Also, the length of the incubation with sensitized erythrocytes was kept short in order to minimize the perturbation of the IgG oligomer–component C1 binding equilibria by the cell-bound immunoglobulin aggregates. This distortion of the complement-fixation curves would be especially serious with dimers because of their intrinsically lower affinity for component C1.

Complement-fixation curves clearly demonstrated that the affinity of the oligomers increased in the order dimer, trimer and tetramer. The steepness of the curves decreased in the same order. The latter feature can be explained by the fact that less tetramers can be accommodated at the component C1 molecule than trimers or dimers. The maximum number of binding sites for IgG protomers is 18. This number can be derived from symmetry considerations (each subcomponent C1q head is composed of three chains), and it was experimentally derived from binding studies with monomeric IgG (Schumaker *et al.*, 1976). If the maximum number of binding sites of subcomponent C1q can be saturated, a maximum number of 9 dimers, 6 trimers or 4 tetramers can bind to component C1. It has to be realized, however, that the maximum number of protomers that can be actually accommodated at the component C1 molecule may be lower than the number of binding sites because of steric hindrance. Also, different binding modes that involved only one or both C_H2 domains of each IgG molecule are possible. Additional steric restrictions will probably be imposed by the cross-links in the IgG oligomers. These are particularly difficult to estimate for our experimental system, since oligomers that are homogeneous in molecular weight may be heterogeneous with respect to the arrangement of cross-links. Care was taken to introduce only a minimum number of cross-links. The average degree of modification was between 1 and 1.5 per IgG protomer (Wright *et al.*, 1980). Part of the excess modification may be due to cross-linker molecules that reacted with one side only or twice with the same molecules. A large decrease of the affinity towards subcomponent C1q was observed when the number of cross-links was increased by higher concentration of the cross-linking reagent (more than 4 mg/ml; see the Materials and Methods

section). Dithiobis(succinimidylpropionate) can react with the many lysine residues at the surface of IgG. When two lysine residues are cross-linked the maximum distance between their α-carbon atoms is 2.2nm (22Å). This is calculated for an extended-chain conformation with average bond distances and bond angles. By using these dimensions of a flexible connection it is concluded that in most of the many possible linearly cross-linked species the C_H2 domains are sufficiently spaced and separated to allow for multiple interactions with the subcomponent C1q heads. The restrictions imposed by the cross-links are probably less severe than may be believed, because of the high flexibility between IgG domains (Huber *et al.*, 1976) and the probable flexibility in subcomponent C1q. For the idealized case in which the dimer is exactly constructed to fit the requirements for multiple interactions with component C1, the binding energy should be almost twice of that for the monomer. Since binding of monomers was observed only at millimolar concentration (Schumaker *et al.*, 1976) and dimers bind in the micromolar range, the binding energy is indeed almost doubled. This supports the view of a flexible arrangement of the binding domains in IgG oligomers and component C1.

In a quantitative treatment of the complement-fixation data, a highly co-operative and a non-co-operative binding mechanism of the oligomers were compared (Table 1). Both models described the general features, such as the increasing steepness of the curves with decreasing oligomer size. The co-operative model yielded a better fit of the high steepness of the dimer and trimer curves than did the non-co-operative model. Even at the highest maximal valency, corresponding to 18 binding sites at subcomponent C1q, the curves generated by the latter model were too flat. The non-co-operative model gave a reasonable fit for the tetramer-fixation curve with a maximum valency of 2. For higher valencies too steep curves resulted for both models. For the tetramer, steric restrictions and the effect of overlapping binding sites (McGhee & van Hippel, 1974) will be most serious. A consistent quantitative fit of all data may be also hampered by the intrinsic difficulties and assumption of the complement-fixation assay (Kabat & Mayer, 1971). For these reasons the quantitative details of their complicated binding mechanism are still open.

The excellent technical assistance of Ms. E. Steffen and the co-operation of Mr. A. Lustig in performing the ultracentrifugation studies are gratefully acknowledged. This work was supported in part by grant no. 3.146.77 (to J.-C. J.) from the Swiss National Science Foundation.

References

Assimeh, S. N., Bing, D. H. & Painter, R. H. (1974) *J. Immunol.* **113**, 225–234

Barandun, S., Kistler, P., Jeunet, F. & Isliker, H. (1962) *Vox. Sang.* **7**, 157–162

Brown, J. C. & Koshland, M. E. (1975) *Proc. Nat. Acad. Sci. U.S.A.* **72**, 5111–5115

Brown, J. C. & Koshland, M. E. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5682–5686

Huber, R., Deisenhofer, J., Colman, P. M., Matsushima, M. & Palm, W. (1976) *Nature (London)* **264**, 415–419

Hyslop, N. E., Jr., Dourmashkin, R. R., Green, N. M. & Porter, R. R. (1970) *J. Exp. Med.* **131**, 783–802

Jaton, J.-C., Huser, H., Braun, D. G., Givol, D., Pecht, I. & Schlessinger, J. (1975) *Biochemistry* **14**, 5312–5315

Jaton, J.-C., Huser, H., Riesen, F. W., Schlessinger, J. & Givol, D. (1976) *J. Immunol.* **116**, 1363–1366

Kabat, E. A. & Mayer, M. M. (1971) *Experimental Immunochemistry*, pp. 135–145, Charles C. Thomas, Springfield

Lancet, D. & Pecht, I. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3549–3553

Levine, L. & van Vunakis, H. (1967) *Methods Enzymol.* **9**, 928–936

Lomant, A. J. & Fairbanks, G. (1976) *J. Mol. Biol.* **104**, 243–261

Matsushima, M., Marquart, M., Jones, T. A., Colman, P. M., Bartels, K., Huber, R. & Palm, W. (1978) *J. Mol. Biol.* **121**, 441–459

McGhee, J. D. & van Hippel, P. H. (1974) *J. Mol. Biol.* **86**, 469–489

Metzger, H. (1974) *Adv. Immunol.* **18**, 169–207

Metzger, H. (1978) *Contemp. Top. Mol. Immunol.* **7**, 119–152

Press, E. M. (1975) *Biochem. J.* **149**, 285–288

Schlessinger, J., Steinberg, I. Z., Givol, D., Hochmann, J. & Pecht, I. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2775–2779

Schumaker, V. N., Calcott, M. A., Spiegelberg, H. L. & Müller-Eberhard, H. J. (1976) *Biochemistry* **15**, 5175–5181

Segal, D. M., Guyer, R. L. & Plotz, P. U. (1979) *Biochemistry* **18**, 1830–1839

Silverton, E. W., Navia, M. A. & Davies, D. R. (1977) *Proc. Natl. Acad. U.S.A.* **74**, 5140–5144

Van Holde, K. E. (1971) *Physical Biochemistry*, pp. 57–64, Prentice–Hall, Englewood Cliffs

Wright, J. K. (1978) *Biochem. Biophys. Res. Commun.* **83**, 1284–1290

Wright, J. K., Jaton, J.-C. & Engel, J. (1978*a*) *Eur. J. Immunol.* **8**, 309–314

Wright, J. K., Brandt, D., Jaton, J.-C. & Engel, J. (1978*b*) *FEBS Lett.* **90**, 79–83

Wright, J. K., Tschopp, J. & Jaton, J.-C. (1980) *Biochem. J.* **187**, 767–774

Ziccardi, R. J. & Cooper, R. N. (1977) *J. Immunol.* **118**, 2047–2052