Molecular modelling of human complement subcomponent C1q and its complex with C1r₂C1s₂ derived from neutron-scattering curves and hydrodynamic properties

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Models for the structures of subcomponent C1q of first component C1 of human complement and its complex with subunit C1r₂C1s₂ are compared with experimental neutron-scattering curves. The length of the C1q collagenous arm is closer to 14.5 nm than to 11.5 nm proposed from electron microscopy, and this is consistent with the primary sequence of C1q. The mean C1q base–arm angle is 40–45° and C1q is found to be flexible: the base–arm angle can vary up to 30° from equilibrium at any moment. The complex of C1r₂C1s₂ and C1q requires a large shape change in C1r₂C1s₂. Ring-like models for C1r₂C1s₂ are not as successful at rationalizing the scattering data as are models that involve C1r₂C1s₂ binding to one side of C1q. Hydrodynamic calculations of the sedimentation coefficients for C1q and C1 are generally consistent with these neutron models.

The first component of complement C1 is a complex of two subunits, C1q and C1r₂C1s₂. C1q binds to IgG and IgM immunoglobulins in immune complexes in the antibody-dependent activation of the classical pathway of complement, whereas C1r₂C1s₂ is an inactive proteinase that becomes activated to C1r₂C1s₂ on the binding of C1q to immune complexes (Reid, 1983). Structural work on C1q and C1r₂C1s₂ will provide insights into their function. A major technique is electron microscopy; however, the resulting structures are observed *in vacuo* after several stages of preparation, staining, dehydration and interpretation procedures that can lead to structural artefacts, so confirmation by the use of other physical methods is required.

Solution scattering and hydrodynamic methods do not enable one to propose a unique structure, but have advantages in the study of macromolecules in the solution state and in narrowing the range of possible structures through the rejection of those that are inconsistent with experiment. This

procedure requires the calculation of scattering curves and sedimentation coefficients from computer models of C1q and C1r₂C1s₂ in which known stereochemical and biochemical constraints can be included. Preliminary calculations have been reported for C1q by Schumaker *et al.* (1976), Liberti & Paul (1978) and Gilmour *et al.* (1980). A rod-like computer model for the neutron curve of C1r₂C1s₂ was developed in Boyd *et al.* (1983), and this is extended here. Neutron-scattering data were presented for C1q and C1 by Perkins *et al.* (1984), and these, in combination with sedimentation data, are presently analysed.

Methods

Experimental data

Neutron-scattering data for C1q and C1 and their analyses were presented in full by Perkins *et al.* (1984); the experimental data of Figs. 4, 5 and 6 in the present study are taken from Fig. 5 of Perkins *et al.* (1984). Hydrodynamic sedimentation coefficients are the literature values (Table 2).

Scattering curves

The method of spheres is used to create models for C1q, C1r₂C1s₂ and C1 and to calculate their neutron-scattering curves (Perkins & Weiss, 1983).

Abbreviations used: the nomenclature of the components of the complement cascade are as recommended by the World Health Organisation (1981).

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This Debye calculation involves a convolution of a form-factor term for the spheres with a term corresponding to their lattice. It breaks down at large Q (where $Q = 4\pi\sin\theta/\lambda$, 2θ is the scattering angle and λ is the wavelength), when either the nominal resolution of the scattering curve becomes comparable with the diameter of the spheres, or at very large Q the Fourier transform of the lattice itself causes irregular intensity artefacts. In this work, the Debye spheres are of diameter 1.477 nm, which are small in contrast with the resolution of 9 nm in Figs. 4, 5 and 6 ($Q = 0.7\text{ nm}^{-1}$). The form-factor term for the spheres diminishes by only 7% over this Q range, and this was shown to have a negligible effect on the calculated curves of $\ln I$ versus Q , where I is the intensity of the scattering curve.

Hydrodynamic simulations

Sedimentation coefficients ($s_{20,w}^0$) are obtained from frictional coefficients by the expression:

$$s_{20,w}^0 = \frac{M_r(1 - \bar{v}\rho)}{N\langle f \rangle}$$

where M_r is the relative molecular mass, $\bar{v}$ is the partial specific volume, ρ is the solvent density, N is Avogadro's number and $\langle f \rangle$ is the frictional coefficient. Three approximate theories exist for the calculation of $\langle f \rangle$ for straight cylindrical rods (Boyd *et al.*, 1983) that correspond to Clr_2Cls_2 , of which the most recent and reliable one gives $\langle f \rangle$ by $3\pi\eta L\{\ln p + [(\gamma_{\parallel} + \gamma_{\perp})/2]\}$ and $p = L/d$, where L is the length of the rod, d is its diameter and η is the solvent viscosity (Garcia de la Torre & Bloomfield, 1981; Tirado & Garcia de la Torre, 1979). The terms $\gamma_{\parallel}$ and $\gamma_{\perp}$ are given by:

$$\begin{aligned}\gamma_{\parallel} &= -0.207 + 0.980p^{-1} - 0.133p^{-2} \\ \gamma_{\perp} &= 0.839 + 0.185p^{-1} - 0.233p^{-2}\end{aligned}$$

Frictional coefficients can also be calculated by using spheres. In principal, the use of spheres minimizes the dependence on semi-empirical expressions as above for $s_{20,w}^0$ and allows an explicit representation of the macromolecular shape to be evaluated. Two approaches are available.

(i) Kirkwood (1954) proposed an expression for the frictional coefficient of n equally sized spheres of hydrodynamic radius r . For structures containing n spheres of different radii r_i , r_s , Bloomfield *et al.* (1967a, b) gave an expression:

$$\langle f \rangle = \frac{6\pi\eta \left(\sum_{i=1}^n r_i^2 \right)^2}{\sum_{l=1}^n r_l^3 + \sum_{l=1}^n \sum_{s=1}^n r_l^2 r_s^2 \langle R_{ls}^{-1} \rangle}$$

where R_{ls} is the distance between subunits l and s and terms in $l=s$ are excluded in the double summation.

(ii) An improved treatment is available through Garcia de la Torre & Bloomfield (1977a), whose style for symbols is followed in the equations below. The Stokes-law frictional coefficient for a given sphere in a multisubunit structure is modulated by the hydrodynamic interactions of the other spheres in the structure, and the latter is allowed for by use of the Oseen tensor. Since the Oseen tensor considered each element as a point source of friction, this is modified in turn to allow for the finite, different sizes of the n elements. The frictional force F_i associated with the i th element of hydrodynamic radius r_i is:

$$F_i = 6\pi\eta r_i(\mathbf{u}_i - \mathbf{v}_i) \quad (i = 1, n)$$

where $\mathbf{u}_i$ is the velocity of the i th element and $\mathbf{v}_i$ is the velocity that the solvent would have at the position of the centre of the i th element if that element were absent. The hydrodynamic interaction tensor, $\mathbf{T}_{ij}$, when modified for spheres of finite sizes and of different hydrodynamic radii, r_i and r_j is:

$$\mathbf{T}_{ij} = \frac{1}{8\pi\eta R_{ij}} \left[\mathbf{I} + \frac{\mathbf{R}_{ij} \mathbf{R}_{ij}}{R_{ij}^2} + \frac{(r_i^2 + r_j^2)}{R_{ij}^2} \left(\frac{1}{3} \mathbf{I} - \frac{\mathbf{R}_{ij} \mathbf{R}_{ij}}{R_{ij}^2} \right) \right]$$

where $\mathbf{I}$ is the unit vector and $\mathbf{R}_{ij}$ is the vector between i and j . The interaction tensor is incorporated with F_i by:

$$\mathbf{v}_i = \mathbf{v}_i^0 - \sum_{j=1}^n \mathbf{T}_{ij} \mathbf{F}_j$$

(excluding terms with $i=j$), where $\mathbf{v}_i^0$ is the unperturbed solvent velocity when the other elements are absent. Computations were performed in this study by using the programs GENTRA and GENDIA kindly supplied by Professor V. A. Bloomfield (Garcia de la Torre & Bloomfield, 1977a,b), in which iterative procedures were used to extract the frictional coefficient $\langle f \rangle$. GENDIA utilizes the diagonal approximation, where the off-diagonal components of the Oseen tensor are usually much lower than the diagonal ones. Since GENTRA is significantly more costly in computer time and yet gave results similar to GENDIA, GENDIA was used routinely and its results were checked with GENTRA when appropriate.

Since the expression for $\mathbf{T}_{ij}$ is valid for $r_i + r_j \leq R_{ij}$, the Debye models for Clr_2Cls_2 and Clq were remodelled with non-overlapping spheres. Fewer spheres are used than in the Debye calculations, since the computer-capacity requirements are large. The total volume of the spheres equals that of the dry macromolecule, and the overall dimensions equal those of the Debye model. Since the

Table 1. Comparison of the results from electron microscopy studies on C1q with the C1q model from neutron scattering

Dimensions (nm) or angle (°)

Parameter	Reference ...	Svehag <i>et al.</i> (1972)	Shelton <i>et al.</i> (1972)	Knobel <i>et al.</i> (1975)	Brodsky-Doyle <i>et al.</i> (1976); Porter & Reid (1979)	Schumaker <i>et al.</i> (1981)	The present neutron study (Fig. 4)
Dimensions of heads		5.5–6	5–7.5	6–7	5 × 7*	—	6.0 × 3.6
Length of arm		10–11	10–13	10–13	11.5	14.2 ± 0.2†	14.3
Width of arm		1.5	1.5	1.5	1.5	—	1.2
Length of base		12.5	10–12.5	9–12	11.2	—	10.7
Width of base		5.5	3–6	4–6	4.5	4.7 ± 0.14	3.6
Angle between arm and base		—	—	—	41 ± 19	50 ± 12	40–45
Overall length		30	28–33	28–33	—	—	—
Overall width		33	27–38.5	35–40	—	—	—

* Length × width.

† This includes the length of half of the head.

Fig. 2. Schematic outline of one-sixth of C1q to indicate the molecular topology of C1q in terms of the spheres employed in modelling the Debye scattering curves

head, and the C1q stalks, using the C1q models with arms of either 11.9 nm or 14.3 nm in length. In all cases, the R_G is seen to be sensitive to the length of the arms. In the case of the C1q stalks and C1q with only one head, the angular dependence of the R_G is low. Hence the length of the C1q arms can be determined on the basis of the experimental radius of gyration at infinite contrast (R_G) for the C1 stalks without a knowledge of the base-arm angle. Experimentally a value of R_G of 11.0 ± 0.5 nm is available for the C1q stalks plus one head (Perkins *et al.*, 1984). Fig. 3(a) shows that this R_G is in better agreement with curve (ii) corresponding to arms of length 14.3 nm rather than curve (v), where the arms are 11.9 nm in length.

The R_G value of C1q depends on both the arm length and the base-arm angle. On the basis of an

arm length of 14.3 nm, the experimental R_G of 12.8 ± 0.2 nm for C1q (Perkins *et al.*, 1984) leads to an average value of about 40° for the base-arm angle from Fig. 3(a). An alternative calculation based on the R_G values of the C1q stalks, the C1q head and C1q, and the C1q arm length gave an estimate of $45 \pm 5^\circ$ (Perkins *et al.*, 1984). This angle is consistent with electron-microscopy data (Table 1). A first calculation of a value of at least 60° by neutron scattering, in apparent conflict with electron microscopy, had relied on C1q arm lengths of 11.5 nm (Gilmour *et al.*, 1980).

Further refinement of the arm length and base-arm angle of C1q utilized the neutron-wide-angle-scattering curve, and simulations of these are shown in Fig. 3(b) for C1q with arms of 14.3 nm length. The use of arms of length 11.9 nm reproduced the form of the curves of Fig. 3(b), but with different peak positions, e.g. the peak at $Q = 0.40 \text{ nm}^{-1}$ for the base-arm angle of 70° is shifted to $Q = 0.46 \text{ nm}^{-1}$. This observation allows a second determination of the C1q arm length to be 14.3 nm, as opposed to 11.9 nm, since experimentally the peak maximum is seen at 0.39 nm^{-1} (Perkins *et al.*, 1984; present Fig. 4). The length of the C1q arms is thus concluded to be in agreement with that calculated from the collagenous sequence, but to be some 3 nm longer than that value observed by electron microscopy. This difference may be the consequence of dehydration and shrinkage effects involved in the preparations for the electron microscope.

The experimental wide-angle curve leads to further information on the C1q base-arm angle. The form of the wide-angle curves of Fig. 3(b) is most sensitive to this angle as this is varied from 20° to 70°; it is also sensitive to the C1q arm-arm angle. None of the theoretical curves of Fig. 3(b) (after the application of smearing corrections to

Fig. 3. Dependence of the scattering properties of C1q on the base-arm angle of C1q in symmetrical models for C1q

(a) Shows the angular dependence of the R_G for (i) intact C1 with arms 14.3 nm in length, (ii) C1q now with six stalks and one head, and (iii) C1q with six stalks only. The broken lines correspond to C1q with arms of 11.9 nm in length, where (iv), (v) and (vi) correspond to (i), (ii) and (iii) in that order. (b) Shows the angular dependence of the unsmeared Debye wide-angle scattering curve of C1q, where the base-arm angles are shown at the top-right corner of each panel.

1984), unless C1q is taken to be a flexible molecule in solution. Three different (but equivalent) good fits were obtained by working with either (a) unequal base-arm angles; (b) unequal arm-arm angles; or (c) a weighted set of symmetrical C1q structures. In model (a) a good fit was obtained from examination of 60 C1q models in which only the base-arm angle was varied. That in Fig. 4(a) was obtained with base-arm angles of 10°, 20°, 40°, 40°, 60° and 70°; another was obtained with angles of 15°, 15°, 40°, 40°, 65° and 65°. The mean angle is 40° as determined above, and two views are shown in Fig. 4. In model (b), a further 60 models were examined where the arm-arm angles were displaced from regular increments of 60°. The best fit (Fig. 4b) was obtained by using arm-arm displacements of 0°, 0°, 0°, 30°, 30° and 30° and a constant base-arm angle of 45°; another involved arm-arm displacements of 0°, 0°, 0°, 0°, 30° and 30° and a constant base-arm angle of 50°. In model (c), the C1q wide-angle curve was summed from the curves of Fig. 3(b) after smearing; each curve was weighted by using a binomial distribution of weights in 10° steps. That centred at a base-arm angle of 50° gave the best agreement; other histograms, including those of Fig. 3 of Schumaker *et al.* (1981), gave comparable fits of slightly lesser quality. In conclusion, the C1q structure is best represented as a flexible structure with an average base-arm angle of 40–45° and arm-arm angle of 60°, each of which is able to fluctuate by up to 30°.

As previously (Perkins & Weiss, 1983), the theoretical curve of Fig. 4(a) was used to test the extent of the Guinier region of the scattering curve of C1q used to determine R_G by using plots of $\ln I$ versus Q^2 . Extended fits in the $Q \cdot R_G$ range between 0.25 and 1.75 satisfied the Guinier approximation within 1% irrespective of whether smearing was applied or not. Fits in the $Q \cdot R_G$ range of 1–2 showed differences of only 2–3% from the expected R_G and $I(0)$ values. These results substantiate the Guinier analyses given in Fig. 2 of Perkins *et al.* (1984), and similar calculations do so for C1.

(c) Computer models and scattering-curve simulations for C1

Neutron data on C1q, C1r₂C1s₂ and C1 show that a large conformational change takes place on the complexing of C1q and C1r₂C1s₂, where either C1r₂C1s₂ or C1q adopts a more compact shape within C1 (Perkins *et al.*, 1984). The individual structures of C1r₂Cs₂ and C1q will determine that of C1. Thus, if C1r₂C1s₂ is unchanged in conformation in the complex, the maximum R_C of bound C1q is 7 nm, in contrast with the experimental R_C of 12.8 ± 0.2 nm for free C1q (Perkins *et al.*, 1984). Fig. 3(a), however,

allow for neutron wavelength spread and beam divergence; Perkins & Weiss, 1983) match the experimental curve (present Fig. 4; Perkins *et al.*,

Fig. 4. Comparison of the experimental wide-angle scattering curve for C1q with the smeared calculated curves for three models of C1q

The experimental curve was obtained in 100% $^2\text{H}_2\text{O}$ buffer at 0.96 mg/ml at sample-detector distances of 2.66 m and 10.66 m and a wavelength of 1.20 nm. The smearing parameters were redetermined and found to be the same as those previously described (Perkins & Weiss, 1983). Curve A corresponds to base-arm angles of 15°, 15°, 40°, 40°, 65° and 65° and arm-arm angles of 60°. This C1q model is depicted on the right in two perspective views using 348 spheres of radius 0.595 nm whose equivalent cubes correspond in volume to that of C1q. C1q is compared with an IgG model of 107 spheres of radius 0.595 nm derived from X-ray structures (Saul *et al.*, 1978; Deisenhofer, 1981; Rajan *et al.*, 1983). Each sphere is approximately equivalent to about 11 amino acids. Curve B corresponds to arm-arm angles that are displaced by 0°, 0°, 0°, 30°, 30° and 30° from 60° and a base-arm angle of 45°. Curve C corresponds to the sum of scattering curves from symmetrical models of C1q with base-arm angles of 20°, 30°, 40°, 50°, 60°, 70° and 80° and weighted binominally (i.e. 1:6:15:20:15:6:1).

shows that such a low R_C for C1q is not possible, thus the model calculations identify the large shape change to take place mostly in C1r₂C1s₂ on complex-formation.

The association of C1r₂C1s₂ with C1q can be considered as one of two major variants. C1r₂C1s₂ can bind as a ring around or within C1q to follow those proposed in Colomb *et al.* (1982), Strang *et al.* (1982) and Poon *et al.* (1983). Alternatively it can bind as an extended or folded rod to C1q. The overall length of C1r₂C1s₂ is about 59 nm (Boyd *et al.*, 1983), one-quarter of which is coincident with the length of the C1q arm or the distance between two arm ends if the base-arm angle is 60°. Thus models for C1r₂C1s₂ bound on C1q can be generated in many ways, and this rules out a definite structure determination for C1. Curve-fitting is also limited by a possible small dissociation of C1 into C1q and C1r₂C1s₂ [discussed by Perkins *et al.* (1984) and below].

Several types of ring models for C1r₂C1s₂ bound to C1q are possible. Here and below, models for C1 are named after the alphabetic character that resembles bound C1r₂C1s₂ the most. Thus, in the O-model, bound C1r₂C1s₂ is a closed ring of inner radius 9.1 nm that touches the arms outside or

inside the C1q arms or is interwoven by the arms. The C-model is an unclosed ring of inner radius up to 20 nm that is positioned outside and touching the C1q arms. Simulations based on a series of O-models and C-models calculated as a function of the C1q base-arm angle showed that even though their R_C can agree with the experimental R_C of 12.6 nm (Perkins *et al.*, 1984), the wide-angle curve was unable to follow the experimental curve beyond the Guinier region. Final ring models were based on structures from electron-microscopic studies of cross-linked C1 (Strang *et al.*, 1982; Poon *et al.*, 1983). These show C1r₂C1s₂ bound in a predominantly circular-like arrangement and most probably interwoven between the arms (present Fig. 5; Fig. 8 of Poon *et al.*, 1983). Calculations using an S-model to reproduce this structure also show poor agreement beyond the Guinier region (Fig. 5). In conclusion, of 40 ring-like models that were tested for the structure of bound C1r₂C1s₂ within C1, none offered the promise of being eventually compatible with the C1 scattering curve. This is because the calculated curves resembled too closely that of C1q and its ring of heads.

Folded-rod models for C1r₂C1s₂ bound to C1q

Fig. 5. Comparison of the experimental scattering curve for C1 with that calculated for the ring-like S-model of C1 (Strang *et al.*, 1982; Poon *et al.*, 1983), which is inset at the top right corner

were found to be more successful in accounting for the R_C and wide-angle curve of C1. Elongated structures were tested for bound $C1r_2C1s_2$ without promise of success. However, when more-compact structures were examined, good curve fits could be readily obtained, and this procedure led to the W-model, P-model and Y-model as possible structures for C1 (Fig. 6). (i) In the W-model, four equal segments of $C1r_2C1s_2$ bind to four arms and four bases at the bend of the C1q stalk. The continuity of $C1r_2C1s_2$ was maintained by the use of a short ring segment between the second and third part of $C1r_2C1s_2$ at its centre (Fig. 6) (although this can alternatively be made between the first and second, and the third and fourth parts of $C1r_2C1s_2$ in what would be called an M-model). Of 50 models that were tested, the best fit (Fig. 6, W-model) was obtained by setting one-sixth of $C1r_2C1s_2$ on the base of C1q (Fig. 6, W-model), and had C1q base-arm angles of 50° . (ii) In the P-model the central two segments of the $C1r_2C1s_2$ were located on the C1q arms and the end segments were used to connect different C1q arms between the C1q heads in one of five different ways. Of 90 models, the best fit of Fig. 6(P-model) shows that $C1r_2C1s_2$ is bound to two adjacent C1q arms and connects three adjacent C1q heads. The three free C1q base-arm angles are 70° and the three bound C1q base-arm angles are 30° ; thus the mean is 50° . (iii)

In the Y-model the two halves of $C1r_2C1s_2$ bind to two stalks on both the arm and base. From the testing of 65 models, a curve that reproduced the form of the C1 scattering curve was obtained; its vertical scale is, however, smaller by 10% from the experimental curve (Fig. 6, Y-model). The parent molecule resembles free C1q (Fig. 4) in which the base-arm angles were set as 10° , 10° , 30° , 30° , 50° and 50° and either two adjacent or adjacent-but-one C1q stalks were involved in $C1r_2C1s_2$ binding. The twelve forms of this model obtained by permutation of the C1q stalks were equally weighted to give the fit of Fig. 6(Y-model).

Final control simulations evaluated the effect of experimental errors. The increasing dissociation of C1 into C1q and $C1r_2C1s_2$ causes the $\ln I$ curve between Q values of 0.3 and 0.8 nm^{-1} to rise in relation to $\ln I$ at zero Q . When free C1q becomes 20% of the total, the minimum at $Q \sim 0.3\text{ nm}^{-1}$ (Fig. 4) begins to be apparent. The curve fits of Fig. 6 are thus compatible with complete or near-complete complexing of C1 in the neutron experiment. The $\ln I$ curve beyond $Q = 0.3\text{ nm}^{-1}$ is also sensitive to the buffer background subtraction. Even though these sources of error can alter the appearance of the $\ln I$ -versus- Q curve, the resulting intensity changes are uniform for Q beyond 0.3 nm^{-1} , and the Y-model is thus within error of the W-model and the P-model.

Fig. 6. Curve-fitting for the W-, P- and Y-models of C1

(a) Shows comparisons of the experimental and calculated scattering curves for the W-model, P-model and Y-model for C1. The experimental curve was measured at 0.3 mg of C1/ml under the same instrumental conditions as those for C1q (Fig. 4). (b) Shows perspective drawings of the 636 spheres used to create the W-, P- and Y-models for C1 which were used in the Debye simulations. The conformation of the bound ClIr_2Cl_2 subcomponent of C1 ($2 \times 3 \times 48 = 288$ spheres equivalent to that described previously; Boyd *et al.*, 1983) is shown separated to the right of each model of C1.

The merit of the three models is to define possible solutions that are improvements on ring-like or interwoven models proposed from biochemical work or electron microscopy. Their common feature is that $\text{C1r}_2\text{C1s}_2$ binds outside C1q on one side, utilizing either two (P-model or Y-model) or four (W-model) C1q stalks. The W-model is located near the centre of C1, whereas the P-model and the Y-model are located on one or the other end of C1q. These models suggest that $\text{C1r}_2\text{C1s}_2$ makes extensive contact with C1q on its collagenous section. In turn this suggests that information about possible models of $\text{C1r}_2\text{C1s}_2$ binding to C1q might be contained in the C1q primary sequence, in view of the known homology of C1r and C1s and the presence of pairs of C1r and C1s. Inspection of the sequence in Fig. 1 for repeats shows that there are two sets of five bands of alternating sign of electrostatic charge densities that are centred on position 39 in the C1q stalk. These might be recognized by $\text{C1r}_2\text{C1s}_2$ for binding purposes in a Y-model or a W-model. Likewise, on the arm of C1q there are two sets of three bands of alternating sign of charge densities that are centred on position 69; this would be relevant to a P-model. What significance if any there is in these observations awaits the primary and tertiary structure determinations of C1r and C1s.

(d) *Hydrodynamic analyses for $\text{C1r}_2\text{C1s}_2$, C1 inh , C1q and C1*

A second independent series of modellings was carried out to calculate sedimentation coefficients for comparison with literature $s_{20,w}^0$ values in order to complement the low-resolution structures determined above and in Boyd *et al.* (1983) for $\text{C1r}_2\text{C1s}_2$, C1q and C1 by using neutron scattering.

Previously $\text{C1r}_2\text{C1s}_2$ was modelled as a hydrated straight cylinder (Tschopp *et al.*, 1980; Boyd *et al.*, 1983; see also the present Methods section). Similar calculations are reported in Table 2 for the subunits of $\text{C1r}_2\text{C1s}_2$. $\text{C1r}_2\text{C1s}_2$ was also remodelled by using hydrodynamic spheres, and this enabled bent cylindrical models to be considered too (Table 2; Fig. 7). The calculated $s_{20,w}^0$ values from cylinders or spheres for C1r and C1r₂, and for C1s and C1s₂, agree to within 5% of the experimental values of C1s is set to be 5 nm longer than C1r. Calculations based on $\text{C1r}_2\text{C1s}_2$ itself using spheres now show that $s_{20,w}^0$ is calculated as 8.5–8.6 for bent-rod models (Boyd *et al.*, 1983), in comparison with a straight-rod model, which gives an $s_{20,w}^0$ of 8.5S, in agreement with the experimental value of 8.7S. Other calculations for $\text{C1r}_2\text{C1s}_2$ using spheres where the C1s and C1r structures above are permuted within $\text{C1r}_2\text{C1s}_2$

give $s_{20,w}^0$ values of 8.4–8.5S, showing that no information on the sequence can be obtained. This sequence is usually assumed to be C1s–C1r–C1r–C1s as in Fig. 7, since C1r exists as a stable dimer in solution, but not C1s (Arlaud *et al.*, 1980). In conclusion, the calculations are consistent with the rod-like model for $\text{C1r}_2\text{C1s}_2$ of length 59 nm from neutron scattering (Boyd *et al.*, 1983), and furthermore show that hydrodynamic spheres can be used to represent $\text{C1r}_2\text{C1s}_2$.

Hydrodynamically, C1 inh can be represented by spheres or cylinders of lengths between 38 and 44 nm (Fig. 7), showing that this has an extended structure, although this cannot be uniquely defined. This structure is compatible with the extended shapes of lengths 33–36 nm from electron microscopy (Odermatt *et al.*, 1981). Other calculations based on the $s_{20,w}^0$ values for the C1 inh complexes with C1r , C1s and $\text{C1r} \cdot \text{C1s}$ (Chesne *et al.*, 1982) are consistent with extended structures that are longer for C1s than they are for C1r, in agreement with Table 2. That C1s can be longer than C1r may be of relevance in the W-model or M-model above in terms of the connectivity between the four segments of $\text{C1r}_2\text{C1s}_2$ when bound to C1q. These models for C1 inh and its complexes also show that C1r and C1s within the structure of C1 inh require a large surface accessibility in order to permit ease of complexing of C1r and C1s to C1 inh , especially in the situation when C1 is bound to immune complexes (Sim *et al.*, 1979; Arlaud *et al.*, 1979). In C1-immune-complex systems, the space within the C1q arms will be sterically obstructed. Similar reasonings apply in consequence of the reversible association–dissociation of $\text{C1r}_2\text{C1s}_2$ to C1q when C1 is bound in immune aggregates (Doekes *et al.*, 1983a), and also likewise for the need for C1s in such systems to be accessible to interact with complement components C4 (Doekes *et al.*, 1983b) and C2. These considerations argue against models for C1 that place $\text{C1r}_2\text{C1s}_2$ within the C1q arms (such as the S-model; Fig. 5) and supports the W-model, P-model and Y-model (Fig. 6), which place $\text{C1r}_2\text{C1s}_2$ outside the C1q stalks.

Early $s_{20,w}^0$ calculations for C1q employed much-simplified C1q shape models (Schumaker *et al.*, 1976; Liberti & Paul, 1978) with the method of Bloomfield *et al.* (1967a,b). The present work employed the explicit models for C1q (Fig. 7) with the improved theory of Garcia de la Torre & Bloomfield (1977a). In continuation of the comparisons between C1q of different arm lengths, models of arm length 11.5 nm and 14.8 nm were studied. Fig. 8(a) shows, that, for a base–arm angle of 40°, the predicted $s_{20,w}^0$ values are 10.1 and 9.03S respectively, to be compared with the experimental value of 10.2S (Reid *et al.*, 1972; Schumaker

Table 2. Calculation of the sedimentation coefficients of Clr_2ClS_2 and its fragments

References: ⁽¹⁾Arlaud *et al.* (1980); ⁽²⁾Tschopp *et al.* (1980); Ziccardi & Cooper (1976), Valet & Cooper (1974) and Arlaud *et al.* (1980); ⁽³⁾Tschopp *et al.* (1980), Sim *et al.* (1977), Valet & Cooper (1974) and Arlaud *et al.* (1980); ⁽⁴⁾Tschopp *et al.* (1980) and Valet & Cooper (1974); ⁽⁵⁾Arlaud *et al.* (1980); ⁽⁶⁾Tschopp *et al.* (1980) and Arlaud *et al.* (1980); ⁽⁷⁾Chesne *et al.* (1982); ⁽⁸⁾using theory (iii) and the parameters of Table 4 in Boyd *et al.* (1983); ⁽⁹⁾using the modified Oseen tensor in the diagonal approximation of Garcia de la Torre & Bloomfield (1977a) (the agreement between experiment and calculation improves with increase in the number of spheres employed).

Complement (sub)component	$10^{-3} \times M_r$	Hydrated volume (mm ³)	Mean experimental $s_{20,w}^0$ (S)	Cylindrical models		Spheres models	
				Diameter and length ($d \times L$ nm)	Calculated $s_{20,w}^0$ (S) ⁽⁸⁾	Diameter of sphere and total length (nm)	Calculated $s_{20,w}^0$ (S) ⁽⁹⁾
Clr	98	174	5.0 ⁽¹⁾	3.8 × 15.3	5.2	4 × 1 = 4 8 × 2 × 2 = 32 12 × 3 × 3 = 108 3.8 } × 15.2 1.9 } 1.266 }	5.8 5.5 5.2
Clr ₂	195	347	7.1 ⁽²⁾	3.8 × 30.6	6.9	8 × 1 = 8 16 × 2 × 2 = 64 3.8 } × 30.4 1.9 }	7.5 7.3
ClS	91	161	4.5 ⁽³⁾	3.8 × 14.2 3.25 × 19.4	5.0 4.5	6 × 1 = 6 12 × 2 × 2 = 48 3.25 } × 19.5 1.625 }	4.9 4.7
ClS ₂	182	322	5.9 ⁽⁴⁾	3.8 × 28.4 3.25 × 38.8	6.7 5.8	12 × 1 = 12 24 × 2 × 2 = 96 12 × 1 = 12 3.8 } × 39.0 1.625 }	6.2 6.0 8.3
Clr ₂ ClS	286	508	7.7 ⁽⁵⁾	3.8 × 44.8	7.9	24 × 2 × 2 = 96 8 × 1 = 14 6 × 1 = 16 3.8 } × 45.6 1.9 } 3.8 } × 49.9 3.2 }	8.1 { 7.9 (mean) 9.1
Clr ₂ ClS ₂	377	669	8.7 ⁽⁶⁾	3.8 × 59.0	8.7	16 × 1 = 16 3.8 } × 60.8 3.8 } × 69.4 3.25 }	8.5 { 8.5 (mean) 4.3
Cl inh	103	174	4.3 ⁽⁷⁾	2.4 × 38.0	4.3	23 × 1 = 23

Fig. 7. Hydrodynamic sphere models for C1 and its subunits and C1ⁱ inh

(a) Models for C1r₂C1s₂, C1q and C1ⁱ inh in terms of 20 spheres of diameters 3.25 nm and 3.8 nm for C1r₂C1s₂, 96 spheres of diameters 1.644 nm and 4.92 nm for C1q, and 23 spheres of diameter 1.9 nm for the C1 inhibitor. The lengths of the C1q base and arm are 9.9 nm and 14.8 nm in comparison with the sequence predictions of 10.7 nm and 14.3 nm respectively. The various possible rod-like models for C1r₂C1s₂ using 60° bends are shown. The use of three spheres to model the base of C1q leads to connectivity gaps and could not therefore be employed. (b) The W-model, O-model and Y-model for C1 are shown. C1r₂C1s₂ is now represented by 16 spheres of diameter 3.8 nm.

et al., 1976; Liberti & Paul, 1978). Flexibility in the C1q structure will, however, increase the predicted $s_{20,w}^0$ values, and this has not been taken into account in the calculations. Thus no choice can be made between the C1q arms of length 11.5 nm or 14.8 nm. Fig. 8(a) also shows that an observed $s_{20,w}^0$ for the C1q stalks of 6.31 S (Siegel & Schumaker, 1983) can be correlated with calculated $s_{20,w}^0$ values of 5.7–5.2 S for reduced base-arm angles of 10°–20° that are reported from electron microscopy (Brodsky-Doyle *et al.*, 1976). Since the calculations for C1q and its stalks are compatible with experiment to within 10–17%, it is seen that hydrodynamic models for C1q can be developed by using 96 spheres (Fig. 7).

The experimental $s_{20,w}^0$ values for C1 are 15.2, 15.9 and 16.2 S (Ziccardi & Cooper, 1977; Siegel *et al.*, 1981; Villiers *et al.*, 1982; Siegel & Schumaker, 1983), thus the frictional ratios for C1q and C1 are

similar at 1.88 and 1.77–1.88 respectively. Hydrodynamic C1 models were developed by combining the 96-sphere model for C1q (Fig. 7) with a 16-sphere model for C1r₂C1s₂, whose calculated $s_{20,w}^0$ value of 8.7 S agreed with the experimental one (Table 2). These were used as a further filter of the W-model, O-model and Y-model for C1 (Fig. 7). From Fig. 8(c), the W-model gives $s_{20,w}^0$ values of 15.6–15.0 S for C1q base-arm angles of 40–50°, which are within the experimental values. The O-model is similar to the W-model (Fig. 8c). The Y-model, however, leads to $s_{20,w}^0$ values that are less than those of the W-model and O-model, unless the base-arm angle is reduced to 30° to give a $s_{20,w}^0$ of 15.4 S to agree with the experiment. This difference is attributed to the extension of C1r₂C1s₂ beyond the base of C1q (Fig. 7). Thus the best hydrodynamic models for C1 are the W-model and the O-model (the P-model is a hybrid of these: Fig.

Fig. 8. Dependence of the calculated sedimentation coefficients of C1q and C1 on the base-arm angle in symmetrical models of C1q

(a) and (b) utilize the theories of Garcia de la Torre & Bloomfield (1977a) and Bloomfield *et al.* (1967a) respectively for C1q. The older 1967 theory leads to $s_{20,w}^0$ values that are uniformly greater than those from the 1977 method. Calculations were made for C1q with arms of length 14.8 nm and 11.5 nm, using the same nomenclature as Fig. 3(a). (c) Indicates the calculated sedimentation coefficients for C1 for the W-model, O-model (broken line) and Y-model for bound $\text{C1r}_2\text{C1s}_2$, using the method of Garcia de la Torre & Bloomfield (1977a). Steric overlap of the $\text{C1r}_2\text{C1s}_2$ ring with the C1q head prevents calculation for base-arm angles of 10° and 20° in the O-model.

6). The Y-model is also compatible if there is a conformational change in C1q, whereas the S-model is disfavoured for reason of steric hindrance in relation to C1^{inh} , C2 and C4.

Discussion

The models for C1q (Figs. 4 and 7) and C1 (Figs. 6 and 7) represent possible solutions of their structure and not unique ones; thus three alternative, but equivalent, structures were developed for each of C1q and C1 to illustrate this. The method is most effective at rejecting unsatisfactory models, as now discussed.

In the case of C1q, the R_c of the C1q stalks and the peak in the C1q wide-angle spectrum shows that C1q arms of length 11.5 nm can be rejected in favour of arms of length 14.3 nm, as predicted from the collagenous sequence of C1q. Since the C1q structure is constrained by its known overall morphology and known primary sequence, and since the C1q wide-angle curve is very sensitive to the structure of C1q (Fig. 3b), further analyses were able to show that C1q is significantly flexible in the solution state. Such flexibility implies a

requirement for a means of preserving a ring-like array of free C1q heads in solution. This may result from electrostatic repulsions between the C1q heads. Analysis of the primary sequence shows the existence of net positive charges, where the net charge of each arm is +3 and each head is +10. Removal of the heads leads to reduced base-arm angles that are seen in electron-microscopic studies of the C1q stalks (Brodsky-Doyle *et al.*, 1976) and suggested from analyses of $s_{20,w}^0$ values (Fig. 8a).

IgG itself is a flexible molecule, where flexibility has been identified at the 'hinge' between the Fab and Fc domains, and at the 'switch' within the Fab domain. Thus the Fab arms of IgG (Fig. 4) would be able to orient themselves to enable IgG to bind on to an irregular antigenic surface. The C1q heads bind to the Fc domain of IgG. This binding would be facilitated by the ability of both the Fc domain and the C1q head and C1q arm to move in order to optimize their contacts. In this sense, C1q flexibility complements that of IgG flexibility at the initiation point of the classical pathway of activation. Once C1q is bound to immune aggregates, C1q flexibility would be diminished.

Biochemical studies of the complex between Clr_2Cls_2 and C1q have already identified the involvement of the C1q collagenous stalks in the binding to Clr_2Cls_2 (Porter, 1980; Reid, 1983). This follows from selective inhibition or competition experiments involving either the C1q stalks, the C1q heads, C1q receptors or C1q inhibitors (Reid *et al.*, 1977; Pâques *et al.*, 1979; Tenner & Cooper, 1981; Silvestri *et al.*, 1981; Lin *et al.*, 1983). Physical studies using ultracentrifugation and electron microscopy indicate likewise (Siegel & Schumaker, 1983; Strang *et al.*, 1982; Poon *et al.*, 1983). Previously (Strang *et al.*, 1982; Poon *et al.*, 1983; Perkins *et al.*, 1984) and here, it is shown that a large conformational change takes place in Clr_2Cls_2 on complexing to C1q. A large difference between the scattering curves of C1q and C1 (Figs. 4 and 6) had to be accounted for in the neutron models. On this criterion, the O-model, C-model and S-model for C1 could be rejected in favour of the W-model, P-model and Y-model, even though all six models had Clr_2Cl_2 bound to the C1q stalks and the O-model is supported by $s_{20,w}^0$ calculations (Fig. 8c). Further examination indicates that the W-model and P-model are in better agreement with the neutron curves and $s_{20,w}^0$ values than is the Y-model; the Y-model is within the limit of experimental error of the neutron and $s_{20,w}^0$ data. The W-model, P-model and Y-model are compatible with the concept of the surface accessibility of Clr_2Cls_2 in C1 that is bound to immune complexes, whereas this is not true of the S-model (Fig. 5), nor of what can be called a 'B-model' that was recently proposed by Colomb *et al.* (1984), where the free ends of Clr_2Cls_2 as in Fig. 5 are folded against the centre of Clr_2Cls_2 .

The W-model, P-model and Y-model for C1 that have emerged from this work involve the binding of Clr_2Cls_2 to one side of C1q. Overall, the C1 complex is assymmetric, hence leading to the removal of the minimum in the C1q neutron curve caused by the ring of C1q heads. A two-fold or four-fold symmetry in C1q is used in these three models; it was not necessary to invoke a six-fold symmetry as postulated in Poon *et al.* (1983). The association constant of C1 is equivalent to a free-energy charge of about -40 kJ/mol , which can be approximated (Perutz, 1978) to the formation of about ten salt bridges between Clr_2Cls_2 and C1q. Thus C1q serves as an extended well-defined template for the weak binding of much-folded Clr_2Cls_2 along its collagenous sections. The process of binding can assist the autoactivation of C1r and/or the activation of C1s within bound Clr_2Cls_2 through the development of proximity relationships in folded Clr_2Cls_2 that bring together the relevant catalytic centres. Clr_2Cls_2 binding can restrict C1q flexibility in addition

to that resulting from C1q binding to immune aggregates. Thus subtle structural or dynamic changes in the conformation of C1q when bound to immune complexes or to Clr_2Cls_2 can also be implicated in the activation of C1.

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