The quaternary structure in solution of human complement subcomponent C1r₂C1s₂

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C1r₂C1s₂ is a subcomponent of first component C1 of the complement cascade. Previously two distinct models for its structure have been described, in which C1r₂C1s₂ is either a linear rod-like assembly of the globular domains found in each of C1s and C1r, or these domains are arranged to form an asymmetric X-shaped structure. These two models were evaluated by using hydrodynamic simulations and neutron scattering. The data on C1s, C1s₂ and C1r are readily represented by straight hydrodynamic cylinders, but not C1r₂ or C1r₂C1s₂. Tests of the X-structure for C1r₂ and C1r₂C1s₂ successfully predicted the experimental sedimentation coefficients, thus supporting this model. Neutron scattering analyses on C1s and C1r₂ are consistent with a linear structure for C1s, but not for C1r₂. An X-shaped structure for C1r₂ was found to give a good account of the neutron data at large scattering angles. The total length of the C1s and C1r monomers was determined as 17–20 nm, which is compatible with electron microscopy. On the basis of the known sequences of C1r and C1s, this length is accounted for by a linear arrangement of a serine-proteinase domain (length 4 nm), two short consensus repeat domains (2 × 4 nm), and a globular entity containing the I, II and III domains (4–7 nm).

INTRODUCTION

In the immune response, the classical pathway of complement activation is initiated by the first component C1 (Cooper, 1985; Reid, 1986; Schumaker *et al.*, 1987). C1 is comprised of two distinct subunits, C1q and C1r₂C1s₂. C1q is the recognition element of C1 and binds to activating substances such as immune complexes. The tetramer C1r₂C1s₂ possesses the enzymic activity of C1 and on activation is able to cleave C2 and C4 of complement. C1r and C1s are homologous proteins. The structure determination of the C1r and C1s subunits and the C1r₂C1s₂ complex is thus central for an understanding of C1 assembly and activation, and also the control of C1 activity.

It has been assumed for many years that C1r₂C1s₂ has an extended, linear rod-like structure on the basis of electron microscopy, hydrodynamic simulations and neutron scattering (Tschoop *et al.*, 1980; Strang *et al.*, 1982; Boyd *et al.*, 1983; Perkins, 1985). That the tetramer is a linear arrangement of C1s·C1r·C1r·C1s in that order has been confirmed by the use of ferritin labelling in electron microscopy (Weiss *et al.*, 1986), and the four serine-proteinase domains have been located at the ends and centre of C1r₂C1s₂ (Villiers *et al.*, 1985; Arlaud *et al.*, 1987a). It is not surprising that the more detailed structure of C1r₂C1s₂ had also been assumed to be a linear end-to-end arrangement of the individual domains found in each of C1r and C1s (Villiers *et al.*, 1985; Arlaud *et al.*, 1987a). That this might not be the case was initially suggested by electron microscopy, where C1r₂C1s₂ was proposed to be X-shaped (Weiss *et al.*, 1986). Structural techniques such as solution scattering (reviewed by Perkins, 1988a,b) and sedimentation

experiments (Perkins, 1989a) have the ability to test alternative molecular models. Here, comparative analyses are presented of the linear and X-shaped domain models for the C1r₂C1s₂ structure in order to make a choice between them.

RESULTS AND DISCUSSION

Hydrodynamic lengths of C1s and C1r

Sedimentation coefficients ($s_{20,w}^0$) have been published for C1r₂C1s₂ and its subunits and are summarized in Table 2 of Perkins (1985). Earlier hydrodynamic simulations which interpreted these data (Tschoop *et al.*, 1980; Boyd *et al.*, 1983; Perkins, 1985) can now be improved on the basis of accurate sequence data (Arlaud & Gagnon, 1983; Journet & Tosi, 1986; Leytus *et al.*, 1986; Arlaud *et al.*, 1987b; Mackinnon *et al.*, 1987; Tosi *et al.*, 1987), and a better appreciation of macromolecular hydration and the Bloomfield–Garcia de la Torre method for calculating sedimentation coefficients (Garcia de la Torre & Bloomfield, 1981; Perkins, 1986, 1989a). The compositions of C1s and C1r are given in Table 1. A good simulation should be within ± 0.2 S of the experimental $s_{20,w}^0$, whereas errors in the experimental values should be generally ± 0.2 S. Table 2 reports the improved calculations for the linear and X-shaped domain models for C1r₂C1s₂ and its subunits using hydrodynamic spheres.

If C1r₂C1s₂ is a linear cylinder in shape, with a circular cross-section, the hydrodynamic lengths, L values, can be obtained from the spheres approach by combining the experimental $s_{20,w}^0$ data with the sequence-determined volumes (Table 1). Thus L values for C1s and C1r are 19

Abbreviations used: EGF, epidermal growth factor; SCR, short consensus repeat; C1r, activated form of C1r.

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Table 1. Comparisons of composition data for C1r and C1s of complement

The carbohydrate content of C1r is that given by Boyd *et al.* (1983), calculated from results of Sim *et al.* (1977), and is in good agreement with the presence of four biantennary complex-type oligosaccharides. That for C1s is calculated by assuming two biantennary complex-type oligosaccharides. This gives a total similar to that given by Sim *et al.* (1977) and Boyd *et al.* (1983), but with a more reasonable *N*-acetylneuraminic acid content. The similarity of the $\bar{v}$ (partial specific volume) and Σb (total of nuclear scattering lengths)/ M_r values for C1r and C1s justify the use of $I(0)/c \cdot T_s$ values (see the text) to calculate M_r values from the neutron data in $^2\text{H}_2\text{O}$ buffers. The absorption coefficients at 280 nm were calculated by the updated Wetlaufer procedure (Wetlaufer, 1962; Perkins, 1986).

Parameter	C1r ₂ C1s ₂	C1r ₂ C1s	C1r	C1s	C1r II
Amino acid residues					
Total ...	2722	2049	688	673	428
M_r ...	306000	231000	78000	75000	50000
Total no. of carbohydrate residues ...	122	100	39	22	19
Total M_r ...	330000	251000	86000	79000	50000
Total dry volume (nm ³) ...	416	316	108	100	64
Hydrated $\bar{v}$ (ml/g) ...	0.723	0.723	0.722	0.724	0.725
Total $\Sigma b/M_r$ in $^1\text{H}_2\text{O}$ (fm) ...	0.2350	0.2348	0.2344	0.2356	0.2333
Total $\Sigma b/M_r$ in $^2\text{H}_2\text{O}$ (fm) ...	0.3953	0.3965	0.3989	0.3915	0.4022
Predicted $A_{280}^{1\%,1\text{ cm}}$...	13.4	13.0	12.3	14.6	15.9

and 17 nm respectively. That for C1s₂ is seen to be doubled at 36 nm, and this shows that C1s₂ is formed by the end-to-end linear connection of two C1s cylinders. However the length of C1r₂ is 28 nm, which is 8 nm shorter than expected. The length of C1r II, the proteolysed form of C1r which has lost 41 % of the parent structure, is 9 nm. The length of C1r₂ II is 15 nm, which is slightly shorter than expected. The length of C1r₂C1s is 47 nm, which is less than the expected value of 54 nm (3 × 18 nm). Finally the length of C1r₂C1s₂ is found to be 58 nm, which is noticeably shorter than the expected length of 72 nm (4 × 18 nm). These length discrepancies

indicate that the model of the linear, end-to-end assembly of C1r and C1s monomers to form C1r₂C1s₂ is not satisfactory. Further calculations based on uniform hydrodynamic cylinders and not spheres [eqn. (77) of Garcia de la Torre & Bloomfield (1981)] as a control confirmed these findings, where very similar lengths were obtained (to within 1–2 nm). This indicates the likely precision of these simulations.

The tests of the X-shaped asymmetric model for C1r₂ (Weiss *et al.*, 1986) were based on the sphere models for C1s, C1r and C1r II as in Table 2(a). As shown in Fig. 1, the separations between the two inner and two outer

Table 2. Calculation of hydrodynamic models for C1r and C1s

L is the length of the model; d is the diameter of the sphere; n is the number of spheres. The experimental $s_{20,w}^0$ values are taken from Arlaud *et al.* (1980), Lacroix *et al.* (1989) and the summary of Table 2 of Perkins (1985). The hydrated volume is the sum of the dry volume and the volume of the electrostricted hydration shell assuming a hydration of 0.3 g of water/g of glycoprotein and a water molecule volume of 0.0245 nm³ (Chothia, 1975; Perkins, 1986), and correcting for the void spaces between the spheres by the factor $\pi d/4$ (Perkins, 1989a). Simulations were based on the program GENDIA (Garcia de la Torre & Bloomfield, 1977a,b).

	Hydrated volume (nm ³)	Hydrodynamic model			$s_{20,w}^0$ (S)	
		L (nm)	d (nm)	n	Calculated	Experimental
(a) Linear sphere models						
Cl _s	142	19	3.8	5	4.5	4.5
Cl _s ₂	284	36	3.9	9	6.0	5.9
Cl _r	154	17	4.2	4	5.1	5.0
Cl _r ₂	307	28	4.6	6	7.2	7.1
Cl _r II	90	9	4.4	2	4.1	3.8
Cl _r ₂ II	180	15	4.9	3	6.0	6.1
Cl _r ₂ Cl _s	449	47	4.3	11	7.6	7.7
Cl _r ₂ Cl _s ₂	591	58	4.4	13	8.7	8.7
(b) X-shaped sphere models						
Cl _r ₂	307	24	4.2	8	7.2	7.1
Cl _r ₂ II	180	11	4.4	4	6.1	6.1
Cl _r ₂ Cl _s	449	39	4.2, 3.8	8, 5	7.8	7.7
Cl _r ₂ Cl _s ₂	591	57	4.2, 3.8	8, 10	8.3	8.7

Fig. 1. Hydrodynamic sphere models for $\text{Clr}_2\text{ClIs}_2$ and its components

These are planar and follow the X-shaped sphere models of Table 2(b). The monomers and dimers of Clr and Clr II are shown. Two models for Clr_2 are shown which give similar $s_{20,w}^0$ values. Models for Clr_2ClIs and $\text{Clr}_2\text{ClIs}_2$ can be developed from these, where the spheres corresponding to ClIs are stippled. The sphere diameters are as follows: Clr , 4.186 nm; Clr II , 4.418 nm; ClIs , 3.786 nm.

Fig. 2. Neutron scattering curve analyses of ClIs (upper) and Clr_2 (lower) in $^2\text{H}_2\text{O}$ buffers

Sample concentrations were: ClIs , 0.54 mg/ml; Clr_2 , 0.39 mg/ml. $Q = 4\pi \sin \theta / \lambda$, where 2θ is the scattering angle and λ is the wavelength. Guinier plots of $\ln[I(Q)]$ versus Q^2 are shown to obtain the R_G values (left). The $Q \cdot R_G$ range is arrowed as shown. The corresponding cross-sectional plots of $\ln[I(Q) \cdot Q]$ versus Q^2 are shown to the right, from the slope of which the cross-sectional radius of gyration, R_{XS} , is obtained. The purifications of Clr_2 and ClIs are described by Arlaud *et al.* (1979). Samples were dialysed into 150 mM-NaCl/10 mM-Tris/HCl, pH 7.4, containing 100% $^2\text{H}_2\text{O}$ with several changes over 48 h. Concentrations were determined before dialysis and rechecked, after data collection, on the basis of the absorption coefficients listed in Table 1. Data were collected at 6 °C using large circular sample cells on the neutron instrument D11 at the Institut-Laue-Langevin, Grenoble, France (Ibel, 1976; Timmins & May, 1981). Detector positions of 2.6 m and 10.6 m were used with beam collimation distances of 2.5 m and 10 m respectively and neutron wavelengths of 0.55 nm or 1.00 nm (wavelength spread of 9% at full-width-half-maximum). Data reduction followed standard procedures (Ghosh, 1981).

ends of the X were set as 7 nm and 13.5 nm to follow Weiss *et al.* (1986). The good agreements of Table 2(b) show that this model is fully consistent with the $S_{20,w}^0$ data and the hydrated volumes. The X-models thus offer an explanation of how two structurally similar monomers can be added together to give nominal hydrodynamic lengths that are not additive, as in Table 2(a). Other control calculations using two-dimensional arrangements of hydrodynamic spheres arranged as ellipsoids were attempted. These were less successful in dealing with the non-additivity of hydrodynamic lengths than those of Fig. 1.

Neutron scattering analyses of C1r_2 and C1s

Further evidence from neutron scattering was used to characterize C1r_2 and C1s in terms of the two models for the arrangement of domains. Since $\text{C1r}_2\text{C1s}_2$ and $\text{C1r}_2\text{C1s}_2$ have similar structures (Boyd *et al.*, 1983), as also do C1r , C1s and C1s (Villiers *et al.*, 1985), proenzymic and activated C1r and C1s are not expected to differ significantly. The Guinier plots at low Q (defined in Fig. 2) were linear (Fig. 2), from which the slope leads to the radius of gyration, R_G , and the intercept at zero Q gives the forward scattering at zero angle, $I(0)$ (Perkins, 1988a,b). As a check, the relative values of $I(0)/c \cdot T_s$ for $\text{C1r}_2\text{C1s}_2$, C1r_2 and C1s (c is concentration; T_s is neutron sample transmission) were compared with the M_r values, where the data for $\text{C1r}_2\text{C1s}_2$ are taken from Boyd *et al.* (1983). In that order, the experimental $I(0)/c \cdot T_s$ ratios of 10:4:2 were found to be in fair agreement with the expected ratios of 10:5:2 from the M_r values. At small Q , the R_G values of C1r_2 and C1s were found to be similar, at 8 ± 1 nm, although the accuracy of these determinations is not considered to be high. According to the linear model for the C1r_2 domains, the length of the dimer should be twice that of the monomer. It can then be shown that the R_G of the C1r_2 dimer should be approximately double that of the C1s monomer. Experimentally, this is not the case. At larger Q , the neutron scattering curves are less prone to possible errors due to aggregation, and here the putative use of the cross-sectional Guinier analyses (Fig. 2) to assess the neutron data shows that the R_{XS} values are 2.2 ± 0.1 nm for C1r_2 and 0.9 ± 0.1 nm for C1s . It is expected from the linear-domain model for $\text{C1r}_2\text{C1s}_2$ that the mean cross-sectional dimensions of C1r_2 and C1s along the length of the macromolecule would be similar. This would mean that the R_{XS} values for the dimer and the monomer should be similar. That this was not found also argues against the linear model for the dimer. The X-model for the dimer is expected to lead to a R_G value that is closer to that of the monomer, since its overall length has been reduced relative to the linear model. Likewise the R_{XS} values of the monomer and dimer are expected to be dissimilar, since the two shorter axes are now unequal. Both predictions are fully consistent with the experimental data.

Comparison of the domains of C1r and C1s with solution data. The hydrodynamic and neutron results are now interpreted in terms of the six protein domains denoted I–VI that have been identified in the sequences of C1r and C1s . Four of these have globular structures, namely the serine proteinase of domain VI (Caporale *et al.*, 1983; Carter *et al.*, 1984), the two short consensus repeats (SCRs) in domains IV and V (Reid *et al.*, 1986;

Perkins *et al.*, 1988), and the epidermal-growth-factor (EGF)-like domain II (Cooke *et al.*, 1987). The 'homologous' domains I and III are of unknown secondary structure. It is reasonable to expect that they will have globular structures as well, on the basis of an averaged Robson secondary-structure prediction (Garnier *et al.*, 1978; Perkins *et al.*, 1988) of the four available sequences. This gives a content of 13% α -helix, 30% β -strand, 37% β -turn and 20% coil (Perkins, 1989b), which is typical for globular proteins.

Domain III in C1r and C1s is readily proteolysed to yield the 68-residue β -fragment (Villiers *et al.*, 1985). Those authors had thus proposed that the β -fragment is random coil (or at least part of it, i.e. from Glu-274 to Thr-286) and so provides the long connection between the two large domains seen in electron micrographs of C1r and C1s (Arlaud *et al.*, 1986, 1987a; see also Busby & Ingham, 1988; Tosi *et al.*, 1987). The structure predictions suggest an improved explanation of this proteolysis. The β -fragment is cleaved in domain III,

Fig. 3. Summary of the domain structure of C1s and C1r .

In the lower part, linear hydrodynamic models for C1s , C1r and C1r II (the B_γ fragment of C1r) are presented using the cylinder model (solid lines) or spheres (Table 2a). That five spheres are used for C1s and two for C1r II results from the modelling procedure in use and has no relation to the substructure of C1s or C1r II . In the upper part, these are compared with the dimensions of the α -carbon co-ordinates of bovine trypsin ('Try'; Bode & Schwager, 1975), the SCR consensus repeat (Perkins *et al.*, 1988) and human EGF (Cooke *et al.*, 1987). The lengthwise arrangement of four distinct substructures in C1s and C1r containing the domains I–VI are compatible with the hydrodynamic lengths.

Fig. 4. Comparisons of the large-angle experimental and simulated neutron scattering curves for models of CIs and Clr_2 .

The Debye models (shown using spheres 1 nm in diameter) are shown in the upper right corners. The total volume corresponds to the unhydrated glycoprotein and was calculated from the data of Chothia (1975) and Perkins (1986). Neutron curves were calculated (Perkins & Weiss, 1983) with smearing corrections based on a wavelength spread of 9% and a beam divergence of 0.011 radians. (a) Linear four-substructure model for CIs based on 100 spheres (R_G 7.8 nm). These substructures were represented by simplified structures of $7 \times 1 \times 6$ spheres (domains I, II and III), $4 \times 2 \times 2$ spheres (each SCR) and $4 \times 4 \times 2$ spheres (proteinase). (b) An X-shaped model for Clr_2 based on 216 spheres and the four substructures (R_G 7.7 nm). The Q ranges used in Fig. 2 for the R_{xs} determinations are indicated, to show that the models can account for their values. Good fits to nominal structural resolution of 4 nm (CIs) and 8 nm (Clr_2) are obtained.

where the prediction shows clear hydrophilic β -turns at Arg-211–Gly-212 and Arg-279–Gly-280 in Clr , and at Lys-195–Gly-196 and Lys-266–Gly-267 in CIs . That β -turns are well-predicted is shown by the presence of a conserved glycine residue at the third position of the turn in seven out of the eight cases in domains I and III (Schulz & Schirmer, 1978; Wilmot & Thornton, 1988). The multiple fragmentation patterns seen between Glu-274 and Thr-286 with different proteinases (Arlaud *et al.*, 1986) occur in a region predicted to contain two β -turns and a β -strand. It is the surface location of β -turns in globular proteins that renders these susceptible to proteolysis. The failure to observe the corresponding proteolyses in domain I can be attributed to the replacement of arginine, lysine and glycine residues at these β -turns by other residues. In conclusion, it is unlikely that random-coil structures play a role in the quaternary structure of $\text{Clr}_2\text{ClIs}_2$.

The sizes of the six globular domains are now compared with the hydrodynamic structures of CIs , Clr and Clr II in Fig. 3. The former are estimated as $4.3 \text{ nm} \times 3.7 \text{ nm} \times 3.3 \text{ nm}$ for the proteinase domain, $4.5 \text{ nm} \times 2 \text{ nm} \times 2 \text{ nm}$ for each SCR domain, and $3.5 \text{ nm} \times 2 \text{ nm} \times 2 \text{ nm}$ for the EGF-like domain. The hydrodynamic length of CIs and Clr is between 17 and 20 nm according to the sphere and cylinder methods. This is reasonably consistent with estimates of 21–26 nm that are inferred for Clr and CIs from the dimensions reported by Villiers *et al.* (1985), and that of 16 nm by Weiss *et al.* (1986). The Clr II hydrodynamic structure is likewise 9–11 nm long. Clr II is formed from domains IV, V and VI. Its length corresponds well to the linear arrangement of the serine-proteinase domain and the

two SCR domains with a total length of up to 13 nm. It is thus not necessary to propose a random-coil structure in order to account for the total length of Clr and CIs (Villiers *et al.*, 1985). By difference, domains I, II and III, which constitute the $\alpha\beta$ -fragment of Clr , are of overall length 4–7 nm. This is compatible with the size of the EGF-like domain II. It is thought that domain II is responsible for the interactions between CIs and Clr in the tetramer $\text{Clr}_2\text{ClIs}_2$ (Tosi *et al.*, 1987). Fig. 3 is compatible also with the most recent proteolytic and calorimetric studies on CIs , which suggest that there are at least four independently folded substructures which correspond to that containing domains I, II and III, the two SCR domains and the proteinase domain (Busby & Ingham, 1988; Medved *et al.*, 1989).

On the basis of these domain dimensions, the proposed arrangement of domains within Clr and CIs can now be explicitly tested against the wide-angle neutron curve (Fig. 4). A linear arrangement of the four substructures identified above leads to a good curve fit for CIs , as expected (Fig. 4a). The Clr_2 curve was investigated by trial-and-error simulations using about 100 models based on this CIs structure. Clr_2 models containing the eight substructures in a linear arrangement deviated markedly from the experimental curve, showing again that the linear model is unsatisfactory. R_G values close to 8 nm were obtained with the X-shaped model. Models of the type shown in Fig. 4(b), where this structure is non-planar, give the most satisfactory curve fit. Good agreement extended to $Q = 0.6 \text{ nm}^{-1}$, which includes both the R_G and R_{xs} regions. The angle between the two pairs of SCRs in the centre of the dimer model is 45° . Conversion of this model into the corresponding hydro-

dynamic model gives an $s_{20,w}^0$ value that is consistent with those calculated above (Table 2). The simulations were extended to consider the Clr_2Cls_2 scattering curve. As expected from the X-model, structures for Clr_2 that were distinct from those of Cls within the tetramer and with more asymmetric cross-sectional axes lead to significantly improved curve fits compared with the linear models of uniform cross-section for Clr_2Cls_2 that were previously used by Boyd *et al.* (1983) (not shown). In conclusion, the neutron curve-fitting was more compatible with the X-shaped domain model for Clr_2 than with a linear one.

Conclusions

The joint analysis of hydrodynamic and neutron data has resulted in an improved understanding of the quaternary structure of Clr_2Cls_2 . This is summarized in Figs. 1, 3 and 4. Both Clr and Cls are similar in shape, and both contain globular domains, as indicated from the primary- and secondary-structure analyses. The two different solution analyses show that the arrangement of these domains in Clr_2 is not linear and that this is manifested also in Clr_2Cls_2 . It was then shown by both methods that the structure of Clr within its dimer is compatible with the asymmetric X-shape model of Weiss *et al.* (1986). It should be noted that the available known structural constraints make such a model quite likely; however, this model is not proven by these analyses. The connection between the monomers in Clr_2 appears to be mediated in either the SCR domain, V, or the proteinase domain, VI (Fig. 3). The structure itself has several implications for the way in which the Cl complex is formed, even though at present there is no agreement on the way in which this complex is formed. Four distinct models for Cl have been proposed, and discussions of current possibilities for the Cl complex are reviewed elsewhere (Schumaker *et al.*, 1987; Perkins, 1989b). In this context, it is interesting that the angle of 45° between the two pairs of SCR domains in Fig. 4(b) is not far from the angle of 60° that separates any two adjacent arms of Clq to which Clr_2 binds in the Cl complex. This may imply that Clr_2 may exist in solution in a pre-formed bent conformation which would facilitate the recognition of its template on the Clq structure. It is hoped that the fine structure of these Cl models can now be more effectively tested by experiment.

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