Gross Conformation of C1q: A Subcomponent of the First Component of Complement†

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ABSTRACT: Hydrodynamic properties of rabbit C1q have been studied in an attempt to determine its gross conformation in solution and the effects of pH, temperature, and calcium on gross conformation. A molecular weight of 415 000 was determined. $s^0_{20,w}$ and $[\eta]$ of C1q were unaffected by calcium concentration up to 0.05 M. These parameters are pH dependent and indicate that translational frictional coefficient and hydrodynamic volume of C1q increase significantly with decreasing pH. They are also temperature dependent and indicate that translational frictional coefficient and hydrodynamic volume increase substantially with increasing temperature. In conjunction with other data the effects of pH and temperature are believed to largely involve conformational changes in the collagen-like regions of C1q. Application of the Simha equations to viscosity results, in conjunction with Scheraga-Mandelkern β values of 2.14–2.17 × 10^6, suggest that an oblate ellipsoid model of about 340 Å major axis is appropriate. From these results and information from electron microscopy on C1q, translational frictional coefficients have been calculated using the Bloomfield, Dalton, and Van Holde equation. Using a planar model which has six peripheral globular subunits placed in the corners of a hexagon and a seventh at the center, f/f_0 values at 20% hydration which are in excellent agreement with experimental results obtain.

Complement is a set of enzyme precursors which is found in normal serum and whose cascading activation is classically initiated by antigen-antibody reaction (Müller-Eberhard, 1969; Osler, 1976). These sequentially activated enzymes of the immune system cause cytotoxic effects and produce mediators which induce various aspects of inflammation. A well-known example of complement mediated activity is the lysis of red blood cells which occurs when cells are incubated with antibodies directed to cell surface components in the presence of fresh normal serum.

The activation of complement via the classical pathway begins with the binding of the first component, C1, to the Fc region of antigen complexed IgM or certain classes of IgG (Müller-Eberhard & Kunkel, 1961; Augener et al., 1971; Cooper, 1971, 1973). At the present time there is evidence that this first component consists of at least three subcomponents, C1q, C1r, and C1s, all of which are bound in a loose macromolecular superstructure whose integrity depends on calcium (Lepow et al., 1963). A fourth subcomponent, C1t, has been implicated due to its ability to enhance hemolytic activity of a mixture of the other three subcomponents (Assimeh & Painter, 1975), but recent evidence has demonstrated this is not the case (Ziccardi & Cooper, 1977).

Subcomponent C1q, which has no apparent enzymatic activity, is believed to be the major Ig binding protein of C1 and is, therefore, the physical link between the antibodies and the complement system (Müller-Eberhard & Kunkel, 1961). The recent development of isolation techniques which result in quantities of C1q sufficient for physical and chemical analysis has been instrumental in studies leading to some understanding of structural aspects of this component (Reid et al., 1972). Current evidence indicates that C1q has a molecular weight between 390 000 and 416 000 and is composed of six subunits each made up of three peptide chains which are either covalently linked by disulfide bonds or associated through noncovalent interactions (Yonemasu & Stroud, 1972; Reid et al., 1972; Heusser et al., 1973; Reid & Porter, 1976). Electron microscopy studies in conjunction with trypsin and collagenase digestion results indicate that C1q has a most unusual gross conformation which has been described as like a bouquet of tulips (Svehag & Bloth, 1970; Svehag et al., 1972; Shelton et al., 1972; Knobel et al., 1975; Reid & Porter, 1976). The results of Svehag et al. (1972) indicate that C1q contains two major types of subunits, possibly two central and six peripheral and that the peripheral subunits are linked to the central part by fiber-like appendages. It is notable that C1q is composed of quantities of the amino acids hydroxylysine, hydroxyproline, and glycine, all of which typically occur in collagen (Müller-Eberhard, 1968). Recent sequence determinations indicate that fiber-like appendages are the collagen-like portions of the molecule (Reid, 1974). Proteolytic digestion studies of C1q by Knobel et al. (1974) suggest that the peripheral globular subunits of C1q are involved in binding to immune complexes. Although the valence of C1q for Ig is not well established, a recent study by Schumaker et al. (1976) presents ultracentrifuge data which are consistent with the possibility of 12 or 18 binding sites for Fc.

In view of the role which C1q may play in the activation of complement, hydrodynamic studies of this macromolecule have been performed in an attempt to assess its gross conformation in solution. In this report measurements of molecular weight, sedimentation coefficients, and intrinsic viscosities of C1q are presented. Hydrodynamic theories have been applied to these results in an effort to ascertain its probable gross conformation in solution. The effect of temperature and pH on the latter coefficients has also been measured to obtain some insight into the heat labile nature of C1q and the dependence of C1q-Ig interaction on pH.

Materials and Methods

All reagents used in the study were of reagent grade or better. C1q was prepared from normal rabbit sera (Pel-Freeze,

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TABLE I: The Effects of pH on Hydrodynamic Parameters of C1q.

pH	$s^0_{20,w\pm}$	k (dL/g)	f/f_{min}	$[\eta]$ (dL/g)	k'	$\beta \times 10^{-6}$
7.4[a]	10.43 ± 0.02	0.15	1.92	0.200	0.80	2.17
6.0[b]				0.211	1.68	
5.3[a]	9.89 ± 0.06	0.28	2.02	0.221	1.72	2.14

[a] Measurements were made in phosphate buffer; $\mu = 0.318$. [b] Measured in 0.05 M cacodylate, 0.15 M NaCl.

Arkansas) by methods described in detail (Paul & Liberti, 1978). Samples ranging from 3 to 12 mg/mL were stored at −70 °C until use. Concentrations of C1q were determined spectrophotometrically using an extinction coefficient of $E_{280nm}^{1\%} = 6.82$, a value determined by gravimetric methods employing a Cahn Micro balance (Cahn Instruments, Paramount, Calif.) and in agreement with the value for C1q determined by Reid et al. (1972).

Molecular Weight Determination. The molecular weight of C1q was determined using the high speed meniscus depletion method of Yphantis (1964). A Spinco Model E ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.) equipped with interference optics was used. Analyses were performed on C1q samples having initial concentrations of 0.3, 0.4, and 0.5 mg/mL equilibrated with 0.05 M cacodylate, pH 6.0, in 0.15 M NaCl. Extreme care was exercised in the aligning of the interference optics and in the reading of the interference patterns.

Sedimentation Velocity Experiments. Sedimentation velocity experiments were performed on the Spinco Model E ultracentrifuge. Schlieren optics (phase plate angle at 60°) and photoelectroscanning absorption were used. During the course of these experiments, the thermistor calibration of the AN-D rotor (Beckman Instruments) used and the temperature-controlling devices were repeatedly checked for calibration. When an experiment involved extremes of temperature, the rotor was either precooled or preheated. Schlieren experiments were done in 12-mm aluminum center piece single-sector cells for concentrations from 1.5 to 10 mg/mL. Double-sector epon filled centerpiece cells were used for absorption experiments. For each scanner experiment, linearity of recorder output with respect to optical density and distance was first determined. The half-concentration level was taken to indicate the boundary position. For both methods, sedimentation coefficients were determined graphically in the usual manner. In addition, linear regression analysis was performed on the data using a program written for the Wang 600 programmable calculator (Wang Instruments, Tewksbury, Mass.; program available upon request). Sedimentation coefficients were corrected to $s^0_{20,w}$ from measured viscosities and densities of the solvents used at appropriate temperatures.

Viscosity Experiments. Viscosity studies were performed in Cannon-Manning, semimicro viscometers, size 75 (Cannon Instrument Co., State College, Pa.), having flow times for water of 150–180 s. Viscometers were cleaned between each experiment with chromic acid cleaning solution and exhaustively flushed with distilled deionized dust free water and subsequently dried in a vacuum oven equipped with an inlet air filter set at 60 °C. In experiments where flow times could not be reproduced within ±0.1 s, the viscometer or the solution being measured was deemed unclean and the run abandoned. Flow times for protein solutions generally ranged from about 15 to 30 s over solvent and could generally be reproduced to ±0.05 s. Specified temperatures were maintained at ±0.05 °C. Protein solutions were extensively dialyzed before use and passed through washed 3-μm filters (Millipore Corp., Bedford, Mass.).

As kinetic energy corrections for the viscometers used proved to be negligible, the average flow time ($\bar{t}_s$) for each concentration of C1q examined was converted to relative viscosity (η_{rel}) by the relationship $\eta_{rel} = D(t_s/t_0)$ where subscripts denote solution and solvent time and D is the density ratio of solution to solvent which was determined in each case. Intrinsic viscosities, $[\eta]$, were determined graphically utilizing the Huggins equation (Huggins, 1942).

$$[\eta]_{sp/C} = [\eta] + [\eta]^2 k' C$$

Intercepts of plots of η_{sp}/C vs. C yield $[\eta]$. Huggins constant k' were determined from slopes.

Results

Two general problems which can affect physiochemical measurements have been observed for C1q preparations. These are: (1) time-dependent protein–protein aggregation; and (2) contamination with proteolytic enzymes which leads to unstable preparations (J. H. Wissler, personal communication). For the C1q studied here there was no indication of time-dependent aggregation nor was there any evidence for enzymatic hydrolysis of samples examined for extended time periods. By a variety of physiochemical methods these preparations were found to be free of contaminants (Paul & Liberti, 1978). The hemolytic activities of preparations were such that 0.5 ng added to RC1q sera resulted in 50% lysis of 5×10^7 optimally sensitized sheep red blood cells. Agglutination of IgG coated latex particles (Hyland Laboratories, Costa Mesa, Calif.) could be detected with concentrations of C1q as low as 0.3 μg/mL.

Molecular Weight of Rabbit C1q. For ultracentrifuge equilibrium experiments done at 8000 rpm, plots of log concentration vs. r^2 were linear over the entire range of concentrations. This result indicates that the preparations were monodispersed and behaved ideally under the conditions chosen (Yphantis, 1964). Analyses performed at 18, 24, and 30 h resulted in graphs which were superimposable. Using an assumed value of $\bar{V}$ of 0.73, a molecular weight of 415 000 ± 1500 was calculated.

Sedimentation Velocity Experiments. The concentration dependence of sedimentation of C1q at pH 7.4 (phosphate buffer, $\mu = 0.318$) was examined from 0.25 to 1.6 mg/mL using absorption optics and from 1.0 to 10 mg/mL with schlieren optics. Over this entire range linearity obtains. Results in pH 6.0, 0.05 M cacodylate, 0.30 M NaCl were similarly linear and were completely unaffected by Ca^{2+} addition up to 0.05 M. Also at pH 6.0 identical results obtain at one-half the saline concentration.

Because rabbit C1q shows excellent solubility properties in the pH 5 range and because C1q–IgG interactions are diminished at this pH, sedimentation analysis was also done at pH 5.3 (phosphate buffer, $\mu = 0.318$). These results along with those at pH 7.4 are tabulated in Table I. As can be seen from this table, the sedimentation coefficient at the lower pH is substantially decreased. Also shown in Table I are frictional coefficient ratios, f/f_{min}, calculated from sedimentation and molecular weight results. These values show that the frictional

TABLE II: Effect of Temperature on $[\eta]$ and $s^0_{20,w}$ of C1q.[a]

Temp (°C)	$s^0_{20,w}$	k (dL/g)	$[\eta]$ (dL/g)	k'
7.6	10.8_0	0.1_6		
20.0	10.4_3	0.1_5		
25.0			0.200	0.80
37.0	10.2_9	0.1_5	0.216[b]	1.70[b]
48.0			0.230	2.52
50.0			0.244	3.28

[a] All measurements were done at pH 7.4 in PBS; $\mu = 0.318$. [b] Similar values obtain for measurements done on samples preincubated at 48 °C for 1 h. Preincubation at 50 °C did not result in similar values.

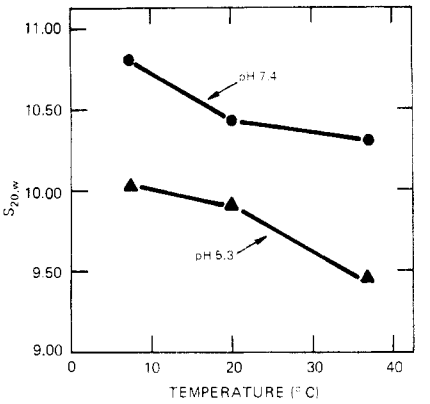

FIGURE 1: The effect of temperature on sedimentation coefficient of rabbit C1q at pH 7.4 and 5.3, phosphate buffer ($\mu = 0.318$).

coefficient ratio is substantially increased at the lower pH. Values of f/f_{min} are a measure of both molecular hydration and asymmetry of shape. For globular proteins typical values range from about 1.0 to about 1.2 (Tanford, 1961). From the value for rabbit C1q it is apparent that the macromolecule is considerably asymmetric and may also include considerable amounts of hydrated solvent.

Intrinsic Viscosities. Table I also lists intrinsic viscosities of rabbit C1q measured at pH 5.3, 6.0, and 7.4. Measurement of viscosities at pH 7.4 and 5.3 in viscometers having considerably different flow times resulted in intrinsic viscosities which were independent of viscometer flow time. This and the fact that low shear velocity gradient viscometers were used indicate the values listed are independent of shear rate.

Intrinsic viscosity is a measure of the hydrodynamic volume of a macromolecule. From Table I it can be seen that intrinsic viscosity values increase with decreasing pH, which is consistent with the f/f_{min} or sedimentation results. Also noteworthy is that, when the pH 6.0 intrinsic viscosity experiment was repeated in the presence of 0.01 M Ca^{2+}, the Huggins plot was identical with that obtained in the absence of Ca^{2+}, indicating Ca^{2+} is without effect on $[\eta]$ as well as sedimentation coefficient.

The Huggins constants, k', listed in Table I, show an increase with decreasing pH. These values, which are a measure of doublet formations, i.e., hydrodynamic interaction (Goring & Sitaramiah, 1963), are related to macromolecular asymmetry and are also consistent with the increases observed in $[\eta]$ and in f/f_{min} with decreasing pH.

Intrinsic viscosity, like f/f_{min}, depends on two quantities, namely, macromolecular asymmetry and hydration. Hence, C1q appears to be considerably symmetric with respect to sedimentation and to viscosity. For globular proteins typical intrinsic viscosities range from 0.030 to 0.060 dL/g (Creeth & Knight, 1965). The intrinsic viscosity values for C1q indicate that its gross conformation is significantly different than that of typical globular proteins.

Consistency of M_r, $s^0_{20,w}$, and $[\eta]$ for Rabbit C1q. The Scheraga-Mandelkern equation, $\beta = s^0_{20,w}\eta_0[\eta]^{1/3}/NM_r^{2/3}(1 - \bar{v}\rho)$, where β is a theoretical parameter related to shape, η_0 is the solvent viscosity, and N is Avogadro's number, has been used successfully to determine the internal consistency of hydrodynamic data. For spherical particles β has a value of 2.12×10^6, for oblate ellipsoids, β ranges from 2.12×10^6 to 2.15×10^6 for semi-major to semi-minor axis ratios, a/b, of up to 300 (β for oblate ellipsoids is therefore essentially insensitive to axial ratio), and for prolate ellipsoids β is 2.16×10^6 for a/b of 3, 2.44×10^6 for a/b of 10, 2.72×10^6 for a/b of 30, and 3.22×10^6 for a/b of 100 (Scheraga & Mandelkern, 1953).

The calculated β values for data obtained at pH 7.4 and 5.3 are listed in the last column of Table I. It is first noted that the listed β values are reasonable, indicating that the C1q results are internally consistent. The β values of 2.14 and 2.17×10^6 suggest that C1q might be a prolate ellipsoid of low axial ratio (about 3:1). However, in combination with the f/f_{min} data and intrinsic viscosity values it would be necessary to conclude that C1q has extremely high degrees of hydration (about 5 g of solvent/g of C1q). On the other hand, the β values are slightly greater than those for oblate ellipsoids. As can easily be shown, small inaccuracies in the results presented or minor adjustments in the assumed $\bar{V}$ results in β values which fall within the theoretical range for oblate ellipsoids.

Another check on data internal consistency can be obtained by calculating the ratio of the coefficient of sedimentation dependence, k, to $[\eta]$. Wales & Van Holde (1954) and Creeth & Knight (1965) have shown that in practice and in theory values of 1.6 result for random coil molecules and for compact spherical macromolecules. Lowered values obtain for asymmetric macromolecules. For the pH 7.4 data (Tables I and II) at 20 and 37 °C $k/[\eta]$ values of 0.75 and 0.7 obtain, respectively. At pH 5.3 and 20 °C the ratio is 1.2. These values in conjunction with the f/f_0 and $[\eta]$ results confirm the asymmetric nature of C1q. The difference in the ratio at pH 7.4 and 5.3 suggests conformationally different states of C1q at these pHs.

Effect of Temperature on $s^0_{20,w}$ and $[\eta]$. Because C1q is a heat labile protein and because the existence of possible effects of protein–protein aggregation was of concern (even though the previous results indicated that not to be the case), sedimentation coefficients and intrinsic viscosities were determined over a range of temperatures.

Table II lists sedimentation $s^0_{20,w}$ values measured at pH 7.4 in the lower portion of the temperature range studies. It can be seen that these values decrease with increasing temperature which indicates that f/f_{min} increases with increasing temperature, or that C1q becomes more asymmetric and/or hydrated with increasing temperature. A similar temperature dependence resulted when measurements were done at pH 5.3. These results along with those at pH 7.4 are depicted in Figure 1.

Viscosity studies done at elevated temperatures are depicted in Figure 2. Since attempts to do intrinsic viscosities at 52 °C resulted in opalescent solutions, it was important to determine at what temperature C1q could be heated and remain native. This was done by performing measurements first at 37 °C, next heating the sample up to some specified temperature for 1 h, cooling back to 37 °C, and repeating the viscosity measurements. As indicated in Figure 2, measurements performed at 37 °C were reproducible on samples which had been incubated for 1 h at 48 °C. On the other hand, when samples were heated

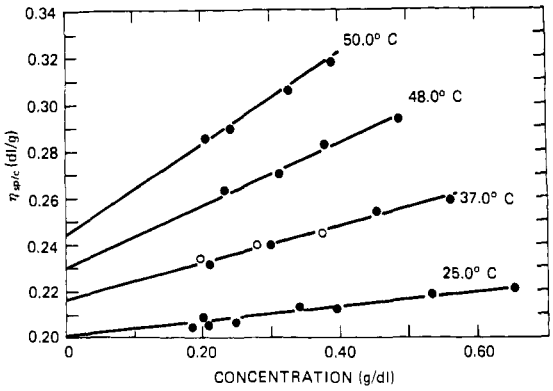

FIGURE 2: The effect of temperature on viscosity of rabbit C1q. Open circles on 37 °C curve represent samples which had been incubated for one hour at 48 °C prior to measurement at 37 °C.

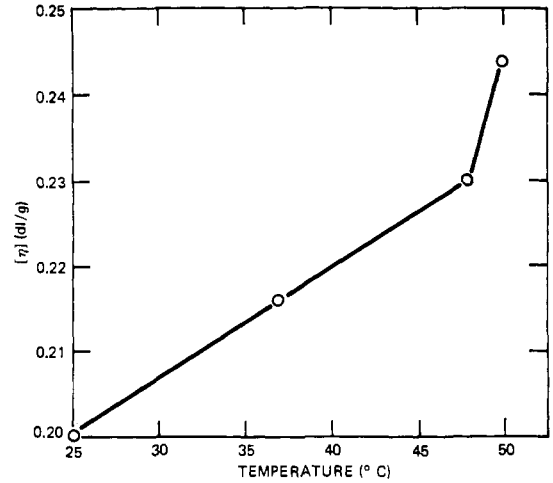

FIGURE 3: The effect of temperature on intrinsic viscosity of rabbit C1q.

to 50 °C for 1 h, cooled to 37 °C, and remeasured, η_{sp}/C values did not fall along the 37 °C curve. This indicates that the viscosity behavior of C1q is reversible within a range of up to 48 °C and becomes partially irreversible above 50 °C. These results also indicate that the C1q preparations studied do not contain aggregated protein or unstable protein, since heating should promote and magnify the effects of their presence. The temperature transition above 48 °C for rabbit C1q can also be seen by the plot of $[\eta]$ vs. temperature depicted in Figure 3. $[\eta]$ values are linear up to that temperature, indicating some smooth transition, but increase sharply above 48 °C. Taken in conjunction with the sedimentation experiments or f/f_{min} values these results indicate that the asymmetry and/or hydration of C1q appear to increase smoothly between 7 and 48 °C.

Discussion

The molecular weight result for rabbit C1q is in good agreement with previous determinations made on C1q (Reid et al., 1972; Calcott & Müller-Eberhard, 1972; Yonemasu & Stroud, 1971). Sedimentation coefficients and their dependence on concentration are in accord with those reported for the human and rabbit subcomponent while the linearity of $s_{20,w}$ with concentration indicates the absence of protein–protein association in these preparations and also that subunit disassociation does not occur in the low concentration range. The viscosity–temperature experiments confirm the absence of C1q aggregates in these preparations. β values calculated from the Scheraga–Mandelkern equation for M_r, viscosity, and sedimentation results indicate that these measurements are internally consistent.

The $[\eta]$ and $s^0_{20,w}$ results show that C1q has increased hydrodynamic volume and translational frictional coefficient with decreasing pH and that those parameters are unaffected by Ca^{2+}. The sedimentation coefficient at pH 6.0 was unaffected by doubling the saline concentration. These results are in complete accord with spectrophotometric and hydrogen exchange studies of C1q (manuscript in preparation). In those experiments, it was found that the conformation of C1q in buffered saline at pH 7.4 and 6.0 did not differ significantly from C1q at twice the saline concentration as determined with circular dichroism, difference spectroscopy and hydrogen exchange. Furthermore, results were unaffected by Ca^{2+} for those methods and additionally Mg^{2+}, Mn^{2+}, or EDTA did not alter hydrogen exchange kinetics. On the other hand, with all the methods used a pH effect was observable between 5.1 and 8.3. At the lower pH, solvent perturbation difference spectroscopy experiments indicate that the equivalent of 10 ± 2 and 6 ± 1 mol of Tyr and Trp, respectively, became exposed. These changes were ascribed to probable alterations in the globular regions of C1q. Circular dichroism spectra near pH 5 suggest changes in Tyr exposure and possible alterations in the collagen-like regions of C1q, while hydrogen exchange results are compatible with a small, but significant, conformational change occurring between pH 5 and 6. Hence by these methods which are all capable of detecting conformational alterations in C1q, along with hydrodynamic results presented, it appears that divalent ions are without effect, ionic strength above saline seems to have no effect, whereas acid pH does result in changes in all cases. These results taken together are compatible with an acid expansion of C1q and small conformational transitions occurring near pH 5. The possible molecular changes indicated by the viscosity and sedimentation results will be considered later.

Since the integrity of the C1 macromolecular complex is known to be calcium dependent and because the collagen-like regions of C1q might be expected to be affected by calcium (Gustavson, 1956; Li et al., 1975), the absence of an effect on the viscosity and sedimentation results is somewhat surprising. As noted above this absence of an effect is in agreement with spectrophotometric and hydrogen exchange results. In view of the rigidity of the collagen triple helix structure and the spacing between charged basic amino acid residues of collagen to which calcium is believed to bind (Li & Katz, 1976), it is possible that calcium induced no dimensional changes in the collagen-like structures of C1q via electrostatic interaction or by destabilizing the triple helix (von Hippel, 1967). Since there is NMR evidence that calcium can occupy the sites of structural water of collagen (Fung & Wei, 1973) thus affecting hydration, some effect on viscosity and sedimentation results would be anticipated, unless asymmetric and hydration effects are of similar but opposite magnitudes. A hydration or localized electrostatic effect would be expected to give rise to some changes in hydrogen exchange kinetics and that also was not the case. However, it is possible that very fast exchanging hydrogens of the collagen-like regions of C1q are affected by calcium but because the dialysis procedure was used to do the exchange studies such effects were not seen.

Taken together these results on the absence of an apparent calcium effect on C1q imply that there is either no effect or, at best, the effect is very small, which is not to imply that a small effect would be unimportant. This tentative conclusion is in keeping with the recent findings of Ziccardi & Cooper

TABLE III: Axial Ratios of Equivalent Oblate and Prolate Ellipsoids Calculated from C1q Intrinsic Viscosity Results.

		$\delta = 0.0$			$\delta = 0.2$			$\delta = 0.5$		
			a/b			a/b			a/b	
pH	$[\eta]$ (dL/g)	ν[a]	Obl[b]	Pro[c]	ν	Obl	Pro	ν	Obl	Pro
5.3	0.221	30.3	44.0	17.3	23.8	34.4	14.8	18.0	25.8	12.3
6.0	0.211	28.9	41.9	16.8	22.7	32.8	14.3	17.2	24.6	11.9
7.4	0.200	27.4	39.7	16.2	21.5	31.0	13.8	16.3	23.3	11.4

[a] Simha factor, ν, calculated from $\nu = 100\,[\eta]/(\bar{v} + \delta v^0)$. [b] Iterated from the Simha equation, $\nu = 16(a/b)/15\,\tan^{-1}(a/b)$, for oblate ellipsoids of $a/b > 10$. [c] Iterated from Simha equation for prolate ellipsoids, $\nu = ((a/b)^2/15[\ln 2a/b - 1.5]) + ((a/b)^2/5[\ln 2a/b - 0.5]) + (14/15)$.

(1976). They have shown that calcium inhibits the 37 °C autoactivation of the C1 subcomponent C1r. Once C1r is activated, calcium has no effect. Their hypothesis is that calcium binds to C1r and stabilizes the proenzyme form. In this concept, a conformational change occurring after C1 binding to an immune complex might be seen as permitting a rearrangement of intramolecular calcium, thus allowing C1r activation.

The experiments on the effect of temperature on sedimentation and viscosity were done to obtain some insight into the heat labile nature of C1q and also as a means of examining the contribution any C1q aggregation might have on viscosity. The experiments dealing with the latter indicate that the viscosity results are not artifactual. Thus the viscosity and sedimentation studies show that C1q conformation is temperature sensitive over the entire range of temperatures examined. The transition temperature (>48 °C) is in good agreement with the results obtained for circular dichroism "melting" studies of C1q by Brodsky-Doyle et al. (1976). They found a midpoint of thermal transition of 48 °C for human C1q and a breadth of transition (1/4 to 3/4 "melting") of 15 °C. By comparison, they observed that lathyritic rat skin collagen had a transition temperature 10 °C less and a breadth of transition of 3 °C. Also notable from their studies is that circular dichroic changes are apparent in C1q starting at the lowest temperature studied (about 26 °C), while for lathyritic collagen there are no apparent dichroic changes until 35 °C is reached (Brodsky-Doyle et al., 1976, Figure 2). Hence not only does the collagen-like nature of C1q differ from collagen with respect to "melting" but their studies and the viscosity and sedimentation results presented indicate temperature conformational dependence over a wide temperature range.

It is tempting to ascribe the apparent hydrodynamic temperature sensitive changes observed for C1q to changes in the collagen-like regions of the molecule since, as noted, circular dichroism results (Brodsky-Doyle et al., 1976) indicate changes in collagen-like regions of C1q at temperatures greater than 26 °C. There is, however, no evidence that temperature affects viscosity or sedimentation behavior of collagen in a fashion similar to that observed for C1q. For the collagen → gelatin transition, viscosity decreases slowly as the transition temperature region is approached and undergoes a sharp decrease at the transition temperature (von Hippel & Wong, 1963). Nonetheless, for a molecule like C1q which has globular and collagen-like regions, it is reasonable to expect little or no change in the globular parts of the molecule in the temperature range examined. On the other hand, the complexities of solvent hydration, hydrogen bonding and molecular packing within a collagen-like structure (Ramachandran, 1967) conceivably could result in significant temperature-dependent conformational alteration particularly if the collagen-like regions of C1q do not possess all the structural stabilizing factors found in collagen. In addition, in structures like collagen, which are essentially unidimensional, small local effects could augment each other while in globular structures they may be self cancelling. Hence, from these considerations, the Brodsky-Doyle et al. (1976) results and in view of the magnitude of change in sedimentation coefficients and intrinsic viscosity results over the temperature range examined it seems likely that structural alterations in the collagen-like regions are involved.

It is of interest to compute hydrodynamic dimensions of C1q from the viscosity results to obtain some insight into the nature of changes observed for pH and temperature and also for the purpose of comparing a hydrodynamic model with that proposed for C1q from electron microscopy studies. Solution dimensions can be obtained from intrinsic viscosity data using the Simha (1940) treatment, which is based on the assumption that macromolecular hydrodynamic properties can be approximated by those due to equivalent prolate or oblate ellipsoids. The Simha equation which relates the dual dependency of $[\eta]$ on macromolecular shape, and volume, is $[\eta] = \nu(\bar{v} + \delta\bar{v}^0)/100$, where ν is the Simha factor, $\delta\bar{v}^0$ is a hydration term (grams of solvent/gram of protein) and $\bar{v}^0$ is the partial specific volume of solvent. The hydration factor is a modification introduced by Oncley (1941). The Simha factor, ν, which is related to particle asymmetry reduces to the Einstein coefficient of 2.5 for spherical particles. In Table III are listed values calculated for the intrinsic viscosity data assuming 0, 20, and 50% hydration. As can be seen from these values, C1q is considerably asymmetric even at 50% hydration.

Using the Simha equations which relate ν to anisotropy of oblate and prolate ellipsoids, semimajor, to semiminor axial ratios, a/b, can be obtained (Simha, 1940). Calculated values of a/b for the prolate and oblate models and for the three degrees of hydration being considered are also tabulated in Table III. Focusing attention on the values of a/b for the prolate model, it can be seen that the lowest values are between 11.4 and 12.3, at the highest degree of hydration. A Scheraga-Mandelkern β value for prolate ellipsoids in this range should be in excess of 2.4×10^6 (Scheraga & Mandelkern, 1953). The values obtained earlier in "results" from the experimental data presented are between 2.14×10^6 and 2.17×10^6.

From the axial data, dimensions of equivalent ellipsoids can be calculated by equating molecular volumes of C1q at appropriate degrees of hydration and equations for volumes of prolate and oblate ellipsoids. Values so obtained are listed in Table IV. From the table the limits of the long dimension, for the prolate ellipsoid approximations, are between 595 and 660 Å. This range is in good agreement with structures reported by Polley (1971) from early electron microscopy studies of C1q. For the oblate ellipsoid model, the calculated major axis values lie between 335 and 350 Å. These values are in excellent agreement with the most recent electron microscopy measurements made in several laboratories (Svehag et al., 1972; Shelton et al., 1972; Knobel et al., 1975). Based on the good agreement between the dimensions of the oblate ellipsoid C1q model and the most recent electron microscopy studies of C1q, on the Scheraga-Mandelkern β values, which are within ex-

TABLE IV: Molecular Dimensions[a] of C1q As a Function of Degree of Hydration.

pH	Model	Degree of hydration (g of solvent/g of C1q) $\delta = 0.0$	$\delta = 0.2$	$\delta = 0.5$
5.3	Oblate	349 × 8[b]	348 × 10	347 × 14
7.4	Oblate	337 × 9	336 × 11	335 × 15
5.3	Prolate	660 × 38[c]	645 × 44	626 × 51
7.4	Prolate	632 × 39	616 × 45	595 × 52

[a] Dimension (Å) calculated from intrinsic viscosity axial ratio data oblate and prolate ellipsoid models. [b] Calculated by equating the volume of an oblate ellipsoid $V_{o.e.} = 4/3\pi ba^2 = 4/3\pi(b/a)a^3$ with the hydrated volume of C1q. [c] Calculated by equating the volume of a prolate ellipsoid, $V_{p.e.} = 4/3\pi ab^2 = 4/3\pi(a/b)b^3$ with the hydrated volume of C1q.

perimental error of those for oblate ellipsoids, it seems reasonable to suggest that C1q in solution approximates an oblate ellipsoid having a major axis of about 340 Å.

Since recent electron microscopy studies give sufficient information to compute reasonable translational frictional coefficients from the shell model theory of Bloomfield et al. (1967) (Schumaker et al., 1976), the planar aspect of the oblate model and its dimensions calculated here can be incorporated with this information to compute frictional ratios which can be compared with experimental values. A reasonable combination of the viscosity and electron microscopy models is a planar structure of C1q composed of six globular subunits arranged at the corners of a hexagon and a seventh globular subunit at the center. The globular subunits are assumed to be spherical and have molecular weights of 51 925 and 103 850 for the peripheral and central subunits, respectively. Assuming 20% hydration, the radii of the peripheral and central subunits are 26.8 and 33.7 Å, respectively. For a pH 5.3 model, the distance of centers of peripheral subunits and the central subunit is 147.2 Å. This corresponds to a maximum dimension of 348 Å in accordance with the viscosity dimension. At pH 7.4, the distance of centers of the peripheral subunits to the central subunit is 141.2 Å corresponding to a maximum dimension of 336 Å. f/f_0 calculated from the appropriately modified Bloomfield et al. equation (eq 23 of Bloomfield et al., 1967) are listed in Table V along with experimental f/f_0's calculated at 20% hydration. As can be seen, for the combined viscosity–electron microscopy model of C1q, theoretical values of f/f_0 result which at pH 5.3 are in exact agreement with experimental values and which differ at pH 7.4 by about 2%. Exact agreement at pH 7.4 requires that the distance between centers of peripheral subunits and the central subunit be decreased from 141.2 to 133.2 Å corresponding to a molecule which has a maximum dimension of 320 Å. This latter value is in excellent agreement with that obtained by Svehag et al. (1972) from electron microscopy studies of C1q.

In view of the adjustment which had to be made in the distance separating the centers of peripheral and central subunits to give theoretical and experimental agreement at pH 7.4, it seems reasonable to suggest that the fibrillar collagenous arms which are believed to connect the globular subunits of C1q are primarily responsible for the changes in intrinsic viscosities and sedimentation coefficients found at pH's 7.4 and 5.3. As was shown above for the theory to agree with experimental values a given connecting strand had to increase from 133.2 Å at pH 7.4 to 147.2 Å at pH 5. This corresponds to an increase of about 10% in length. It is notable that the collagen-like regions of human C1q have a considerable excess of basic to acidic amino

TABLE V: Comparison of Theoretical and Experimental Translational Frictional Coefficients of C1q at 20% Hydration.

pH	f/f_0(experimental)	f/f_0(theoretical)[a]
5.3	1.86	1.86
7.4	1.77	1.82

[a] Calculated from the Bloomfield et al. equation (1967) modified to include f_0, $f/f_0 = (R_0^{-1}[\Sigma^n_{i=1} r_i^2])/(\Sigma^n_{i=1} r_i^3 + \Sigma^n_{i=1}\Sigma^n_{j=1} r_i r_j (R_{ij})^{-1})$, where r_i and r_j are radii of spherical subunits and R_{ij} is the distance between centers of subunits. R_0 is the equivalent hydrated radius of the entire molecule assuming it to be spherical.

acid residues (Reid, 1974). Hence, an acid expansion which effects the collagen-like regions of C1q is reasonable. It seems also reasonable to suggest that the temperature dependence of C1q conformation, apparent in the sedimentation and viscosity results, may also derive from changes in the collagen-like connecting regions of the molecule, since the magnitude of changes in those parameters indicates substantial dimensional alteration.

In spite of the excellent agreement apparent from the previous calculations and the indications that C1q in solution approximates a planar macromolecule, in a recent study by Schumaker et al. (1976) translational frictional coefficients of C1q were calculated using assumptions similar to those presented here, but from a quite different geometrical arrangement of subunits. In their model, six peripheral subunits were arranged at the vertices of a regular octahedron and the seventh subunit at the center. Distances between the peripheral and central subunit were adjusted until frictional coefficients were obtained which agreed with sedimentation results they had obtained for human C1q. Their calculations show that placement of the centers of the peripheral subunits 135.5 Å from that of the central subunit, which corresponds to a molecule having a span of 320 Å between two opposite peripheral subunits, yields appropriate frictional coefficients. This model which also agrees with electron microscopy information has three-dimensional symmetry which intuitively is attractive, while the planar model which derives from calculations based on $[\eta]$ and β has two-dimensional symmetry. It is of interest to note that the Schumaker model is not asymmetrical in the normal sense.

In summary, the intrinsic viscosity sedimentation coefficient and molecular weight results on C1q suggest that C1q is a planar molecule approximating an oblate ellipsoid. The data and theoretical treatment results suggest that the gross conformation of C1q in solution is in accord with that visualized by electron microscopy. pH and temperature have significant effects on the gross conformation of C1q which are believed to involve the collagen-like regions of the molecule, while calcium appears to be without effect. Studies of the effects on C1q conformation upon interaction with immune complexes are in progress.

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