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Assembly of subcomponents C1r and C1s of first component of complement: Electron microscopic and ultracentrifugal studies

(hydrodynamic shape/domain structure)

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ABSTRACT **Monomeric C1s (M_r, 85,000; $s_{20,w}$, 4.3 S), a subcomponent of first component of complement (C1), the dimer (M_r, 170,000; $s_{20,w}$, 6.7 S) of C1r, another subcomponent, and the tetrameric complex $(C1r,C1s)_2$ (M_r, 340,000; $s_{20,w}$, 8.7 S) are elongated molecules. Hydrodynamic equivalents of cylindrical shape have a diameter of 3.3 nm and lengths of 20 nm for C1s, 36 nm for $(C1r)_2$, and 64 nm for $(C1r,C1s)_2$. In electron micrographs the C1r,C1s complex appears as a chain composed of six to eight globular domains with a contour length of 51 nm. A structure is proposed in which $(C1r)_2$ forms a core to which C1s protomers are associated at both ends. The C1 complex ($s_{20,w}$, 16.3 S) reconstituted from C1q, C1r, and C1s dissociates under the conditions used for electron microscopy. Some features of the C1 complex are revealed in the dissociation products.**

The first component of complement (C1) is a complex of the binding protein C1q and two zymogens, C1r and C1s (1, 2). The zymogens are activated on binding of immune complexes, IgG aggregates (3), or chemically crosslinked IgG protomers (4) to the heads of C1q. In the classical pathway the activation of C1 is the first step in a sequence of activation steps of other components of the complement system. This sequence finally leads to the lysis of cells recognized by the antibodies. The structure of C1q has been elucidated by chemical (1, 2, 5), electron microscopic (6–8), and spectroscopic (8) studies. It consists of six globular heads held together by collagen stems, in a structure resembling a flower bouquet.

Much less is known about the structures of C1r and C1s. They are similar homologous proteins. From gel electrophoresis, chain molecular weights ranging from 83,000 (9) to 95,000 (10) were derived for C1r and from 83,000 to 87,000 for C1s (9, 10). Native C1r is known to dimerize in the absence or presence of Ca^{2+}; C1s dimerizes only in the presence of Ca^{2+} (11). In the presence of Ca^{2+} C1r combines with C1s to form a 1:1 complex which probably contains two protomers of C1r and of C1s (1, 11, 12). Most likely the zymogens combine in the form of this complex with C1q to yield functional C1 (13). On activation, C1r and C1s are split to two disulfide-linked peptide chains of molecular weights 27,000 and 56,000 (9, 10, 14, 15).

The present communication reports the shapes of $(C1r)_2$, C1s, and the complex $(C1r,C1s)_2$ as estimated from sedimentation constants and molecular weights. In addition we studied the morphology of $(C1r,C1s)_2$ by electron microscopy.

MATERIAL AND METHODS

C1r and C1s were prepared (16) from fresh human blood plasma (Blutspendezentrum, Basel). Diisopropyl fluorophosphate (iPr_2P-F) was added after plasma clotting and at various steps during the preparation. In addition, purified C1r and C1s were extensively dialyzed against 5 mM iPr_2P-F/10 mM Tris·HCl, pH 7.4/150 mM NaCl. The preparations obtained in this way contained 10–30% cleaved but iPr_2P-F-inactivated (14, 15) material as judged by $NaDodSO_4$/polyacrylamide gel electrophoresis. The 70–90% nonactivated C1r (or C1s) could be completely activated in the presence of C1q and IgG dimers (17). C1 reconstituted from C1q, C1r, and C1s showed little self-activation during an incubation period of 20 min as monitored by the Cooper and Ziccardi (18) test. C1q was prepared according to Assimeh *et al.* (19). Protein concentrations were determined by amino acid analysis.

Sedimentation Analysis. Sedimentation studies were performed in a Beckman model E ultracentrifuge in 10 mM Tris·HCl, pH 7.4/150 mM NaCl/5 mM $CaCl_2$ or 5 mM EDTA at 56,000 rpm at 20°C in an An-D rotor. Epon double-sector centerpieces were used. Protein concentrations were 0.2–0.5 mg/ml. Equilibrium sedimentation was carried out at 8000–12,000 rpm. The absorbance of the protein at 280 nm was measured in the cell by means of a photoelectric scanner. The molecular weight was calculated from a least squares fit of $\log(A)$ versus r^2. The partial specific volume, calculated (20) from amino acid composition and sugar content (9), was 0.711 and 0.714 cm^3/g for C1r and C1s, respectively.

Electron Microscopy. Solutions of the complexes in Tris·HCl, pH 7.4/150 mM NaCl/ 1 mM $CaCl_2$ were diluted with 150 mM ammonium acetate, pH 7.2/5 mM $CaCl_2$ to a protein concentration of 20–40 μg/ml. The dilution was completed within 3–5 sec in order to minimize a possible dissociation of the complexes. Samples were rapidly applied to thin carbon films which had been rendered hydrophilic by glow discharge. The adsorption time was 20–30 sec and was reduced to 5 sec in the case of the C1 complex. Specimen were negatively stained with 1% aqueous uranyl formate at pH 4.1 (21). A Philips 301 electron microscope was used at 80 kV with a 200-nm condenser aperture and a 30-nm objective aperture. Pictures were taken at a magnification of 77,000 and enlarged 4-fold for reproduction.

RESULTS

Molecular Weights and Sedimentation Coefficients. In the presence of 5 mM Ca^{2+}, both C1r and C1s formed dimers with a weight average of 170,000 (Table 1). The relatively small sedimentation coefficients compared with the molecular weights indicate that both dimers had an elongated shape. When the Ca^{2+} concentration was <3 mM, $(C1s)_2$ begins to dissociate. In the presence of 5 mM EDTA which removed even traces of Ca^{2+}, a molecular weight of 85,000 and a sedimentation coefficient of 4.3 S were measured for C1s. C1r persisted

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Abbreviations: C1, first component of complement; C1r and C1s, subcomponents of C1; iPr_2P-F, diisopropyl fluorophosphate.

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Table 1. Sedimentation coefficients and molecular weights of C1r, C1s, and an equimolar mixture of C1r and C1s in 10 mM Tris·HCl, pH 7.4/150 mM NaCl at 20°C

Protein	Buffer containing	$s_{20,w}$, S	Weight average M_r
C1r	5 mM Ca^{2+}	6.7 ± 0.4	170,000 ± 10,000
	5 mM EDTA	6.7 ± 0.4	170,000 ± 10,000
	4 M Gdn·HCl	–	85,000 ± 5,000
C1s	5 mM Ca^{2+}	5.7 ± 0.4	170,000 ± 10,000
	5 mM EDTA	4.3 ± 0.3	85,000 ± 5,000
	4 M Gdn·HCl	–	85,000 ± 5,000
C1r + C1s	5 mM Ca^{2+}	8.7 ± 0.5	430,000 ± 70,000

The protein concentration was 0.2–0.5 mg/ml. The error limits were estimated from four to six measurements. Gdn·HCl, guanidinium chloride.

as a dimer in the absence of Ca^{2+} (in the presence of 5 mM EDTA). In 4 M guanidine·HCl, both proteins were monomers with molecular weights of 85,000.

When C1r and C1s were mixed in a 1:1 molar ratio in the presence of 5 mM Ca^{2+} most of the material sedimented in a sharp boundary with a sedimentation coefficient of 8.7 S (Fig. 1). The slightly curved plateau region indicates the presence of small amounts of faster sedimenting material. In accordance with earlier findings (1, 9, 11), the 8.7S boundary is assumed to reflect the formation of a $(C1r,C1s)_2$ complex with a molecular weight of 340,000. The observed weight average molecular weights for 1:1 mixtures of C1r and C1s were higher (Table 1) and the log(A) versus r^2 plots were curved. The minimal molecular weight was estimated to be 350,000 from the slope near the meniscus. The data are consistent with the formation of a $(C1r,C1s)_2$ complex and some further association. No free $(C1r)_2$ or $(C1s)_2$ was detectable in the sedimentation velocity runs at concentrations of the components of about 1 μM. This indicates that a very stable 1:1 complex with a dissociation equilibrium constant of less than 0.1 μM was formed.

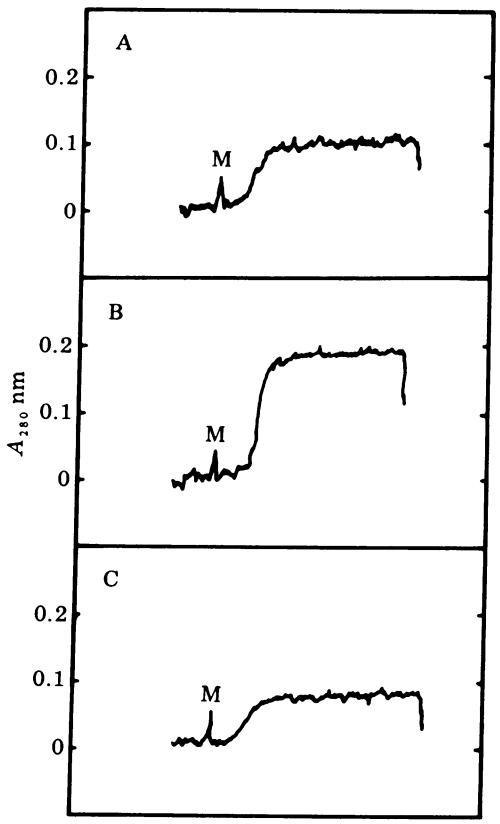

FIG. 1. Sedimentation profiles of $(C1s)_2$ (A) and $(C1r)_2$ (C) in 10 mM Tris·HCl, pH 7.4/150 mM NaCl/5 mM Ca^{2+} at 20°C scanned at 280 nm. Sharp single boundaries with $s_{20,w}$ = 5.7 and 6.7 S were observed for $(C1r)_2$ and $(C1s)_2$, respectively. When the two proteins were mixed (B) at an equal molar ratio in the same buffer, a single boundary with $s_{20,w}$ = 8.7 S was observed, indicating complete formation of the (C1r,C1s) complex. Protein concentrations were 1 μM per chain. Scans were recorded 8 min after the rotor attained a speed of 52,000 rpm. Menisci are indicated by M.

Estimate of Shape from the Hydrodynamic Properties. Because of the elongated shape of the particles, cylinders of length L and diameter d were used as the hydrodynamic equivalents. The sedimentation constant s^0 for particles of this shape is given by (22):

$$s^0 = \frac{M(1 - v_2\rho)}{6\pi\eta N_A L}\left[2\ln\left(\frac{2L}{d}\right) - 0.2316 + \frac{d}{L}\right] \quad [1]$$

in which M is the molecular weight, v_2 is the partial specific volume of the protein, η and ρ are viscosity and density of the solvent, respectively, and N_A is Avogadro's number. The volume of a cylinder with a degree of hydration δ_1 is (23);

$$\frac{\pi}{4}d^2L = \frac{M(v_2 + \delta_1 v_1^0)}{N_A} \quad [2]$$

in which v_1^0 is the specific volume of the solvent. At 20°C and for water $\eta \approx 0.01$ cP, $\rho \approx 1$ g/ml, and $v_1^0 \approx 1$ ml/g. Furthermore, a degree of hydration $\delta_1 = 0.3$ was assumed (23). The sedimentation coefficients measured at protein concentrations of 0.2–0.5 mg/ml were multiplied by an estimated correction factor of 1.05 to obtain the sedimentation constant. From Eqs. 1 and 2 the following mean (±SD) dimensions were calculated: for $(C1r,C1s)_2$, L = 64 ± 4 nm and d = 3.4 ± 0.1 nm, for $(C1r)_2$, L = 36 ± 4 nm and d = 3.3 ± 0.1 nm; and for C1s, L = 20 ± 5 nm and d = 3.3 ± 0.2 nm.

The electron micrographs (Fig. 2) revealed abundant amounts of elongated particles with a uniform contour length of 51 ± 2 nm (mean ± SD). This is somewhat shorter than the length estimated for $(C1r,C1s)_2$ from its hydrodynamic properties in solution. In view of the approximate nature of such estimates and the possible systematic errors for dimensions taken from electron micrographs (because of shrinkage, etc.) the agreement is satisfactory. The size of the particles is consistent with the molecular weight of 340,000. This is demonstrated by a comparison with the size of a C1q molecule (molecular weight, 420,000) shown in Fig. 4.

The $(C1r,C1s)_2$ particles appear as linear chains of globular domains (Fig. 2). Their normal appearance is that of elongated question marks. Of 100 particles, 64 had this appearance, only 1 had the opposite curvature and appeared as an elongated S, 19 were more or less straight, and 16 had distorted shapes. This preferred adsorption indicates that the complexes are not symmetrical but have two sides with different affinities for the carbon film. At both ends a globular structure connected by a small stem to the central part can be clearly distinguished. This stem is often bent away from the long axis of the particle. Individual domains are less clearly distinguished in the central part than at the ends. In favorable cases, four to six globular structures can be seen in this region. An idealized, hypothetical view of the molecule is shown in Fig. 3.

Reconstitution of the C1 Complex. C1q (0.5 μM) was incubated with an excess of C1r and C1s in 10 mM Tris·HCl, pH 7.4/150 mM NaCl/5 mM $CaCl_2$ for 20 min at 20°C. In the analytical ultracentrifuge a rapidly sedimenting boundary of 16.3 S appeared, indicating the formation of the C1 complex (18). For electron microscopic inspection, the solution of the reconstituted complex had to be diluted 1:20. The time needed

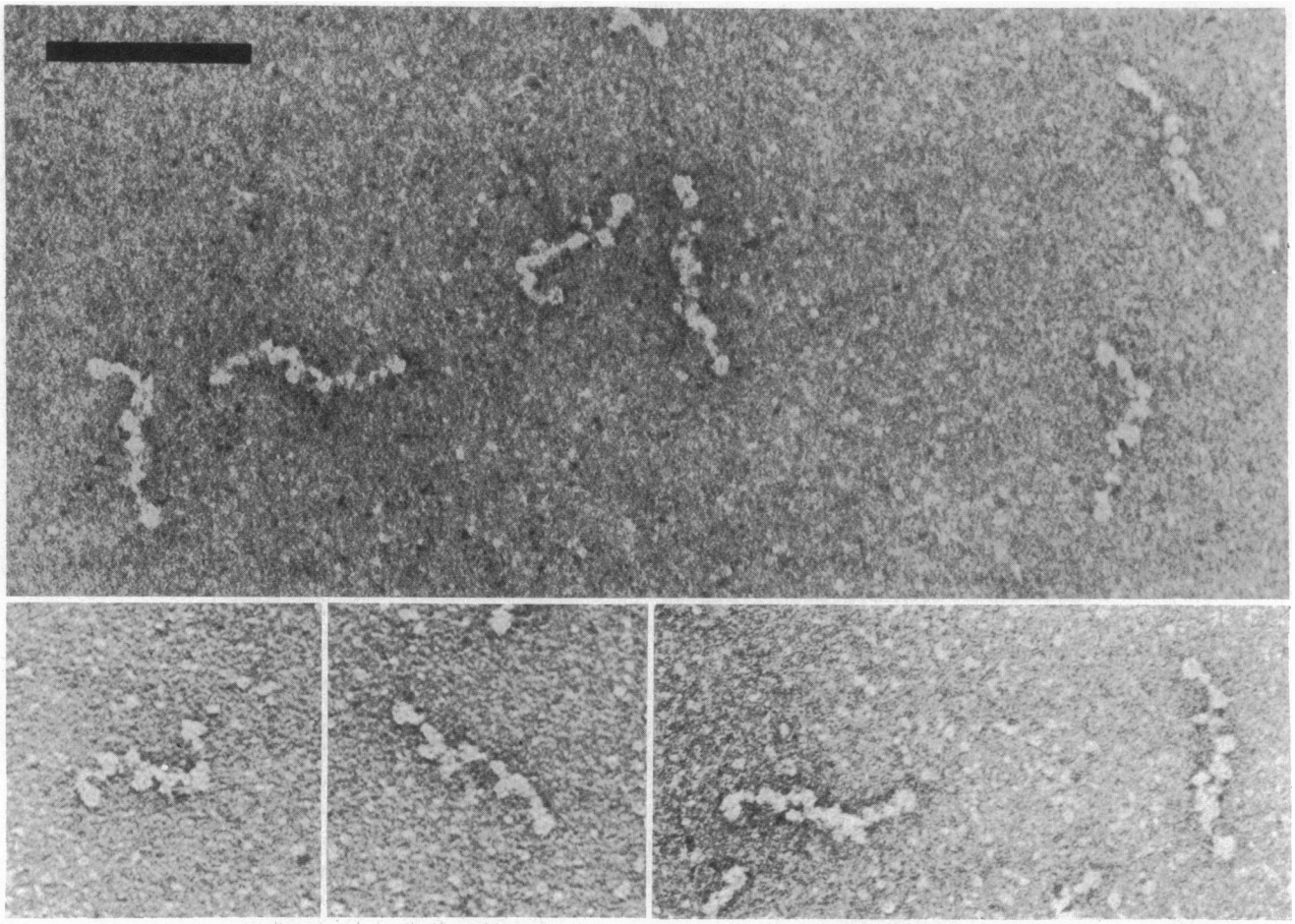

FIG. 2. Electron micrograph of the C1r,C1s complex. Bar corresponds 50 nm.

for dilution and adsorption to the carbon grids was decreased to a total of 10 sec in order to minimize dissociation of the C1 complex at the low concentration. In spite of this precaution the complex apparently dissociated or disrupted during specimen preparation. This was evident from the large amount of free C1q molecules that were clearly resolved in the electron micrographs (Fig. 4). A large fraction of $(C1r,C1s)_2$ particles was also seen. A number of ring-like structures were visible and about half had a C1q molecule in the ring. They were not observed when the $(C1r,C1s)_2$ complex was reconstituted from C1s and C1r in the absence of C1q or in the C1q preparation used for the reconstitution experiments. The ring-like structures are tentatively interpreted as dimers or trimers of $(C1r,C1s)_2$. Their circumferences varied from 100 nm for the small rings to 150 nm for the largest. This may reflect the presence of two or three $(C1r,C1s)_2$ molecules of length 50 nm per ring. Some particles were observed in which the (C1r,C1s) complex was connected to the heads of C1q (Fig. 4). In no case was the complex located at the stem of C1q.

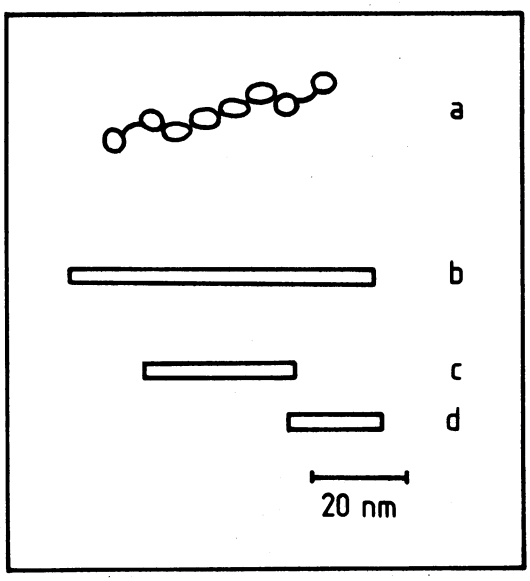

FIG. 3. The arrangements of the globular domains in the $(C1r,C1s)_2$ complex (a) is idealized and partially speculative (see text). The hydrodynamic equivalents are cylinders of diameter 3.3 nm and lengths 64 nm for $(C1r,C1s)_2$ (b), 36 nm for (C1r) (c), and 20 nm for C1s (d).

DISCUSSION

The molecular weights determined for C1s, C1r, $(C1r)_2$, and $(C1s)_2(C1s)_2$ by sedimentation equilibrium runs confirm earlier values derived from $NaDodSO_4$/polyacrylamide gel electrophoresis and column chromatography (9). Our mean (±SD) value of $s_{20,w} = 6.7 \pm 0.4$ S for $(C1r)_2$ is smaller than the value derived from density gradient centrifugation (9). Our sedimentation coefficient of 8.7 ± 0.5 S for $(C1r,C1s)_2$ is somewhat lower than the value, 10 S, obtained by sucrose gradient centrifugation (11).

Monomeric C1s, the $(C1r)_2$ dimer, and the (C1r,C1s) complex are elongated molecules. Their sedimentation properties can be described by hydrodynamic equivalents with cylindrical shape, a diameter of 3.3 nm and lengths of 20 nm for C1s, 36

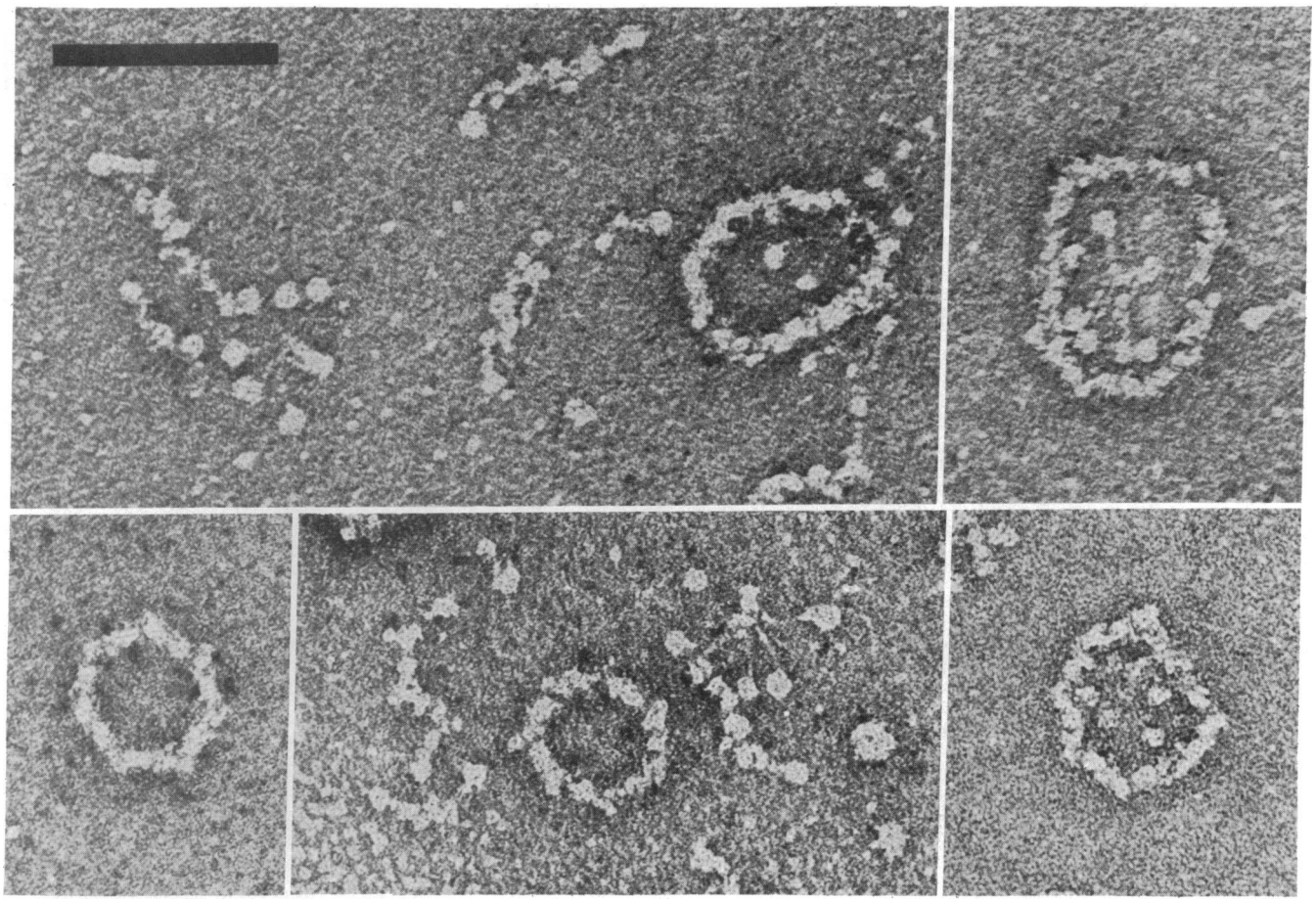

FIG. 4. Electron micrograph of the dissociation products of the C1 complex reconstituted from C1q, C1r, and C1s. Bar corresponds to 50 nm.

nm for $(C1r)_2$, and 64 nm for $(C1r,C1s)_2$. The dimensions of $(C1r,C1s)_2$ derived in this way are similar to the dimensions of the (C1r,C1s) particles seen in the electron microscope (Fig. 2). This indicates that the electron microscopic structure resembles the structure in solution. The fact that the (C1r,C1s) complex does not dissociate at the low protomer concentrations (20–40 nM) used in the electron microscopic studies suggests that the binding constants for the assembly of the complex are larger than $10^9\ M^{-1}$. The assembly of $(C1r)_2$ with two C1s is linear. Otherwise, the extremely elongated shape of the complex cannot be constructed with the dimensions of the constituent molecules (Fig. 2).

The electron microscopic appearance of the (C1r,C1s) complex as chains of domains is best explained by the assumption that each of the four protomers is composed of two domains. Two globular morphological units located at both ends of the particles can be clearly distinguished. The structural details in the central part of the complex are less clear and in this respect the model proposed in Fig. 3 is tentative. No direct information is available on the location of C1r and C1s in the complex. The much higher stability of the $(C1r)_2$ dimer as compared to $(C1s)_2$ suggests that $(C1r)_2$ forms a core to which C1s protomers are associated at both ends.

An electron microscopic study of the mode of C1r and C1s binding to C1q was hampered by the low stability of the C1 complex. Apparently, this complex dissociated at the low concentrations and perhaps under the influence of shear forces during the preparation of the electron microscopic specimen. It may be speculated that the ring-like structures are composed of two or three $(C1r,C1s)_2$ units and are dissociation products of the C1 complex. A complex $C1q[(C1r,C1s)_2]_3$ would be in accordance with the 3-fold symmetry of the C1q molecule but is difficult to reconcile with the stoichiometry of C1 (1, 10, 16, 18). It is hoped that further studies with C1 complexes in which dissociation of the components is prevented by chemical crosslinking will help to clarify this question.

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