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Skeletal Structure of Clathrin Triskelion in Solution: Experimental and Theoretical Approaches†

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ABSTRACT: The physicochemical properties of the clathrin triskelion were determined by dynamic and static light-scattering and sedimentation analyses in Tris and triethanolamine (TEA) buffers of about pH 8, in which the clathrin triskelion has been found to be in different conformational states by electron microscopy [Heuser, J., & Kirchhausen, T. (1985) *J. Ultrastruct. Res. 92*, 1-27]. Dynamic light-scattering measurements provided diffusion coefficients ($D^0_{20,w}$) of 1.22×10^{-7} and 1.23×10^{-7} cm^2/s, and ultracentifugal analysis gave sedimentation coefficients ($s^0_{20,w}$) of 8.39 and 8.32 S in Tris and TEA buffer, respectively. The average Stokes radius of the protein was determined to be 175 Å from its diffusion and sedimentation coefficients and its molecular weight. Static light-scattering analysis provided molecular weights of 6.58×10^5 and 6.41×10^5 and radii of gyration of 311 and 301 Å in the respective buffers. These results indicate that the clathrin triskelion has a similar conformation in the two buffers. For clarification of the skeletal structure of the clathrin triskelion in solution, the physicochemical parameters were calculated by using two models in which the clathrin arms are bent at various angles in a plane, on the basis of the Bloomfield approximation and a formula derived to estimate the radius of gyration of proteins consisting of various structural units. Values for the Stokes radius, diffusion and sedimentation coefficients, and radius of gyration in the ranges of 178-170 Å, $(1.20\text{-}1.26) \times 10^{-7}$ cm^2/s, 8.26-8.66 S, and 316-266 Å, respectively, were obtained with these models with the arms bent in the range of 0-60°. Comparison of these experimental and theoretical values suggests that, in weakly alkaline solution, the clathrin triskelion adopts a pinwheel-like structure with the arms more extended than in the polyhedral lattice, where the arms are bent at an angle of 60°.

In most cells, receptor-mediated endocytosis and intracellular protein transport are known to be mediated by coated pits and coated vesicles (Goldstein et al., 1985). These pits and vesicles are coated with a polyhedral lattice of a coat protein, clathrin. The assembly unit of this clathrin coat, termed a triskelion, has a molecular weight of 6.5×10^5 and forms a pinwheel-like structure consisting of three heavy and three light chains (Keen, 1985; Pearse & Crowther, 1987; Brodsky, 1988). Recently, the amino acid sequences of the heavy (Kirchhausen et al., 1987a) and light (Jackson et al., 1987; Kirchhausen et al., 1987b) chains have been deduced from the sequences of their cDNA clones.

The three arms of the clathrin triskelion are believed usually to be bent to some extent. However, electron-microscopic observations have indicated that in Tris buffer the arms are bent clockwise, whereas in triethanolamine (TEA)[1] buffer they are bent counterclockwise and are more extended (Heuser & Kirchhausen, 1985; Kirchhausen et al., 1986). Since an evaporation step is included in the procedure for preparation of samples for electron microscopy, the images may not reflect the "true" conformation of the clathrin triskelion in solution. Therefore, we attempted to determine the exact structure of the clathrin triskelion in solution. In the present study, we examined the physicochemical properties of the clathrin triskelion at about pH 8 in Tris and TEA buffers, mainly by a light-scattering technique, compared the experimental results with theoretical estimations, and found that the clathrin triskelion forms a pinwheel-like structure with rather extended arms and that its structure is the same in the two buffers.

Experimental Procedures

Clathrin Preparation. Clathrin was purified from crude coated vesicles of bovine brain as described previously

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[1] Abbreviations: TEA, triethanolamine; DTT, dithiothreitol.

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(Yoshimura et al., 1987). Purified clathrin in 0.5 M Tris-HCl (pH 7.5) was stored in ice and again subjected to Sepharose CL-4B gel chromatography in Tris buffer (20 mM Tris, 100 mM NaCl, 0.2 mM DTT, 0.1 mM EDTA, pH 8.2) or TEA buffer (50 mM TEA, 0.2 mM DTT, 0.1 mM EDTA, pH 8.3) just before use. This preparation was confirmed to consist of monodispersed clathrin triskelion by light-scattering measurements as described later and to be stable in these buffers at room temperature for at least three days. The concentration of clathrin was determined spectrophotometrically by using an extinction coefficient of $E^{1\%}_{1cm} = 10.9$ at 280 nm (Nandi et al., 1980).

Light-Scattering Measurement. Dynamic and static light-scattering measurements were performed at 20 °C with a photon-counting laser light-scattering photometer model LS-601 (Otsuka Electronics, Co., Osaka), equipped with a laser light source at a wavelength of 632.8 nm. The scattering angles were set at 45° for the dynamic mode and at 30–120° at intervals of 10° for the static mode.

The autocorrelation function of the scattered electric field, $g^{(1)}(\tau)$, was deduced from the homodyne photon-counting correlation function, $c(\tau)$, by the following formula (Berne & Pecora, 1976):

$$c(\tau) = A(1 + B|g^{(1)}(\tau)|^2) \quad (1)$$

where A and B are constants independent of the correlation time, τ. The correlation function $c(\tau)$ was obtained in a correlation and probability analyzer model SAI-43A, equipped with a photon-counting interface (Nihon Kogaku Kogyo, Co., Suita). The constant A was determined from the base line of the measured correlation function.

For evaluation of the z-averaged translational diffusion coefficient of clathrin, D, $g^{(1)}(\tau)$ was analyzed by the cumulant method using the following equation (Koppel, 1972):

$$\ln |g^{(1)}(\tau)| = -Dq^2\tau + \mu^2q^4\tau^2/2 \quad (2)$$

$$q = (4\pi n/\lambda_0) \sin (\theta/2) \quad (3)$$

where μ is the dispersion of the distribution of D, and λ_0, θ, and n are the wavelength, the scattering angle, and the refractive index of the solution, respectively.

The molecular weight, M, and the root-mean-square average radius of gyration, R_G, of clathrin were estimated by the following equation (Tanford, 1961):

$$[Kc/R(\theta)]_{c\to 0} = (1/M)[1 + (R_G^2/3)(4\pi n/\lambda_0)^2 \sin^2 (\theta/2) + ...] \quad (4)$$

$$[Kc/R(\theta)]_{\theta\to 0} = 1/M + 2A_2c + ... \quad (5)$$

where K is an optical constant, c is the concentration of clathrin in g/cm^3, $R(\theta)$ is the reduced scattering intensity, and A_2 is the second virial coefficient. K is given by $[2\pi n_0(dn/dc)]^2/(N\lambda_0^4)$ for vertically polarized incident light where n_0, dn/dc, and N are the refractive index of the solvent, the refractive index increment of the solution, and Avogadro's number, respectively. The value of dn/dc for the clathrin solution was determined with a differential refractometer model RM 102 (Otsuka Electronics, Co., Osaka). $R(\theta)$ is expressed as $[I(\theta)/I_0][r^2/V(\theta)]$, where $I(\theta)$ and I_0 are the intensities of the scattered and incident lights, respectively, r is the distance from the scattering to observation point, and $V(\theta)$ is the scattering volume. The intensity of light scattered by clathrin $[I(\theta)]$ was measured by static analysis, and $r^2/[I_0 \cdot V(\theta)]$ was determined from the intensity of light scattered by benzene at a scattering angle of 90° and the value of $R(90)$ for benzene, which is 11.84×10^{-6} (Pike et al., 1975). Values of $Kc/R(\theta)$ measured at various scattering angles and various clathrin concentrations were plotted against c and $\sin^2 (\theta/2)$, respectively, and $[Kc/R(\theta)]_{c\to 0}$ and $[Kc/R(\theta)]_{\theta\to 0}$ were obtained by extrapolation of the two plots of $Kc/R(\theta)$ to zero clathrin concentration and to zero scattering angle, respectively. The values of M and R_G for clathrin were determined from the combination of the plot of $[Kc/R(\theta)]_{c\to 0}$ against $\sin^2 (\theta/2)$ and the plot of $[Kc/R(\theta)]_{\theta\to 0}$ against c.

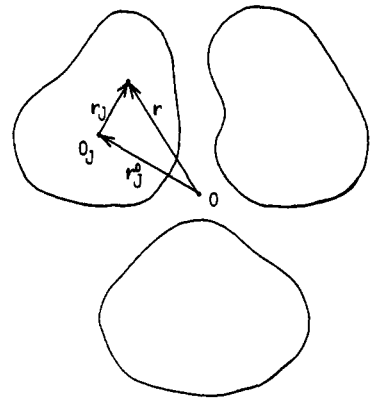

FIGURE 1: Diagram of a protein molecule consisting of different structural units. The points o and o_J indicate the centers of mass of the whole molecule and the Jth unit, respectively.

All measurements were performed in Tris buffer (20 mM Tris, 100 mM NaCl, 0.2 mM DTT, 0.1 mM EDTA, pH 8.2) or TEA buffer (50 mM TEA, 0.2 mM DTT, 0.1 mM EDTA, pH 8.3).

Sedimentation Measurement. Sedimentation velocity experiments in the two buffers were carried out at about 20 °C in a Hitachi model 282 analytical ultracentrifuge, equipped with a photoelectric absorption scanner. The rotor speed was set at 50 000 rpm.

Theoretical Estimation of the Stokes Radius. The Stokes radius of the clathrin triskelion, R_H, was estimated theoretically by using the following equation described by Bloomfield et al. (1967a,b):

$$R_H = (\sum_{i=1}^{n} R_i^2)^2/[\sum_{i=1}^{n} R_i^3 + \sum_{i=1}^{n}\sum_{j=1}^{n}{}'(R_i^2R_j^2/D_{ij})] \quad (6)$$

where R_i and R_j are the radii of the ith and jth spherical shells, respectively, and D_{ij} is the center-to-center distance between the ith and jth spherical shells.

Theoretical Estimation of the Radius of Gyration. To estimate the radius of gyration of the clathrin triskelion theoretically, we derived a general equation for the radius of gyration of a protein composed of different units. Assuming that a protein molecule is composed of N structural units, the inertial moment of the Jth unit (I_J) with respect to the center of mass of the whole molecule (o) is expressed by

$$I_J = \int_{V_J} \rho(\mathbf{r})|\mathbf{r}|^2 dv \quad (7)$$

where $|\mathbf{r}|$ is the distance of the arbitrary point in the Jth unit from o and $\rho(\mathbf{r})$ is the density of the Jth unit at the distance $|\mathbf{r}|$. Considering the position of the center of mass of the Jth unit (o_J), $\mathbf{r}$ can be described by the sum of vectors $\mathbf{r}^o_J$ and $\mathbf{r}_J$ (Figure 1). This yields

$$I_J = \int_{V_J} \rho(\mathbf{r}_J)|\mathbf{r}^o_J + \mathbf{r}_J|^2 \, dv \quad (8)$$

$$= |\mathbf{r}^o_J|^2 \int_{V_J} \rho(\mathbf{r}_J) \, dv + \int_{V_J} \rho(\mathbf{r}_J)|\mathbf{r}_J|^2 \, dv + 2\left[\mathbf{r}^o_J \cdot \int_{V_J} \rho(\mathbf{r}_J)\mathbf{r}_J \, dv\right] \quad (9)$$

Since

$$(R_G^2)_J = \int_{V_J} \rho(\mathbf{r}_J)|\mathbf{r}_J|^2 \, dv / \int_{V_J} \rho(\mathbf{r}_J) \, dv,$$

$$M_J = \int_{V_J} \rho(\mathbf{r}_J) \, dv$$

and

$$\left| \int_{V_J} \rho(\mathbf{r}_J)\mathbf{r}_J \, dv \right| = 0$$

then

$$I_J = M_J|\mathbf{r}_J^0|^2 + M_J(R_G^2)_J \qquad (10)$$

where M_J and $(R_G^2)_J$ are the mass and the mean-square average radius of gyration of the Jth unit, respectively. Thus, the mean-square average radius of gyration of the whole molecule composed of N structural units is given by

$$R_G^2 = \sum_{J=1}^{N} I_J / \sum_{J=1}^{N} M_J = \sum_{J=1}^{N} M_J[|\mathbf{r}_J^0|^2 + (R_G^2)_J] / \sum_{J=1}^{N} M_J \qquad (11)$$

This equation means that the radius of gyration for a complex structure constructed from well-defined structural units can be calculated from the individual radii of gyration of the units.

RESULTS

Experimental Evaluation of the Physicochemical Properties of Clathrin. To evaluate the overall structure of the clathrin triskelion in solution, we measured its diffusion coefficient by the dynamic (quasi-elastic) light-scattering technique. Figure 2 shows typical plots of the natural logarithm of the autocorrelation function as a function of the delay time at a clathrin concentration of 0.77×10^{-3} g/cm^3 in Tris and TEA buffers of about pH 8, in which clathrin is known to exist as a triskelion. Almost linear plots were obtained in both solutions. The apparent diffusion coefficients of the protein were estimated to be 1.25×10^{-7} and 1.22×10^{-7} cm^2/s, respectively, by using eqs 2 and 3, in which μ/D was 0.11 in both conditions. The diffusion coefficients of the clathrin triskelion at various concentrations in the two buffers were then measured. Scarcely any concentration dependence was observed: the slopes of the two plots of the apparent diffusion coefficient against the protein concentration were negligible, being -0.02×10^{-7} and -0.01×10^{-7} cm^2/s per 10^{-3} g/cm^3 by least-squares analysis, and the diffusion coefficients at infinite dilution ($D^0_{20,w}$) were almost identical, being $(1.22 \pm 0.04) \times 10^{-7}$ and $(1.23 \pm 0.02) \times 10^{-7}$ cm^2/s, respectively (Table I). In all cumulant analyses, μ/D values were within 0.1 ± 0.05.[2]

The sedimentation coefficient of the clathrin triskelion at various concentrations in Tris and TEA buffers was measured by analytical ultracentrifugation. The protein sedimented as a single symmetrical boundary in the ultracentrifugal field. The values were almost independent of the protein concentration: least-squares analysis provided slopes of -0.07 and -0.21 S per 10^{-3} g/cm^3 in the plots of the apparent sedimentation coefficient vs the protein concentration, and the respective sedimentation coefficients at infinite dilution ($s^0_{20,w}$) were determined to be 8.39 ± 0.11 and 8.32 ± 0.06 S (Table I), that is, almost identical in the two buffers.

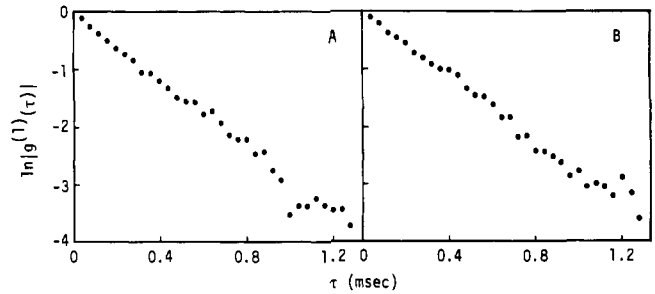

FIGURE 2: Natural logarithm of the autocorrelation functions as functions of the delay time for the clathrin triskelion in Tris (A) and TEA (B) buffer. The protein concentration was 0.77×10^{-3} g/cm^3.

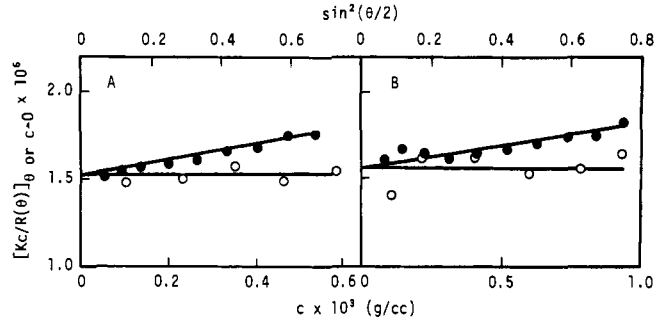

FIGURE 3: Light-scattering data for the clathrin triskelion in Tris (A) and TEA (B) buffer. Open and closed circles show the plots of $[Kc/R(\theta)]_{\theta \to 0}$ vs clathrin concentration (c) and $[Kc/R(\theta)]_{c \to 0}$ vs $\sin^2(\theta/2)$, respectively.

From these diffusion and sedimentation coefficients, the molecular weight of the clathrin triskelion was calculated to be 6.52×10^5 in Tris buffer and 6.42×10^5 in TEA buffer (Table I). We also calculated the Stokes radius of the clathrin triskelion (R_H) from the diffusion coefficient and from both the sedimentation coefficient and molecular weight. As shown in Table I, values of 174–177 Å were obtained, and there was no appreciable difference in the values in Tris and TEA buffers.

The radius of gyration of the clathrin triskelion was measured by the static light-scattering technique. The intensity of light scattering was measured with clathrin solutions of various protein concentrations in Tris and TEA buffers at various scattering angles of 30–120° at intervals of 10°. The resulting plots of $[Kc/R(\theta)]_{c \to 0}$ against $\sin^2(\theta/2)$ and $[Kc/R(\theta)]_{\theta \to 0}$ against c are shown in Figure 3. The molecular weight and the root-mean-square average radius of gyration were estimated from the combination of these two plots by using eqs 4 and 5, in which the second virial coefficient (A_2) was negligible. Thus, these parameters were determined to be 6.58×10^5 and 311 Å in Tris buffer and 6.41×10^5 and 301 Å in TEA buffer, as shown in Table I.

Theoretical Estimation of the Hydrodynamic Parameters of Clathrin. To clarify the overall structure of the clathrin triskelion in solution, we calculated its Stokes radius theoretically according to the Bloomfield approximation, regarding the protein molecule as an assembly of nonidentical spherical shells. We first assumed that each of the proximal portions of the arms is surrounded by a light chain. This assumption is consistent with the idea that the heavy and light chains form a coiled-coil structure in their binding regions (Kirchhausen et al., 1987a,b; Jackson & Parham, 1988; Brodsky, 1988). As shown in Figure 4, we next designed a model consisting of an assembly of five different types of spherical shells in a plane, which correspond to structural domains such as the proximal and distal domains of the arms, the link region, and the ter-

[2] This value might not be negligible if the correlation functions could be measured precisely. However, although at a clathrin concentration of 0.95 mg/mL μ/D was an almost constant value of 0.06, at lower concentrations the values were variable: for instance, at a protein concentration of 0.46 mg/mL μ/D was 0.06 in one measurement but 0.14 in another measurement. Nevertheless, the calculated diffusion coefficients were almost the same. Since the scattering intensities of the sample were not so strong at lower protein concentrations, the mean μ/D value of 0.1 seems to be due to the decrease in the signal-to-noise ratio in the measured correlation functions or due to the effect of trace amounts of unremovable mixed particles. It is thus evident that the protein was almost monodispersed in Tris and TEA buffers during the measurement of its diffusion coefficient.

Table I: Experimental Values for Diffusion and Sedimentation Coefficients, Molecular Weight, Stokes Radius, and Radius of Gyration of the Clathrin Triskelion

buffer	$D^0_{20,w}$[a] (cm²/s)	$s^0_{20,w}$[a] (S)	R_H (Å)	M SD[b]	M LS[c]	R_G (Å)
Tris	(1.22 ± 0.04) × 10^{-7}	8.39 ± 0.11	175[d] 175[e]	6.52 × 10^5	6.58 × 10^5	311
TEA	(1.23 ± 0.02) × 10^{-7}	8.32 ± 0.06	174[d] 177[e]	6.42 × 10^5	6.41 × 10^5	301

[a] $D^0_{20,w}$ and $s^0_{20,w}$ were calculated from plots of the apparent value vs the protein concentration by the least-squares method. [b] Determined by a combination of the two equations given below, that is, the Svedberg equation. [c] Determined from the combination of the two plots based on eqs 4 and 5. [d] Determined from the diffusion coefficient by the following equation on the frictional coefficient, f (Tanford, 1961): $f = RT/(ND^0_{20,w}) = 6\pi\eta R_H$ where R, T, and N are the gas constant, the absolute temperature, and Avogadro's number, respectively, and η is the viscosity of water at 20 °C, which is 1.0019 × 10^{-2} P. [e] Determined from the sedimentation coefficient by the following formula (Tanford, 1961): $f = M(1 - \bar{v}\rho)/(Ns^0_{20,w}) = 6\pi\eta R_H$ where $\bar{v}$ is the partial specific volume of clathrin, which is assumed to be 0.744 mL/g (Pretorius et al., 1981), ρ is the density of the solvent, and M is the molecular weight of clathrin, which is calculated to be 6.53 × 10^5 from the amino acid sequence of clathrin (see Appendix I).

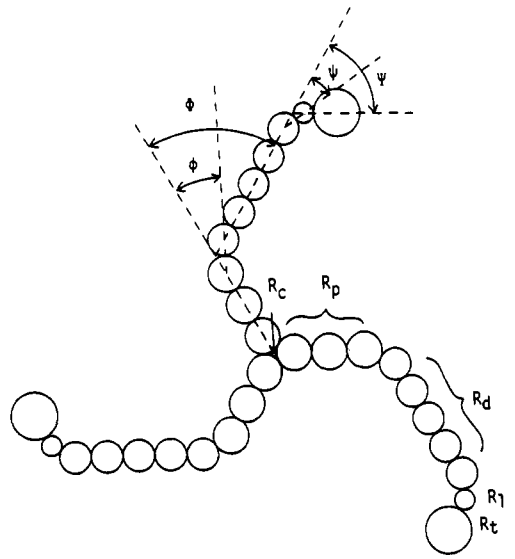

FIGURE 4: Model of the clathrin triskelion composed of five types of spherical shells. The radii of the spherical shells are R_c = 4.3 Å, R_p = 27.7 Å, R_d = 24.1 Å, R_l = 16.2 Å, and R_t = 35.0 Å (see text).

minal domain, and calculated their radii from the volumes of the corresponding domains (Appendix I). The proximal and distal portions of the arms were replaced by three spherical shells each with a radius of R_p = 27.7 Å and five spherical shells each of R_d = 24.1 Å. The radius of the spherical shells at the center of the protein molecule (R_c) was given by R_p(sec 30° − 1), being 4.3 Å, since this spherical shell is circumscribed by the three spherical shells with the radius (R_p) that are closely apposed to each other. The link region was represented by a spherical shell with a radius (R_l) of 16.2 Å. An intrinsic value of 35 Å was taken as the radius of the terminal domain (R_t) as described in Appendix I.

We also defined the angles between the proximal and distal parts of the arms (Φ) and between the distal parts of the arm and the part including both the link region and the terminal domain (Ψ) (Figure 4) and varied these angles in calculations. To keep the lengths of the proximal and distal parts of the arms almost constant, irrespective of the angles Φ and Ψ, the angles between the proximal part of the arm and the line connecting the centers of the two spherical shells at the position of the "kink" (ϕ) and between the distal part of the arm and the analogous line at the position of the "link" (ψ) were taken into account and defined as described in Appendix II. The relations of Φ and ϕ and Ψ and ψ are expressed as follows:

$$\tan\phi = \{[(1 + F_1)^2 + 4F_1 \tan^2\Phi]^{1/2} - (1 + F_1)\}/(2\tan\Phi) \quad (12)$$

$$\tan\psi = \{[(1 + F_2)^2 + 4F_2 \tan^2\Psi]^{1/2} - (1 + F_2)\}/(2\tan\Psi) \quad (13)$$

where $F_1 = R_d/R_p$ and $F_2 = R_l/R_d$.

Table II: Theoretical Values for the Stokes Radius and the Diffusion and Sedimentation Coefficients of the Clathrin Triskelion

parameters	Φ (deg)	Ψ (deg) 0	15	30	45	60
R_H (Å)	0	178.0	177.9	177.6	177.0	176.3
	15	177.6	177.5	177.1	176.6	175.8
	30	176.6	176.4	176.0	175.4	174.5
	45	174.9	174.6	174.1	173.5	172.6
	60	172.3	172.0	171.4	170.7	169.7
$D^0_{20,w}$ (× 10^7 cm²/s)[a]	0	1.204	1.205	1.207	1.211	1.216
	15	1.207	1.208	1.210	1.213	1.219
	30	1.214	1.215	1.218	1.222	1.228
	45	1.226	1.227	1.231	1.236	1.242
	60	1.244	1.246	1.250	1.256	1.263
$s^0_{20,w}$ (S)[a]	0	8.256	8.261	8.275	8.299	8.333
	15	8.272	8.279	8.295	8.322	8.359
	30	8.319	8.329	8.348	8.378	8.418
	45	8.403	8.415	8.438	8.471	8.515
	60	8.527	8.543	8.570	8.608	8.657

[a] Calculated by using the same equations and values as described in Table I.

Using eq 6, we calculated the Stokes radii of the clathrin triskelion (R_H) with various values for Φ and Ψ at intervals of 15° (Table II). The values for the Stokes radius decreased with an increase in Φ or Ψ and were maximal (178 Å) and minimal (170 Å) in conformations with the arms fully extended and bent at 60° at both Φ and Ψ, respectively. As also shown in Table II, diffusion and sedimentation coefficients in the ranges of (1.20–1.26) × 10^{-7} cm²/s and 8.26–8.66 S, respectively, were obtained with both Φ and Ψ in the range of 0–60° from the Stokes radii. The observed values for the Stokes radius and diffusion and sedimentation coefficients were roughly within these theoretical ranges, suggesting that the clathrin triskelion has a pinwheel-like structure in solution and that its arms are probably more extended than in the polyhedral lattice, in which they are bent at an angle of 60° (Keen, 1985; Pearse & Crowther, 1987).

Theoretical Estimation of the Radius of Gyration of Clathrin. Using eq 11 derived here, we calculated the root-mean-square average radius of gyration of the clathrin triskelion. We again assumed that the proximal part of each arm is surrounded by a light chain and designed a model for the calculation in which (1) the proximal part of the arm consists of a cone, a cylinder, and a hemisphere, (2) the distal arm is a semispherocylinder, (3) the link region is composed of a hemisphere and a cylinder, (4) the terminal domain is a sphere, and (5) these regions are in a plane (Figure 5). We then calculated the lengths, radii, and masses of these structural units from the actual volumes, lengths and densities of the structural domains, as described in Appendix I. The calculation yielded a radius (R_p) of units such as the cone, cylinder, and hemisphere in the proximal part of the arm of 23.6 Å, a length (L_c) of the cone of 13.6 Å, which is given by $L_c = R_p$ tan 30°, a residual length (L_p') of the structural units in the

Table III: Square Distances of the Centers of Mass from That of the Whole Molecule and Mean-Square Average Radii of Gyration of Structural Units Included in the Domains of the Clathrin Triskelion

domain	structural unit	square distance of the center of mass from that of the whole molecule ($\|r_j^0\|^2$)	mean-square average radius of gyration (R_G^2)
proximal domain of the arm	cone	$(3L_c/4)^2$	$3(L_c^2/80 + R_p^2/10)$
	cylinder	$[L_c + (L_p' - R_p)/2]^2$	$(L_p' - R_p)^2/12 + R_p^2/2$
	hemisphere	$[L_c + (L_p' - R_p) + 3R_p/8]^2$	$147R_p^2/320$
distal domain of the arm	semispherocylinder	$[L_c + (L_p' - R_p) + (R_p + R_d)\cos\phi + L_s\cos\Phi/2]^2 + [(R_p + R_d)\sin\phi + L_s\sin\Phi/2]^2$ [a]	$(L_s^3/12 + R_dL_s^2/3 + R_d^2L_s + 4R_d^3/5)/(L_s + 4R_d/3)$
link region	hemisphere	$[X - 3R_l\cos(\Phi + \Psi)/8]^2 + [Y - 3R_l\sin(\Phi + \Psi)/8]^2$ [b]	$147R_l^2/320$
	cylinder	$[X + (L_l - R_l)\cos(\Phi + \Psi)/2]^2 + [Y + (L_l - R_l)\sin(\Phi + \Psi)/2]^2$	$(L_l - R_l)^2/12 + R_l^2/2$
terminal domain	sphere	$[X + (L_l - R_l + R_t)\cos(\Phi + \Psi)]^2 + [Y + (L_l - R_l + R_t)\sin(\Phi + \Psi)]^2$	$3R_t^2/5$

[a] $L_s = L_d - 2R_d$. [b] $X = L_c + (L_p' - R_p) + (R_p + R_d)\cos\phi + (L_d - 2R_d)\cos\Phi + (R_d + R_l)\cos(\Phi + \psi)$; $Y = (R_p + R_d)\sin\phi + L_d - 2R_d\sin\Phi + (R_d + R_l)\sin(\Phi + \psi)$.

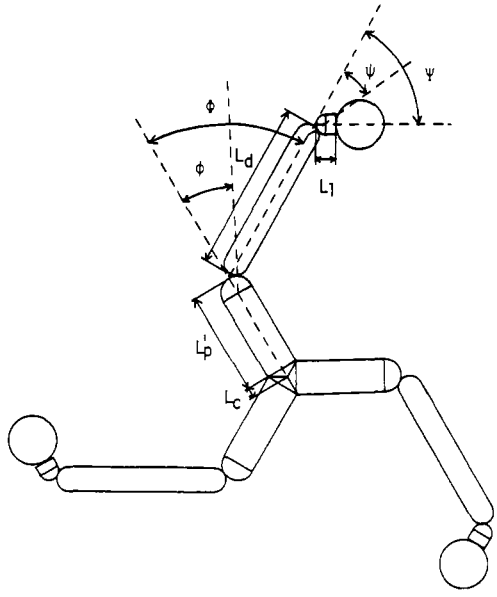

FIGURE 5: Model of the clathrin triskelion composed of proximal and distal domains of arms, link regions, and terminal domains. These domains are represented as cylinders and hemispheres (R_p = 23.6 Å; L_c = 13.6 Å; L_p' = 156.4 Å), semispherocylinders (R_d = 18.9 Å; L_d = 250.0 Å), hemispheres and cylinders (R_l = 15.1 Å; L_l = 30.0 Å), and spheres (R_t = 35.0 Å), respectively (see text).

proximal part of the arm of $170 - L_c$ = 156.4 Å, and masses of the cone, cylinder, and hemisphere of 2.35, 68.96, and 8.17 kDa, respectively, the sum of which is close to the sum of the masses of the proximal part of the arm and light chain (54.0 + 25.6 = 79.6 kDa, see Appendix I). The radii of the semispherocylinder in the distal part of the arm (R_d) and the structural units in the link region (R_l) were calculated to be 18.9 and 15.1 Å, respectively, and the masses of the hemisphere and cylinder in the link region were calculated to be 2.14 and 3.17 kDa, respectively, the sum of which is also very close to the mass of the link region (5.3 kDa, see Appendix I). The length (L_d) and mass of the semispherocylinder in the distal part of the arm, the total length of the structural units in the link region (L_l), and the radius (R_t) and mass of the sphere in the terminal domain are the same as those in Appendix I.

As shown in Figure 5, we also defined the angles, Φ, Ψ, ϕ, and ψ at the positions of the "kink" and "link" as described for estimation of the Stokes radius of the clathrin triskelion (Figure 4; Appendix II) and varied these angles in calculations. Table III summarizes the square distances between the centers of the masses of these structural units and the center of mass of the whole molecule ($|r_j^0|^2$) and their mean-square average radii of gyration [$(R_G^2)_j$] (Appendix III). The center of mass of the whole molecule is defined as the center of "vertex". By

Table IV: Theoretical Values for Mean-Root-Square Average Radius of Gyration of the Clathrin Triskelion [R_G (Å)]

	Ψ (deg)				
Φ (deg)	0	15	30	45	60
0	315.5	314.9	313.0	310.1	306.1
15	313.4	312.2	309.8	306.3	301.9
30	307.1	305.3	302.4	298.4	293.6
45	296.8	294.5	291.0	286.7	281.6
60	282.7	279.9	276.0	271.3	266.0

introducing these equations and parameters into eq 11, we calculated the root-mean-square average radius of gyration (R_G) of the protein molecules with various values for Φ and Ψ at intervals of 15° (Table IV). Decrease in Φ and Ψ resulted in decrease in R_G, and the values for the conformation with fully extended arms and with bends of 60° at both Φ and Ψ were maximal (316 Å) and minimal (266 Å), respectively. The experimental values were within the theoretical ranges, again suggesting that the clathrin triskelion has a pinwheel-like structure in solution and that the arms are more extended in solution than in the polyhedral lattice.

Discussion

In the present study, we determined the physicochemical properties of the clathrin triskelion at about pH 8 in Tris and TEA buffers by dynamic and static light-scattering and sedimentation analyses. The diffusion coefficients were similar to a value of 1.27×10^{-7} cm^2/s, computed for the diffusion coefficient of human brain clathrin triskelion from its molecular weight and sedimentation coefficient (Nandi & Wahl, 1988). The sedimentation coefficients were in good agreement with reported values of 8.1 and 8.4 S (Pretorius et al., 1981; Ungewickell & Branton, 1981), and the Stokes radii were also close to the value of 160 Å estimated by gel filtration (Keen et al., 1979). The molecular weights obtained were in good accord with each other and with the values estimated from sedimentation equilibrium experiments [(6.1 or 6.3) $\times 10^5$] (Pretorius et al., 1981; Ungewickell & Branton, 1981) and the value calculated from the amino acid sequence (6.5×10^5) (Kirchhausen et al., 1987a,b; Jackson et al., 1987).

There is a report that in Tris buffer the clathrin arms are bent clockwise, whereas in TEA buffer they are bent counterclockwise and are more extended (Heuser & Kirchhausen, 1985; Kirchhausen et al., 1986). However, we obtained almost identical values for the diffusion and sedimentation coefficients, the Stokes radius, and the radius of gyration in Tris and TEA buffers, indicating that the clathrin triskelion has a similar conformation in the two buffers.

Does the clathrin triskelion have a bent or extended conformation in solution? To clarify the overall structure of the clathrin triskelion in solution, we estimated the Stokes radius

and the radius of gyration theoretically using two types of models for the calculation. For a model consisting of an assembly of five differently sized spherical shells and a model composed of different structural units, Stokes radii of 170–178 Å based on the Bloomfield approximation and radii of gyration of 266–316 Å were obtained, respectively, assuming that the clathrin arms are bent at the positions of the kink and link with angles of less than 60° or are fully extended in a plane. The values for R_H and R_G determined experimentally were found to be roughly within the range of theoretical values. In particular, the bent conformation with angles of 60° at the positions of the kink and link, which corresponds to the clathrin conformation in the basket or polyhedral lattice (Keen, 1985; Pearse & Crowther, 1987), seems unlikely in solution, because the sedimentation coefficient (8.66 S) and the radius of gyration (266 Å) calculated theoretically for this conformation were very different from the observed values. Thus, these results suggest that in weakly alkaline solution, the clathrin triskelion adopts a pinwheel-like structure with the arms more extended than in the polyhedral lattice.

However, one may wonder whether the theoretical values of the physicochemical parameters of the clathrin triskelion are sufficiently strict to be closely comparable with the experimental values, because the geometry of the molecule is very complex. On estimation of the Stokes radius, we assumed that the volumes of the four structural domains are constant, but similar results were obtained even when the radii of four different types of spherical shells were assumed to be equal to the original radii of the four domains in the cylindrical and spherical structure: the Stokes radii were calculated to be 180.2, 178.4, 174.4, and 171.7 Å at Φ and Ψ of both 0°, 0° and 60°, 60° and 0°, and both 60°, respectively, for a model consisting of one spherical shell with R_c = 3.5 Å, four with R_p = 22.4 Å, seven with R_d = 18.5 Å, one with R_l = 13.8 Å, and one with R_t = 35.0 Å. On estimation of the radius of gyration, the variance was also slight provided the total length of the clathrin arm was fixed. For example, we obtained a similar R_G value of 314.6 Å only in the fully extended conformation for the original structure of the clathrin triskelion composed of a spherical terminal domain, cylindrical proximal, distal, and link regions, and a cylindrical light chain that is in juxtaposition with the proximal portion of the heavy chain. It can thus be said that the theoretical values are quite specific even if different types of models are designed.

In vitro, clathrin triskelions are known to disassemble reversibly from coated vesicles and assemble reversibly into a polyhedral lattice structure in a pH-dependent manner (Keen, 1985); they assemble and disassemble below and above pH 7, respectively. Although the clathrin arms are rigid in the assembled form, the link and kink regions in the arm are thought to be flexible in the disassembled state (Kirchhausen et al., 1986). Assembly-competent clathrin is reported to display a random arrangement of arms on rotary shadowing (Winkler & Stanley, 1983). Thus, the present results together with these findings suggest that, in solution, the arms could be rigid and also bent below pH 7 but flexible and apparently extended above pH 7. This change in conformation might be one of the factors regulating the pH-dependent association–dissociation of the clathrin molecule. Indeed, Heuser (1989) found that cytoplasmic acidification alters the structure of the clathrin lattices on the inside of the plasma membrane and speculated that pH alterations at a local level could modulate the dynamics of individual coated vesicles.

In relation to the assembly and disassembly of the clathrin coat on the membrane forming coated pits and coated vesicles, we have investigated the interaction of clathrin with phospholipid vesicles, found that the protein is capable of inducing fusion of phospholipid membranes at acidic pH (Hong et al., 1985), and shown that exposure of hydrophobic domains of the protein through conformational change occurs in the pH region inducing membrane fusion (Yoshimura et al., 1987; Maezawa et al., 1989). Very recently, we found that the terminal domain of clathrin has no ability to induce membrane fusion but that the residual proximal part of the protein has fusogenicity (Maezawa & Yoshimura, 1990). Thus, the flexibility of the clathrin arms might be important for the fusogenic properties of clathrin.

Appendix I

The clathrin arms are known to consist of different structural domains: distal and proximal portions, a link region, and a terminal domain. The total length of one arm, the lengths of the proximal part and the link region, and the diameter of the terminal domain were determined to be 520, 170, 30, and 70 Å, respectively, by electron microscopy (Kirchhausen & Harrison, 1984; Heuser & Kirchhausen, 1985; Kirchhausen et al., 1986, 1987a). Thus, the length of the distal part of the arm is calculated to be 250 Å, which is reasonable because the distal segment is about 260 Å long (Kirchhausen & Harrison, 1984). The masses of the heavy chain and the terminal domain are reported to be 192 and 53.3 kDa, respectively (Kirchhausen et al., 1987a). The average mass of the light chain is estimated to be 25.6 kDa, because the light chains a and b have masses of 26.7 and 25.1 kDa, respectively, and are present in a molar ratio of 1:2 (Jackson et al., 1987). The mass of the link region is estimated to be 5.3 kDa from its amino acid sequence (Kirchhausen et al., 1987a).

For determination of the volumes of the four domains of the clathrin arms and the light chains, we assumed that (1) these domains have the same density and (2) the terminal domain is a sphere. We obtained masses of the distal and proximal domains of the arms of 79.4 and 54.0 kDa, respectively, assuming that their total masses were 133.4 (= 192 − 5.3 − 53.3) kDa and that the ratio of their masses was equal to that of their lengths (250:170). The density of the terminal domain was calculated from its mass and volume (1.80×10^5 Å^3). Thus the volumes of the proximal and distal parts of the arms, the link region, and the light chain were calculated to be 1.82, 2.68, 0.18, and 0.86×10^5 Å^3, respectively, from their masses.

Appendix II

The relationships of Φ and ϕ and Ψ and ψ were obtained by a general formula. As shown in Figure 6, the tangent at the point of contact of the two spherical shells with radii of R_1 and R_2 is denoted as AB, the point of intersection of AB with the line connecting the centers of the spherical shells (or with the axis of the arm) with a radius of R_1 (CD) is denoted as P, and the angle (α) between CD and the line connecting the centers of the spherical shells (or the axis of the arm) with a radius of R_2 (O_2F) is set so that O_2F intersects AB at P. When the angle between the line connecting the centers of the two spherical shells with radii of R_1 and R_2 (O_1E) and CD is denoted as β, the relation between α and β is expressed as

$$R_1 \tan \alpha = R_2 \tan (\alpha - \beta)$$

Since $\tan (\alpha - \beta) = (\tan \alpha - \tan \beta)/(1 + \tan \alpha \tan \beta)$, the above equation leads to

$$\tan \alpha \tan^2 \beta + (1 + F) \tan \beta - F \tan \alpha = 0$$

where $F = R_2/R_1$. As $\beta < 60°$ in our treatment, we obtain

$$\tan \beta = \{[(1 + F)^2 + 4F \tan^2 \alpha]^{1/2} - (1 + F)\}/(2 \tan \alpha)$$

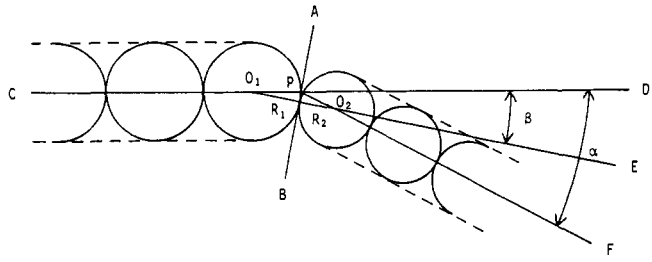

FIGURE 6: Diagram of the relative positions of two types of spheres or arms with radii of R_1 and R_2. Indicated by α and β are the angles between the lines connecting the centers of the two types of spheres (or the axes of the arms) and between the line connecting the centers of the two spheres and one of the lines connecting the centers of spheres (or the axis of the arm), respectively.

APPENDIX III

The mass (M) and the inertial moment (I) for molecules with various shapes are given by the equations shown below. In these equations, ρ is the density of the molecule, r is the distance of a point in the molecule from the axis of the molecule, l and x are minimum distances of the point from the center of mass and from the axis of the molecule, respectively, and R and L are the radius and length of the molecule, respectively.

Sphere

$$M = \rho \int_0^R dr \int_0^{2\pi} d\psi \int_0^{\pi} r^2 \sin\theta \, d\theta = 4\pi\rho R^3/3$$

$$I = \rho \int_0^R dr \int_0^{2\pi} d\psi \int_0^{\pi} l^2 r^2 \sin\theta \, d\theta = 4\pi\rho R^5/5$$

where $l^2 = r^2$.

Hemisphere

$$M = \rho \int_0^R dr \int_0^{2\pi} d\psi \int_0^{\pi/2} r^2 \sin\theta \, d\theta = 2\pi\rho R^3/3$$

$$I = \rho \int_0^R dr \int_0^{2\pi} d\psi \int_0^{\pi/2} l^2 r^2 \sin\theta \, d\theta = 49\pi\rho R^5/160$$

where $l^2 = (r \sin\theta)^2 + (r \cos\theta - 3R/8)^2$.

Cylinder

$$M = \rho \int_{-L/2}^{L/2} dx \int_0^R dr \int_0^{2\pi} r \, d\theta = \pi\rho R^2 L$$

$$I = \rho \int_{-L/2}^{L/2} dx \int_0^R dr \int_0^{2\pi} l^2 \, r \, d\theta = \pi\rho R^2 L(L^2/12 + R^2/2)$$

where $l^2 = x^2 + r^2$.

Semispherocylinder

$$M = 2M_{\text{hemisphere}} + M_{\text{cylinder}} = \pi\rho R^2(4R/3 + L_s)$$

where $L_s = L - 2R$.

$$I = 2I_{\text{hemisphere}} + I_{\text{cylinder}} =$$

$$2\rho \int_0^R dr \int_0^{2\pi} d\psi \int_0^{\pi/2} l^2 r^2 \sin\theta \, d\theta + I_{\text{cylinder}}$$

$$= \pi\rho R^2(L_s^3/12 + RL_s^2/3 + R^2 L_s + 4R^3/5)$$

where $l^2 = (L_s/2 + r\cos\theta)^2 + (r \sin\theta)^2$.

Cone

$$M = \rho \int_{-3L/4}^{L/4} dx \int_0^{(x+3L/4)R/L} dr \int_0^{2\pi} r \, d\theta = \pi\rho R^2 L/3$$

$$I = \rho \int_{-3L/4}^{L/4} dx \int_0^{(x+3L/4)R/L} dr \int_0^{2\pi} l^2 r \, d\theta =$$

$$\pi\rho R^2 L(L^2/80 + R^2/10)$$

where $l^2 = x^2 + r^2$.

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