Molecular Properties of the Reassembled Coat Protein of Coated Vesicles†

Pradip K. Nandi, Harold T. Pretorius, Roland E. Lippoldt, Michael L. Johnson,‡ and Harold Edelhoch*

ABSTRACT: Clathrin has been prepared from human and bovine brains by a rapid technique which does not require sucrose gradient centrifugation. The protomer molecule which is obtained has the ability to polymerize and form protein coats, i.e., so-called cages or baskets, which resemble the structures observed in coated vesicles. The polymerization of clathrin to form cage structures in 0.2 M ammonium acetate, pH 6.8, results in two distributions of sedimenting particles in the ultracentrifuge, one centered near 300S and the other near 150S. Equilibrium sedimentation gives molecular weights of the 150S and 300S particles near 25 million and 100 million, respectively. The turbidities of the two species have been measured during centrifugation in the ultracentrifuge. When the turbidity values are combined with the molecular weight values, the radii of the 150S and 300S species can be obtained, assuming a hollow sphere as a model for the clathrin polyhedral molecules.

Interest in clathrin has been stimulated since its identification as the major coat protein of coated pits and coated vesicles (Pearse, 1975, 1976; Goldstein et al., 1979). These structures were noted long ago in electron micrographs (Roth & Porter, 1964). Coated vesicles appear to arise from the coated pit and bristle coat regions of cellular membranes. It is only recently that a whole new area of dynamic cellular physiology which involves coated pits and coated vesicles as the principal organelles has developed to help explain such processes as intracellular protein translocation (Ockleford & Whyte, 1977), specific exocytosis (Franke et al., 1976), membrane recycling (Heuser & Reese, 1973), and receptor-mediated endocytosis (Goldstein et al., 1979; Brown & Goldstein, 1979). While the coated vesicles possess a bilayer membrane composed of phospholipids (Pearse, 1975), current evidence favors the protein coat as the major determinant of their size and structure (Pearse, 1978; Schook et al., 1979; Keen et al., 1979).

It is now clear that clathrin is the major protein in the coat structure. We have shown that mild extraction of coated vesicles results in a preparation of clathrin, i.e., 8S (H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, and H. Edelhoch, unpublished results), that is quite stable near physiological values of pH, temperature, and ionic strength. Moreover, this material is fully competent to polymerize to form characteristic basket-like molecules very similar in structure to those seen in coated vesicles. This report presents the results of equilibrium ultracentrifugation and turbidity studies of the two polymerized species obtained from the stable form of clathrin.

Materials and Methods

Chemicals. Tris-HCl, GdmCl,[1] and ammonium sulfate were Ultrapure grade from Schwarz-Mann. $NaDodSO_4$ was specially pure grade from BDH Poole Ltd. Coomassie Blue R250 was electrophoresis grade from Bio-Rad, as were the ammonium persulfate, bisacrylamide, and acrylamide used for gel electrophoresis. Deuterium oxide of 99.7% isotopic purity was from Merck Sharp & Dohme. Phenylmethylsulfonyl fluoride (PMSF) was the Boehringer-Mannheim crystallized product. Dithiothreitol and dithioerythreitol from Sigma were used interchangeably and gave within 5% of theoretical thiol content, as measured with 5,5′-dithiobis(2-nitrobenzoic acid), also from Sigma. All other chemicals were reagent grade.

Buffers. Three buffers were routinely used and are designated with letters for ease of reference.

Buffer A contained 0.10 M NaMES, 1.0 mM $MgCl_2$, 0.5 mM sodium ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid, 3 mM NaN_3, 1.5 mM PMSF, and 0.11 M ethanol (0.5%), pH 6.5. Buffer B contained 50 mM Tris-HCl, 1 mM EDTA, and 3 mM NaN_3, pH 8.0. Buffer C contained 0.25 M NaCl, 10 mM Tris-HCl, and 3 mM NaN_3, pH 7.5.

In some cases the dithiothreitol was also included in buffer A and the PMSF and EDTA were included in buffer C. While the inclusion of these reagents seemed to improve the stability of clathrin isolated as outlined below, we found no essential differences when they were omitted, provided that fresh (less than 3 days at room temperature or 7 days at 4 °C) samples were compared.

Computations. Most calculations were done on the National Institutes of Health DEC 10 system, taking particular advantage of the system's capacity for nonlinear least-squares analysis (Knott & Reese, 1972). The uncertainties obtained with this system correspond approximately to standard errors.

$NaDodSO_4$–Gel Electrophoresis. This procedure was the same as described by Laemmli (1970) and already reported (H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, and H. Edelhoch, unpublished results).

Absorbance. Ultraviolet absorbance was measured on a Cary 14 (double beam) spectrophotometer, which was equipped with an expanded slide wire for measurements of optical density differences less than 0.2.

Absorbance was also measured on the analytical ultracentrifuge, and the spectral properties of this instrument were compared to the Cary 14. At the slit widths used, the ratios of protein absorbance at 280 and 288 nm agreed to within 3% in the two instruments and the peak absorbance values agreed to within 1%.

Fluorescence. Emission spectra were recorded on a Perkin-Elmer MPF3 spectrofluorometer equipped with a Hitachi QPD 33 recorder. Temperature was controlled at 25 °C by circulating water from a controlled water bath through a jacket around the fluorescence cell.

† From the Clinical Endocrinology Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received March 26, 1980.*

‡ Present address: Department of Pharmacology, University of Virginia, School of Medicine, Charlottesville, Virginia 22901.

[1] Abbreviations used: GdmCl, guanidinium chloride; $NaDodSO_4$, sodium dodecyl sulfate; NaMES, sodium 2-(*N*-morpholino)ethanesulfonic acid; EDTA, disodium ethylenediaminetetraacetate.

Protein Concentration. Two basic assumptions were used in measuring protein concentration. One was that the ultraviolet absorbance of clathrin in the region from 205 to 220 nm is similar to that of a series of reference protein spectra obtained in this laboratory (Edelhoch & Chen, 1980). The validity of this assumption depends mainly upon the observation that protein absorbance in the far-ultraviolet is dominated by absorbance from a single functional group, the peptide bond. Whenever turbidity of samples interfered with direct absorbance measurements, the samples were diluted into concentrated (about 6 M) GdmCl, or, in some cases, the absorbance was estimated by subtracting an extrapolated (log turbidity assumed proportional to log wavelength) turbidity determined from measurements at longer wavelengths where absorbance was negligible.

The second assumption is that the refractive index increment of clathrin is typical of the values observed for most proteins in dilute salt and nearly neutral pH. The values of the refractive index increment at a given wavelength are very similar for most proteins (Timasheff, 1976). We have taken the typical value of 0.188 mL/g as the refractive index increment for clathrin at 546 nm in dilute salts near neutral pH (such as buffer C). Where necessary, the refractive index and refractive index increment at other wavelengths were calculated from

$$n(\lambda) = 1.334(0.9922 + 2310\ \text{nm}^2/\lambda^2)$$

$$dn(\lambda)/dc = 0.188\ \text{mL/g}(0.925 + 22000\ \text{nm}^2/\lambda^2)$$

as given by Camerini-Otero & Day (1978).

The clathrin extinction coefficient at 280 nm was found to be $A^{1\%}_{cm} = (10.9 \pm 0.6)\ cm^{-1}$ based on its far-ultraviolet absorbance, i.e., 205–220 nm and $(10.5 \pm 0.6)\ cm^{-1}$ from interference fringes measured simultaneously with absorbance in the analytical ultracentrifuge. The extinction coefficient of clathrin dissolved in 6.0 M GdmCl was 4% less at 280 nm than in dilute salts and buffers, such as buffer C.

Turbidity. Berkowitz & Day (1980) have developed a method of measuring the turbidity of viruses during ultracentrifugation from the transmission of light through the ultracentrifuge cell. We have applied the same basic technique to the measurement of the turbidities of two different size particles present in a solution. The turbidity of clathrin polymers present in the 150S and 300S sedimenting boundaries was evaluated from their optical densities at 335 nm. The choice of 335 nm for the turbidity measurement was dictated, in part, by the existence of a high intensity of light at this wavelength from the high-pressure mercury vapor light source of the analytical centrifuge and by the fact that this wavelength is not far from the wavelength giving the largest signal which may be measured and ascribed entirely to turbidity. The concentrations of the two boundaries were obtained from their optical density changes at 288 nm after correction for the contribution of the turbidities. The corrections for each species were made by adjusting the turbidity measured at 335 nm to the value it should have at 288 nm. This was accomplished by extrapolating to 288 nm the straight line obtained of a plot of the logarithm of the measured optical density of each boundary against the logarithm of the wavelength when measured above 310 nm in the ultracentrifuge. Slopes of 4.0 and 3.8 were found for the 150S and 300S species, respectively.

The optical density scanner pattern in the ultracentrifuge cell was measured consecutively at 288 and 335 nm. The optical density values of each species at both wavelengths were then corrected for radial dilution, i.e., multiplying by $(r/r_0)^2$, where r is the midpoint of the boundary position and r_0 is the position of the meniscus. Thus, the initial turbidity and concentration of each species could be evaluated from scans at the two wavelengths.

The relation between the molecular weight and the turbidity (Camerini-Otero & Day, 1978) is given by eq 1 if we can ignore the second virial coefficient. Thus, the turbidity ($-\ln (I/I_0)$) gives a value for MQ. If either M or Q is known, the other parameter can be calculated. Since we have obtained M for 150 and 300S, we can calculate Q and from Q the particle size

$$\tau = HcMQ = 2.3(\text{OD}) = -\ln (I/I_0) \qquad (1)$$

where

$$H = \left(\frac{32}{3N}\right)^3\left(\frac{n_0^2}{\lambda 4}\right)\left(\frac{dn}{dc}\right)^2 = 3.53 \times 10^{-6}$$

This is a reasonable assumption since it has been shown that numerous viruses show little or no concentration dependence in the region of protein concentration we are employing (Berkowitz & Day, 1980). C is the concentration (grams/milliliter), N is the Avogadro constant, n_0 is the refractive index of the solvent (1.34), dn/dc is the refractive index increment at constant chemical potential, i.e., 0.211 mL/g, at a wavelength, λ, of 335 nm, and Q is the transmittance equivalent of the particle scattering factor P and is given by eq 2 (Camerini-Otero & Day, 1978). On the basis of electron microscopic observations by others (Pearse, 1978; Schook et al., 1979; Keen et al., 1979) and ourselves, we have assumed the shape of the 150S and 300S polymer molecules to be an optically isotropic hollow sphere. In this case (Pecora & Aragon, 1974)

$$Q = \frac{3}{8}\int_0^{\pi} P(\theta)(1 + \cos^2\theta)\sin\theta\, d\theta \qquad (2)$$

$$P(x,1) = \left[\left(\frac{3}{x^3(1-m^3)}\right)\sin x - \sin xm - x\cos x + xm\cos xm\right]^2 \qquad (3)$$

where $x = bq$, $q = [4\pi/\lambda]\sin(\theta/2)$, $m = a/b$, and b is the outer radius and a the inner radius of the spherical shell. Values of Q were determined by numerical integration with the aid of a computer. A graph of Q vs. b is shown in Figure 4 for the case of $b - a = 5.0$ nm, which is the approximate thickness of the empty coated vesicles as seen by electron micrography (Crowthers et al., 1976).

Sedimentation. A Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner was used with either 12 or 30-mm optical path length double-sector cells. The temperature control unit of this centrifuge was manufactured by Arden Instruments.

Sedimentation equilibrium data analysis was done by nonlinear least-squares methods (see Computations) by using the exponential form of the concentration distribution:

$$C = \sum_i C_{i0}\exp[\sigma_i(r_i^2/2 - r_0^2/2)] \qquad (4)$$

where

$$\sigma_i = \frac{M_i(1 - \bar{v}\rho)\omega^2}{RT} \qquad (5)$$

C is the total concentration of solutes at radius, r, C_{i0} is the concentration of the ith solute at a reference radius, r_0, ω is the angular velocity of the rotor, ρ is the density of the solution, R is the ideal gas constant; T is the absolute temperature, M_i

is the molecular weight of the ith solute, and $\bar{v}$ is the apparent partial specific volume.

Isolation of Clathrin. Most attempts to prepare clathrin have focused upon the initial purification of coated vesicles, followed by extraction of the coat protein (Pearse, 1975; Blitz et al., 1977; Keen et al., 1979). This approach seemed reasonable since the initial view was that most of the protein present in coated vesicles was clathrin (Pearse, 1975); however, it has since been shown by $NaDodSO_4$–gel electrophoresis that numerous proteins other than clathrin are present in preparations of coated vesicles (Pearse, 1978; Woodward & Roth, 1978; Blitz et al., 1977). We therefore modified the technique of Pearse (1975) by omitting the time-consuming sucrose gradients which were designed to purify coated vesicles. Instead, the crude vesicle pellet, which negative staining electron microscopy shows to contain many membrane fragments in addition to coated vesicles, was homogenized in extracting buffer containing 50 mM Tris-HCl and 1.0 mM EDTA, pH 8.0 (buffer B). The soluble protein extract obtained by extraction of the crude vesicle pellet with buffer B was separated from the membrane fragments and other high molecular weight material by centrifugation at 230000g for 1 h. Clathrin was concentrated and further purified by precipitation from the supernatant with 30% saturated ammonium sulfate. The precipitate was taken up in 0.25 M NaCl, 50 mM Tris-HCl, and 1.5 M urea, pH 7.5 or 8.0, and dialized for about 4 h against this buffer. A second dialysis back into buffer C was done prior to loading the sample onto a Sepharose CL-4B column in buffer C.

The elution pattern from the Sepharose column shows three peaks by monitoring the effluent concentration by tryptophan emission intensity (Figure 1A). A similar distribution of protein was observed by absorption either at 280 or 220 nm. Peak I was in the excluded volume. When light scattering was used as one of the parameters, most of the scattering appeared in peak I, but a small but constant level was also present in half of peak II (Figure 1A). Negative staining electron microscopy shows that peak I contains membrane fragments. The similarity of the $NaDodSO_4$–gel electrophoresis pattern of peak I with that of the crude vesicle pellet or the membrane pellet obtained after extraction of clathrin from the crude vesicle pellet is thus not unexpected. $NaDodSO_4$–gel electrophoresis of various fractions from the column shows that clathrin is present in both peaks I and II (Figure 1B). Peak II is largely clathrin, but small amounts of several other proteins are also present.

Early preparations following the procedure of Keen et al. (1979) were used for some polymerization studies. Later preparations according to the above procedure gave similar results.

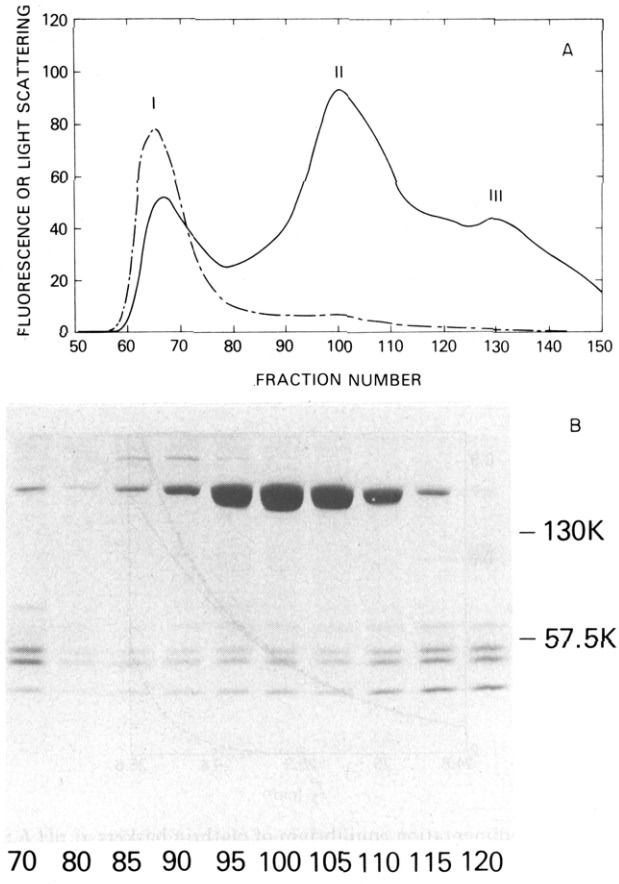

FIGURE 1: (A) Fractionation of coated vesicle proteins on sepharose CL-4B. Column buffer contained 0.25 M NaCl, 10 mM Tris-HCl, 3 mM NaN_3, and 0.50 mM EDTA, pH 7.5. The sample loaded was 5–10 mL of 1% protein extract. Fractions of 3.7 mL each were collected at 20 mL/h. The elution pattern was monitored by fluorescence at 335 nm, excited at 290 nm. The column void volume is at peak I, as defined by coelution with phage ($M = 3 \times 10^7$). Thyroglobulin dimers ($M = 1.3 \times 10^6$) elute at fraction 106, thyroglobulin monomers ($M = 665\,000$) at fraction 120, catalase ($M = 232\,000$) at fraction 141, and aldolase ($M = 158\,000$) at 143. The bed volume, defined by elution of *N*-acetyl-L-tryptophanamide, was at fraction 179. The solid line is the relative fluorescence intensity. The dashed line is the light scattered at 90° at wavelength 436 nm. (B) $NaDodSO_4$–gels analysis. Polyacrylamide–$NaDodSO_4$ gel electrophoresis. The direction of migration is from top to bottom. The numbers below each lane denote the fraction number from (A). Equal volumes of each fraction were applied to the gel. The markers shown are for β-galactosidase (135 000) and catalase (57 500). From plots of standard molecular weight of cross-linked hemocyanin ($M = 70\,000$) vs. log of migrated distance gives the clathrin monomer a molecular weight of 177 000 ● 10 000.

Results

Our preparation of bovine clathrin sediments as a single symmetrical boundary ($s^0_{20,w} = 8.2$). We have shown that this molecular species may be considered as the native form of clathrin since it is stable over a considerable range of pH (6–9), temperature (5–35 °C at pH 7.5), and salt concentration (0.05–0.50 M NaCl at pH 7.5) (H. T. Prectorius, P. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, and H. Edelhoch, unpublished results).

Sedimentation Equilibrium. We have evaluated the average molecular weight of the 150S and 300S species by sedimentation equilibrium experiments. Two different preparations of polymerized clathrin were sedimented to equilibrium at 1200 and 1000 rpm, respectively. The clathrin concentration in the experiment at 1000 rpm was measured by both absorption scanner and interference optics. It should be noted that the interference fringes measure the actual concentrations whereas the scanner absorption measures the sum of the turbidity and absorption. The equilibrium concentrations obtained by interference optics were first fit to a single exponential function. This fit curve gave very poor agreement with the experimental data. A very good fit was obtained with a two-exponential function (and integration constant). This analysis requires a five constant fit, and the correlation between constants becomes strong. From this analysis, however, it became evident that the 300S species was depleted from the meniscus region and that the concentration of 8S species was essentially constant throughout the cell. Thus, the data in the meniscus region of the cell would be expected to fit a single exponential function, i.e., a three-parameter fit. In fact, a good fit of the data to a single exponential representing the 150S distribution in the meniscus region of the cell was found. With the two

Table I: Turbidity and Sedimentation Coefficients of Clathrin Baskets[a]

	150 S				300 S					
expt	C (mg/mL)	τ (cm^{-1})	$10^{-6}M^b$	$s_{20,w}$ (S)	C (mg/mL)	τ (cm^{-1})	$10^{-6}M^b$	$s_{20,w}$ (S)	T (°C)	l (mm)
2477	0.110	0.0830	22.9	170	0.113	0.138	37.2	335	15	12
2448	0.147	0.0884	18.4	155	0.121	0.148	37.2	290	15	12
2452	0.0765	0.0382	15.3	125	0.182	0.205	34.1	325	4	30
2453	0.036	0.0161	13.6	145	0.0726	0.0756	31.9	320	6	30
2466	0.343	0.151	13.6	145	0.609	0.832	41.5	285	25	12

[a] The pH of all solutions was changed from 7.5 to 6.8 by dialysis for 1–4 h at 25 °C and then kept at the temperature of the experiment for about 20 h. All final solutions at pH 6.8 in 0.20 M ammonium acetate, 0.5–1.0 mM Ca^{2+}, and 3 mM azide. Experiment 2452 contained 0.10 M ammonium acetate. Preparation 2453 and 2452 contained only 150S and 300S sedimenting protein since 8S protein was removed on a Sepharose A-50 column. In preparation 2466, the absorption was measured at 296 nm instead of 288 nm due to the high concentration of clathrin used in this experiment. The readings were corrected to 288 nm by the ratio of the absorption at the two wavelengths. [b] Values of M are uncorrected for Q; i.e., assume $Q = 1$.

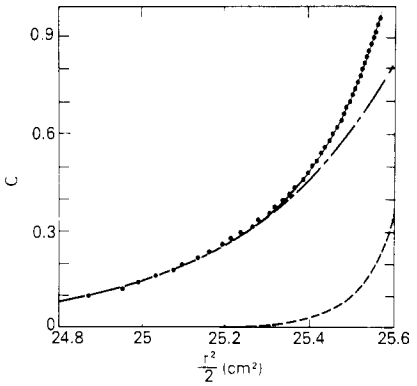

FIGURE 2: Sedimentation equilibrium of clathrin baskets at pH 6.8 in 0.20 M ammonium acetate, 0.5 mM $CaCl_2$, and 3 mM sodium azide at 11 °C. A 30-mm cell was used and the rpm was 1000. The (—-) curve represents the distribution of clathrin by a least-squares fit to the 150S species; the (– –) curve applies to the 300S species. (●) Represent the experimental points; the line through these points is the sum of the two best-fit curves to the 150S and 300S species. The ordinate (c) is in units of mg/mL.

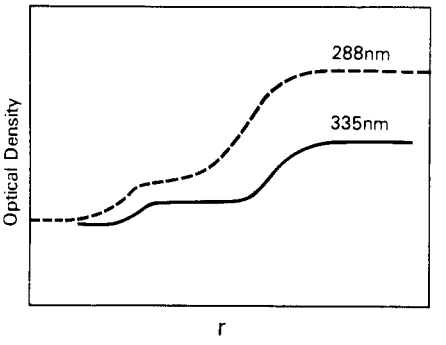

FIGURE 3: Scanner transmittance profile at wavelengths 288 and 335 nm of polymerized clathrin (2452) observed during velocity centrifugation. The region separating the 150S and 300S species is almost a plateau. Solution at pH 6.8 in 0.10 M ammonium acetate and 3.0 mM sodium azide at 4 °C.

constants for the 150S form available, a second analysis was performed with a two-exponential function by using the data throughout the cell. The fit of the two-exponential functions to the equilibrium data is shown in Figure 2. A value of (99.7 ± 8.8) × 10^6 was obtained for the 300S and (24.1 ± 0.6) × 10^6 for the 150S sedimentating components. The concentration of the three components (300, 150, and 8 S) in the cell could now be calculated. These were in approximate agreement with that observed by velocity sedimentation.

The same procedure was used to evaluate the molecular weight of the 150S polymer from the optical density pattern obtained by scanner optics of the second preparation. A very good fit was found for the optical density values in the meniscus region of the cell with M_r = (26.8 ± 0.6) × 10^6. The base region of the cell containing the 300S could not be analyzed directly since the turbidity contribution of the 300S to its optical density is different from that of the 150S.

Turbidity. The molecular weights of large polymers and viruses have been obtained from their optical densities measured during sedimentation in the ultracentrifuge (Berkowitz & Day, 1980; Day & Mindich, 1980). This procedure allows the simultaneous analysis of the molecular weight of several sedimenting species on very small amounts of solute. In addition, the problems of clarifying solutions are avoided.

In Figure 3 can be seen the scanner tracing of the optical densities of the 150S and 300S sedimenting boundaries. In this preparation, the separation between the two boundaries is quite clear. The spreading of both the 150S and the 300S boundaries is much greater than that due to diffusion alone and arises from the heterogeneity of both species (H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, H. Edelhoch, unpublished results). Without a clear flat region between the two boundaries, resolution of the two boundaries is uncertain. In the absence of a clear plateau region, we selected a point between the two boundaries closer to the 150S boundary since the spreading of the 300S boundary was almost always much greater than that of the 150S boundary.

We have measured the turbidities of the 150S and 300S species of five different preparations of clathrin (Table I). The average of the molecular weights calculated from the turbidities (see Materials and Methods) for the five preparations without correcting for the transmittance equivalent of the particle scattering factor Q is (16.8 ± 4.0) × 10^6 and (36.4 ± 3.6) × 10^6 for the 150S and 300S species, respectively. These two values are 0.70 ± 0.17 and 0.37 ± 0.04 of the molecular weight values we found by equilibrium sedimentation; consequently, Q is 0.70 ± 0.17 for the 150S particles and 0.37 ± 0.04 for the 300S particles. From these values and the graph of Q vs. b in Figure 4, the average particle diameters of the two species are 64.0 ± 15 and 112 ± 11 nm. These two sizes are in rough agreement with those reported from electron microscopy by Pearse (Pearse, 1976, 1978; Crowthers & Pearse, 1976) for coated vesicles from several tissues and by Friend & Farquhar (1967) from renal tubular epithelium.

Discussion

A comparison of various methods of preparing clathrin reveals some aspects of the clathrin interaction with the phospholipid membrane of coated vesicles. In our procedure, clathrin was dissociated from membranes by exposure to low (less than 50 mM Tris-HCl) ionic strength, mild alkali (pH 8.0), and removal of divalent metal ions (1.0 mM EDTA). A slightly more complete extraction of clathrin is afforded by

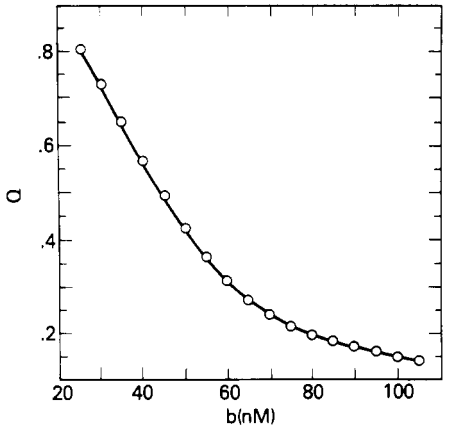

FIGURE 4: Plot of Q vs. b calculated from eq 3 in text.

exposure to 0.5 M Tris-HCl at neutral pH in the presence of 0.25 mM $MgCl_2$ (Keen et al., 1979). Since 0.5 M Tris-HCl was much more effective than 0.5 M NaCl or 0.5 M concentrations of several other amines, a specific amine effect was suggested. A nearly complete extraction of clathrin was also found after exposure to moderate alkali, such as 0.015 M Na_2CO_3, pH 10 (Keen et al., 1979). Since pH 10 has only a small effect upon the circular dichroic spectrum of isolated clathrin (H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, and H. Edelhoch, unpublished results), exposure to alkali would appear preferable to other techniques in terms of maximum yields of native clathrin. It appears, however, that preparations of clathrin of greater purity are obtained when only mildly alkaline (pH 8.0) buffers are used.

Nonionic detergents, such as Tween (Keen et al., 1979), are quite ineffective in solubilizing clathrin from membranes. An ionic detergent (0.5–1% cholate) appears about equally effective as 0.5 M Tris-HCl (Pearse, 1978) although the subsequent separation of solubilized clathrin from lipid, which is also solubilized by the detergent, may be worse with cholate. Taken together, these data suggest that the binding of clathrin to the phospholipid membrane components of coated vesicles is relatively weak and dominated by ionic effects.

We have reported elsewhere on the formation from 8.2S clathrin of two high polymeric species, i.e., 150 S and 300 S, which have lattice structures by electron microscopic examination which closely resemble similar structures found in coated vesicles (H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, and H. Edelhoch, unpublished results). Moreover, two studies on coated vesicles have reported the presence of two discrete size ranges (Friend & Farquhar, 1967; Pearse, 1978).

The formation of the high polymeric forms of clathrin was normally observed in the ultracentrifuge after reducing the pH from 7.5 to 6.8 in 0.20 M ammonium acetate, 0.5–1.0 mM $CaCl_2$, and 3 mM NaN_3 at 4 °C and allowing the solution to stand overnight. In addition to the 150S and 300S species, varying amounts of unpolymerized clathrin were also observed. The rates of polymerization depend on clathrin concentration, pH, ionic strength, and $CaCl_2$ concentration. By overnight incubation, the polymerization reaction was complete.

Numerous experiments were also performed at pH values between 6.2 and 6.4 in 0.20 M ammonium acetate, with and without 0.5 mM calcium and magnesium ions. At these lower pH values, significant amounts of polymerized clathrin with sedimentation rates greater than the 300S boundary were observed which were not observed at pH 6.8. In these cases, the scanner optical density tracing continued to increase linearly ahead of the normal 300S boundary. At pH values below 6.0, clathrin was no longer soluble and precipitated from solution. The rates of formation of 150 S and 300 S were considerably faster at the lower pH values.

We have found by sedimentation equilibrium experiments the average molecular weights of the two high polymeric species formed by the self-association of 8.2S clathrin at pH values slightly below neutral. Examination of these polymeric molecules in the electron microscope reveals a lattice structure and size range similar to that observed with the protein coat of coated vesicles (Pearse, 1975, 1978; Schook et al., 1979; Friend & Farquhar, 1967; (H. T. Pretorius, P. K. Nandi, R. E. Lippoldt, M. L. Johnson, J. H. Keen, I. Pastan, and H. Edelhoch, unpublished results).

Turbidity measurements of each of the high polymeric species obtained during sedimentation give particle diameters consistent with those observed by electron microscopy for coated vesicles. It should be noted that sedimentation velocity experiments reveal that both the 150S and 300S species contain a range of molecular sizes (or shapes). It appears that clathrin can be readily reassembled in high yields at pH values between 6 and 7 to give two species of cage or basket molecules which resemble in size and structure the protein coat of coated vesicles.

References

Berkowitz, S. A., & Day, L. A. (1980) *Biochemistry 19*, 2696–2702.

Blitz, A. L., Fine, R. E., & Toselli, P. A. (1977) *J. Cell Biol. 75*, 135–147.

Brown, M. S., & Goldstein, J. L. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*.

Camerini-Otero, R. D., & Day, L. A. (1978) *Biopolymers 17*, 2241–2249.

Crowthers, R. A., Finch, J. T., & Pearse, B. M. F. (1976) *J. Mol. Biol. 103*, 785–798.

Day, L. A., & Mindich, L. (1980) *Biochemistry* (in press).

Edelhoch, H., & Chen, R. F. (1980) *Horm. Proteins Pept. 9*, 109–173.

Franke, W. W., Luder, M. R., Kartenbeck, J., Zerban, H., & Keenan, T. W. (1976) *J. Cell Biol. 69*, 173–195.

Friend, D. S., & Farquhar, M. G. (1967) *J. Cell Biol. 35*, 357–376.

Goldstein, J. L., Anderson, R. G. W., & Brown, M. S. (1979) *Nature (London) 279*, 679–685.

Heuser, J. E., & Reese, T. S. (1973) *J. Cell Biol. 57*, 315–344.

Keen, J. H., Willingham, M. C., & Pastan, I. H. (1979) *Cell 16*, 303–312.

Knott, G. D., & Reese, D. K. (1972) *Proc. ONLINE 1972 Int. Conf. 1*, 497.

Ockleford, C. D., & Whyte, A. (1977) *J. Cell Sci. 25*, 293–312.

Pearse, B. M. F. (1975) *J. Mol. Biol. 97*, 93–98.

Pearse, B. M. F. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1255–1259.

Pearse, B. M. F. (1978) *J. Mol. Biol. 126*, 803–812.

Pecora, R., & Aragon, S. R. (1974) *Chem. Phys. Lipids 13*, 1.

Roth, T. F., & Porter, K. R. (1964) *J. Cell Biol. 20*, 313–332.

Schook, W., Puszkin, S., Bloom, W., Ures, C., & Kochwa, S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 116–120.

Timasheff, S. N. (1970) in *CRC Handbook of Biochemistry* (Sober, H. A., Ed.) 2nd ed., p C67, Chemical Rubber Co., Cleveland, OH.

Woodward, M. P., & Roth, T. F. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4394–4398.