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Polymerization of Clathrin Protomers into Basket Structures†

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ABSTRACT: The effects of pH, ionic strength, temperature, and protein concentration on the rate of clathrin (8 S) polymerization to form coat (or basket) structures (~300 S) have been measured by turbidity. The extent of polymerization has also been evaluated under the same experimental conditions by analytical centrifugation. The characteristic polygonal structure of the re-formed coat was confirmed by electron microscopy. The rate of polymerization is sensitive to all the variables investigated. The reaction is very slow at pH ~7 and becomes very rapid by pH ~6. The polymerization is readily reversed by increasing the pH slightly. The time dependence of the polymerization does not conform to either a first- or a second-order reaction but to a higher order. Increasing temperature increases the rate but decreases the extent of reaction. Increasing the salt concentration decreases the rate. The effects of several salts on the rate follow the Hofmeister ranking, with the exception of sulfate.

The coated pit regions of mammalian plasma membranes have been implicated as the site of receptor-mediated endocytosis for numerous proteins and hormones (Schlessinger et al., 1978; Maxfield et al., 1978; Goldstein et al., 1979; Brown & Goldstein, 1979). It has also been shown that the protein which forms the coat of these pits is the same as that found around coated vesicles inside cells (Pearse, 1975, 1976). These vesicles probably play a major role in intracellular transfer among organelles (Ockleford & Whyte, 1977; Rothman et al., 1980). Pearse (1975, 1976, 1978) and others (Ockleford & Whyte, 1977; Blitz et al., 1977; Woods et al., 1978; Woodward & Roth, 1978; Bloom et al., 1980) have isolated coated vesicles from various cell types and shown that one protein is the major constituent present in the coats of these vesicles. This protein, which was named clathrin by Pearse (1975), is capable of re-forming the coat structure observed in coated vesicles (Pearse, 1978; Schook et al., 1979; Keen et al., 1979; Woodward & Roth, 1979).

Clathrin has been isolated from coated vesicles of human and bovine brain by similar procedures and further characterized in this laboratory. A protomer with a molecular weight of 610000 and a sedimentation constant of 8.1 S has been isolated (Pretorius et al., 1981). It was shown to polymerize at pH 6.8 to yield two populations of clathrin baskets (coat structure) with average sedimentation rates of 150 S and 300 S and molecular weights of 25×10^6 and 100×10^6, respectively (Nandi et al., 1980).

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In this report, we show that the kinetics of polymerization of clathrin can be conveniently followed by turbidity measurements, i.e., either absorbance or light scattering. By using these two procedures, we have evaluated the effects of protein concentration, pH, salt, and temperature on the rate of clathrin polymerization. We have also measured the extent of conversion of the protomer to 300S baskets by centrifugation. For the first time, the rate and extent of this reaction have been evaluated by quantitative procedures where all the components are accounted for. With these results, it should be possible to assay the effects of various molecules, e.g., metabolites, drugs, and proteins, which may interact with clathrin to regulate endocytosis.

Methods

Preparation of Clathrin. Bovine brain was used to prepare clathrin. A modification of the method of Schook et al. (1979) was employed as previously reported by us (Nandi et al., 1980). Five brains were generally used in one preparation. The protein was extracted from crude coated vesicles (230000*g* pellet) with 50 mM Tris-HCl (pH 8) and concentrated by precipitation with 30% ammonium sulfate. It was then chromatographed on Sepharose CL-4B (Nandi et al., 1980). About 30–60 mg of clathrin was obtained per preparation. The polypeptide compositions of the fractions containing 8S clathrin were routinely analyzed by $NaDodSO_4$[1] gel electrophoresis by using the procedure of Laemmli (1970) with 8% gels and 5% stacking gels. As observed by Nandi et al. (1980) and also by Schook et al. (1979) (Blitz et al., 1977; Woodward & Roth, 1979; Keen et al., 1979), small amounts (~10%) of other proteins copurified with 8S clathrin. All fractions contained a major 175000 molecular weight band, representing the monomer of clathrin and several other bands.

The fractions containing 8S clathrin were stored in the column elution buffer (0.01 M Tris-HCl, 0.25 M NaCl, and 5 mM NaN_3, pH 8.0) at 4 °C until used. In general, they were not stored for more than 1 week although in numerous instances the activity remained intact even after several weeks. Immediately before polymerization studies, clathrin preparations were dialyzed against a pH 8 solution with weak buffering capacity (usually ammonium acetate) to facilitate rapid adjustment of the pH in order to initiate the polymerization reaction.

Preparations of Solutions for Optical Measurements. Considerable care was necessary in adjusting the pH of clathrin solutions when rates of polymerization were measured. This was important because of the large pH dependence of the rate of polymerization. In most cases, the pH was adjusted by the careful addition, with stirring, of about 50 μL of a 1 M Mes buffer, varying from pH 6.0 to 6.3, to a 1-mL solution of clathrin at pH 8 in a 1-cm^2 cuvette. The exact amount of Mes needed to achieve the final pH was determined in a separate experiment. The pH of the solution was routinely measured at the end of the polymerization in order to verify the reaction pH. All turbidity measurements were routinely made near 25 °C unless otherwise specified.

Turbidity. In the absence of absorption (and a second virial coefficient), the relationships between turbidity, absorbance, and molecular weight are the following:

$$\tau = -\ln (I/I_0) = HcMQ = 2.3A \qquad (1)$$

where

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

and A is the absorbance in a 1-cm path-length cuvette. Q is the transmittance equivalent of the particle scattering factor, P, and is given by Camerini-Otero & Day (1978).

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

For an optically isotropic hollow sphere (Pecora & Aragon, 1974)

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

where

$$x = bq$$

$$q = \frac{4\pi}{\lambda} \sin(\theta/2)$$

$$m = a/b$$

and b is the outer radius and a the inner radius of the hollow sphere. Values of Q for clathrin baskets have been calculated and reported previously (Nandi et al., 1980). We have found values of Q of 0.70 and 0.37 for the 150S and 300S basket species, respectively. Using these values, we can relate the absorbance to the molecular weight by eq 1 and 2. Absorbance measurements were made in a 1-cm^2 cuvette in a Cary Model 14 or Model 219 spectrophotometer.

Light Scattering. The relation between turbidity and light scatter at angle θ is

$$\tau = \frac{8\pi}{3}\frac{Ir^2}{I_0} = HcMP(\theta)$$

The quantity that is measured is Ir^2/I_0, the Rayleigh ratio. We have measured the scattered light routinely at 45° in a Brice Phoenix Universal light-scattering photometer equipped with a blue filter (435 nm). A few times, measurements were made at 90° and 45° on the same polymerization reaction. We found that about twice as much light was scattered at 45° as at 90°. A greater ratio would clearly be observed at still lower angles.

We have found that a polished cylindrical cell (r = 5 mm) gave reproducible scattering values for unpolymerized clathrin solutions. These were purchased from Precision Cell, Inc. Protein samples were routinely centrifuged at 20000*g* for 30 min before measurements. A small magnetic stirring bar was used to rapidly mix (~7 s) the protein solution with Mes buffer when polymerization was started by lowering the pH. The increase in scattering intensity from that of the unpolymerized 8S clathrin solution gives a measure of the extent of polymerization. We have not given molecular weights by this method since an independent method of relating light scatter of this instrument to molecular weight would be necessary.

Analytical Ultracentrifugation. In order to verify that the turbidimetric changes represented 300S clathrin baskets, the solutions were analyzed by ultracentrifugation. A Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner was used. A wavelength of 320 nm was normally used (Pretorius et al., 1981). At this wavelength, all

[1] Abbreviations used: Mes, 2-(*N*-morpholino)ethanesulfonic acid, sodium salt; $NaDodSO_4$, sodium dodecyl sulfate.

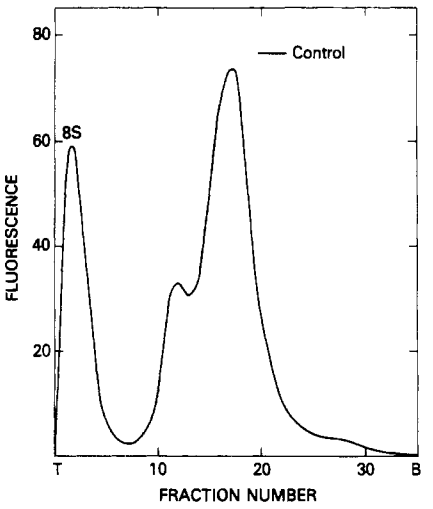

FIGURE 1: Sucrose gradient centrifugation on a linear 10–30% gradient of clathrin polymerized at pH 6.5 for 48 h at 4 °C. Protein concentration determined by tryptophan emission intensity. T and B are top and bottom of gradient tubes, respectively.

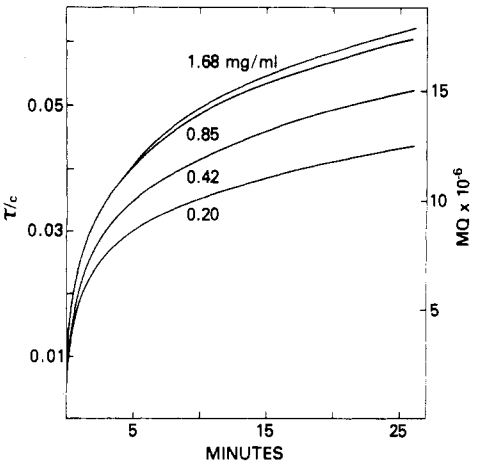

FIGURE 2: Effect of protein concentration on the rate of clathrin polymerization as measured by absorbance at 320 nm. All solutions contained 0.10 M ammonium acetate and 0.045 M Mes, pH 6.32–6.34, $T \simeq 23$ °C. Values of M may be obtained by using a value of 0.37 for Q (see Methods).

the absorbance is due to turbidity. In the same experiment, the amount of unpolymerized clathrin was measured by increasing the rotor speed from 7200 to 40 000 rpm and scanning at 280 nm. A detailed analysis of the sedimentation patterns of clathrin baskets has been reported elsewhere (Pretorius et al., 1981). It is necessary to point out the importance of correlating turbidimetric changes with ultracentrifugal characterization of 300S baskets before relying solely on the turbidimetric assay. Turbidity does not distinguish between specific and nonspecific association, and care must be exercised in using this type of assay. When electron microscopical examination is used to investigate clathrin polymerization, it is also necessary to realize that without considerable effort one is not certain what percent of 8 S has polymerized to 300S clathrin baskets. A quantitative measure of the extent of conversion is provided, however, by ultracentrifugation, since the amount of unpolymerized 8S clathrin can be measured direclty and the size distribution of the basket population can be evaluated (Pretorius et al., 1981).

Sucrose Gradient Centrifugation. A Beckman Model L2-65 ultracentrifuge with an SW 27 rotor was used. Linear gradients were formed by mixing equal volumes of 10 and 30% sucrose solutions (w/w) in the same buffer as used for polymerizing clathrin. A 1-mL sample of polymerized clathrin was layered on top of the gradients. After centrifugation at 4 °C, 1-mL fractions were collected from the bottom of the tubes. An LKB peristaltic pump set at 2 mL/min was used. Protein concentration was monitored by tryptophan fluorescence in a Perkin-Elmer MFP-3 fluorometer. Samples were excited at 280 nm, and the emission was monitored at 340 nm.

We have also assessed the size distribution of baskets by band sedimentation on a linear sucrose gradient (10–30%). A standard preparation of 8S clathrin was polymerized at pH 6.5 (0.7 mg/mL in 0.1 M ammonium acetate) for 48 h at 4 °C. The sedimentation profile is shown in Figure 1, where a small trailing peak is seen as a shoulder of the principal band. By comparison of this pattern with those obtained by scanner optics of sedimenting boundaries, it is evident that the small and main peaks represent the 150S and 300S species, respectively.

Hydrogen Ion Uptake. The uptake of protons during clathrin polymerization was followed with a pH stat. The pH was brought to the reaction value with 0.01 M acetic acid. A radiometer PHM 26 pH meter was used which was equipped with an automatic titrator, an Ole Dich recorder, and an Agla micrometer syringe. A micro glass electrode with a calomel reference was used to measure the pH. The reaction was carried out at 25 °C by circulating water through a jacket surrounding the cell and with nitrogen passing over the solution.

Protein Concentration. A value of 10.9 was used for $E^{1\%}_{1cm}$ at 280 nm for 8S clathrin (Nandi et al., 1980).

Analysis of Kinetic Data. The kinetic data have been analyzed according to a first-order reaction

$$\log (1 - p) = -kt$$

and according to high-order reaction rates indicated by Powell (1953)

$$\left(\frac{1}{n-1}\right)\left(\frac{1}{C_0^{n-1}}\right)\left(\frac{1}{(1-p)^{n-1}} - 1\right) = kt$$

where C_0 is the initial concentration, p is the fraction of clathrin (8 S) reacted (or the extent of reaction), k is the velocity constant, and n is the order of the reaction.

Electron Microscopy. Routine electron microscopic examination of the characteristic polygonal coat structure of polymerized clathrin was carried out by Dr. B. Kramarsky. Negative staining with 1% uranyl acetate was used. Typical electron micrographs of the population of clathrin baskets have been presented elsewhere (Nandi et al., 1980).

Results

Effect of Clathrin Concentration. The rate of increase in the absorbance at 320 nm (i.e., $\tau/2.3$) was measured at four concentrations of clathrin, ranging from 1.77 to 0.215 mg/mL. The final pH was carefully controlled between 6.32 and 6.34 (see Methods) since the rate is very sensitive to pH (vide infra). The results, expressed in units of reduced turbidity, are shown in Figure 2. The values of MQ are also given in order to indicate the range of molecular weights represented by the turbidity values. A striking and unexpected result is that the initial rate of polymerization changes much slower than the square of the concentration as would be expected for a second-order reaction. In fact, it is not very different from a first-order reaction.

In order to ascertain whether the polymer molecules observed turbidimetrically had the molecular properties which characterize clathrin baskets, all four solutions were examined

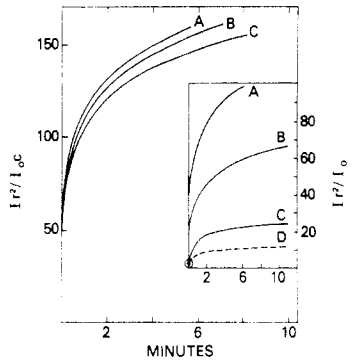

FIGURE 3: Effect of protein concentration on the rate of clathrin polymerization as measured by light scatter at 435 nm. All solutions contained 0.10 M ammonium acetate and 0.045 M Mes, pH 6.02–6.04, $T \simeq 23$ °C. Insert gives observed light-scattering values at different concentrations of clathrin. The relationship between $I_r^2/(I_0C)$ to M is given under Methods. Protein concentrations of A, B, C, and D were 0.56, 0.35, 0.14, and 0.07 mg/mL, respectively.

by velocity sedimentation after 16 h of polymerization at 23 °C. In each case, the sedimentation pattern revealed a major boundary with an average sedimentation rate near 300 S. The patterns resemble those reported earlier (Nandi et al., 1980) for clathrin baskets except there was much more of the 300S than the 150S boundary. The extent of formation of baskets was determined from the amount of 8 S which disappeared by polymerization. This was obtained by increasing the rotor speed from 7200 to 40 000 rpm and measuring the absorption at 280 nm of the unpolymerized 8S boundary. About 40% of the 8S boundary was unpolymerized at the three lowest clathrin concentrations while about 20% of 8 S was left at the highest concentration.

The light lost in the transmission experiments discussed above could be observed directly by the amount of light scattered. This method provides an alternative procedure which can be useful in more dilute solutions where the turbidity is too small to measure by transmission. The same preparation of clathrin was used to evaluate the concentration dependence of the rate of polymerization. The light scatter and reduced scatter for several concentrations of clathrin are reported in Figure 3. The reduced scattering values observed after 16 h were about twice as large as the values reported after 6–8 min in Figure 3. The data are consistent with those shown by absorbance measurements in Figure 2 in revealing a rather small dependence of initial rate on concentration.

The solutions were examined also by velocity centrifugation after standing for 16 h at 4 °C. In this case, at pH 6.02–6.04, the extent of conversion of 8S to 300S polymer was much larger than at pH 6.3 and reached about 90% at all four concentrations. The almost complete conversion of 8S to 300S species suggests that practically all of the protein present in solution is active in forming baskets.

pH Dependence. The rate of polymerization of clathrin becomes readily measurable by absorbance measurements near pH 6.65 and increases rapidly with decreasing pH to ~6.0 (Figure 3). Below pH ~6.0, clathrin aggregates to larger particles and precipitates from solution. The rate of increase in absorbance was measured continuously for ~2 h and then after 24 and 60 h. When these solutions were centrifuged after 24 h, the average sedimentation constant of the polymerized clathrin was near 300 S. The amount of unpolymerized 8 S decreased linearly from 53 to 7% with decreasing pH from 6.65 to 6.25. It can be seen in Figure 4 that the absorbance remains constant between 24 and 60 h at pH 6.65 whereas it continues to increase slowly at pH 6.44 and 6.37 in the same time interval. It is evident, therefore, that the incomplete

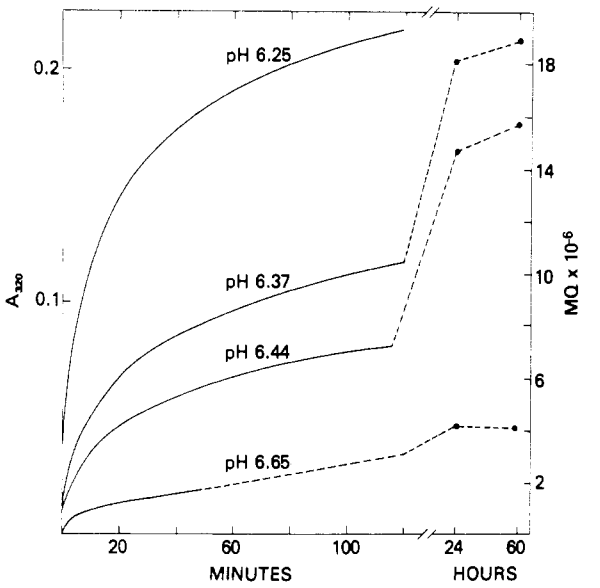

FIGURE 4: Effect of pH on the rate of clathrin polymerization as measured by absorbance. All solutions contained 0.20 M ammonium acetate, 0.02 M phosphate, and 1.30 mg/mL clathrin. $T \equiv 23$ °C.

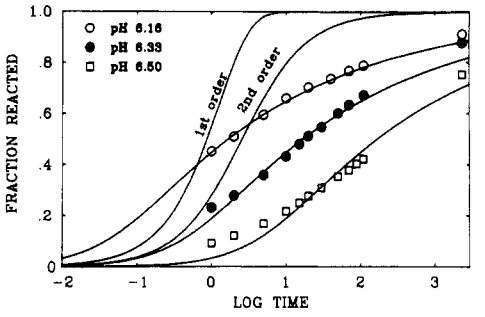

FIGURE 5: Kinetic analysis of the polymerization of clathrin at three different pH values. Polymerization was followed at 7 °C by the increase in absorbance at 320 nm (0.79 mg/mL) in 0.10 M ammonium acetate and 0.46 M Mes. The experimental points at each pH do not fit either a first- or a second-order reaction, but a much higher order.

conversion of 8 S to baskets at pH 6.65 represents an equilibrium value while at pH 6.44 and 6.37 the incomplete conversion may mean that more time is needed for the reaction to reach completion.

The data in Figure 4 are not amenable to kinetic analysis since the polymerization did not go to completion. It was observed, however, that essentially complete disappearance of 8 S occurred when the temperature was near 5 °C. The polymerization reaction was therefore performed at 7 °C at pH values of 6.16, 6.33, and 6.50.

Clathrin was polymerized very slowly at pH 6.43 in 0.10 M ammonium acetate in order to determine whether intermediate size species, i.e., between the protomer (8 S) and the baskets, were present. Aliquots were examined by ultracentrifugation at various times during which 10–50% of 8 S disappeared. No intermediate species were observed. Since only two components, i.e., protomer and baskets, were found, the data at pH 6.16, 6.33, and 6.50, where the conversion was almost complete, could be analyzed by the method of Powell (1953). The kinetic data were evaluted by plotting the fraction of clathrin reacted as a function of the logarithm of the time. This plot gives a characteristic slope for a given order. For orders higher than the first, different concentrations shift only the location of the curve on the log time axis without changing the shape. The data, plotted in this manner in Figure 5, can

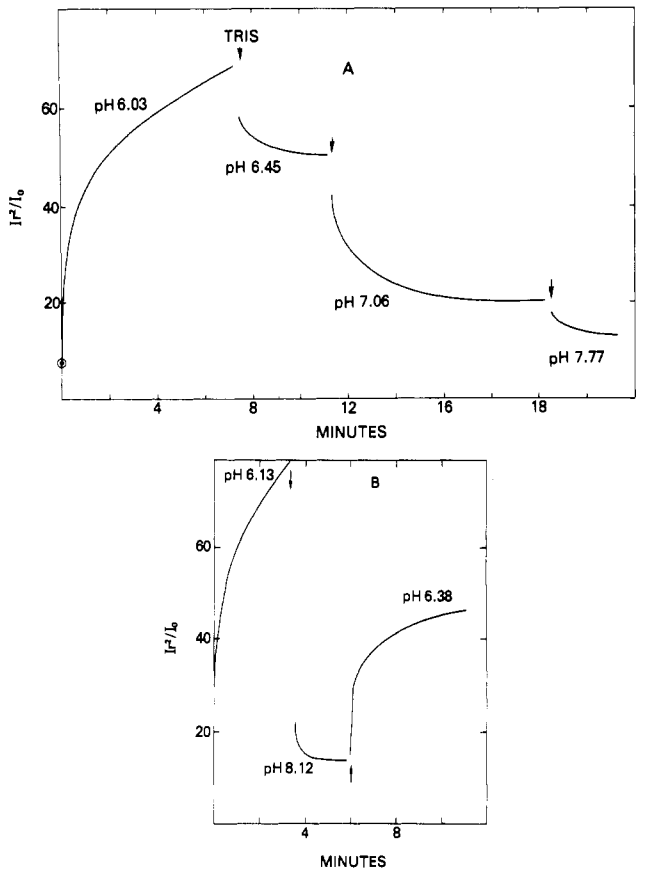

FIGURE 6: (A) Effect of pH on the depolymerization of clathrin baskets. A solution of clathrin (0.52 mg/mL) in 0.01 M NaCl and 0.001 M Tris was brought to pH 6.03 with the addition of Mes to a final concentration of 0.045 M. Three aliquots of 25 μL of 1 M Tris were added to bring the solution to pH 6.45, 7.06, and 7.77. Each addition of Tris is shown by arrows. The Tris concentration at pH 7.77 was 0.07 M. (B) Effect of pH on the depolymerization and repolymerization of clathrin. A solution of clathrin (0.76 mg/mL) in 0.01 M NaCl and 0.001 M Tris was brought to pH 6.13 with the addition of Mes to a final concentration of 0.045 M. Depolymerization to approximately the initial light-scattering value was obtained at pH 8.12 by addition of 100 μL of 1 M Tris (0.087 M final concentration). Subsequent addition of 50 μL of 1 M Mes (pH 5.9) to a final concentration of 0.087 M decreased the pH to 6.38 and initiated repolymerization. The final solution was analyzed by analytical ultracentrifugation after 16 h at 4 °C. About 30% of the clathrin was not polymerized into 300S baskets.

be fitted approximately with a sixth-order dependence on concentration. The characteristic curves for first- and second-order reactions are also shown in Figure 5 to indicate that they have very different slopes. It is apparent that a simple formulation of the aggregation reaction does not apply to the polymerization of clathrin. The higher order reaction implies a more complex mechanism.

The reversibility of the polymerization reaction was also shown by increasing the pH slightly. When the pH of a partially polymerized solution of clathrin was increased from 6.03 to 6.45 by the addition of a small volume of 1 M Tris, a very rapid initial decrease in light scatter occurred which reached a constant value after several minutes (Figure 6A). Further addition of 1 M Tris increased the pH to 7.06 and resulted in a second time-dependent decrease in light scatter. The light-scattering value was almost reduced to that of the starting value by raising the pH further to 7.77 (Figure 6A). When this solution was centrifuged, the major sedimenting species was 8 S, and only a small amount of 300 S was left. Intermediate size polymers were not observed. It has been reported that only 150S and 300S species were found after polymerization of 8 S for 24 h at pH 6.8 (Nandi et al., 1980).

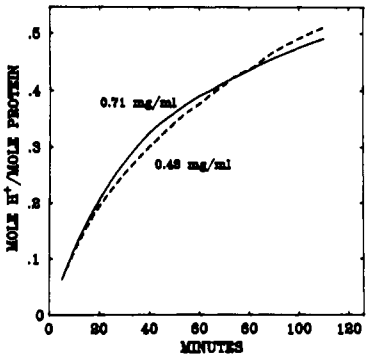

FIGURE 7: Rate of proton uptake during the polymerization of clathrin at pH 6.31 in 0.001 M NaCl. The reaction was started by adding, with stirring, a small amount of 0.01 M acetic acid to a pH 7.5 solution.

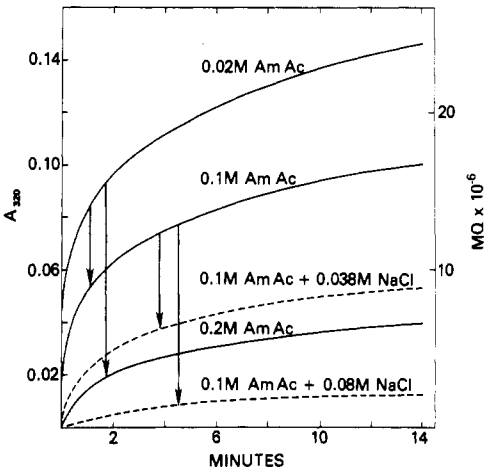

FIGURE 8: Effects of ammonium acetate and NaCl concentrations on the rate of polymerization of clathrin at pH 6.33. Clathrin concentration was 0.7 mg/mL in all solutions. The pH was adjusted with 50 μL of 1 M Mes to a final concentration of 0.045 M.

In the present study, we have found much smaller amounts of 150 S.

Increasing the pH from 6.13 to 8.12 in a single change reduced the light scattering of clathrin baskets to that of the 8S species in 1–2 min. Decreasing the pH of the same solution from 8.12 to 6.38 resulted in a rapid increase in light scatter (Figure 6B). As was expected, the extent of polymerization was less at pH 6.38.

From the strong pH dependence of polymerization, it was expected that a considerable number of protons may be involved in the formation of clathrin baskets. We have measured the rate of proton consumption during the polymerization by a pH stat. The rates of proton uptake at two concentrations of clathrin at pH 6.31 are shown in Figure 7. It can be seen that the initial rates do not show much dependence on concentration as was found by absorbance and light scatter. The number of protons taken up is, however, surprisingly small. After 2 h of reaction, only 0.5 mol of H^+ was consumed per mol of 8S clathrin. The extent of conversion of 8 S to baskets should be about 40–50% in this case. Consequently, complete conversion would require the uptake of about one proton per mole of 8 S at pH 6.31.

Salt Effects. In early experiments where the polymerized clathrin was analyzed by electron microscopy for the presence of its characteristic polygonal structure, ammonium acetate was used as a neutral salt because it is volatilized under vacuum. It was found that increasing the concentration of ammonium acetate from 0.02 to 0.10 M decreased the rate of polymerization at pH 6.33 (Figure 8). Further increase of ammonium acetate concentration to 0.20 M decreased the

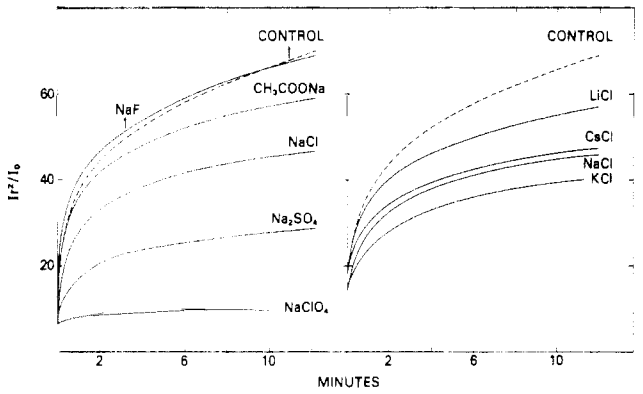

FIGURE 9: Effects of various anions (left panel) and cations (right panel) on the rate of polymerization of clathrin at pH 6.03 as measured by light scatter. All solutions contained 0.01 M NaCl, 0.001 M Tris, 0.045 M Mes, and 1.0 mg/mL clathrin, as well as 0.036 M of the individual salts, $T \simeq 23$ °C. The light scatter of unpolymerized clathrin was 6.5 units.

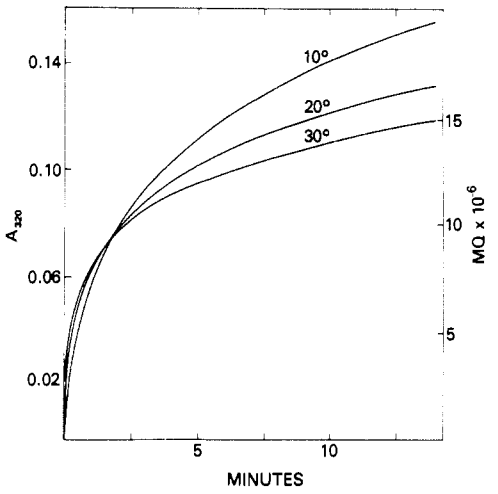

FIGURE 10: Effect of temperature on the rate of polymerization of clathrin at pH 6.33. All solutions contained 0.10 M ammonium acetate, 0.045 M Mes, and 0.91 mg/mL clathrin.

rate even further. Addition of 0.038 M NaCl to a 0.10 M ammonium acetate solution had about the same effect in decreasing the rate as adding 0.10 M ammonium acetate. Increasing the total NaCl concentration to 0.080 M further decreased the rate (Figure 8). It is evident that NaCl was considerably more effective than ammonium acetate on a molar basis in reducing the rate.

To evaluate the effects of different salts, we have compared the rate of clathrin polymerization at pH 6.03 in the presence of a constant amount of NaCl (i.e., 0.01 M) and 0.036 M of the second salt. The data are shown in Figure 9. It can be seen that whereas fluoride had little or no effect perchlorate almost completely inhibited the reaction. Acetate, chloride, and sulfate had increasing inhibitory effects. With the exception of sulfate, the anions follow the Hofmeister ranking (von Hippel & Schleich, 1969). It appears that sulfate has a more specific effect in that it does not fit into its normal Hofmeister position and cannot be explained as an ionic strength effect.

Several alkali metals were compared in the form of their chlorides. There was relatively little difference among them, with lithium showing less and potassium slightly greater inhibition (Figure 9). The alkali cations show a much smaller range of inhibitory effects than the anions studied.

Effect of Temperature. The rate of clathrin polymerization was measured (by absorbance) at 10, 20, and 30 °C at pH 6.33 in 0.10 M ammonium acetate. Although the initial rate increased, the extent of reaction decreased with increasing temperature (Figure 10). The apparent negative enthalpy of the reaction observed by absorbance was confirmed by sedimentation analysis. A clathrin solution similar to one of the above solutions was allowed to polymerize for 15 min at 23 °C and was then divided into two parts. One part was kept at 4 °C and the other part at 23 °C for 16 h. The percent of unpolymerized clathrin (8 S) was 10% at 4 °C an 35% at 23 °C. Thus, by two independent methods, it was found that the extent of the polymerization reaction decreases with increasing temperature.

Discussion

This report is the first attempt to measure the rate of clathrin polymerization to baskets. Two different turbidimetric procedures have been employed which give equivalent results. Bloom et al. (1980) have measured the turbidity of clathrin baskets between pH 7.6 and 6.0 but have not reported any rate measurements or the extent of conversion of reactants to products. We have not observed any filamentous bundles which Bloom et al. (1980) report, presumably because the concentrations of clathrin that we have used were much smaller.

The formation of baskets (or coat structures) has been reported by several investigators using preparations of clathrin which resemble ours. Only Woodward & Roth (1979) and Bloom et al. (1980) appear to have reduced the nonclathrin components, i.e., proteins visible on $NaDodSO_4$ gels, somewhat, and still obtained typical baskets. In all of the studies on the formation of baskets, except those of Woodward & Roth (1979), no attempt was made to determine the extent of basket formation, and the only identification of baskets was made by electron microscopy. Woodward & Roth (1979) have sedimented their polymerized preparation of clathrin on a sucrose gradient and found a very broad sedimenting band with considerable protein accumulating at the bottom of the gradient. We have observed both by boundary sedimentation with scanner optics (Pretorius et al., 1981) and by band sedimentation on a sucrose gradient (Figure 1) a major homogeneous boundary with an average sedimentation coefficient near 300 S and a minor component sedimenting at 150 S. Although we have made quantitative measurements of the rates and extent of reaction, there are many questions concerning the mechanism which remain unanswered. We have, however, attempted to define the basic parameters of the polymerization reaction and to provide methods so that the mechanism can be studied in greater detail in the future.

Our results indicate that the rate of polymerization depends very importantly on all the variables studied. The dependence on pH is very strong, and this variable must be tightly controlled in order to obtain reproducible results. There are also rather large inhibitory effects with most of the salts tested. The relative influence of the salts follows the Hofmeister ranking, with only sulfate out of order. In general, therefore, the rate appears to depend, in part, on electrostatic factors, as seen in the strong pH dependence, as well as on hydrophobic interactions since Hofmeister ranking has been shown to be a property of the interaction of nonpolar moieties with water (von Hippel & Schleich, 1969).

The absence of a threshold or critical concentration for clathrin polymerization clearly distinguishes the clathrin self-assembly system from many other proteins which self-associate to form high polymers, i.e., tubulin (Gaskin et al., 1974) and sickle cell hemoglobin (Hofrichter et al., 1974). The

polymerization of these proteins is characterized by both a critical concentration and a kinetic lag period.

It is apparent from Figures 2, 3, and 5 that a simple kinetic formulation of clathrin basket formation does not apply to the polymerization reaction. While the dependence of initial rates on concentration appears to be closer to a first- than to a second-order process, the reaction as a function of time fits neither of these two orders. The analysis of the data as a function of time needs a mechanism more complex than a first- or second-order process, presumably involving consecutive or competitive second-order processes.

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Isolation and Partial Characterization of Secretory Protein I from Bovine Parathyroid Glands†

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ABSTRACT: Secretory protein I, a protein that is cosecreted with parathormone, has been isolated from bovine parathyroid tissue. The purification procedure was aided by the inclusion in the starting material of fresh tissue that had been incubated with radioactive amino acids to label the newly formed secretory protein I. The isolation of the secretory protein I was then followed by locating the radioactive species. Later, purification was also followed by radioimmunoassay. The procedures included salt fractionation, gel filtration, and two steps of ion-exchange chromatography, yielding a 96-fold purification of secretory protein I. The final product contained two species that were shown to be related by comparison of their tryptic peptides and the release of only a single major residue at each step of the Edman degradation. On the basis of amino acid analysis, secretory protein I contains about 30% acidic amino acid residues, contributing to an isoelectric point of 4.5, and has a minimum molecular weight of about 70 000. It contains 2.6% carbohydrate. A radioimmunoassay was established for secretory protein I. A partial amino acid sequence spanning the first 32 residues of the amino-terminal region was obtained. This portion of the structure appeared to be unrelated to those of the known parathyroid hormonal peptides.

In vitro study of the parathyroid indicates that the tissue secretes at least two major proteins that are rapidly synthesized (Kemper et al., 1974; Morrissey et al., 1978). The synthesis, metabolism, and secretion of one of them, namely, parathormone, have been the subject of intensive study (Habener, 1979; Cohn & MacGregor, 1981). A cohesive picture of its processing has emerged. In general, the hormone is synthesized on rough endoplasmic reticulum as a large precursor molecule that is converted to the native form during its passage through the cisternae of the endoplasmic reticulum and the Golgi complex prior to secretion (MacGregor et al., 1976; Chu et

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