Snyder, R. G., Cameron, D. G., Casal, H. L., Compton, D. A. C., & Mantsch, H. H. (1981) *Biochim. Biophys. Acta* (in press).
Taraschi, T., & Mendelsohn, R. (1979) *J. Am. Chem. Soc. 101*, 1050–1052.
Taraschi, T., & Mendelsohn, R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2362–2366.
Taylor, M. G., & Smith, I. C. P. (1980) *Biochim. Biophys. Acta 599*, 140–149.
Thomas, G. J., & Barylski, J. R. (1970) *Appl. Spectrosc. 24*, 463–464.
Umemura, J., Cameron, D. G., & Mantsch, H. H. (1980) *Biochim. Biophys. Acta 602*, 32–44.
Van Zoelen, E. J. J., Verkleij, A. J., Zwaal, R. F. A., & Van Deenen, L. L. M. (1978) *Eur. J. Biochem. 86*, 539–546.
Warren, L. (1959) *J. Biol. Chem. 234*, 1971–1975.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.

Effect of Basic Compounds on the Polymerization of Clathrin†

P. K. Nandi, P. P. Van Jaarsveld, R. E. Lippoldt, and H. Edelhoch*

ABSTRACT: The effects of several divalent cations, various polybasic amines, and lysozyme on the rate of polymerization of 8S clathrin to the 300S coat structure have been evaluated by turbidimetric procedures. Ca^{2+} and Mn^{2+} strongly enhance the rate of polymerization. Only spermine among the naturally occurring polybasic amines had an important effect. Of the several basic proteins evaluated, only lysozyme stimulated the rate of polymerization. Some of these substances were able to increase the rate sufficiently so that polymerization occurred at physiological pH values. Without these compounds, clathrin will only polymerize at pH values of 6.8 or less.

The coated pit regions of plasma membranes have been reported to be the site of endocytosis of lipoproteins (Goldstein et al., 1979) and glycoproteins (Wall et al., 1980), as well as peptide (Schlessinger et al., 1978; Maxfield et al., 1978; Fitzgerald et al., 1980; Salisbury et al., 1980) and nonpeptide (Cheng et al., 1980) hormones. Coated vesicles have been shown to be involved in the secretion of glycoproteins (Rothman et al., 1980) and in membrane exchange processes (Heuser & Reese, 1973). The coated pit regions of membranes and coated vesicles are readily identifiable by electron microscopy since they appear as very characteristic polygonal structures (Kanaseki & Kadota, 1969; Heuser, 1980). The coat protein which is responsible for this appearance has been isolated by Pearse and called clathrin (Pearse, 1975, 1976, 1978). Purified preparations of clathrin have been shown to be capable in the absence of membrane of re-forming the coat structure by self-association (Pearse, 1978; Schook et al., 1979; Keen et al., 1979; Woodward & Roth, 1978, 1979; Nandi et al., 1980; Pretorius et al., 1981). The polygonal coat structure (devoid of membrane) will be referred to as baskets.

Although the endocytotic process resulting in the pinching off of coated pits into vesicles is now well documented, very little is known of the cellular regulation of this process. It is possible that regulation could be controlled either by modifying the structure of clathrin (or other proteins) in coated pits or by interfering with the recycling of clathrin from its soluble, cytoplasmic form to its network structure in pit regions of the membrane. It has been postulated by Kanaseki & Kadota (1969) that the formation of curvature in the coats of pit regions results from the introduction of pentagons into the hexagonal structure of coated pits. Thus, a rearrangement of clathrin interactions could lead to the formation of coated vesicles from pits. Heuser (1980) has presented electron microscopical evidence that this type of geometrical switch occurs in coated pit regions of membranes.

We have reported that the rate of polymerization of protomeric clathrin, i.e., 8 S, to form baskets, i.e., 300 S, can be conveniently followed by light scatter or absorption measurements (Van Jaarsveld et al., 1981). In this paper, we present the results obtained with three types of basic molecules, (a) divalent bases (cations), (b) polybasic amines, and (c) a basic protein, which accelerate the rate of clathrin polymerization.

Materials and Methods

Materials

$CaCl_2$, $MgCl_2$, and $MnCl_2$, and ammonium acetate were certified grade and supplied by Fisher Scientific Co. Putrescine, cadaverine, dansylcadaverine, spermidine, and spermine were purchased from Sigma Chemical Co. and used without further purification. Lysozyme was obtained from Miles Laboratories. It was dialyzed to remove salt and lyophilized.

Methods

Preparation of Clathrin. Bovine brains were used for preparing clathrin by a procedure described previously (Nandi et al., 1980) which is a mofidication of the method of Schook et al. (1979). The 8S protomer of clathrin is obtained by extracting a crude vesicle preparation with 0.05 M Tris (pH 8.0) followed by ammonium sulfate precipitation (30%) and gel chromatography on Sepharose 4B-Cl in 0.02 M Tris, 0.25 M NaCl, and 5 mM NaN_3 (pH 8.0). Three distinct peaks are obtained in the gel chromatographic separation. The major band (peak II) contains 8S clathrin. This peak was analyzed routinely by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Pretorius et al., 1981) in order to check the uniformity of the polypeptide composition of the 8S clarthrin preparation. In all cases, about 80–90% of the total protein migrated with the mobility of clathrin.

Measurement of Polymerization Rates. In order to follow the rate of polymerization of clathrin, we have used light scatter measurements as described previously (Van Jaarsveld et al., 1981). A Brice Phoenix Universal light scatter pho-

† From the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received March 31, 1981.*

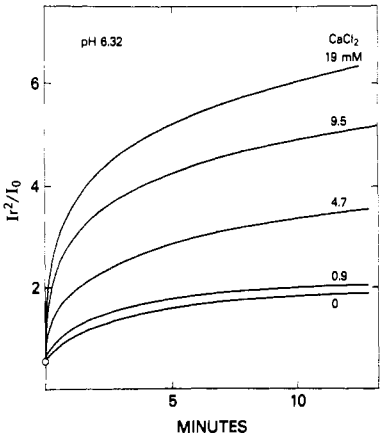

FIGURE 1: Effect of $CaCl_2$ concentration on the rate of clathrin polymerization as followed by the increase in light scatter at 45 °C. Small volumes (1–20 μL) of 1 M $CaCl_2$ in 0.1 M ammonium acetate were added to 1-mL solutions of clathrin (0.42 mg/mL) at pH 8.0 in 0.10 M ammonium acetate. No increase in light scatter was observed at pH 8.0. The pH was then adjusted to 6.32 ± 0.02 by addition of 50 μL of 1 M Mes buffer in order to initiate polymerization.

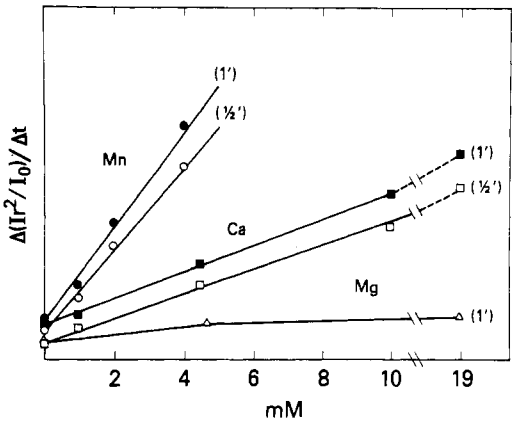

FIGURE 2: Relative stimulatory effects of Mn^{2+}, Ca^{2+}, and Mg^{2+} concentrations on the initial rates of clathrin polymerization. The points shown for Mn^{2+} and Ca^{2+} were obtained by taking the 0.50- and 1.0-min increases in light scatter. Since the increase in light scatter was much smaller with Mg^{2+}, we have shown only the 1-min points.

tometer equipped with a blue filter (435 nm) was used, and the measurements were made at 45°. Solutions (1 mL) of clathrin (~0.3–0.6 mg/mL) were used in a polished cylindrical cell obtained from Precision Cell, Inc. Small volumes (1–100 μL) of buffer or salt solutions were added to the clathrin solution and mixed rapidly (~7 s) by a magnetic stirring bar in the cell. Since the rate of clathrin polymerization is very sensitive to salt concentration, we have routinely dialyzed all fractions against 0.1 M ammonium acetate (pH 8.0), except as otherwise stated, for 3 h before experiments were performed. Clathrin preparations were then centrifuged at 30000*g* for 30 min in order to eliminate any aggregates. Ammonium acetate was a convenient salt to use since the pH of the clathrin solutions can be reduced from 8 to 6.0–6.9 by addition of a small volume of buffer, i.e., 50 μL of 1 M 2-(*N*-morpholino)ethanesulfonic acid (Mes) buffer (pH 6.0–6.3).

Sucrose Density Gradient Centrifugation. A Beckman Model L2-65 ultracentrifuge was used for sucrose density gradient centrifugation. All centrifugations were performed with the SW27 rotor at a temperature setting of 5 °C. Linear gradients were formed by mixing equal volumes of 10 and 30% sucrose solutions (w/w) in the same buffer used for polymerizing clathrin. Solutions (1 mL) of polymerized clathrin were layered on top of the gradients. After centrifugation, the gradients were fractionated by pumping 1-mL fractions from the bottom of the tubes with the aid of a needle lowered through the gradient. An LKB peristaltic pump set at 2 mL/min was used for this purpose. The protein concentration was monitored by measuring tryptophan fluorescence in a Perkin Elmer MPF-3 fluorometer. Samples were excited at 280 nm, and the emission was monitored at 340 nm.

Results

Divalent Bases (Cations). We have shown that monovalent salts decrease the rate of clathrin polymerization. The anions follow the Hofmeister ranking whereas the alkali cations (Na^+, K^+, and Cs^+) behave similarly at the same concentration (Van Jaarsveld et al., 1981). The effects of three divalent cations were found to be different from those of the alkali cations since they dramatically increased the rate of polymerization and accomplished this stimulation in the presence of much higher concentrations of ammonium acetate.

Ca^{2+} has been reported to facilitate basket formation (Keen et al., 1979). The influence of $CaCl_2$ on the rate of polymerization of 8S clathrin at pH 6.32 ± 0.02 can be seen in Figure 1. It is necessary to control the pH very carefully since clathrin polymerization is strongly pH dependent between pH 6.0 and 6.8. A plot of the increase in light scatter for either 30 or 60 s after the start of the reaction results in a linear dependence of rate on Ca^{2+} concentration (Figure 2). In order to determine the effect, if any, of Ca^{2+} on the size and heterogeneity of clathrin baskets, the solutions studied by light scattering (see Figure 1) were also analyzed by ultracentrifugation after 24 h of incubation at 4 °C.

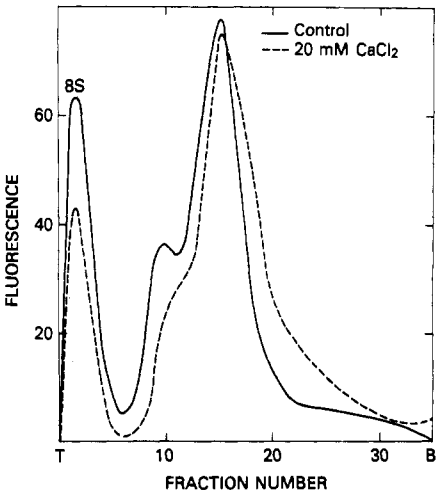

FIGURE 3: Sucrose density gradient centrifugation of clathrin polymerized in the absence and presence of 20 mM $CaCl_2$. Solutions of 1 mL of clathrin (0.7 mg/mL in 0.1 M ammonium acetate, pH 8.0) were polymerized by addition of 50 μL of 1 M Mes buffer to a final pH of 6.50. The reaction mixtures were incubated for 48 h at 4 °C before they were centrifuged on 35-mL linear 10–30% sucrose gradients for 90 min in the SW27 rotor of a Spinco ultracentrifuge.

We have examined the sedimentation profile on sucrose gradients of clathrin polymerized in the absence and presence of 20 mM Ca^{2+} at pH 6.50 in order to evaluate whether the distribution of clathrin baskets was affected (Figure 3). It has been shown by sedimentation velocity that solutions of clathrin baskets contain two sedimenting components with coefficients near 150 and 300 S (Pretorius et al., 1981). It can be seen that the principal band in the sedimentation pattern, i.e., 300 S, did not change significantly when Ca^{2+} was present. There is a diminution in the 150S band and the appearance of a faster sedimenting band as a shoulder of the 300S band. The slowest band is the clathrin protomer (i.e., 8 S). Overall, the change in the distribution of sedimenting

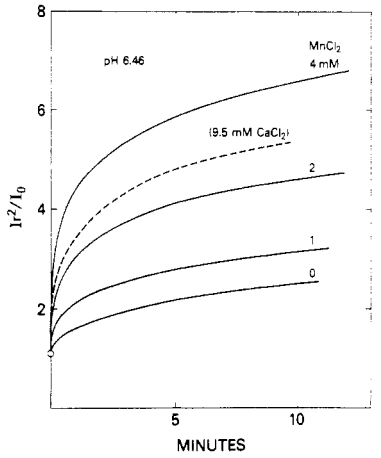

FIGURE 4: Effect of $MnCl_2$ on the rate of clathrin (0.43 mg/mL) polymerization. The experimental conditions were similar to those in Figure 1 except the final pH of all solutions was 6.46 ± 0.02. The effect of 9.5 mM $CaCl_2$ on the same preparation is also included to provide a direct comparison of the two salts.

components is relatively minor. When 40 mM $CaCl_2$ was added to a solution of clathrin baskets preformed at pH 6.46 (2 h at 23 °C and 48 h at 4 °C), essentially no change was found in the sedimentation coefficient of the 300S component although a small increase in the amount of faster sedimenting baskets was observed.

$MgCl_2$ has been shown to inhibit the dissociation of clathrin from coated vesicles and to promote reassembly after dissociation (Woodward & Roth, 1979). $MgCl_2$ enhanced the rate of clathrin polymerization but was much less effective than $CaCl_2$. The initial rates obtained at pH 6.45 ± 0.02 are also included in Figure 2. In contrast to $CaCl_2$, only a small further increase in rate was observed between 4.7 and 19 mM $MgCl_2$. Increasing the $MgCl_2$ concentration to 45 mM completely inhibited the polymerization. A large part of the inhibition is probably attributable to the increase in ionic strength that occurs. It has been reported that increasing salt concentration decreases the rate of polymerization (Van Jaarsveld, et al., 1981). There may still be some inhibition by Mg^{2+}, however, since the increase in ionic strength should not completely inhibit polymerization.

$MnCl_2$ showed a larger enhancement of the rate of clathrin polymerization than did $CaCl_2$ (Figure 4). The initial rates for 0.5 and 1-min time intervals are reported in Figure 2. The slopes of the $MnCl_2$ data are about 4-fold greater than those for $CaCl_2$. The polymerization experiments were at pH 6.46 ± 0.02. A direct comparison was made under identical conditions of the effects of $CaCl_2$ and $MnCl_2$. It can be seen in Figure 4 that the rate of polymerization in 9.5 mM $CaCl_2$ falls between that of 2 and 4 mM $MnCl_2$.

The pronounced stimulation produced by $MnCl_2$ at pH 6.46 suggested that clathrin could be polymerized at physiological pH where the rate would otherwise be too slow to measure. This proved to be the case since significant rates were observed at physiological pH values and above. In fact, the rate of polymerization observed at pH 7.95 with 10 mM $MnCl_2$ was about 25% greater than that with 10 mM $CaCl_2$ at pH 6.45. It is evident that relatively small amounts of ligand can increase the rate of polymerization sufficiently so that polymerization can occur even at slightly alkaline pH values. When the $MnCl_2$ concentration was reduced to 5 mM, the rate at pH 7.95 fell to about 10% of that observed with 10 mM $MnCl_2$.

Basic Polyamines. The fluorescent derivative of cadaverine, dansylcadaverine, has been shown to be very effective in inhibiting the clustering of α_2-macroglobulin on the surface of Chinese hamster ovary cells (Davis et al., 1980), normal rat kidney cells (Haigler et al., 1980a), and 3T3 fibroblasts (Haigler et al., 1980b). We therefore investigated the effects of dansylcadaverine and some naturally occurring oligoamine bases on the rate of clathrin polymerization.

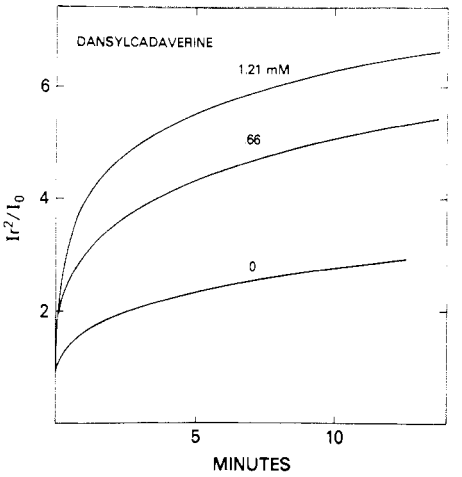

FIGURE 5: Effect of dansylcadaverine on the rate of polymerization of clathrin (1 mL of 0.7 mg/mL solutions in 0.1 M ammonium acetate; initial pH 8.0). Small volumes of a 7.6 mM dansylcadaverine stock solution in 0.1 M phosphate buffer (pH 6.3) were added to the clathrin solution, and the final pH of the reaction was brought to 6.33 by the addition of 50 μL of 1 M Mes buffer.

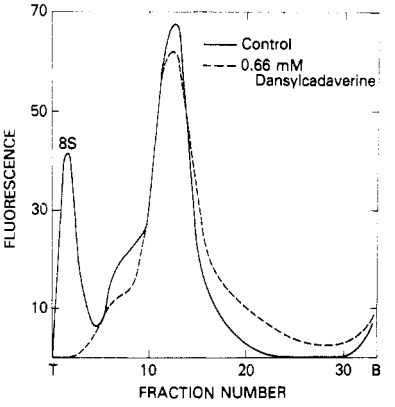

FIGURE 6: Sucrose density gradient centrifugation of clathrin polymerized in the absence and presence of 0.66 mM dansylcadaverine. The polymerization was carried out as described in Figure 5. The reaction was further allowed to proceed for 16 h at 4 °C before it was centrifuged for 60 min at 24 000 rpm on a 35-mL linear 10–30% sucrose gradient in the SW27 rotor of a Spinco centrifuge. The same buffer in which the polymerization took place was used for the gradients.

The effect of two levels of dansylcadaverine on clathrin polymerization at pH 6.32 ± 0.1 is shown in Figure 5. Higher concentrations of dansylcadaverine could not be investigated because of its limited solubility.

Solutions which were polymerized in the presence of dansylcadaverine (Figure 5) were also sedimented on sucrose gradients after incubation for 16 h at 4 °C. The sedimentation pattern (Figure 6) was similar to that observed with Ca^{2+}. The principal band, i.e., 300 S, sedimented at the same rate as the control. There was also a small loss in the 150S species, and some species sedimenting faster than 300 S appeared.

Of the common oligoamine bases found in cells (i.e., spermine, spermidine, putrescine, and cadaverine), only spermine had an appreciable effect on the rate of clathrin polymerization. The increase in rate produced by 120 and 300 μM spermine at pH 6.03 is seen in Figure 7. Under the same

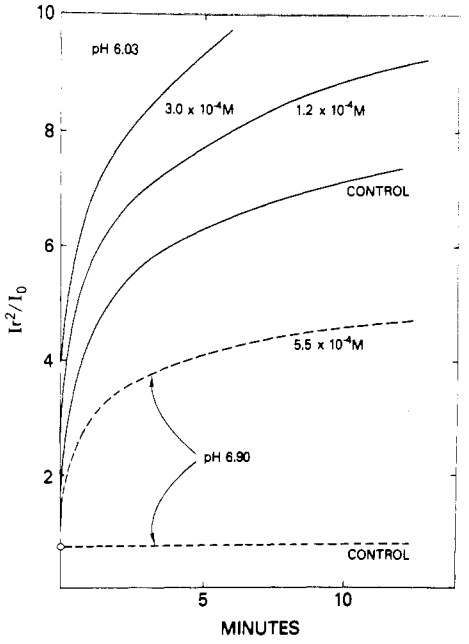

FIGURE 7: Effect of spermine concentration on the rate of clathrin polymerization at pH 6.03 (—) and 6.90 (---). Spermine concentrations are 0.12 and 0.30 mM at pH 6.03 and 0.55 mM at pH 6.90. Clathrin concentrations are 0.38 mg/mL at pH 6.03 and 0.33 mg/mL at pH 6.90. At pH 6.90, clathrin does not polymerize at this concentration.

conditions, 2 mM spermidine increased the rate only marginally, and 10 mM putrescine or cadavarine had no effect.

At pH 6.90, where the rate of clathrin polymerization in the absence of spermine is too slow to measure, 550 μM spermine was effective in polymerizing clathrin at an observable rate (Figure 7). Spermidine, however, at the same concentration had no effect.

Basic Proteins. Since some bivalent cations and polybasic amines are able to enhance the rate of clathrin polymerization, we also investigated the effects of several basic proteins and of several acidic proteins as a control group. The acidic proteins, i.e., α-lactalbumin, serum albumin, and thyroglobulin, had no effect on the rate of clathrin polymerization at pH 6.25. When similar amounts of lysozyme, ribonuclease, and cytochrome *c* were added to clathrin at pH 6.25, ribonuclease and cytochrome *c* had no effect on the rate of polymerization whereas clathrin was precipitated by lysozyme. At somewhat higher pH values (~7.5), where the complex did not precipitate, the rate of polymerization was sufficiently reduced so that the dependence on lysozyme concentration could be measured over a range of concentrations.

The kinetics of clathrin polymerization at pH 7.70, over a 5-fold range of lysozyme concentration, are shown in Figure 8. The initial rates at 0.5 and 1 min are seen in the inset in Figure 8. There is very little difference in shape between the two curves at 0.5 and 1 min, and both are sigmoidal. A sigmoidal curve with a greater lag period was observed in a similar series of experiments performed with a different preparation of clathrin at pH 8.0. In this series, no evidence of polymerization was observed with a lysozyme concentration of 3.4 μM although a significant rate was observed with twice this amount.

We have analyzed the structure of the clathrin baskets formed in alkaline pH (7.9) by lysozyme. In the presence of 0.04 M NaCl and 0.20 M ammonium acetate, electron microscopic pictures gave clear evidence of polygonal molecules resembling those seen at lower pH values in the absence of lysozyme. Ultracentrifugal analysis of clathrin solutions after polymerization at pH 8.0 in the presence of lysozyme showed sedimentation patterns (scanner optics) similar to those for clathrin baskets. At a molar ratio of lysozyme to clathrin of 6, about half the clathrin present in solution sedimented with a rate similar to that of clathrin polymerized at pH 6.3, i.e., ~300 S. This result was found with clathrin solutions, 1 h after addition of lysozyme. After 15 h of reaction, the sedimenting boundary was broader, and the average rate had increased to ~450 S. It appears that association of clathrin baskets occurs as a slower secondary reaction after their formation. A similar set of reactions was also observed at molar ratios of lysozyme to clathrin of 7.8 and 9.0. Due to the complexity of each stage of the reaction, no further elaboration was attempted at this time.

The effect of lysozyme on the rate of clathrin polymerization at pH 8.0 in 0.20 ammonium acetate could be reduced in half by concentrations of NaCl as low as 0.015 M. The rate was inhibited even more strongly by higher concentrations of NaCl. Unfortunately, the rate of clathrin polymerization in the absence of lysozyme at pH 6.3 is also markedly inhibited by NaCl. Consequently, it is difficult to known whether the lysozyme-induced polymerization of clathrin is as much affected by NaCl concentration as is its self-association in the absence of lysozyme.

Discussion

It has been reported (vide infra) that two types of drugs containing structurally similar basic moieties inhibit receptor-mediated endocytosis with several different types of cells. We have shown that these drugs significantly enhance the rate of polymerization of clathrin to baskets (P. P. Van Jaarsveld, P. K. Nandi, R. E. Lippoldt, and H. Edelhoch, unpublished data). In this report, we have investigated the effects of several rather different types of basic substance in order to delineate the nature of the basic group which is effective in stimulating clathrin polymerization and studied whether these basic substance can raise the pH threshold of clathrin polymerization and initiate the reaction at physiological pH values.

The kinetics of clathrin polymerization have been evaluated by an empirical procedure since the reaction kinetics have been shown to be complex and, at present, too difficult to analyze adequately (Van Jaarsveld et al., 1981). With this method, a linear dependence of the initial rates was found over a sufficient range of Ca^{2+} and Mn^{2+} concentrations to give some credence to the method of analyzing the data.

Clathrin polymerizes at pH values below ~6.8 with the absorption of about one proton per mole of clathrin (Van Jaarsveld et al., 1981). The binding of other positively charged substances at the proton binding site(s) could also initiate polymerization. In this case, the reaction would preceed at more basic pH values than normal. Ca^{2+} and Mn^{2+} are probably chelated to the same site on clathrin since their relative effectiveness in polymerizing clathrin corresponds to their relative binding affinities to such chelating reagents as EDTA, etc.

Ca^{2+} and Mn^{2+} not only accelerate the rate of polymerization at pH values where clathrin spontaneously polymerizes, i.e., 6.8–6.0, but also induce polymerization at physiological pH values and above. It has been reported elsewhere that depolymerization of clathrin baskets is readily accomplished by raising the pH to values slightly above those used to polymerize clathrin. It is also possible to control the polymerization reaction by changes in Ca^{2+} concentration since the polymerization can be reversed by adding a complexation agent such as EDTA.

We have shown by sucrose-gradient analysis that the size

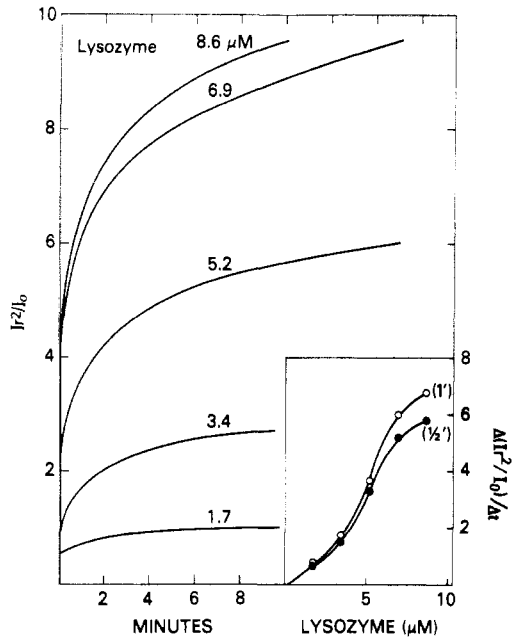

FIGURE 8: Effect of lysozyme concentration on the rate of clathrin (0.30 mg/mL) polymerization at pH 7.7 in 0.10 M ammonium acetate. Inset: Initial rates of increase in light scatter at 0.5 and 1 min as a function of lysozyme concentration.

distribution of clathrin baskets formed in the presence of Ca^{2+} molecules does not change significantly. Thus, the rate and not the mechanism of basket formation appears to be modified by cations.

The results obtained with the polyamine bases and dansylcadaverine afford us a somewhat different perspective into the structure of the binding site regulating clathrin polymerization. Only the polybases with three or four amines are active, with the latter type being considerably more active than the former. The two dibasic amines (cadaverine and putrescine) were inactive at levels considerably higher than those at which spermidine was active. It is evident from the relative effectiveness of the polyamine bases that the additional nitrogen bases of spermine and spermidine enhance their binding (or their activity) in promoting clathrin polymerization. It is likely that the binding constants of putrescine and cadaverine are not strong enough for them to be bound at the concentrations used. When a dansyl group is added to cadaverine, however, polymerization occurs. It appears, therefore, that a polybase with only two amine groups (separated by three to four methylene groups) is sufficient for binding if a hydrophobic moiety also contributes to the interaction. Thus, the extra alkylamine groups of spermine and spermidine could act as hydrophobic moieties. In fact, a structure of this type seems to be needed since two antimalarial drugs (quinacrine and chloroquine) and two phenothiazine drugs (chlorpromazine and trifluoperazine), which have both basic and hydrophobic components, enhance clathrin polymerization at the same or lower concentrations than that of spermine (P. P. Van Jaarsveld, P. K. Nandi, R. E. Lippoldt, and H. Edelhoch, unpublished data). These drugs have been shown to inhibit receptor-mediated endocytosis by coated pits with different types of cells (Fitzgerald et al., 1980; Salisbury et al., 1980; Haigler et al., 1980a). The structure of dansylcadavarine would also belong to this class except that it contains more than three to four methylene groups, i.e., five in the cadaverine moiety.

The marked stimulation of the rate of clathrin polymerization produced by lysozyme is presumably due to the presence of basic groups on its surface which are separated by the appropriate distance. A rather specific structure is needed since two other basic proteins (ribonuclease and cytochrome *c*) which were tested were ineffective. The basicity of lysozyme is not enough to explain its effects, and other factors, such as steric and hydrophobic, are required to act in concert with the basic groups.

It appears, therefore, that the three groups of basic substances we have employed, though differing structurally, have similar effects on clathrin polymerization. The basicity is clearly a common feature and may serve a role equivalent to that of protonation (at a lower pH) in initiating polymerization. It is likely that two separate loci for binding exist on the surface of clathrin which interact with two of the basic groups of spermine and the two basic groups of the drugs tested. This basic structure presumably also is present on the surface of lysozyme.

References

Cheng, S.-Y., Maxfield, F. R., Robbins, J., Willingham, M. C., & Pastan, I. H. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3425–3429.

Davies, P. J. A., Davies, D. R., Lenitzki, A., Maxfield, F. R., Milhand, P., Willingham, M. C., & Pastan, I. H. (1980) *Nature (London) 283*, 162–167.

Fitzgerald, D., Morris, R. E., & Saelinger, C. B. (1980) *Cell (Cambridge, Mass.) 21*, 867–873.

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

Haigler, H. T., Willingham, M. C., & Pastan, I. (1980a) *Biochem. Biophys. Res. Commun. 94*, 630–637.

Haigler, H. T., Maxfield, F. R., Willingham, M. C., & Pastan, I. (1980b) *J. Biol. Chem. 255*, 1239–1241.

Heuser, J. (1980) *J. Cell Biol. 84*, 560–583.

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

Kanaseki, T., & Kadota, K. (1969) *J. Cell Biol. 42*, 202–220.

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

Maxfield, F. R., Schlessinger, J., Shechter, Y., Pastan, I., & Willingham, M. J. (1978) *Cell (Cambridge, Mass.) 14*, 805–810.

Nandi, P. K., Pretorius, H. T., Lippoldt, R. E., Johnson, M. L., & Edelhoch, H. (1980) *Biochemistry 19*, 5917–5921.

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.

Pretorius, H. T., Nandi, P. K., Lippoldt, R. E., Johnson, M. L., Keen, J. H., Pastan, I., & Edelhoch, H. (1981) *Biochemistry 20*, 2777–2782.

Rothman, J. E., Bursztyn-Pettegrew, H., & Fine, R. E. (1980) *J. Cell Biol. 86*, 162–171.

Salisbury, J. L., Condeelis, J. S., & Satir, P. (1980) *J. Cell Biol. 87*, 132–141.

Schlessinger, J., Shechter, Y., Willingham, M. C., & Pastan, I. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2659–2663.

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

Van Jaarsveld, P. P., Nandi, P. K., Lippoldt, R. E., Saroff, H., & Edelhoch, H. (1981) *Biochemistry 20*, 4129–4135.

Wall, D. A., Wilson, G., & Hubbard, A. L. (1980) *Cell (Cambridge, Mass.) 21*, 79–93.

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

Woodward, M. P., & Roth, T. F. (1979) *J. Supramol. Struct. 11*, 237–250.