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Properties of Clathrin Coat Structures†

G. Irace, R. E. Lippoldt, H. Edelhoch,* and P. K. Nandi

ABSTRACT: Clathrin polymerizes to form characteristic coat structures (baskets) closely resembling those found on coated vesicles. Two sizes of baskets are formed from clathrin, depending on the purity of the preparation and on other factors. A protein of M_r 110 000 has been separated from clathrin by lysine–Sepharose chromatography which is needed for the formation of 150S baskets. In its absence, polymerization results in the larger size baskets, i.e., 300S. Addition of Ca^{2+} or Mg^{2+} stimulates 300S formation in the presence of the 110 000 protein.

The coated pit regions of plasma membranes have been shown to be the site of localization of receptor-bound ligands (conjugated and nonconjugated proteins, nonprotein hormones, etc.) prior to internalization (Goldstein et al., 1979; Brown & Goldstein, 1979; Willingham & Pastan, 1980; Wall et al., 1980). The coated pits have also been implicated in other cellular processes involving membrane surfaces: in membrane recycling (Heuser & Reese, 1973), in intracellular protein translocation (Ockleford & Whyte, 1977; Pearse & Bretscher, 1981), and in exocytosis of newly synthesized protein (Rothman et al., 1980). The transfer of ligands has been assumed to occur by the intermediary of coated vesicles pinched off from coated pits (Goldstein et al., 1979; Brown & Goldstein, 1979; Pearse, 1980, 1982). The details of this processing have been reexamined recently, and a modified mechanism has been suggested (Willingham & Pastan, 1980).

The characteristic coat structure of coated pits and vesicles has been shown by Pearse to be formed principally by one protein called clathrin (Pearse, 1975, 1976; Crowther et al., 1976). The conformation of clathrin is well adapted to form the pentagons and hexagons of the coat since it has three equal arms radiating from a central locus (Ungewickell & Branton, 1981). Clathrin has a sedimentation constant near 8 S and a molecular weight of 610 000 (Pretorius et al., 1981; Ungewickell & Branton, 1981). It is readily released from coated vesicles and can be polymerized to form a polygonal structure closely resembling that found on coated vesicles (Pearse, 1978; Schook et al., 1979; Keen et al., 1979; Woodward & Roth, 1979; Nandi et al., 1980). The re-formed coat structure, in the absence of membrane, is usually referred to as baskets. A second, much smaller protein may form a part of the coat structure (Lisanti et al., 1981; Kurchhausen & Harrison, 1981). A tryptic fragment of clathrin of M_r 110 000 has been shown to be capable of self-associating to form baskets (Schmid et al., 1982).

We have reported elsewhere that when native clathrin is polymerized, two sizes of baskets, i.e., 150S and 300S, are produced (Pretorius et al., 1981) with average molecular weights of 25×10^6 and 100×10^6, respectively (Nandi et al., 1980). We have now tried to determine the factors and conditions that influence the size of the basket formed by polymerization of clathrin in slightly acidic solutions. With the present method of isolating coated vesicles, a preparation of clathrin is obtained which gives only 150S. We have fractionated clathrin on a lysine–Sepharose column and isolated a protein fraction which appears to be needed for the formation of the 150S basket.

Materials and Methods

Preparation of Clathrin. Coated vesicles were prepared by a procedure based on that published by Pearse (1975), which, however, avoided high concentrations of sucrose; it is to be described in another report on the properties of coated vesicles (Nandi et al., 1982). The vesicles gave a single, symmetrical band on sucrose gradients (10–30%) and sedimented with an average $s_{20,w} \simeq 200$ S.

Clathrin was solubilized from this homogeneous preparation of coated vesicles by extracting in a 2 M urea solution, pH 8.0, 10 mM Tris,[1] and 5 mM NaN_3 for 2 h. The solution was then dialyzed for 4 h against 10 mM Tris, pH 8.0, and 5 mM NaN_3 in order to remove urea and then centrifuged at 150000*g* for 70 min. Velocity centrifugation of the supernatant showed a single moving boundary with a sedimentation coefficient ($s_{20,w}$) typical of native clathrin, i.e., ~8 S. The protein composition of this preparation was analyzed by $NaDodSO_4$ gel electrophoresis in overloaded gels (50–100 μg of protein) (Figure 1, gel on left) by the technique reported previously with 8% gels and 5% stacking gels (Nandi et al., 1980). Smaller amounts of nonclathrin components were present than in earlier clathrin preparations.

Lysine–Sepharose Column. Clathrin could be fractionated somewhat further by chromatography on a lysine–Sepharose

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[1] Abbreviations: Mes, 2-(*N*-morpholino)ethanesulfonic acid; LS-clathrin, clathrin isolated from lysine–Sepharose column in 0.075 M NaCl; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate.

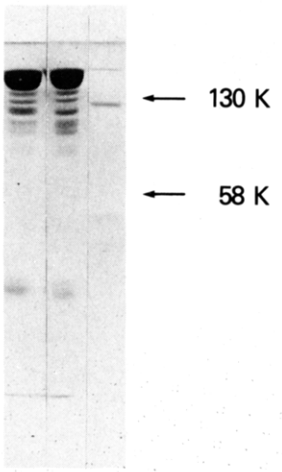

FIGURE 1: Polyacrylamide–NaDodSO$_4$ (8%) gel electrophoresis of clathrin (left gel), of LS–clathrin (middle gel), and of a band eluting in 1 M NaCl (right gel). The major band eluting in the right gel is missing in the middle gel.

4B column (15 × 1 cm) (referred to as LS–clathrin) which was equilibrated with 10 mM Tris, pH 8.0, and 5 mM NaN_3 solution. Clathrin was eluted from the column by the equilibrium buffer after bringing it to 0.075 M NaCl. A small band representing less than 5% of the total fluorescence intensity was eluted in 0.075 M NaCl before the clathrin peak. Increasing the NaCl concentration to 1.0 M (in the same buffer) eluted a second, small band (about 5% of the total fluorescence). The NaDodSO$_4$ gel electrophoretic patterns of LS–clathrin and of the band eluting in 1.0 M NaCl are shown in Figure 1 (gel in center and on right, respectively).

Polymerization of Clathrin. Both the clathrin and lysine–Sepharose-purified clathrin (LS–clathrin) polymerized to form typical polygonal structures characteristic of clathrin baskets when observed by negative-staining electron microscopy. Polymerization was usually carried out by dialysis for 18 h at 23 ± 2 °C of a clathrin solution (pH 8.0, 10 mM Tris and 5 mM NaN_3) against a large volume of 0.10 M Mes[1] buffer adjusted to the final pH. A second method of polymerization was also used in which the pH was adjusted by direct titration with small amounts of a concentrated Mes buffer (1 M) to the desired value. The latter procedure is described in a paper on the kinetics of clathrin polymerization (Van Jaarsveld et al., 1981).

Analytical Ultracentrifugation. (1) Boundary Centrifugation. The molecular composition of polymerized solutions of clathrin has been evaluated by ultracentrifugal techniques. In this way, a quantitative measure of the extent of polymerization was obtained.

A Beckman Model E analytical ultracentrifuge, equipped with a photoelectric scanner, was used with 12-mm optical path-length double-sector cells. A detailed analysis of the sedimentation patterns of clathrin baskets has been reported elsewhere (Pretorius et al., 1981). The 280-nm absorbance values are due to both protein absorption and turbidity. The turbidity of the 300S species is considerably larger than that of the 150S species. Consequently, the 280-nm absorbance values will be larger for the 300S species than for the same concentration of 150S. The sedimentation coefficients ($s_{20,w}$) were obtained from the half-heights (50%) of the moving boundaries of each species, using scanner optics, and represent average values. The amount of unpolymerized 8S clathrin was evaluated from the height of its sedimenting boundary after the rotor speed was increased from 7200 rpm, the rpm used to sediment clathrin baskets, to 40 000 rpm where native clathrin (8S) sediments at a convenient rate.

(2) Band Centrifugation in Sucrose Gradients. A Beckman Model L2-65 ultracentrifuge with an SW27 rotor was used. Linear gradients were formed by mixing equal volumes of 10 and 30% sucrose solutions (w/w). The same buffers were used for the 10 and 30% sucrose solutions as were used in the polymerization solutions. One milliliter of polymerized clathrin was layered on the top of the gradients. Solutions were sedimented usually for 110 min at 24 000 rpm, 20 °C, in a SW27 rotor; 1-mL fractions were collected from the bottom of the tubes. An LKB peristaltic pump, set at 2 mL/min, was used to collect the fractions. Protein concentration was monitored by tryptophan fluorescence in a Perkin-Elmer MPF-3 fluorometer. Samples were excited at 280 nm, and the emission was observed at 340 nm.

Protein Concentration. Clathrin concentration was determined by absorptin by using $E^{1\%}_{1cm}$ = 10.9 at 280 nm (Nandi et al., 1980). Absorbance was measured with either a Cary 14 or a Beckman DU spectrophotometer.

Electron Microscopy. Routine electron microscopic examination by negative staining with 1% uranyl acetate was performed by Dr. B. Kramarsky (Electro Nucleonics, Silver Spring, MD).

Results

Effect of pH on the Size of Clathrin Baskets. The formation of 150S and 300S baskets was followed by moving boundary centrifugation experiments. The polymerization was usually carried out by dialyzing clathrin solutions (pH 8.0, 10 mM Tris) for about 18 h at 23 °C against a large volume of 0.10 M Mes buffer adjusted at the final pH value. The sedimentation boundary pattern of solutions dialyzed against pH 6.60 and 6.40 Mes buffers showed only a single boundary (average $s_{20,w}$ values of several experiments were 130–160 S) aside from that of unpolymerized 8S clathrin. The patterns obtained with solutions dialyzed against pH 6.20 and 6.06 Mes buffers revealed, in addition to the above species, a second, faster sedimenting boundary (average $s_{20,w}$ values of several experiments were 300–340 S).

In order to obtain more precise values of the molecular composition, the same solutions were also analyzed by band sedimentation on linear sucrose gradients (10–30%) (Figure 2). In moving boundary centrifugation, using scanner optics, the concentration of each component is not strictly proportional to its height (as measured by absorbance) since the 150S and 300S species have measurable and different turbidities (which contribute to the absorption) (Nandi et al., 1980). In addition, Johnston–Ogston effects could also modify the heights of the various boundaries. None of these effects enters into the proportionality between fluorescence intensity and protein concentration as observed in sucrose gradients since polymerization of clathrin does not change its emission intensity.

It is evident by comparing the protein profiles on sucrose gradients (Figure 2) with those recorded by scanner optics of sedimenting boundaries that the three peaks in the former correspond to unpolymerized 8S, and 150S and 300S baskets. It can be seen in the sucrose gradients (and in the scanner plots) that the extent of conversion of native 8S to baskets increases strongly with decreasing pH with little 8S left at pH 6.06.

The results of electron micrography of polymerized clathrin were in accord with those obtained by ultracentrifugal analysis. Negatively stained images of polymerized clathrin obtained by dialyzing native clathrin solutions, pH 8.0, against 0.10 M Mes, pH 6.50 buffer solutions consisted essentially of small baskets with an average diameter near 80 nm (±10%) (Figure

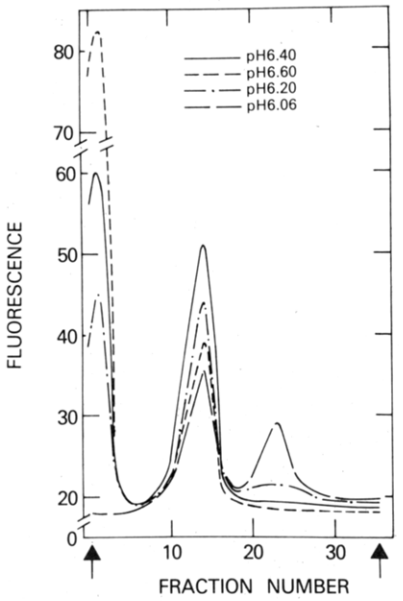

FIGURE 2: Sucrose gradient centrifugation on a linear 10–30% gradient of the clathrin solutions dialyzed at pH values 6.60–6.06. Protein concentration was determined by tryptophanyl emission intensity.

3, top). Similar size baskets have been obtained recently by Ungewickell & Branton (1981) in a very high yield after polymerization at pH 6.5. The baskets formed by dialzing against 0.10 Mes, pH 6.00, were a mixture of two sizes; the smaller size baskets were similar to that observed at pH 6.50 while the larger size baskets had an average diameter near 130 nm (±10%) (Figure 3, bottom). Diameter values slightly smaller than these were obtained for the 150S and 300S species, respectively, for turbidity measurements (Nandi et al., 1980).

Since the molecular weight of 300S is about 4 times that of 150S (Nandi et al., 1980), it was possible that the former was a polymer of the latter. We therefore tried increasing the concentration of native clathrin at pH 6.40 to see if we could produce 300S baskets as well as 150S baskets. When polymerization was carried out by dialyzing clathrin solutions (between 0.40 and 1.04 mg/mL) against 0.10 M Mes, pH 6.40, for 16 h at 23 °C, only a single polymerization product, i.e., 150S, was observed by centrifugation on sucrose gradients. As seen in Figure 4, the extent of conversion of 8S to 150S increased with clathrin concentration without formation of 300S. A similar experiment, performed at pH 6.05, at clathrin concentrations of 0.31 and 0.62 mg/mL, did not indicate any change in the relative amounts of 150S and 300S, which was near a ratio of 2.0 in each solution.

Stability of 150S and 300S. In order to evaluate the stability of the two basket species, we have tried to transform one species into the other. We therefore isolated the 150S species by sucrose gradient centrifugation after polymerization at pH 6.40 (see Figure 2). Reducing the pH of solutions containing only 150S baskets (after dialyzing out the sucrose) from 6.40 to 6.06 by dialysis for 16 h against 0.10 M Mes buffer did not result in the formation of any 300S and left the 150S species intact. Therefore, 150S cannot be considered as an intermediate in the formation of 300S baskets.

It was also shown that the 300S does not form 150S baskets. Clathrin was polymerized at pH 5.80 (0.1 Mes), with results similar to those obtained at pH 6.06 (Figure 2). Dialysis of this solution for 18 h at 23 °C against a pH 6.50 (0.10 M Mes) solution did not alter the ratio of the two species of baskets, as analyzed in sucrose graidents. At pH 6.50, direct polymerization from 8S gave only the 150S species (see above). It

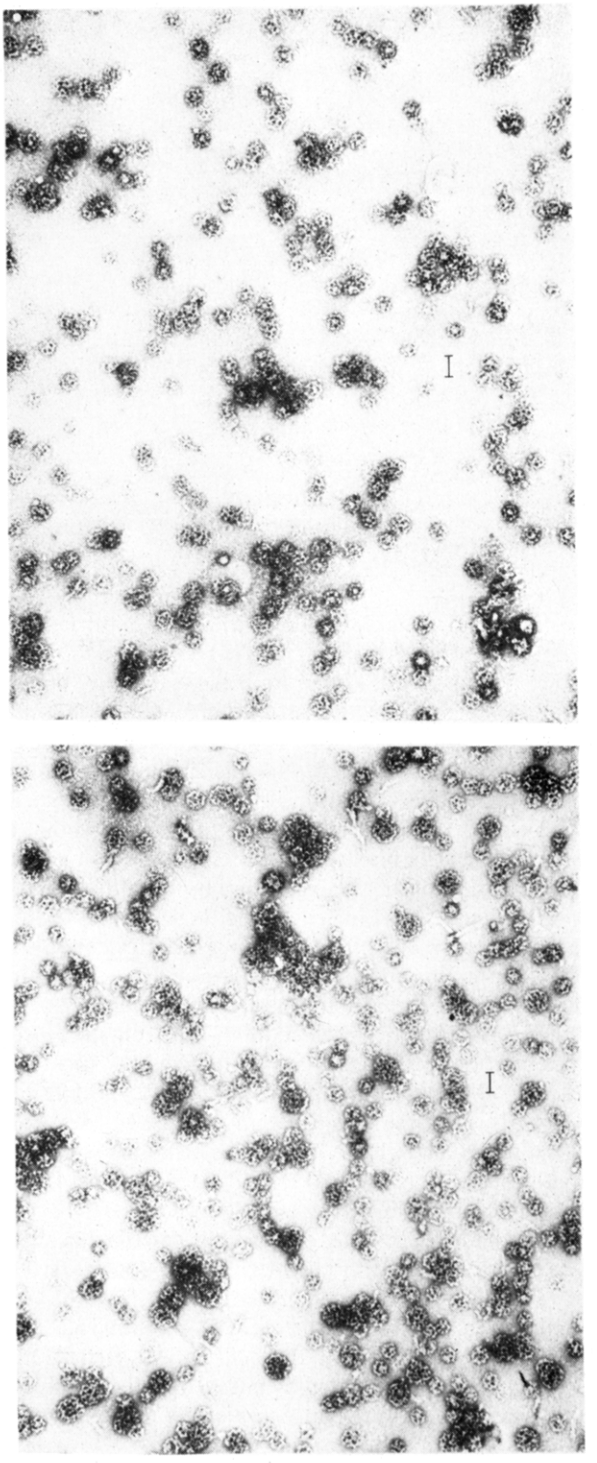

FIGURE 3: Electron micrographs of clathrin polymerized under different conditions. (Top) Dialysis against pH 6.5, 0.10 M Mes buffer for 16 h at 23 °C. (Bottom) Dialysis against pH 6.0, 0.10 M Mes buffer for 16 h at 23 °C. Scale bar represents 100 nm.

appears therefore that once formed, 300S baskets are stable structures and cannot be dissociated to 150S baskets.

Factors That Influence the Formation of the 150S and 300S Baskets. We noticed that when clathrin solutions in 0.01 M Tris, pH 8.0, were titrated with Mes buffer to pH ~6.0 instead of being dialyzed against Mes buffer for 18 h, little or no 300S was observed by gradient analysis. Electron micrography confirmed the absence of the 300S species. We have performed several different types of experiments in order to evaluate the factors that can affect the size of the basket formed by polymerization.

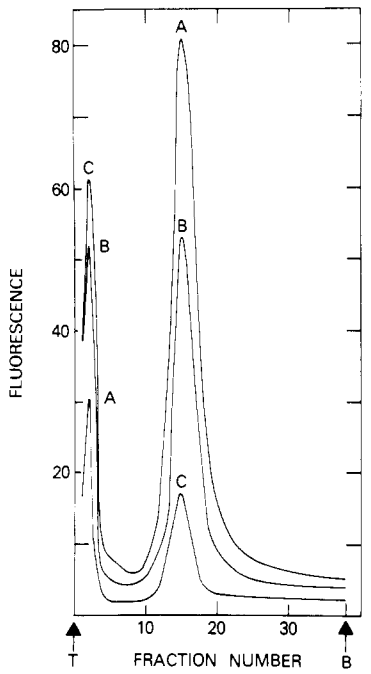

FIGURE 4: Effect of concentration on the polymerization of clathrin at pH 6.40 in 0.10 M Mes. Sucrose gradient centrifugation was on a linear 10–30% gradient. Native clathrin concentrations were (A) 1.04, (B) 0.69 and (C) 0.40 mg/mL (before dialysis). Protein concentration was evaluated by tryptophanyl fluorescence intensity. T and B are top and bottom of the gradient tubes, respectively.

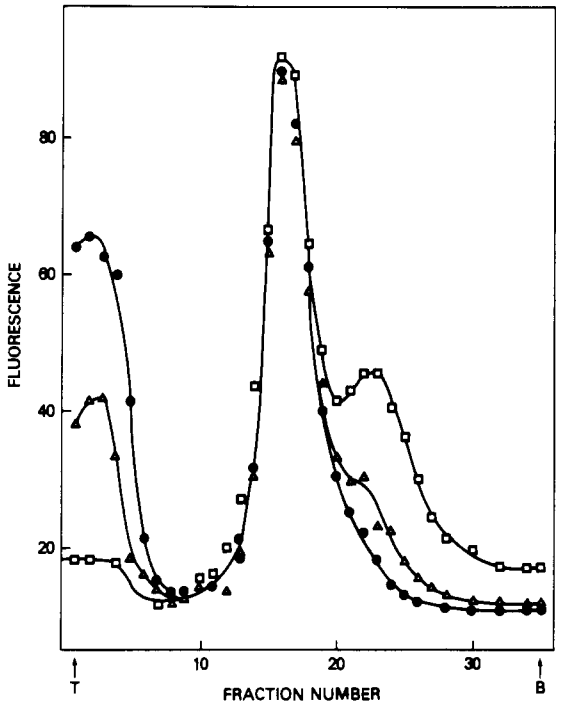

FIGURE 5: Effect of Ca^{2+} on the formation of 300S baskets. Clathrin (0.88 mg/mL) was titrated to pH 6.45 with Mes buffer after addition of Ca^{2+}. Control (●); 1 mM Ca^{2+} (△); 5 mM Ca^{2+} (□). Sucrose gradient, 10–30%, 24000 rpm, 110 min, 20 °C.

(*1*) *Ca^{2+}*. When clathrin solutions (pH 8.0, 0.01 M Tris) containing Ca^{2+} were titrated to pH 6.45, 300S was formed. Without Ca^{2+}, following the same procedure, only 150S was observed (Figure 5). The effects of 1 and 5 mM Ca^{2+} on the sucrose gradient pattern are seen in Figure 5. In the presence of 5 mM Ca^{2+}, the ratio of the 300S to the 150S peaks is about 2/5. In a similar experiment, using 10 mM Ca^{2+} performed with a different preparation of clathrin, the ratio was 2/3; 10 mM Mg^{2+} had only a slightly smaller effect than 10 mM Ca^{2+} and gave a ratio of 1/2.

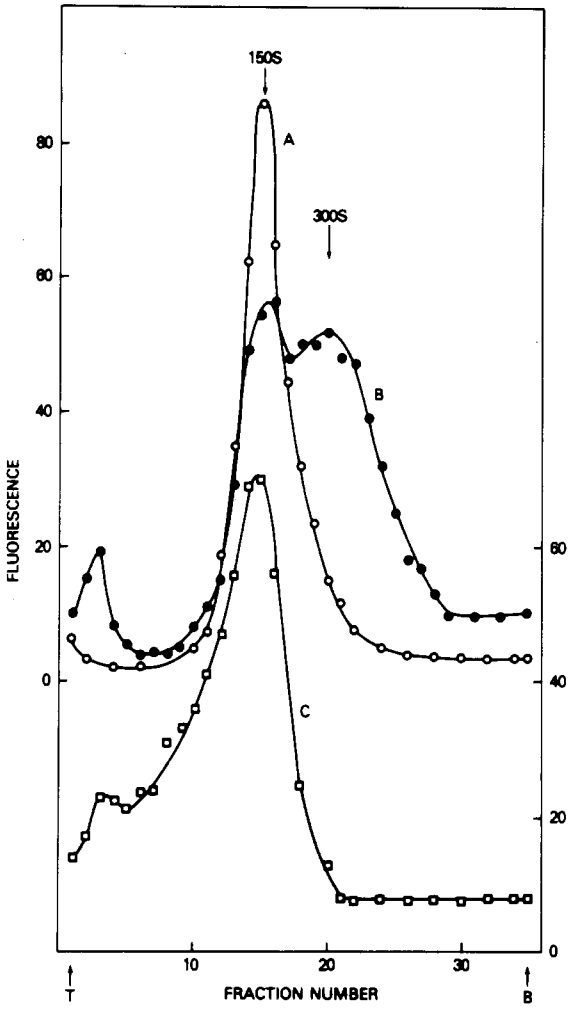

FIGURE 6: Sucrose gradient analysis of polymerized clathrin obtained by titrating a pH 8.0, 0.01 M Tris solution (1 mg/mL) to pH 5.90 with Mes buffer (final Mes concentration is about 0.10 M). (Curve A) Native clathrin; (curve B) LS–clathrin; (curve C) LS–clathrin plus 110000 protein eluted from lysine–Sepharose column in 1 M NaCl. Sucrose gradient, 10–30%, 24000 rpm, 100 min, 20 °C.

(*2*) *Clathrin Isolated from a Lysine–Sepharose Column.* Clathrin was fractionated further on a lysine–Sepharose column (see Materials and Methods). Polymerization was carried out by *titrating* the chromatographed LS–clathrin solution to pH 5.90 with Mes buffer. Sedimentation on a 10–30% sucrose gradient revealed about equal amounts of 150S and 300S (Figure 6, curve B). Electron micrography showed a mixture of small and large baskets. When this preparation of clathrin was titrated to pH 5.90 before chromatography on lysine–Sepharose, little or no 300S was observed (Figure 6, curve A). However, when the protein eluting from the lysine–Sepharose column in 1.0 M NaCl (see Materials and Methods) was added to clathrin at pH 8.0, only 150S and no 300S were formed after titration to pH 5.90 with Mes buffer (Figure 6, curve C).

The $NaDodSO_4$ gel electrophoretic pattern of LS–clathrin was similar to that of the starting material except that a band with a molecular weight near 110000 was no longer present (Figure 1). Electrophoretic analysis of the band eluting in 1 M NaCl revealed that the missing 110000 band was the most prominent one and represented more than half of the protein stained by Coomassie Blue (Figure 1).

(*3*) *$NaDodSO_4$ Gel Electrophoretic Analysis of 150S and*

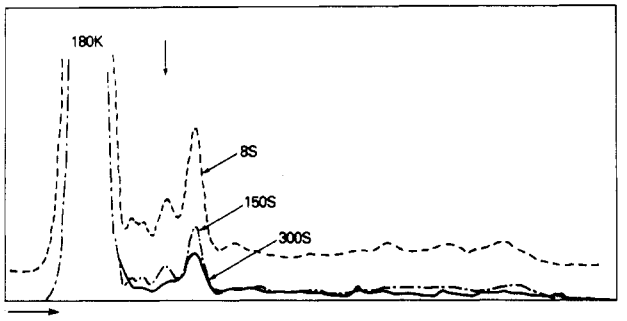

FIGURE 7: Densitometric tracing of $NaDodSO_4$ (7%) electrophoresis of 8S clathrin, 150S baskets, and 300S baskets. The 150S and 300S baskets were obtained from 10–30% sucrose gradients.

300S Baskets. We have analyzed for the protein composition of 150S and 300S baskets to see if a minor protein component was present in the 150S basket and not in the 300S basket which was related to the 110000 protein isolated from clathrin on the lysine–Sepharose column. Purified 150S and 300S species were obtained from 10–30% sucrose gradients after polymerization of 8S clathrin by dialysis at pH 6.0. After removal of sucrose and concentration of the protein, $NaDodSO_4$ electrophoresis on a 7% cross-linked polyacrylamide gel gave the results shown in Figure 7. (The 7% cross-linked gel led to a better resolution than an 8% of the two principal minor bands.) The densitometric tracings of native 8S and 150S resemble each other closely, taking into account the somewhat higher concentration of 8S. In contrast to these species, all the minor components are reduced in the 300S, with the 110000 (see arrow in Figure 7) reduced more than its neighbor on its right. Little can be said concerning the two very minor components on its left since their areas are comparable with the background density value. Its position corresponds to the component eluted in 1 M NaCl on the lysine–Sepharose column with M_r 110000.

Discussion

(1) The polygonal network structure of coated pits and vesicles has been thoroughly documented by electron microscopic studies (Crowther et al., 1976; Woodward & Roth, 1979; Heuser, 1980; Crowther & Pearse, 1981). (2) It has been shown that a bimodal distribution of baskets is formed when clathrin is polymerized (Nandi et al., 1980; Pretorius et al., 1981). (3) The two sizes of baskets may be related to those reported by Friend & Farquhar (1967), who observed two discrete sizes (i.e., >1000 Å and <750 Å) of coated vesicles in epithelial cells of the rat vas deferens and found that only the larger size was involved in endocytosis of horseradish peroxidase. (4) Two sizes of coated vesicles have also been observed in preparations from adrenal medulla, pig brain, and lymphoma cells (Crowther et al., 1976; Pearse, 1980).

In earlier studies when bovine clathrin was prepared from a less highly purified preparation of CV, we observed both the 150S and 300S baskets at pH 6.8, 0.10 M ammonium acetate, by moving boundary ultracentrifugation (Nandi et al., 1980). In a subsequent study by sucrose gradient analysis, it was found that the 300S species was the major product formed at pH 6.5, 0.10 M ammonium acetate and 0.05 M Mes (Van Jaarsveld et al., 1981). With the current preparation, the 150S baskets are exclusively formed either at pH values above 6.40 by dialysis or at lower pH values by titration.

We have shown that the 150S basket could not be converted to the 300S basket by increasing the concentration of native clathrin during polymerization at pH 6.4. Reducing the pH from 6.5 to 6.0 also had no effect on the stability of 150S baskets. Similarly, the 300S could not be dissociated into the 150S basket by raising the pH from 6.0 to 6.5. Thus, once formed, both types of basket structures appear to be stable and not to be in equilibrium with each other.

We have attempted to resolve some of the factors responsible for the formation of the two sizes of baskets. There appear to be a variety of factors which modify the ratio of the two species of baskets, including Ca^{2+} and a protein(s) present in clathrin preparations. We have isolated from our clathrin preparation, by lysine–Sepharose column chromatography, a high molecular weight protein (M_r 110000) which enhances the formation of the 150S basket. It can be surmised that this component, when bound to 8S clathrin, modifies the interactions between the arms of clathrin so as to favor the formation of 150S. In its absence, another conformation prevails and 300S results. Consequently, the variability in our earlier results with respect to the ratio of these two baskets could be due, at least in part, to the extent to which this 110000 protein was present.

In contrast to clathrin solutions that have been dialyzed to pH 6.0, those which werre titrated showed only one size basket, i.e., the 150S. Since the clathrin obtained from lysine–Sepharose column gives 300S baskets by titration and lacks the 110000 protein, we expected that the 300S basket obtained by dialysis should also be missing this component. $NaDodSO_4$ gel electrophoresis of 300S obtained by dialyzing to pH 5.9, 0.10 Mes for 16 h and isolating on sucrose gradients indicated that a minor component corresponding to the 110000 protein was either reduced in concentration or missing. It appears that this component may be involved in controlling the formation of 150S when 8S clathrin is polymerized. In its absence, the 300S basket is formed. The formation of 300S baskets when clathrin solutions are dialyzed against buffer can be explained by a loss of the 110000 protein during dialysis. Its release from clathrin would also have to be pH dependent, becoming significant at pH values below 6.4.

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Purification of Human Liver Cytosolic Epoxide Hydrolase and Comparison to the Microsomal Enzyme†

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ABSTRACT: Epoxide hydrolase (EC 3.3.2.3) was purifed to electrophoretic homogeneity from human liver cytosol by using hydrolytic activity toward *trans*-8-ethylstyrene 7,8-oxide (TESO) as an assay. The overall purification was 400-fold. The purified enzyme has an apparent monomeric molecular weight of 58 000, significantly greater than the 50 000 found for human (or rat) liver microsomal epoxide hydrolase or for another TESO-hydrolyzing enzyme also isolated from human liver cytosol. Purified cytosolic TESO hydrolase catalyzes the hydrolysis of *cis*-8-ethylstyrene 7,8-oxide 10 times more rapidly than does the microsomal enzyme, catalyzes the hydrolysis of TESO and *trans*-stilbene oxide as rapidly as the microsomal enzyme, but catalyzes the hydrolysis of styrene 7,8-oxide, *p*-nitrostyrene 7,8-oxide, and naphthalene 1,2-oxide much less effectively than does the microsomal enzyme. Purified cytosolic TESO hydrolase does not hydrolyze benzo[*a*]pyrene 4,5-oxide, a substrate for the microsomal enzyme. The activities of the purified enzymes can explain the specific activities observed with subcellular fractions. Anti-human liver microsomal epoxide hydrolase did not recognize cytosolic TESO hydrolase in purified form or in cytosol, as judged by double-diffusion immunoprecipitin analysis, precipitation of enzymatic activity, and immunoelectrophoretic techniques. Cytosolic TESO hydrolase and microsomal epoxide hydrolase were also distinguished by peptide mapping. The results provide evidence that physically different forms of epoxide hydrolase exist in different subcellular fractions and can have markedly different substrate specificities.

Epoxide hydrolase (EC 3.3.2.3) is an enzyme found in a variety of mammalian tissues as well as in lower species (Guengerich, 1982; Lu & Miwa, 1980; Oesch, 1980). Activity has been demonstrated toward a wide variety of epoxides (Guengerich, 1982). Such activity is of importance because many epoxides are capable of irreversibly binding to nucleophilic sites present in tissue macromolecules and may initiate toxic or carcinogenic conditions. The action of epoxide hydrolase presumably serves to protect against the effects of epoxides which can enter the body or be produced from the action of monooxygenases on olefins and aromatic compounds (El-Tantawy & Hammock, 1980).

Early studies indicated that this activity is localized in microsomal fractions of tissues of experimental animals (Oesch, 1973; Oesch et al., 1971). More recently, activity has also been demonstrated in nuclear (Bornstein et al., 1979; Mukhtar et al., 1979), mitochondrial (Gill & Hammock, 1981), and cytoplasmic fractions (Gill & Hammock, 1979, 1980; Ota & Hammock, 1980). The current literature suggests that the epoxide hydrolases in nuclear and microsomal fractions are similar (Thomas et al., 1979a). However, Hammock and his associates have reported that the epoxide hydrolase activity in the cytosolic and mitochondrial fractions differs from that found in microsomal fractions in terms of pH optima and substrate specificity (Gill & Hammock, 1979–1981; Gill et al., 1974; Ota & Hammock, 1980). The cytosolic epoxide hydrolase has been reported to act on TESO[1] but not SO or BP-4,5-oxide, in contrast to the specificity of microsomal fractions (Gill & Hammock, 1981; Mullin & Hammock, 1980; Oesch & Golan, 1980). Antibodies raised against rat liver microsomal epoxide hydrolase precipitated microsomal BP-4,5-oxide hydrolase activity but not cytosolic TESO hydrolase activity in mouse and rat liver samples (Guenthner et al., 1981).

While microsomal epoxide hydrolase has been purified to electrophoretic homogeneity from rat and human liver in

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[1] Abbreviations: TESO, *trans*-8-ethylstyrene 7,8-oxide; CESO, *cis*-8-ethylstyrene 7,8-oxide; PNSO, *p*-nitrostyrene 7,8-oxide; SO, styrene 7,8-oxide; BP, benzo[*a*]pyrene; $NaDodSO_4$, sodium dodecyl sulfate; IgG, immunoglobulin G; EDTA, ethylenediaminetetraacetic acid.

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