Roussel, M. F., Dull, T. J., Rettenmier, C. W., Ralph, P., Ullrich, A., & Sherr, C. J. (1987) *Nature 325*, 549–552.
Shackelford, D. A., & Trowbridge, I. S. (1986) *J. Biol. Chem. 261*, 8334–8341.
Takayama, S., White, M. F., Lauris, V., & Kahn, C. R. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7797–7801.
Takayama, S., White, M. F., & Kahn, C. R. (1988) *J. Biol. Chem. 263*, 3440–3447.
Tornqvist, H. E., Pierce, M. W., Frackelton, A. R., Nemenoff, R. A., & Avruch, J. (1987) *J. Biol. Chem. 262*, 10212–10219.
Tornqvist, H. E., Gunsalus, J. R., Nemenoff, R. A., Frackelton, A. R., Pierce, M. W., & Avruch, J. (1988) *J. Biol. Chem. 263*, 350–359.
Turner, R. S., Kemp, B. E., Su, H., & Kuo, J. F. (1985) *J. Biol. Chem. 260*, 11503–11507.
Ullrich, A., Bell, J. R., Chen. E. Y., Herrera, R., Petruzzelli, L. M., Dull, T. J., Grunfeld, C., Rosen, O. M., & Ramachandran, J. (1985) *Nature 313*, 756–761.
White, M. F., Shoelson, S. E., Kentmann, H., & Kahn, C. R. (1988) *J. Biol. Chem. 263*, 2969–2980.
Whittaker, J., Okamoto, A. K., Thys, R., Bell, G. L., Steiner, C. F., & Hofmann, C. A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5237–5241.
Yu, K.-T., & Czech, M. P. (1984) *J. Biol. Chem. 259*, 5277–5286.
Yu, K.-T., Werth, D. K., Pastan, I. H., & Czech, M. P. (1985) *J. Biol. Chem. 260*, 5838–5846.

Determination of the Regions of the Clathrin Molecule Inducing Membrane Fusion†

Shigenori Maezawa and Tetsuro Yoshimura*
Institute for Enzyme Research, University of Tokushima, Tokushima 770, Japan
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ABSTRACT: Clathrin induces fusion of liposome membranes containing phosphatidylserine at acidic pH [Maezawa, S., Yoshimura, T., Hong, K., Düzgüneş, N., & Papahadjopoulos, D. (1989) *Biochemistry 28*, 1422–1428]. The regions of the clathrin molecule inducing membrane fusion were determined by examining the fusion abilities of clathrin fragments obtained by limited proteolysis of clathrin cages with thermolysin. Membrane fusion was assessed by resonance energy transfer assay in terms of the dilution of fluorescent phospholipids in liposome membranes. Proteolysis of clathrin decreased the fusion rate and the amount of protein but did not affect the specific fusion rate (i.e., the fusion rate per unit of protein), indicating that clathrin fragments retain the ability to induce fusion. Of the two proteolytic fragments of the clathrin heavy chain, the terminal domain and the residual proximal part, which were separated by ultracentrifugation or gel chromatography, only the proximal part showed fusion activity. Light chains seemed to have no role in membrane fusion, since they are susceptible to proteolytic digestion. The terminal domain induced reversible liposome membrane aggregation, which was also induced by the residual proximal part of the heavy chain and the whole molecule of clathrin. These results suggest that the terminal domain and the proximal portion of clathrin have critical roles in the steps of close apposition and fusion of membranes, respectively.

Recently, proteins have been recognized to participate in membrane fusion processes in biological systems, such as exocytosis, fertilization, myoblast fusion, virus infection, and intracellular transport (Lucy, 1984; Hong et al., 1987). Detailed studies have been made on the roles of various proteins in membrane fusion, such as those of envelope proteins in virus membrane fusion (White et al., 1983) and those of various proteins and peptides in fusion of liposome membranes (Lucy, 1984; Hong et al., 1987).

Previously, we found that the protein clathrin induces fusion of liposomes containing phosphatidylserine (PS)[1] below pH 6 (Hong et al., 1985) and that the hydrophobic domains of the protein molecule are exposed in this pH region (Yoshimura et al., 1987). Moreover, from studies on the relation of clathrin-induced membrane fusion with membrane binding, the conformational state, and the hydrophobicity of clathrin, we proposed a possible mechanism for induction of membrane fusion by this protein (Maezawa et al., 1989).

Clathrin is not globular, but has a unique three-legged pinwheel-like structure termed a triskelion, which is a high molecular weight protein of 650K composed of three heavy and three light chains (Keen, 1985; Pearse & Crowther, 1987). Each leg is about 500 Å long (Heuser & Kirchhausen, 1985; Kirchhausen et al., 1986) and consists of a terminal domain and distal and proximal segments (Kirchhausen & Harrison, 1984; Pearse & Crowther, 1987). For elucidation of the mechanism of clathrin-induced membrane fusion at the molecular level, it was essential to determine whether the entire clathrin leg or part of this leg induced membrane fusion. Kirchhausen and Harrison (1984) reported that limited proteolysis of clathrin-assembled coat structures, named cages,

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* To whom correspondence should be addressed.

[1] Abbreviations: DTT, dithiothreitol; LUV, large unilamellar vesicle(s); MES, 2-(*N*-morpholino)ethanesulfonic acid; NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PMSF, phenylmethanesulfonyl fluoride; Rh-PE, *N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TES, *N*-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid.

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by thermolysin, elastase, or subtilisin results in separation of the clathrin heavy chain into fragments, three terminal domains and a proximal portion consisting of the proximal segment and part of the distal segment, and complete digestion of light chains. In the present study, we examined the fusion abilities of clathrin fragments obtained by limited proteolysis of clathrin cages with thermolysin and found that only the proximal portion of the protein molecule induced membrane fusion.

Experimental Procedures

Materials. Thermolysin, phosphoramidon, and DTT were purchased from Sigma (St. Louis, MO), Protein Research Foundation (Osaka, Japan), and Nacalai Tesque (Kyoto, Japan), respectively. Bovine brain PS, egg PC, NBD-PE, and Rh-PE were obtained from Avanti Polar Lipids (Birmingham, AL). All other reagents were commercial products of reagent grade.

Preparation of Clathrin. Clathrin was purified from crude coated vesicles of bovine brain as described previously (Yoshimura et al., 1987). Purified clathrin in 0.5 M Tris (pH 7.5) was stored in ice and again subjected to Sepharose CL-4B gel chromatography in 0.5 M Tris, 0.5 mM EGTA, 0.25 mM $MgCl_2$, 0.01% NaN_3, and 0.0025% PMSF, pH 7.5, followed by dialysis against 10 mM TES, 100 mM NaCl, and 0.1 mM EDTA, pH 7.4, just before use. The concentration of clathrin was determined spectrophotometrically using an extinction coefficient of $E^{1\%}_{1cm} = 10.9$ at 280 nm (Nandi et al., 1980).

Preparation of Clathrin Cages. Clathrin cages were prepared essentially as described by Kirchhausen and Harrison (1984). Clathrin at 0.8 mg/mL was dialyzed overnight against 25 mM MES (pH 6.5) containing 0.5 mM DTT and 0.02% NaN_3 for several hours against the same buffer of pH 6.3, and for 2 days against the same buffer (pH 6.3) containing 2 mM $CaCl_2$ (assembly buffer). Dialysis was performed at about 4 °C.

Proteolysis of Clathrin Cages. The procedure used was essentially as described by Kirchhausen and Harrison (1984). Clathrin cages (about 0.8 mg/mL) were digested at 25 °C with thermolysin at a weight ratio of clathrin to thermolysin of 100:1 in assembly buffer. Digestion was terminated at various times by addition of about 20 μg/mL phosphoramidon.

Separation of Clathrin Fragments. Clathrin fragments, which were obtained by limited proteolysis with thermolysin as described above, were separated at 4 °C by centrifugation at 190000*g* for 90 min in a Hitachi preparative ultracentrifuge, Model SCP70H. The resulting precipitates were resuspended at their original volume in assembly buffer containing 20 μg/mL phosphoramidon. Large amounts of the terminal domain of clathrin were obtained by the same centrifugation procedure after cleavage of clathrin cages (2 mg) with thermolysin for 100 min, and concentration of the resulting supernatant. All the samples obtained were used for membrane fusion and aggregation assays, protein assay, and SDS–PAGE after their solubilization by adjusting the pH to 8.5 with Tris.

Clathrin fragments were also separated by gel chromatography. For this, clathrin cages (2 mg) were treated with thermolysin for 120 min as described above, and the resulting fragments were solubilized by raising the pH to 8.5 with Tris and applied to a Sepharose CL-4B column (0.7 × 50 cm) equilibrated with a solution of 0.1 M Tris, 0.2 M NaCl, 0.5 mM DTT, 0.1 mM phosphoramidon, and 0.02% NaN_3, pH 8.5. Materials were eluted with the same buffer.

Preparation of Liposomes. PS/PC (2:1) LUV and PS/PC (2:1) LUV labeled with both NBD-PE and Rh-PE at 1 or 0.1 mol % each were prepared in 10 mM TES (pH 7.4) containing 100 mM NaCl and 0.1 mM EDTA by the reverse-phase evaporation method (Szoka & Papahadjopoulos, 1978) with the modifications described by Wilschut et al. (1980) and then filtered through polycarbonate membranes of 0.1-μm pore size (Olson et al., 1979). The vesicle concentration was determined by measuring total lipid phosphorus by the method of Bartlett (1959).

Membrane Fusion Assay. Membrane fusion was measured at 25 °C by the resonance energy transfer assay described previously (Maezawa et al., 1989) in a Hitachi 650-60 fluorescence spectrophotometer, equipped with a constant-temperature cell holder and stirrer. Fusion was initiated by addition of clathrin or clathrin fragments to a mixture of PS/PC (2:1) LUV, labeled with both NBD-PE and Rh-PE at 1 mol % each, and unlabeled PS/PC (2:1) LUV at a molar ratio of 1:9, in 10 mM acetic acid (pH 4.75) containing 100 mM NaCl and 0.1 mM EDTA. The 100% fusion level was set at the fluorescence intensity of LUV containing the two fluorescence probes at 0.1 mol % each, which were prepared separately.

Membrane Aggregation Assay. Membrane aggregation was assayed by measuring the turbidity at 380 nm. Clathrin or clathrin fragments were added to PS/PC (2:1) LUV in 10 mM acetic acid (pH 4.8) containing 100 mM NaCl and 0.1 mM EDTA, and the turbidity change was monitored at 25 °C in a Hitachi 624 digital double-beam spectrophotometer, equipped with a constant-temperature cell holder.

Other Procedures. The concentration of thermolysin-treated clathrin was determined by the method of Bradford (1976) with bovine serum albumin as a standard. SDS–PAGE was carried out essentially as described by Laemmli (1970) in a 7.5% disk gel.

Results

Induction of Membrane Fusion by Thermolysin-Treated Clathrin. For determination of the regions of the clathrin molecule inducing membrane fusion, we first examined the effect of limited proteolysis of clathrin with thermolysin on its induction of fusion. Figure 1A shows the SDS–PAGE profiles of clathrin after different times of treatment of clathrin cages with thermolysin. During treatment, intact clathrin disappeared with concomitant appearance of species of 52, 59, 90, and 105 kDa. This result was consistent with that of Kirchhausen and Harrison (1984): they reported that the 52- and 59-kDa species were fragments containing the terminal domain of the clathrin heavy chain, while the 90- and 105-kDa species were the residual proximal parts of the protein consisting of the proximal segment and part of the distal segment of the heavy chain.

Results on the abilities of these thermolysin-treated samples (hereafter referred to as digest) to induce fusion of liposome membranes are shown in Table I. The rate of fusion and the amount of protein decreased with time. However, the specific fusion rate, that is, the rate of fusion per unit of protein, did not change with time. Since intact clathrin gradually disappeared and light chains are completely digested (Kirchhausen & Harrison, 1984), this result indicates that the terminal domain and/or the residual proximal part of the clathrin heavy chain retained fusion activity.

Induction of Membrane Fusion by Clathrin Fragments. Kirchhausen and Harrison (1984) reported that the terminal domain of clathrin can be isolated from clathrin cages as a supernatant fraction by high-speed centrifugation after proteolysis. To determine which domain is fusogenic, we separated the two fragments by centrifugation of the digest of clathrin cages with thermolysin and tested the abilities of the

Table I: Fusion Activity of Thermolysin-Treated Clathrin[a]

fractions	incubation time (min)	total fusion rate (% min^{-1})	total protein (μg)	specific fusion rate (% min^{-1} μg^{-1})
digest	0	51.0	7.6	6.71
	5	48.3	6.8	7.10
	25	45.8	6.3	7.27
	60	40.0	5.9	6.78
	180	29.4	4.4	6.68
supernatant	0	0.00	0.0	0.00
	5	0.00	0.0	0.00
	25	0.06	1.0	0.06
	60	0.12	2.0	0.06
	180	0.10	2.0	0.05
precipitate	0	49.6	7.4	6.70
	5	48.1	6.6	7.29
	25	43.2	5.5	7.85
	60	37.2	4.1	9.07
	180	32.2	3.1	10.4

[a] Fractions were obtained as described for Figure 1 and solubilized by adjusting the pH to 8.5 with Tris. Samples of the digest (15 μL), supernatant (50 μL), or precipitate (15 μL) fraction were added to a mixture (50 μM phospholipid) of LUV labeled with both NBD-PE and Rh-PE at 1 mol % each and unlabeled LUV in a molar ratio of 1:9, and the fluorescence increase was monitored at 25 °C with excitation and emission wavelengths of 475 and 530 nm, respectively. Aliquots of the three fractions (25, 100, and 25 μL, respectively) were used for protein assay. The total fusion rate and total protein were expressed as values per 15 μL of sample.

terminal domain and the residual proximal part to induce membrane fusion. The results of SDS–PAGE confirmed that the supernatant fraction contained the terminal domain only (Figure 1B). Scarcely any protein was detected in the supernatant fraction after proteolysis for 5 min, but a significant amount of protein was detected after proteolysis for 25 min (Figure 1B, Table I). The rates of fusion induced by these supernatant fractions were extremely low (Table I): only 0.1–0.3% of the fusion activity of the digest was recovered in the supernatant, and the specific fusion rate was only 0.7–0.9% of that of the digest. These results indicate that the terminal domain of clathrin has no ability to induce membrane fusion.

We then examined the fusion ability of the precipitated fraction. As shown in Figure 1C, this fraction contained the whole molecule of clathrin and the residual proximal part of the heavy-chain trimer. The residual proximal part, like the terminal domain, was detected in the precipitate after proteolysis for 25 min. Results on the fusion activities of these domains are also shown in Table I. Both the total rate of fusion and the amount of protein decreased with time, like those of the digest. However, the total fusion rate of the precipitated fraction was the same as that of the digest at each time, indicating that all the fusion capacity was recovered in the proximal portion of the heavy-chain trimer. The specific rate of fusion increased with time, being 160% of the initial rate after cleavage for 180 min, suggesting that the fusion activity of clathrin is enhanced by removal of the terminal domain. However, this increase might be due to increase in the number of molecules of the proximal portion per unit of protein, because the molecular mass of the proximal portion of the heavy-chain trimer is 270 or 320 kDa, which is about half that of the whole molecule (650 kDa).

We next separated the fragments of the thermolysin-treated clathrin sample by Sepharose CL-4B gel chromatography. Without thermolysin treatment, clathrin was eluted as a single peak in fraction 24 (Figure 2A), but after thermolysin treatment for 120 min, three peaks were obtained (Figure 2B). SDS–PAGE showed that the first peak was composed of aggregates of the residual proximal part of the heavy-chain trimer, the second peak was a mixture of the residual proximal part and the whole molecule, and the third peak was mainly the terminal domain. The first and second fractions showed

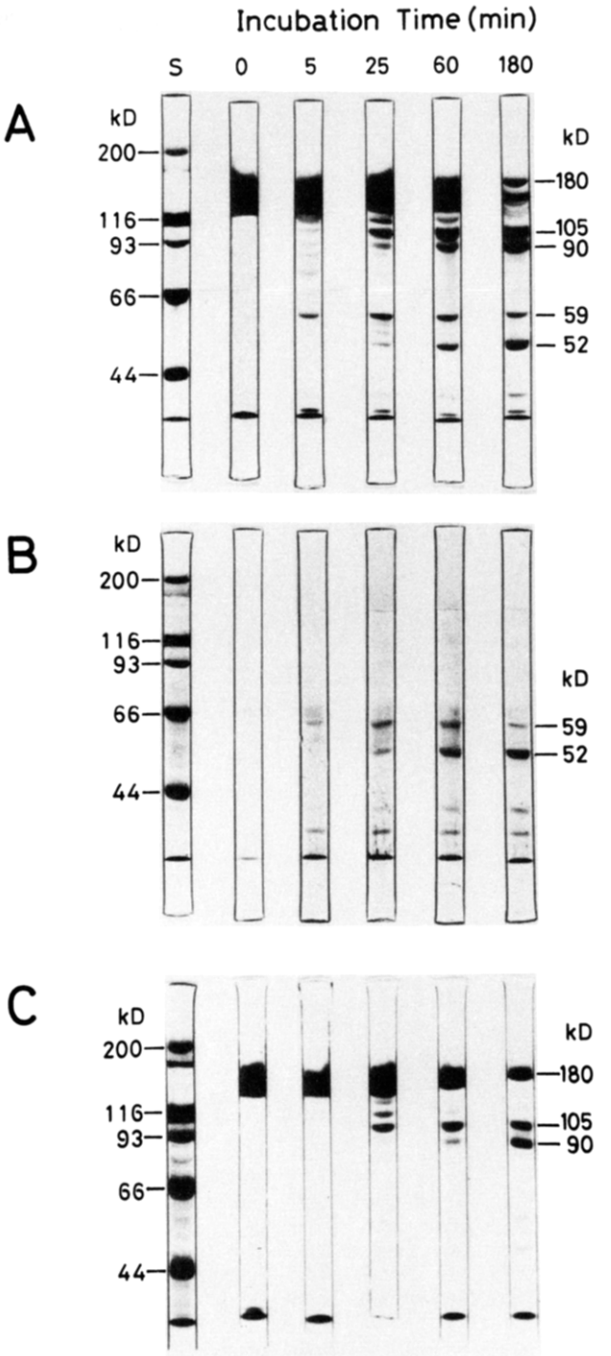

FIGURE 1: SDS–PAGE profiles of thermolysin-treated clathrin samples (digest) (A) and the supernatant (B) and precipitate (C) fractions obtained by centrifugation of the digest. A 0.5-mL solution of clathrin cages (0.4 mg) was treated at 25 °C with thermolysin at a weight ratio of clathrin to thermolysin of 100:1 in 25 mM MES (pH 6.3) containing 2 mM $CaCl_2$, 0.5 mM DTT, and 0.02% NaN_3 (assembly buffer). Digestion was terminated after the indicated times by addition of 20 μg/mL phosphoramidon, and samples (digest) were centrifuged at 190000g for 90 min. The precipitates were resuspended in 0.5 mL of assembly buffer containing 20 μg/mL phosphoramidon. A 60-μL aliquot of each fraction, solubilized by raising the pH to 8.5 with Tris, was subjected to SDS–PAGE. The SDS–PAGE profiles of standard proteins are shown on the left (S).

fusion activity, whereas the third fraction showed little fusion activity (Figure 2B). The second fraction was eluted in fraction 27, slightly after that of the intact form.

The results of these two experiments indicate that the ability of clathrin to induce membrane fusion is localized in the proximal portion of its heavy-chain trimer.

Abilities of Clathrin Fragments To Induce Membrane Aggregation. Clathrin induces liposome aggregation as well

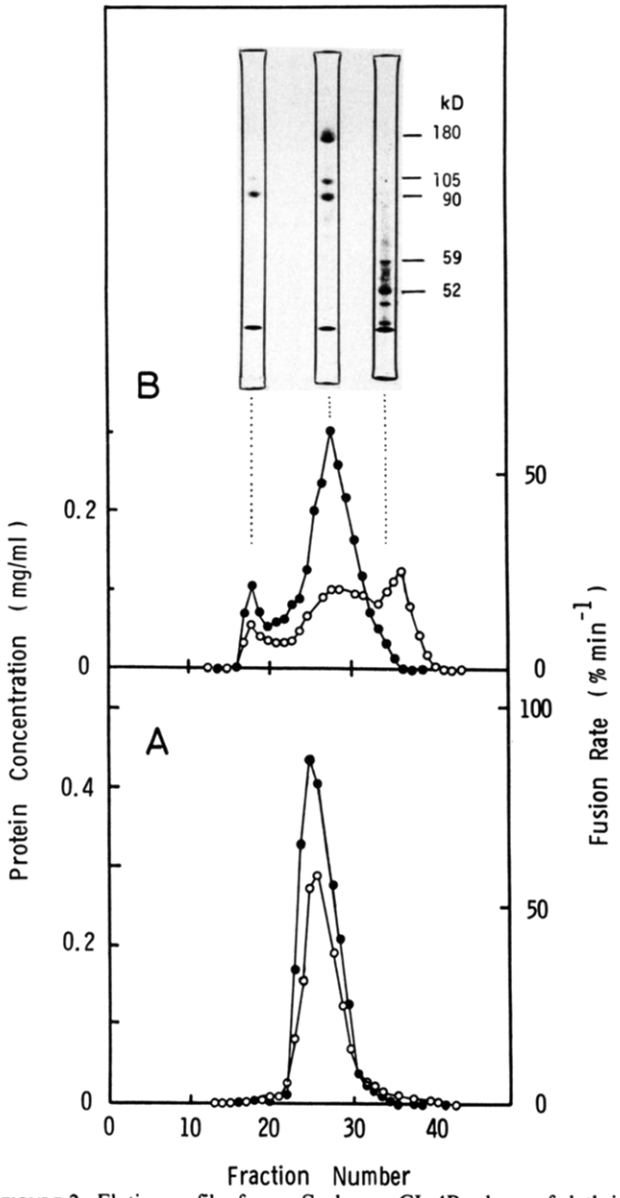

FIGURE 2: Elution profiles from a Sepharose CL-4B column of clathrin untreated (A) and treated with thermolysin for 120 min (B). Gel chromatography was performed as described under Experimental Procedures at a flow rate of 2 mL/h, and the eluate was collected in 0.5-mL fractions. The fusion rate (●) was measured as described for Table I using a 50-μL aliquot of each fraction. The protein concentration of each fraction (○) was determined by the method of Bradford (1976). Fractions 18, 27, and 34 were subjected to SDS-PAGE.

as liposome fusion (Hong et al., 1985). As shown in Figure 3, the turbidity at 380 nm increased at pH 4.8 on addition of the intact clathrin and decreased on raising the pH of the medium to 7.2, indicating liposome aggregation. However, the turbidity change was not restored completely, because some liposome fusion occurred.

We examined the effects of the terminal domain and the proximal portion of the clathrin heavy chain on liposome aggregation. For this, the turbidity increase at 380 nm was measured after addition of the supernatant or precipitate obtained by high-speed centrifugation of the digest of clathrin cages with thermolysin (Figure 3). At pH 4.8, the precipitated fraction caused increase in turbidity, which was not restored completely by changing the pH to 7.2, as with the whole molecule. Interestingly, turbidity change was also observed at this pH on addition of the supernatant fraction, though to a lesser extent, and was restored to its original level on adjusting the pH to neutrality. The turbidity in the steady state was proportional to the amount of protein of the supernatant fraction added (data not shown). These results indicate that the terminal domain of clathrin can induce reversible membrane aggregation, although it does not induce fusion.

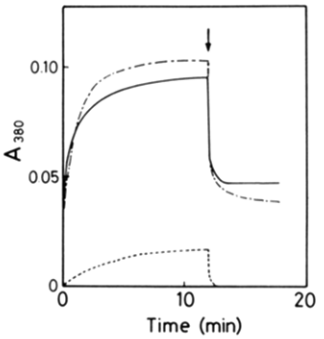

FIGURE 3: Time course of turbidity change induced by clathrin or clathrin fragments. About 10 μg of clathrin (—) or the supernatant (- - -) or precipitated (–·–) fraction was added to PS/PC (2:1) LUV (48 μM phospholipid) at pH 4.8, and the turbidity change was monitored at 25 °C. After 12 min (arrow), the pH of the medium was adjusted to 7.2 by addition of a trace amount of 0.2 N NaOH.

Discussion

In the present study, we separated the clathrin molecule into its terminal domain and residual proximal part by treatment of clathrin cages with thermolysin and examined the abilities of these two portions to induce membrane fusion. We found that the terminal domain did not induce fusion and that the fusion activity was completely recovered in the residual proximal part of the heavy chain. Light chains seemed to have no role in membrane fusion, as they are susceptible to proteolytic degradation (Kirchhausen & Harrison, 1981, 1984). Thus, we concluded the fusogenicity of clathrin is localized in the proximal portion of the heavy-chain trimer.

The terminal domain was found to induce liposome membrane aggregation, but not fusion. However, the extent of membrane aggregation induced by the terminal domain was much less than that induced by the residual proximal part of the heavy-chain trimer or the whole molecule of clathrin. This lower efficiency of membrane aggregation may be attributable to a difference in molecular size and shape: the terminal domain is a compact globular protein with a molecular mass of only 50 kDa, while the proximal part and whole clathrin molecule have molecular masses of 300 and 650 kDa, respectively, and a three-legged pinwheel-like structure, which must attach to the membrane surface with wider occupancy to facilitate membrane aggregation.

On the basis of experimental evidence, we previously speculated that membrane fusion induced by clathrin is caused by the following two steps: (1) close apposition of membranes due to reduction of surface charge density or bridge formation between adjacent membranes by the extended arms of the clathrin triskelion; (2) actual fusion of membranes due to a strong perturbation of their bilayers by penetration of the exposed hydrophobic domains of the protein (Hong et al., 1985; Yoshimura et al., 1987; Maezawa et al., 1989). The existence of these two steps in clathrin-induced membrane fusion has also been suggested by Blumenthal et al. (1983). In coated vesicles, the terminal domains appear to contact the vesicle membranes through a shell formed by the assembly polypeptide complexes (Pearse & Crowther, 1987). The proximal portion of the clathrin heavy chain was found to be more hydrophobic than the terminal domain by the hydropathy test (S. Maezawa and T. Yoshimura, unpublished results) and is

reported to contain 47 uncharged amino acid residues in its carboxyl-terminal region (Kirchhausen et al., 1987). Thus, the present results suggest that the terminal domain and the proximal portion of the protein have critical roles in the processes of close apposition and fusion of membranes, respectively.

Recently, natural and synthetic peptides, such as melittin (Eytan & Almary, 1983; Morgan et al., 1983), insulin (Farías et al., 1985), a synthetic peptide with the same sequence as the N-terminal segment of influenza virus hemagglutinin (Murata et al., 1987), and synthetic amphiphathic peptides (Parente et al., 1988), have been found to induce fusion of liposome membranes. Virus envelope proteins, such as the F protein of Sendai virus, the HA protein of influenza virus, and the G protein of vesicular stomatitis virus, have short hydrophobic segments at their N-termini, which induce membrane fusion when they are exposed by the action of proteases or pH-dependent conformational changes of the proteins (Spear, 1987). The membrane-penetrating segments of proteins such as α-lactalbumin (Kim & Kim, 1986) and ovalbumin (Yun & Kim, 1989) have been identified, and membrane fusion is supposed to be caused by penetration of these segments, which are exposed to the aqueous environment (Yun & Kim, 1989). Thus, a small segment in the proximal portion of the clathrin heavy chain might be involved in membrane fusion.

In the receptor-mediated endocytosis pathway, coated vesicles are generated by pinching off of coated regions of the surface membranes (coated pits), the clathrin coats disassemble from the vesicles, and the resulting uncoated vesicles fuse with each other to form endosomes. In coated vesicles, the proximal portion of clathrin, which has the potential to induce membrane fusion, cannot come in direct contact with the vesicle membranes (Pearse & Crowther, 1987). This fact is consistent with the idea that the clathrin coat inhibits fusion of the vesicles and its removal allows fusion to occur (Altstiel & Branton, 1983). However, Heuser (1989) found that cytoplasmic acidification alters the structure of clathrin lattices on the inside of the plasma membrane and speculated that pH alterations at a local level could modulate the dynamics of individual coated vesicles. Moreover, Levin et al. (1986) reported that clathrin might cause a structural rearrangement in the bilayer of coated and uncoated vesicles, synaptic membranes, and liposomes composed of synthetic PC. Thus, under particular conditions where the local pH, ion flux, ATP energy supply, or the membrane potential is altered, a conformational change could occur in the disassembled clathrin molecule that might result in its reassociation with uncoated vesicles and direct contact of the proximal portion with their membranes that are closely apposed through bridge formation by the terminal domain, and this could cause fusion of uncoated vesicles. Identification of the peptide in the proximal portion of the heavy chain with fusogenicity is important in establishing the role of clathrin in membrane fusion at the physiological level.

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REFERENCES

Altstiel, L., & Branton, D. (1983) *Cell 32*, 921–929.
Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.
Blumenthal, R., Henkart, M., & Steer, C. J. (1983) *J. Biol. Chem. 258*, 3409–3415.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Eytan, G. D., & Almary, T. (1983) *FEBS Lett. 156*, 29–32.
Farías, R. N., Viñals, A. L., & Morero, R. D. (1985) *Biochem. Biophys. Res. Commun. 128*, 68–74.
Heuser, J. (1989) *J. Cell Biol. 108*, 401–411.
Heuser, J., & Kirchhausen, T. (1985) *J. Ultrastruct. Res. 92*, 1–27.
Hong, K., Yoshimura, T., & Papahadjopoulos, D. (1985) *FEBS Lett. 191*, 17–23.
Hong, K., Düzgüneş, N., Meers, P. R., & Papahadjopoulos, D. (1987) in *Cell Fusion* (Sowers, A. E., Ed.) pp 269–284, Plenum Press, New York.
Keen, J. M. (1985) in *Endocytosis* (Pastan, I., & Willingham, M. C., Eds.) pp 85–130, Plenum Press, New York.
Kim, J., & Kim, H. (1986) *Biochemistry 25*, 7867–7874.
Kirchhausen, T., & Harrison, S. C. (1981) *Cell 23*, 755–761.
Kirchhausen, T., & Harrison, S. C. (1984) *J. Cell Biol. 99*, 1725–1734.
Kirchhausen, T., Harrison, S. C., & Heuser, J. (1986) *J. Ultrastruct. Mol. Struct. Res. 94*, 199–208.
Kirchhausen, T., Harrison, S. C., Chow, P., Mattaliano, R. J., Ramachandran, K. L., Smart, J., & Brosius, J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8805–8809.
Laemmli, U. K. (1970) *Nature 227*, 680–685.
Levin, I. W., Vincent, J. S., Blumenthal, R., & Steer, C. J. (1986) *Biophys. J. 49*, 111–113.
Lucy, J. A. (1984) *FEBS Lett. 166*, 223–231.
Maezawa, S., Yoshimura, T., Hong, K., Düzgüneş, N., & Papahadjopoulos, D. (1989) *Biochemistry 28*, 1422–1428.
Morgan, C., Willamson, H., Fuller, S., & Hudson, B. (1983) *Biochim. Biophys. Acta 732*, 668–674.
Murata, M., Sugahara, Y., Takahashi, S., & Ohnishi, S. (1987) *J. Biochem. (Tokyo) 102*, 957–962.
Nandi, P. K., Pretorius, H. T., Lippoldt, R. E., Johnson, M. L., & Edelhoch, H. (1980) *Biochemistry 19*, 5917–5921.
Olson, F., Hunt, C. A., Szoka, F. C., Vail, W. J., & Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta 557*, 9–23.
Parente, R. A., Nir, S., & Szoka, F. C., Jr. (1988) *J. Biol. Chem. 263*, 4724–4730.
Pearse, B. M. F., & Crowther, R. A. (1987) *Annu. Rev. Biophys. Biophys. Chem. 16*, 49–68.
Spear, P. G. (1987) in *Cell Fusion* (Sowers, A. E., Ed.) pp 3–32, Plenum Press, New York.
Szoka, F. C., & Papahadjopoulos, D. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4194–4198.
White, J., Kielian, M., & Helenius, A. (1983) *Q. Rev. Biophys. 16*, 151–195.
Wilschut, J., Düzgüneş, N., Fraley, R., & Papahadjopoulos, D. (1980) *Biochemistry 19*, 6011–6021.
Yoshimura, T., Maezawa, S., & Hong, K. (1987) *J. Biochem. (Tokyo) 101*, 1265–1272.
Yun, C.-H., & Kim, H. (1989) *J. Biochem. (Tokyo) 105*, 406–411.