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Biochemical and immunological studies on clathrin light chains and their binding sites on clathrin triskelions

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Clathrin light chains from bovine brain tissue (LC_α and LC_β) are monomeric proteins with an average mol. wt. of ~33 000, as determined by sedimentation equilibrium. Solution studies on purified light chains indicate a large Stokes radius (R_e = 3.3 nm) and little defined secondary structure. Both light chains bind specifically and with high affinity (K_A ~5 x 10^7/M) to overlapping sites on clathrin heavy chains. These binding sites are contained within a 125 000 dalton heavy chain fragment that forms truncated triskelions with legs, 15 nm shorter than those of intact triskelions. As judged by immuno-electron microscopy, light chain-specific IgG molecules bind mostly to the center of triskelions, but there are also sites that are scattered some 16 nm along the proximal part of triskelion legs. From heterologous binding experiments using human placenta light chains and heavy chain fragments from bovine brain clathrin, it is concluded that the domains of light and heavy chains that are involved in the interaction are conserved across tissue and species boundaries.

Key words: clathrin/immuno-electron microscopy/light chains/triskelions

Introduction

The protein clathrin is the major constituent of the polyhedral lattice that surrounds coated vesicles and that underlies coated pits (Pearse, 1975). Coated vesicles have been shown to play an important role in the initial stages of the specific transfer of membrane within eucaryotic cells (Goldstein *et al.*, 1979; Bretscher *et al.*, 1980). The assembly units of the coat are three-legged structures, termed clathrin triskelions (Ungewickell and Branton, 1981). They are constructed from three clathrin heavy chains (mol. wt. ~180 000) and three clathrin light chains (mol. wt. ~30 000–36 000). The electrophoretic mobility of light chains on SDS-polyacrylamide gels is, unlike that of heavy chains, species- and tissue-specific. Clathrin heavy chains, judged by one-dimensional polypeptide analysis (Pearse, 1976) and by immunological criteria (Kartenbeck *et al.*, 1981), are in evolutonary terms highly conserved polypeptides.

With the aim of clarifying the biological role of the clathrin light chains, I have undertaken a more detailed characterization of these molecules, prepared from bovine brain, and their binding sites on the heavy chains. Here I describe the purification of individual α and β light chains and some of their physical properties, and present biochemical and immunological evidence to show that they bind in the proximal regions of the triskelion legs nearest the vertex. I also describe the heterologous interaction between clathrin light chains derived from human placental tissue and heavy chains from bovine brain.

Results

Purification and preliminary characterization of clathrin light chains

During the initial stages of this work clathrin light chains were dissociated from triskelions by means of strongly denaturing media such as 8 M urea or 6 M guanidine hydrochloride, and they were then separated from heavy chains by gel filtration (Keen *et al.*, 1981; Ungewickell *et al.*, 1982). In the course of surveying different dissociation conditions, I observed that the light chain with the lower apparent mol. wt. (LC_β; ~33 000) is already released from the heavy chains at a urea concentration of 3.6 M, whereas the α light chain (mol. wt. ~36 000) requires at least 4.2 M urea for its dissociation (data not shown). This observation probably reflects a difference between the light chains rather than between their binding sites (see below). For some of the work presented here, light chain preparations were obtained by the more convenient heat denaturation procedure of Lisanti *et al.* (1982), applied with minor modifications. Light chains dissociated by either method were then subjected to ion-exchange chromatography on DE 52, which gives partial fractionation of α and β light chains (Figures 1 and 2). In general, the purest light chain preparations were obtained when triskelions from highly purified coated vesicles were used as the starting material, rather than those from crude coated vesicles as suggested by Lisanti *et al.* (1982).

A purified mixture of α and β light chains was used to calibrate the colour response of Coomassie Brilliant Blue after electrophoresis in SDS-polyacrylamide gels. The colour values derived for heavy and light chains were found to differ

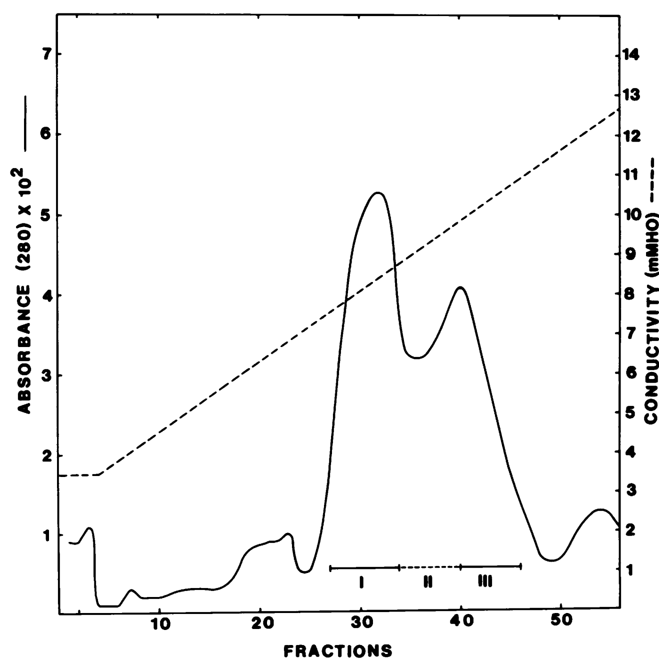

Fig. 1. Column chromatography of clathrin light chains on DEAE-cellulose. Separately pooled fractions are indicated by horizontal lines.

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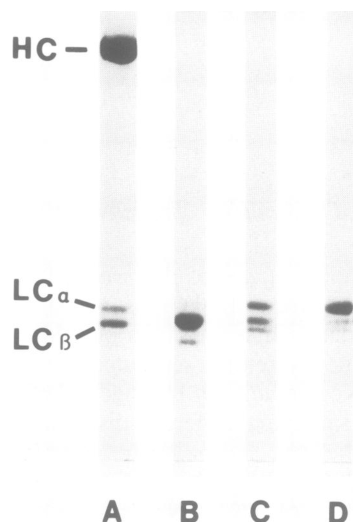

Fig. 2. SDS-PAGE analysis of pooled ion-exchange column fractions. **(A)** Original clathrin triskelions; **(B)**, **(C)** and **(D)** correspond to pooled fractions I, II and III, respectively. HC: heavy chains; LC: light chains.

by <10% from each other (data not shown). With the aid of this calibration, the stoichiometry of light and heavy chains in different triskelion preparations could be estimated and it was found to vary between 0.7 and 0.9 mol light chain per mol heavy chain. The reason for ratios of less than unity is likely to be the high susceptibility of the light chains to proteolysis (Kirchhausen and Harrison, 1981), which could cause partial destruction in the course of the preparation of coated vesicles.

Apparent Stokes radii were determined for the light chains by gel filtration on a G-100 chromatographic column, calibrated with appropriate protein standards. Values were obtained by interpolation on a plot of Stokes radius against $\mathrm{erf}^{-1}(1 - K_D)$ (Ackers, 1970), where the partition coefficient K_D is derived from the elution volume (Figure 3). Both light chains have an apparent Stokes radius of 3.3 nm, which is nearly twice that for a globular protein of the mol. wt. of the light chains, inferred from SDS-gel electrophoresis. To ensure that the light chains were not self-associated under the conditions of the experiment, sedimentation equilibrium experiments were undertaken on preparations containing both light chains. The equilibrium distribution (Figure 4) gives a mol. wt. of 33 000, assuming a partial specific volume from their amino acid composition of 0.72 ml/g. They are thus monodisperse and monomeric. The sedimentation coefficient was determined to be 2.2S. Combining this with the Stokes radius according to the relation:

$$S = \frac{M(1 - \bar{\nu}\varrho)}{Nf}$$

in which the frictional coefficient $f = 6\pi\,\eta_o\,R_e$; η_o is the absolute viscosity of the solvent; R_e the Stokes radius; N the Avogadro number; $\bar{\nu}$ the partial specific volume and ϱ the solvent density, one obtains a mol. wt. of 31 000. The hydrodynamically derived mol. wts. are slightly lower than the average for the two light chains, of 34 000, determined by SDS-gel electrophoresis, using standard polypeptides as references. The discrepancy may be due to anomalous SDS binding to light chains. It is, however, clear that the light chains are monomers that are characterized by a large Stokes radius. This indicates that they either possess an open, flexible

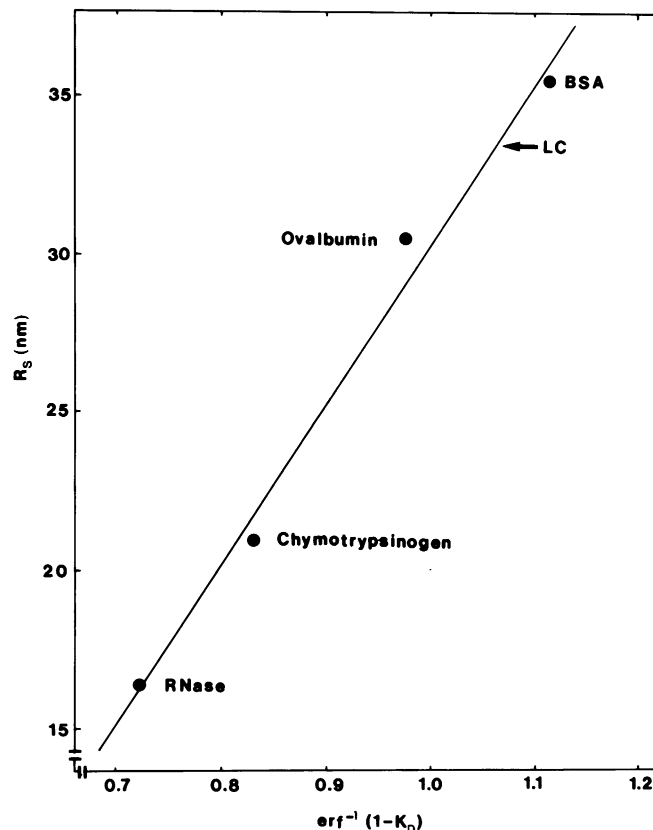

Fig. 3. Stokes radius determination for clathrin light chains. The arrow indicates the elution position of both clathrin light chains which corresponds to an apparent Stokes radius of 3.3 nm. R_e: Stokes radius; K_D: partition coefficient for the protein between mobile and stationary phase.

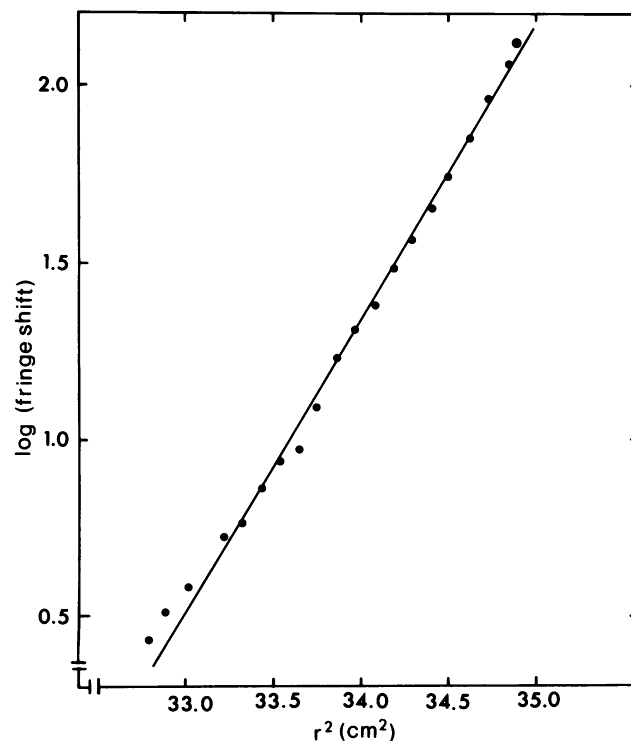

Fig. 4. Sedimentation equilibrium distribution of clathrin light chains. The mol. wt. of light chains was determined by the high speed method (Yphantis, 1964). At a rotor speed of 30 000 r.p.m. equilibrium was approached after 20 h. Measurements were made on plates taken after 30 h. r: distance from center of rotation.

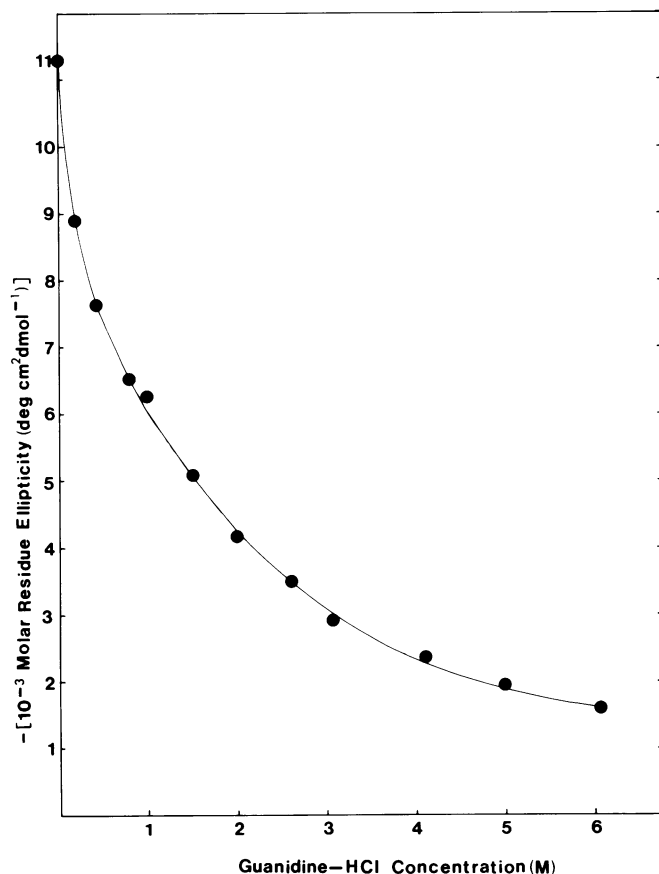

Fig. 5. Unfolding of light chains in guanidine hydrochloride solutions. The value of the molar residue ellipticity at 222 nm in the absence of denaturant corresponds to an α-helical content of ~30% which drops to ~4% in 6 M guanidine hydrochloride.

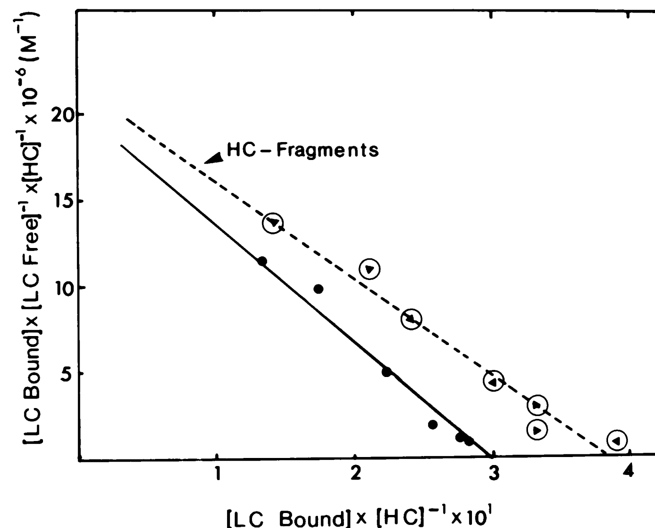

Fig. 6. Scatchard plot of the binding of [^{3}H]light chains to intact clathrin heavy chains (●——●) and to a mixture of 112 000 and 125 000 dalton heavy chain fragments (○- - - -○). The lines were drawn to fit a linear least square regression.

coil-like structure, or are highly asymmetric. Thus, for example, if the molecules can be described as a prolate ellipsoid of revolution, then with a hydration of 0.4 g water per g protein (Kuntz and Kautzmann, 1974) the axial ratio would be 8, and the length of the major axis ~17 nm. To distinguish between the two models, denaturant-induced unfolding profiles were measured by c.d. In aqueous solution at 20°C, the molar residue ellipticity at 222 nm was found to be −11 000 deg cm^2/dmol, corresponding to an α-helix content of ~30%. On addition of guanidine hydrochloride, the helicity drops smoothly with denaturant concentration, reaching ~4% at 6 M (Figure 5). Thus, the structure is labile in the absence of denaturant and does not show the sigmoidal denaturation profiles that are characteristic of structured proteins (Tanford, 1962; or see Pont and Woods, 1971, for fibrous proteins of defined structure). These results strongly suggest, though they do not prove, that the free clathrin light chains in solution behave as flexible coils. Such a model readily explains their susceptibility to proteases and it may also be the reason for their solubility in boiling water.

Binding of light chains to heavy chains

We have recently shown that clathrin light chains reassociate specifically with heavy chains (Ungewickell *et al.*, 1982). Using a similar binding assay, based on the co-sedimentation of light chains with light chain-depleted clathrin cages, I set out to determine whether light chains are also capable of interacting with heavy chain fragments that are known to be derived from the central region of triskelions. Trypsin treatment of clathrin cages truncates the legs of triskelions by ~15 nm, and produces two, in sequence overlapping, heavy chain fragments with relative mol. wts. of 125 000 and 112 000 (Schmid *et al.*, 1982; Ungewickell *et al.*, 1982; Hanspal *et al.*, in preparation). Under the conditions of proteolysis, the truncated triskelions remain organized in sedimentable cage-like structures. For some of the binding experiments I used a mixture of α and β light chains that had been labelled with tritium by reductive methylation (Tack *et al.*, 1980). The label was found to be proportionally distributed between α and β light chains. Other mild labelling procedures, such as iodination with Bolton-hunter reagent, significantly affected the ability of light chains to interact with heavy chains (data not shown). When I attempted to iodinate light chains *in situ* (on triskelions) hardly any of the label became incorporated into the light chains. This observation suggests that the lysine side chains are implicated in the interaction with heavy chains. The binding of tritiated light chains to heavy chains is fast (complete within 15 min) and it showed no detectable temperature dependence in the range of 0–20°C. The binding was analyzed by means of Scatchard plots (Scatchard, 1949) which show (Figure 6) that light chains attach to both intact and truncated heavy chains with an affinity of at least 5 x 10^7/M. Because of the modified lysine residues, actual affinities may be even higher. The observed low site occupancy at saturation of 0.3–0.4 mol rebound light chain per mol heavy chain or heavy chain fragment could be due to incomplete removal of endogenous light chain fragments that failed to dissociate from the clathrin cages and/or to the dissociation of some of the cages into triskelions that would bind added light chains, but would fail to sediment significantly under the conditions employed in this experiment. In any case we may infer from the data that both binding sites are contained within the 125 000 dalton heavy chain fragment and thus lie within 28 nm of the triskelion center. The same conclusion also emerges from a more qualitative binding experiment: unlabelled light chains from bovine brain and human placenta were incubated in the presence and absence of assembled heavy chain fragments

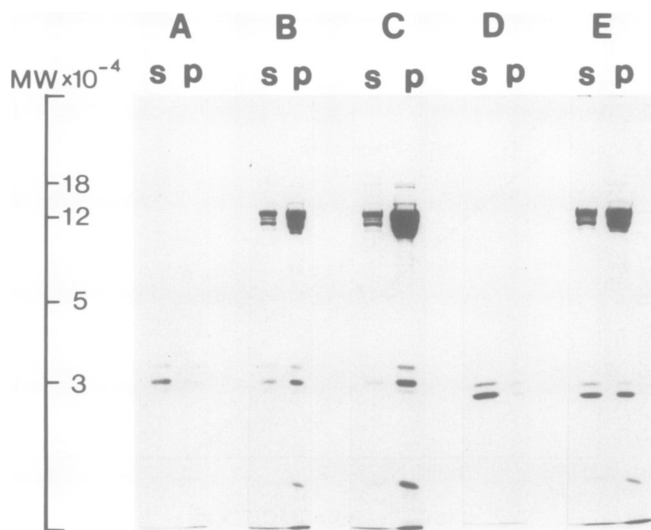

Fig. 7. Binding of bovine brain and human placenta light chains to bovine brain heavy chain fragments. Supernatants (s) and pellets (p) of different incubation mixtures were analyzed by SDS-PAGE on 7.5% gels. **(A)** Bovine brain light chains (B-LC) without added heavy chains, **(B)** B-LC incubated with 12 μg heavy chain fragments, **(C)** B-LC incubated with 50 μg heavy chain fragments, **(D)** human placenta light chains (H-LC) without added heavy chains, **(E)** H-LC incubated with 50 μg heavy chain fragments.

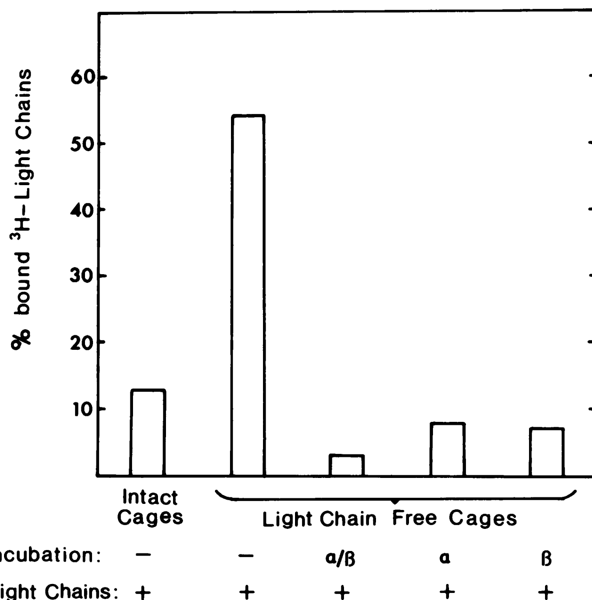

Fig. 8. The effect of preincubating light chain-free cages with either α, β, or α and β light chains on the binding of radiolabelled α and β light chains (for details see Materials and methods).

and the mixture was then centrifuged in a Beckman airfuge. The pellets and supernatants were analyzed by SDS-PAGE. Figure 7 shows that both light chains interact with the tryptic heavy chain fragments, since the amount of the light chains carried down is a function of the concentration of heavy chain fragments in the incubation mixture. These results further show that human placenta light chains are capable of binding to heavy chain fragments from bovine brain clathrin, and the interaction is thus not constrained by tissues and species boundaries (Figure 7, lanes D and E).

To test if α and β light chains from bovine brain share overlapping binding sites, heavy chains were incubated with a

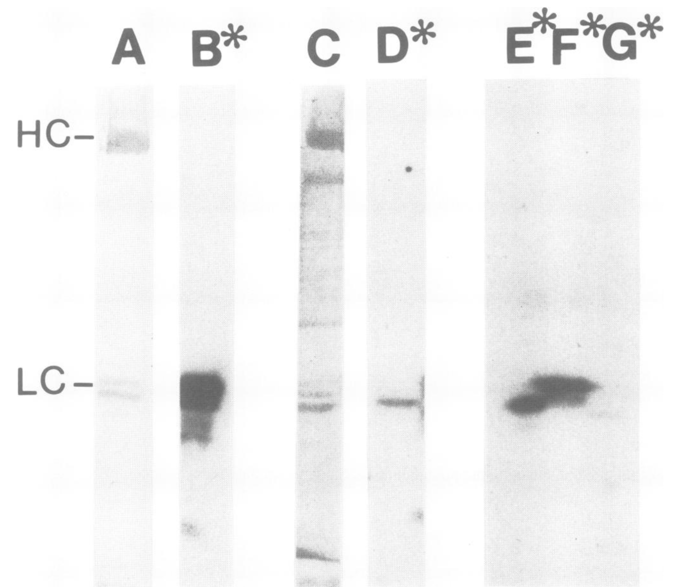

Fig. 9. Nitrocellulose replicas of 10% SDS-polyacrylamide gels. **Lanes A** and **C** are original nitrocellulose sheets, which were stained for protein with Ponceau S (Serva). Lanes denoted by asterisks are autoradiographs of nitrocellulose sheets, stained with light chain-specific antibodies. **(A)** Purified bovine brain triskelions and **(B*)** corresponding autoradiograph; **(C)** Triton X-100-extracted coated vesicle from human placenta (Pearse, 1982) and **(D*)** the corresponding autoradiograph. **(E*)**, **(F*)** and **(G*)** are autoradiographs of solubilized tissue extracts from bovine kidney, bovine brain and human placenta, respectively.

3-fold excess of either α, β or a mixture of both prior to the addition of a mixture of labelled light chains. Strong and quantitatively comparable inhibition of attachment of labelled light chains was observed in all cases (Figure 8), showing that the α and β light chains compete for the same sites on the triskelions.

Localization of light chains on clathrin triskelions by immuno-electron microscopy

In addition to the evidence from binding experiments presented above, we know from an earlier study (Ungewickell *et al.*, 1982), based on electron microscopy with the use of a biotin-avidin-ferritin bridge, that biotinyl-light chains bind to triskelions near their mid-points. To obtain direct information on the positions of light chains on triskelions and to improve on the precision of the localization, I raised polyclonal antibodies against bovine brain light chains. Affinity-purified antibodies were prepared by passing either whole serum or partially purified IgGs through a column of immobilized light chains that had been purified by preparative SDS-PAGE and covalently attached to a Sepharose matrix. On immuno-blots of either total brain extracts or purified clathrin triskelions the antibodies reacted only with light chains and with some lower mol. wt. material that probably represents breakdown products of light chains (Figure 9). In blots of solubilized bovine kidney extracts, the antibodies recognized only one band of mol. wt. ~32 000 and reacted only weakly with the β light chain of human placenta clathrin. Thus, in immunological terms, bovine brain light chains differ significantly from those of human placenta and even somewhat from those of bovine kidney tissue.

The specificity of the antibodies was also tested by solid phase immunoassays. Triskelions with and without light chains were immobilized on sheets of nitrocellulose paper

Table I. Binding of light-chain specific antibodies to triskelions

Competition with		% Binding to		
Light chains (μg)	Triskelions (μg)	Triskelions[a]	Triskelions treated with elastase	Triskelions treated with trypsin
0	0	100	7	6
0.005	0.02	51 (63)	–	–
0.01	0.05	48 (58)	–	–
0.05	0.25	24 (42)	–	–
0.1	0.52	23 (27)	–	–
0.2	0.99	17 (27)	–	–

[a]Values in parentheses represent % binding of antibodies in the presence of added free triskelions.

Table II. Distribution of IgG binding sites on triskelions

Distance of IgG binding sites from the triskelion center (nm)	Observed number of bound IgG
0 – 5.0	30
5.1 – 10.0	17
10.1 – 15.0	16
15.1 – 20.0	6

Table III. Number of triskelion-IgG complexes in the absence and presence of added free light chains

	Triskelions with attached IgG	Unlabelled triskelions	Small aggregates (ϕ 200 – 300 nm)	Uninterpretible objects
Triskelions plus light-chain specific antibodies[a]	40	51	69	45
Triskelions plus free light chains plus light chain-specific antibodies[b]	10	124	40	57

[a]205 objects
[b]231 objects

which were then incubated with the antiserum in a spot assay. Binding of light chain-specific antibodies to intact immobilized triskelions was readily inhibited by mixing free light chains with the antibody solution before it was spotted on to the triskelions (Table I). Light chains that are still attached to clathrin triskelions were slightly less effective competitors than free light chains. The important conclusion from this control is that triskelions *per se* are no better competitors than free light chains, which indicates that heavy chains do not react with anti-light chain antibodies. Moreover, elastase- and trypsin-treated triskelions, which are largely devoid of light chains, bound hardly any antibody.

To visualize immuno-complexes in the electron microscope, intact triskelions were incubated with an excess of affinity-purified antibodies. Unbound antibodies were removed by gel filtration after 1 h of incubation at room temperature. Fractions corresponding to the void volume were immediately processed for electron microscopy. A field of rotary-shadowed triskelions that had been incubated with antibodies is shown in Figure 10. Among large immuno-aggregates and some free triskelions, one can discern many triskelions with IgG molecules attached to the proximal parts of their legs. When I attempted to quantify the distribution of IgG binding sites along triskelion legs, I found that >50% of the sites lay within 5 nm of the triskelion center, but the number of triskelions bearing antibody molecules further from their mid-points also appears significant (Table II). The largest observed distance between antibody binding sites is 16 nm, which suggests that bound light chains must be no shorter than this.

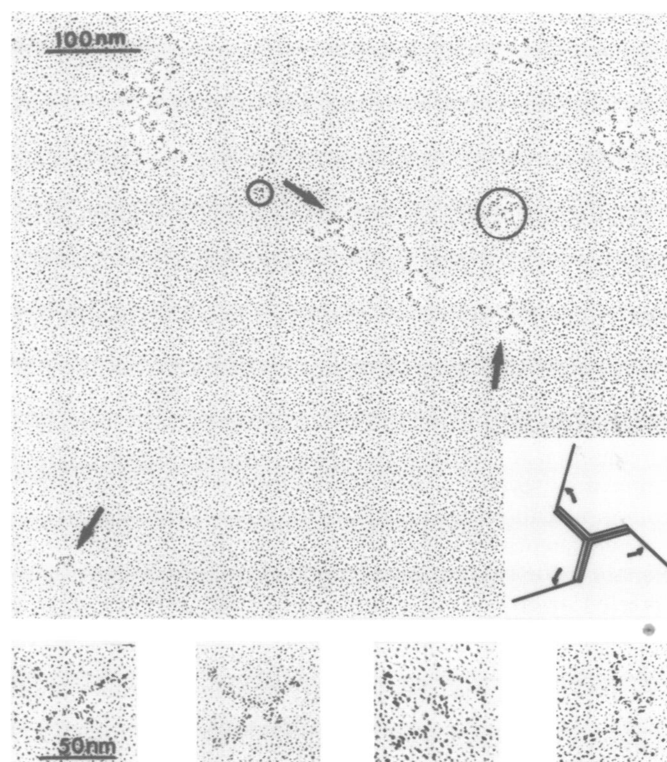

Fig. 10. Electron micrograph of rotary-shadowed clathrin triskelions, which had been incubated with light chain-specific antibodies. Arrows indicate IgG molecules attached to triskelions. The circles denote free IgG and IgM molecules. Inset shows schematically the location of clathrin light chains on a triskelion. All binding sites for light chain-specific antibodies were confined to the proximal parts of the legs (in the boxes). The arrows indicate the positions of trypsin cuts that result in the removal of most of the distal parts of the legs. The residual truncated triskelions bind light chains normally.

Upon mixing free light chains with triskelions and antibodies the number of labelled triskelions dropped significantly (Table III), which indicates that under the conditions of this experiment, as elsewhere, the antibodies attached solely to light chains.

Discussion

The biochemical and immuno-electron microscopic data demonstrate that clathrin light chains bind to the proximal parts of triskelion legs (Figure 10, inset). This finding corroborates and extends our earlier observations, in which we used electron microscopy to localize biotin-labelled light

chains near the triskelion center after labelling them with ferritin-conjugated avidin (Ungewickell *et al.*, 1982). Because of the smaller size of the IgG molecule compared with the ferritin label, it is now also possible to distinguish between several different antibody binding sites. Most antibodies appear to bind within 5 nm of the center of the triskelion, but I also observed several near the kink in the legs. Since the average distance between kink and center is 16 nm (Crowther and Pearse, 1982) and because α and β light chains have common binding sites, it can be concluded with some assurance that bound light chains are at least 16 nm long. In this case, they would be no more than 2 nm in diameter. However, on the basis of the data one cannot entirely rule out a model whereby they extend from the kink of one leg, through the triskelion apex, to the kink of another leg. Very recently, Kirchhausen *et al.* (1983) showed by immuno-electron microscopy that several α light chain-specific monoclonal antibodies bind to a determinant at the triskelion center.

The presence of light chains at the kinks provides an explanation for observed structural distortions of light chain-depleted triskelions. Usually, triskelions are very regular structures with their legs bent in a preferred direction (Ungewickell and Branton, 1981), in marked contrast to light chain-free triskelions and indeed, too, triskelion-antibody complexes (Figure 10), in which particularly the distal leg segments are more randomly oriented (Schmid *et al.*, 1982; Ungewickell *et al.*, 1982). It may therefore be concluded that one function of the light chains is to confer rigidity on this region of the triskelion leg.

Free light chains in solution are monomeric proteins with very little or no defined tertiary structure. This is probably one reason for their 'heat resistance', which they share with other proteins such as calmodulin (Cheung, 1980), tropomyosin (Bailey, 1948) and microtubule-associated proteins (Herzog and Weber, 1978). Heat stability and presumably similarities in SDS-polyacrylamide gel electrophoresis encouraged Brodsky *et al.* (1983) to resurrect speculations on a possible relationship between clathrin light chains and tropomyosin. To our original arguments against any relationship between these proteins (Ungewickell and Branton, 1981) one may now add the following: tropomyosin is a rigid coiled-coil with an α-helical content of >90% (Caspar *et al.*, 1969). The light chains of clathrin are neither rigid nor rich in α-helix. Furthermore, tropomyosins contain no prolines (Stone and Smillie, 1978), because this amino acid cannot be accommodated in an α-helical conformation. In contrast, the clathrin light chains possess at least 17 prolines (data not shown). Finally, tropomyosins are necessarily dimers with a mol. wt. of ~66 000, whereas clathrin light chains are monomers with a mol. wt. of 33 000.

The electrophoretic mobility of light chains, in contrast to that of heavy chains, is species- and tissue-specific (Pearse, 1976, 1978). For example, human placental light chains migrate on SDS-polyacrylamide gels with apparent mol. wts. of 33 000 and 31 000. Moreover, antibodies directed against bovine brain light chains cross-react only rather poorly with the 31 000 dalton placental light chain and not at all with the larger one. But despite all these differences both of the placental light chains are capable of binding to bovine brain heavy chains, thus indicating the conservation of relevant parts of both the light chains and heavy chains across species and tissue boundaries. Differences between light chains reveal themselves not only between such remote sources as bovine brain and human placenta, but also between bovine brain and kidney, for the anti-brain light chain antibody recognizes only one light chain in solubilized bovine kidney extracts. This species migrates in SDS gels slightly more rapidly than the brain β light chain.

Currently it is unclear if the observed tissue specificity of the light chains has a functional significance. Apart from a stabilizing effect on the overall structure of triskelions and clathrin cages (Branton and Ungewickell, unpublished data) no other functions of the light chains are known. However, judged by their location on the triskelion, light chains may be capable of regulating polyhedral angles within the lattice of the coat and may thus determine the curvature of the lattice. Furthermore, they are placed so that they might be conjectured to modulate clathrin-membrane interactions which have been shown to be localized within the proximal part of triskelion legs (Unanue *et al.*, 1981).

Materials and methods

Purification of clathrin light chains

Clathrin triskelions were purified according to Ungewickell and Branton (1981) and used as starting material for the preparation of light chains (LC). To dissociate LC from heavy chains (HC), triskelions were dialyzed overnight at 5°C against 30 mM Tris-HCl, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), 0.02% NaN_3, 6 M guanidine-HCl (BRL). Free LC were separated from HC by gel filtration on a 2.5 x 100 cm Sepharose C1-6B column (Pharmacia), which was equilibrated in the same solution. Typically, 5 – 10 ml clathrin at 1 mg/ml were applied. Protein-containing fractions were identified by absorbance at 280 nm. For polypeptide analysis by SDS-PAGE according to Laemmli (1970), 50 µl aliquots of the desired fractions were desalted by low speed centrifugation through small G-25 columns, packed in 1 ml Eppendorf pipette tips. For ion-exchange chromatography on DE 52 (Whatman), pooled light chain-containing fractions were dialyzed for 24 h with three changes against 10 mM Tris-HCl, 0.2 mM EDTA, 0.2 mM DTT, 0.02% NaN_3, pH 7.5 (buffer I).

Alternatively, LC were prepared by heat inactivation, essentially according to Lisanti *et al.* (1982): 2 ml aliquots of clathrin (1 mg/ml) in 0.1 M morpholino-ethanesulfonic acid (MES), 1 mM EGTA, 0.5 mM $MgCl_2$, 0.02% NaN_3, pH 6.5 (buffer II) were heated for 3 min in a waterbath at 90°C. Precipitated protein was removed by centrifugation for 20 min at 12 000 *g* in a Sorvall HB4 rotor. The supernatant was dialysed against buffer I. 2 – 3 mg/ml LC in buffer I were applied to a 0.8 x 20 cm DE 52 column, also equilibrated in buffer I. Proteins were eluted with a 200 ml linear 40 – 300 mM NaCl gradient at a constant flow rate of 20 ml/h. Protein-containing fractions were dialyzed against buffer II and stored frozen in aliquots at –70°C.

For the preparation of clathrin LC from human placenta, coated vesicles from placenta were prepared according to Pearse (1982). All subsequent purification steps were identical to those employed for bovine brain LC.

Protein concentrations

All concentrations were determined spectrophotometrically. For clathrin a specific absorbance at 280 nm of $E^{1\%}_{1\,cm}$ = 11.9 was used (Unanue *et al.*, 1981). LC concentrations were determined by measuring the absorbance of their peptide bonds at 215 nm and 225 nm, which is substantially independent of amino acid composition and protein conformation. Concentrations were calculated according to the relation c (mg/ml) = 0.144 ($A^{215\ nm}$ – $A^{225\ nm}$) (Wadell, 1956).

Stoke radius determination

A G-100 superfine column (1.5 x 100 cm) was calibrated with a series of protein standards of known Stokes radius (Pharmacia). The void volume was determined from the elution profile of Dextran Blue 2000 (Pharmacia) and the included volume from that of Bromophenol Blue. All volumes were determined by weighing. ^{3}H-Labelled LC were run together with two standards (ovalbumin and RNase A). The flow rate was maintained at 5 ml/h.

Ultracentrifugation

Sedimentation coefficients and mol. wts. were determined in a Beckman model E analytical ultracentrifuge. For determination of sedimentation coefficients, 0.5 mg/ml LC in 5 mM sodium phosphate, 0.1 M NaCl, pH 7.0, were centrifuged at 20°C in a synthetic boundary cell at 60 000 r.p.m. using the Schlieren optical system. The mol. wt. of LC was determined in the same

solvent by the high-speed meniscus depletion method (Yphantis, 1964), using the Rayleigh interference optical system. The protein concentration was 0.4 mg/ml and the temperature 20°C.

C.d. measurements

C.d. measurements were made in a Cary 61 instrument at room temperature. The LC concentration was 0.36 mg/ml and a cell of 2 mm pathlength was used. For denaturation experiments, weighed amounts of solid guanidine HCl (BDH) were added to the cell. The guanidine concentration and volume changes were calculated using the data of Kawahara and Tanford (1966); percentage α-helix was estimated from the molar residue ellipticity (θ) at 208 nm using the relationship given by:

$$\% \ \alpha\text{-helix} = \frac{(\theta)_{280\ \mathrm{nm}} - 4000 \times 100}{33\,000 - 4000} \quad \text{(Greenfield and Fasman, 1969)}$$

Binding experiments

LC were radioactively labelled with tritium by reductive methylation to a specific activity of 0.1 mCi/mg (Tack *et al.*, 1980). [^{3}H]Sodium borohydride with a specific activity of 10 Ci/mmol was obtained from Amersham.

Light chain-free triskelions were prepared by exposing clathrin cages to elastase under conditions that cause selective proteolysis of LC (Kirchhausen and Harrison, 1981). 125 000 and 112 000 dalton HC fragments were prepared by trypsin digestion of clathrin cages according to Ungewickell *et al.* (1982).

For a typical binding experiment, 5 μg clathrin cages were incubated with labelled LC in 0.1 ml buffer II that contained 1 mg/ml bovine serum albumin (BSA). The mixture was incubated on ice for 1 h. Prior to ultracentrifugation the volume was brought to 0.3 ml with buffer II and two aliquots of 10 μl each were removed for determination of radioactivity. The remaining sample was centrifuged for 10 min at 60 000 *g* in a Beckman Ti 50 rotor, using 0.6 ml nitrocellulose tubes with appropriate adaptors. After centrifugation, 0.2 ml of the supernatant were removed and mixed with Aquasol (NEN) and counted in a Beckman scintillation counter.

For binding experiments with unlabelled bovine brain or placenta LC, 12 μg LC were incubated with a variable amount of HC fragments in 0.1 ml of buffer II. After 1 h incubation the samples were centrifuged for 10 min in a Beckman airfuge at 20 psi. The polypeptide composition of supernatants and pellets was analyzed by SDS-PAGE.

In competition experiments, clathrin HC were incubated for 10 min with a 3-fold molar excess of unlabelled LC prior to addition of tritium-labelled LC.

Antibodies against light chains

Two LOU rats were each injected s.c. with 50 μg LC, emulsified in complete Freund's adjuvant. The animals were given three similar doses in incomplete Freund's adjuvant at biweekly intervals. To test the titer of the serum, 0.5 ml blood were taken from the tails of the animals. The titer was determined by a nitrocellulose solid phase spot assay, based on a procedure of Fisher *et al.* (1982), as modified by Amos and Kilmartin (personal communication). In brief, 0.1 μg aliquots LC in 5 mM sodium phosphate, pH 7.0, were spotted onto a grid pattern that had been previously outlined with a pencil on a sheet of nitrocellulose paper (BA 85, 0.45 μm, Schleicher and Schüll). After air-drying the protein binding capacity of the paper was saturated by incubating it for 30 min in 0.05 M Tris-HCl, pH 7.5, 0.15 M NaCl, 5 mM EDTA, 0.25% gelatin, 0.05% Nonidet P40 (Shell), 0.02% NaN_3 (buffer III) containing 3% BSA. The paper was then briefly rinsed in buffer III to remove excess BSA and air-dried. Dilutions of the antisera were then spotted onto the antigen and air-dried. Unbound antibody was removed during periods of extensive washes with buffer III containing 0.1% SDS and 0.5% Nonidet P40. The paper was then incubated for 1 h with iodinated goat anti-rat antibodies in buffer III. After extensive washings in SDS-containing buffer III to remove unbound radioactivity, the paper was subjected to autoradiography according to Laskey and Mills (1977).

Affinity purification of antibodies

100 μg LC, electrophoretically eluted from preparative SDS gels (Mendel-Hartvig, 1982), were coupled to 1 ml CNBr-activated Sepharose 4B (Pharmacia). Purified IgGs were applied to the column at room temperature and after extensive washings with 5 mM sodium phosphate, 0.5 M NaCl, pH 7.0, LC-specific antibodies were eluted in the cold with 0.2 M glycine, pH 2.6. After neutralization and addition of BSA to a final concentration of 1 mg/ml, the titer was tested in the spot assay. If necessary the antibody solution was concentrated in an A-75 Minicon concentrator (Amicon).

Nitrocellulose gel replicas

After SDS-PAGE, polypeptides were transferred electrophoretically from the polyacrylamide gel to the nitrocellulose paper (Towbin *et al.*, 1979). Prior to staining with antibodies, the paper was incubated with 3% BSA in buffer III as described above. For staining with antibodies the paper was incubated at room temperature for 1 – 2 h with appropriate dilutions of sera. All subsequent washes and incubations were performed as described for the spot assay.

Immuno-electron microscopy

Triskelions in 30 mM Tris-HCl, 100 mM NaCl, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.8, were incubated with a 3-fold molar excess of affinity-purified LC-specific antibodies. After 1 h at room temperature, unbound antibody and BSA were removed by gel filtration on Ultrogel ACA 34 (LKB), packed in a disposable 1 ml syringe. The gel was equilibrated with 0.1 M ammonium acetate, pH 8.0. Incubation volumes were generally varied between 30 μl and 50 μl. Fractions of ~50 μl were collected and those corresponding to the void volume of the column were immediately mixed with an equal volume of glycerol (Tyler and Branton, 1980); 5 – 10 μl aliquots of the samples were sucked into 10 μl disposable glass pipettes which were mounted with plasticine on top of the nozzle of an atomizer (Cam Lab), and sprayed onto freshly cleaved mica. The mica was immediately transferred to an Edwards high vacuum unit, in which the molecules were dried down and rotary-shadowed at an angle of 10° with platinum/tungsten. Resistance heating was used to evaporate the alloy. Replicas were stabilized by a thin carbon film and examined in a Philips electron microscope at 80 kV.

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